Reference Type: Journal Article
Record Number: 312
Author: Wegst, W.; Lingens, F.
Year: 1981
Title: [Degradation of L-phenylalanine and of aromatic carboxylic acids by chloridazon-degrading bacteria. Combination of side chain degradation and dioxygenase pathway]
Journal: Hoppe Seylers Z Physiol Chem
Volume: 362
Issue: 9
Pages: 1219-27
Abstract: Strain N of Chloridazon-degrading bacteria degrades phenylalanine via cis-2,3-dihydro-2,3-dihydroxyphenylalanine,2,3-dihydroxyphenylalanine aspartate and 4-hydroxy-2-oxovalerate [Hoppe-Seyler's Z. Physiol. Chem. 360, 957--969, (1979); Biochem. J. 194, 679--684 (1981)]. cis-2,3-Dihydro-2,3-dihydroxyphenylalanine and 2,3-dihydroxyphenylalanine as well as phenylpyruvate, cis-2,3-dihydro-2,3-dihydroxyphenylpyruvate, 2,3-dihydroxyphenylpyruvate, cis-2,3-dihydro-2,3-dihydroxyphenylacetate, 2,3-dihydroxyphenylacetate and 2,3-dihydroxybenzaldehyde are detectable in the medium of strain E during growth on phenylalanine. Incubation with phenylacetate, 3-phenylpropionate or 4-phenylbutyrate leads to the accumulation of the corresponding cis-2,3-dihydro-2,3-dihydroxyphenyl derivatives. These compounds are transformed with dihydrodiol dehydrogenase to 2,3-dihydroxyphenylacetate, 3-(2,3-dihydroxyphenyl)propionate and 4-(2,3-dihydroxyphenyl)-butyrate, 3-(2,3-dihydroxyphenyl)propionate is attacked by a catechol 2,3-dioxygenase and the meta-cleavage product is again cleaved by a hydrolase yielding succinate. In a similar reaction sequence the degradation of 4-phenylbutyrate leads to the formation of glutarate. From the growth medium of strain E on phenylacetate also small amounts of 2-, 3- and 4-hydroxyphenylacetate were isolated. Resting cells were shown to metabolize 3- and 4-hydroxyphenylacetate via homogentisate and 3,4-dihydroxyphenylacetate. In the culture medium of strain K2AP benzoate could be detected. Pathways for the degradation of phenylalanine and aromatic carboxylic acids in chloridazon degrading bacteria are proposed.

Reference Type: Journal Article
Record Number: 313
Author: Wegst, W.; Tittmann, U.; Eberspacher, J.; Lingens, F.
Year: 1981
Title: Bacterial conversion of phenylalanine and aromatic carboxylic acids into dihydrodiols
Journal: Biochem J
Volume: 194
Issue: 3
Pages: 679-84
Abstract: Strain E of chloridazon-degrading bacteria, when grown on L-phenylalanine accumulates cis-2,3-dihydro-2,3-dihydroxyphenylalanine. In experiments with resting cells and during growth the bacterium converts the aromatic carboxylic acids phenylacetate, phenylpropionate, phenylbutyrate and phenyl-lactate

into the corresponding cis-2,3-dihydrodiol compounds. The amino acids L-phenylalanine, N-acetyl-L-phenylalanine and t-butyloxycarbonyl-L-phenylalanine were also transformed into dihydrodiols. All seven dihydrodiols, thus obtained, were characterized both by conventional analytical techniques and by the ability to serve as substrates for a cis-dihydrodiol dehydrogenase.

Reference Type: Journal Article
Record Number: 311
Author: Furukawa, I.; Kurooka, S.; Arisue, K.; Kohda, K.; Hayashi, C.
Year: 1982
Title: Assays of serum lipase by the "BALB-DTNB method" mechanized for use with discrete and continuous-flow analyzers
Journal: Clin Chem
Volume: 28
Issue: 1
Pages: 110-3
Abstract: We successfully adapted the dimercaprol (BAL) tributyrate-5,5'-dithiobis(2-nitrobenzoic acid) method (J. Biochem. 81: 361, 1977) for assay of lipase in human serum to a discrete analyzer (the TBA 880) (I) or a continuous-flow analyzer (AutoAnalyzer, Type II) (II). In both, BAL-tributyrate is used as substrate, in combination with serum esterase inhibitors and a chromogenic reagent for the SH group of the liberated BAL. Serum lipase activities of patients with pancreatic diseases, measured at 90 or 40 samples per hour by I or II, respectively, correlated well with those measured by the corresponding manual method or by Kaplan's radioassay (Anal. Biochem. 33: 213, 1970). The correlation coefficients were all greater than 0.95, and the coefficients of variation were less than 8%, showing the practical usefulness of these procedures.

Reference Type: Journal Article
Record Number: 310
Author: Rick, W.; Hockeborn, M.
Year: 1982
Title: [Determination of lipase catalytic activity with 2,3-dimercapto-1-propanol-tributyrate as substrate]
Journal: J Clin Chem Clin Biochem
Volume: 20
Issue: 8
Pages: 537-52
Abstract: The two point test for the determination of lipase by Kurooka et al. (J. Biochem. Tokyo 81, 361-369 (1977)) was studied in detail. This procedure uses 2,3-dimercapto-1-propanol-tributyrate as substrate and Ellman's reagent as an acceptor for the released thiol groups. The time course of the enzymic hydrolysis of the substrate showed a pronounced lag phase, which can be influenced by sodium glycocholate. There is no proportionality between the quantity of added serum and the concentration of released thiol groups. Preincubation of the sample with the esterase inhibitor, phenylmethylsulphonylfluoride, as recommended by the authors, does not completely inhibit the serum esterase activity. The action of sodium dodecyl sulphate, which is included in the system, is not explained; in the continuous titrimetric test with triolein

as substrate, it acts as a powerful lipase inhibitor. Using 104 serum samples, significant differences were found between the results from this method and those obtained by the titrimetric determination of lipase. Possible fundamental improvements of this test system, using thioesters as substrate, are discussed.

Reference Type: Journal Article
Record Number: 309
Author: Sariaslani, F. S.; Sudmeier, J. L.; Focht, D. D.
Year: 1982
Title: Degradation of 3-phenylbutyric acid by Pseudomonas sp
Journal: J Bacteriol
Volume: 152
Issue: 1
Pages: 411-21
Abstract: Pseudomonas sp. isolated by selective culture with 3-phenylbutyrate (3-PB) as the sole carbon source metabolized the compound through two different pathways by initial oxidation of the benzene ring and by initial oxidation of the side chain. During early exponential growth, a catechol substance identified as 3-(2,3-dihydroxyphenyl)butyrate (2,3-DHPB) and its meta-cleavage product 2-hydroxy-7-methyl-6-oxononadioic-2,4-dienoic acid were produced. These products disappeared during late exponential growth, and considerable amounts of 2,3-DHPB reacted to form brownish polymeric substances. The catechol intermediate 2,3-DHPB could not be isolated, but cell-free extracts were able only to oxidize 3-(2,3-dihydroxyphenyl)propionate of all dihydroxy aromatic acids tested. Moreover, a reaction product caused by dehydration of 2,3-DHPB on silica gel was isolated and identified by spectral analysis as (--)-8-hydroxy-4-methyl-3,4-dihydrocoumarin. 3-Phenylpropionate and a hydroxycinnamate were found in supernatants of cultures grown on 3-PB; phenylacetate and benzoate were found in supernatants of cultures grown on 3-phenylpropionate; and phenylacetate was found in cultures grown on cinnamate. Cells grown on 3-PB rapidly oxidized 3-phenylpropionate, cinnamate, catechol, and 3-(2,3-dihydroxyphenyl)propionate, whereas 2-phenylpropionate, 2,3-dihydroxycinnamate, benzoate, phenylacetate, and salicylate were oxidized at much slower rates. Phenylsuccinate was not utilized for growth nor was it oxidized by washed cell suspensions grown on 3-PB. However, dual axenic cultures of Pseudomonas acidovorans and Klebsiella pneumoniae, which could not grow on phenylsuccinate alone, could grow syntrophically and produced the same metabolites found during catabolism of 3-PB by Pseudomonas sp. Washed cell suspensions of dual axenic cultures also immediately oxidized phenylsuccinate, 3-phenylpropionate, cinnamate, phenylacetate, and benzoate.

Reference Type: Journal Article
Record Number: 308
Author: Hathaway, J. A.; Kitt, D.; Wingate, B.
Year: 1983
Title: A comparison of currently used serum lipase and amylase procedures in the serial detection of enzyme elevations in acute pancreatitis
Journal: Clin Chim Acta
Volume: 133

Issue: 3
Pages: 327-30
Abstract: Twenty-eight patients having acute pancreatitis were followed during convalescence with serum amylase and lipase determinations. Starch and p-nitrophenyl-oligosaccharide substrates were used for amylase. Dimercaptotributyrate and triolein were employed for lipase. The extreme sensitivity of the lipase procedure using the tributyrate detected a persistent elevation of lipase when other parameters of measurement had returned to normal.

Reference Type: Journal Article
Record Number: 307
Author: Potempska, A.; Loo, Y. H.; Wisniewski, H. M.
Year: 1984
Title: On the possible mechanism of phenylacetate neurotoxicity: inhibition of choline acetyltransferase by phenylacetyl-CoA
Journal: J Neurochem
Volume: 42
Issue: 5
Pages: 1499-501
Abstract: The influence of phenylacetate, phenylbutyrate, and phenylacetyl-CoA on the activity of choline acetyltransferase and S-acetyl-CoA synthetase was investigated in vitro. Phenylacetyl-CoA was found to be a very potent inhibitor of choline acetyltransferase, competitive for acetyl-CoA with Ki of 3.1 X 10(-7)M. In contrast, millimolar concentrations of phenylacetate and phenylbutyrate were required to inhibit the activity of the enzyme. Activity of S-acetyl-CoA synthetase was affected only slightly by the three agents in concentrations of 10(-3)-10(-2)M. At this time, results are interpreted to suggest that in phenylketonuria, phenylacetate exerts its neurotoxic action through its metabolic product, phenylacetyl-CoA, which could severely decrease the availability of acetyl-CoA.

Reference Type: Journal Article
Record Number: 305
Author: Furukawa, Y.; Fujita, S.; Yamada, H.; Kanaya, T.; Kunita, S.; Kawanishi, M.; Nakamura, M.; Yoshida, S.; Kajiyama, G.
Year: 1986
Title: [A new simplified colorimetric assay of colipase in duodenal juice using BALB as substrate and its clinical application]
Journal: Nippon Shokakibyo Gakkai Zasshi
Volume: 83
Issue: 7
Pages: 1367-75

Reference Type: Journal Article
Record Number: 306
Author: Jones, T. R.; Smithers, M. J.; Betteridge, R. F.; Taylor, M. A.; Jackman, A. L.; Calvert, A. H.; Davies, L. C.; Harrap, K. R.
Year: 1986

Title: Quinazoline antifolates inhibiting thymidylate synthase: variation of the amino acid
Journal: J Med Chem
Volume: 29
Issue: 6
Pages: 1114-8
Abstract: Five new analogues (1c-g) of the antifolate N10-propargyl-5,8-dideazafolic acid (1a) are described in which the benzoyl-L-glutamate moiety was replaced by benzoic acid (desglutamyl-N10-propargyl-5,8-dideazafolic acid), benzoyl-L-aspartate, 4-phenylbutyrate, benzoylglycine, and benzoyl-L-alanine. The esters of the appropriate 4-aminophenyl (benzoyl) starting materials were sequentially alkylated upon nitrogen, first with a propargyl halide and then with 2-amino-6-(bromomethyl)-4-hydroxyquinazoline hydrobromide. Saponification of the antifolate esters so produced gave the desired analogues. The new derivatives (1c-g) and also the known diethyl ester of 1a (1b) were tested for their inhibition of purified L1210 thymidylate synthase (TS) and for their inhibition of the growth of L1210 cells in culture. The TS inhibition of the analogues 1b-g was estimated by calculating the inverse relative potency, defined as the ratio IC50(compound)/IC50(1a). The results obtained were as follows: greater than 62, 84, 9, 333, 21, and 5, respectively. All were thus less inhibitory than 1a. None of the compounds improved upon 1a in inhibiting the growth of L1210 cells in culture.

Reference Type: Journal Article
Record Number: 304
Author: Riley, T. V.
Year: 1987
Title: A note on hydrolysis of tributyrin by Branhamella and Neisseria
Journal: J Appl Bacteriol
Volume: 62
Issue: 6
Pages: 539-42
Abstract: Sixty-three strains of Branhamella and Neisseria were tested by two methods for their ability to hydrolyse glycerol tributyrate. After the conventional plate test, gas liquid chromatographical (GLC) analysis of the agar medium was carried out to detect the hydrolysis product, butyric acid, and other volatile fatty acids. All strains of Branhamella catarrhalis, Neisseria caviae, N. cuniculi and N. ovis but no other Neisseria spp. gave positive results with the conventional test. With GLC, however, most strains of Branhamella and Neisseria were shown to liberate butyric acid. In addition, some strains liberated acetic and isovaleric acids. Greater amounts of butyric acid were produced by clinical strains, in particular B. catarrhalis, compared with reference strains. It was concluded that the conventional plate test for tributyrin hydrolysis differentiates B. catarrhalis, N. caviae, N. cuniculi and N. ovis from other Neisseria.

Reference Type: Journal Article
Record Number: 303
Author: Peeters, P. A.; Claessens, C. A.; Eling, W. M.; Crommelin, D. J.
Year: 1988

Title: Immunospecific targeting of liposomes to erythrocytes
Journal: Biochem Pharmacol
Volume: 37
Issue: 11
Pages: 2215-22
Abstract: Immunoliposomes were made by covalently linking Fab' fragments (from rabbit antimouse erythrocyte IgG) to reverse-phase evaporation vesicles (REV) via maleimido-4-(p-phenylbutyrate) phosphatidylethanolamine (MPB-PE) as anchor molecule. These immunoliposomes were characterized in terms of size, charge, stability and antigen binding capacity. The effect of the liposomal Fab' density on the interaction with the target cell was studied. Two isolation methods were tested to separate free Fab' from liposomally bound Fab'. The necessity of deactivation of remaining reactive sites with dithiothreitol preincubation to increase the specificity of immunoliposome target cell interactions was demonstrated.

Reference Type: Journal Article
Record Number: 302
Author: Peeters, P. A.; Oussoren, C.; Eling, W. M.; Crommelin, D. J.
Year: 1988
Title: Immunospecific targeting of immunoliposomes, F(ab')2 and IgG to red blood cells in vivo
Journal: Biochim Biophys Acta
Volume: 943
Issue: 2
Pages: 137-47
Abstract: In this report a model to study the fate of target cells in the blood circulation after injection of appropriate immunoliposomes is discussed. The effect of intravenous administration of antimouse RBC immunoliposomes, F(ab')2 or IgG on the fate of intravenously injected 51Cr-labelled mouse RBC (Cr-mRBC) in the mouse and, particularly, in the rat was studied. The immunoliposome was of the Fab'-MPBPE-REV type (Fab'-fragments covalently linked to reverse phase evaporation vesicles by maleimido-4-(p-phenylbutyrate)phosphatidylethanolamine). In the rat model a high blood level (80%) of the injected dose of target cells, Cr-mRBC, was maintained for several hours. The elimination by Fab'-liposomes, F(ab')2 or IgG of Cr-mRBC, and subsequent uptake into liver and spleen was dose dependent. Administration of Fab'-liposomes or F(ab')2 resulted in a preferential uptake into the spleen (above a certain dose also, but much lower, uptake into the liver was observed), while after IgG administration 51Cr-label was mainly recovered in the liver. At equal protein doses (+/- 130 micrograms) Fab'-liposomes induced a faster elimination of the Cr-mRBC and a higher uptake into the spleen than F(ab')2. The potential advantage of the use of drug-loaded immunoliposomes to eliminate target cells from the blood stream and to induce a certain pharmacological effect in the target cells, in comparison with the free antibody administration of F(ab')2 or IgG is discussed.

Reference Type: Journal Article
Record Number: 300
Author: Pieroni, G.; Fourneron, J. D.

Year: 1990
Title: Lipases catalyse hydrolysis of fatty acid anhydrides
Journal: Eur J Biochem
Volume: 193
Issue: 1
Pages: 249-53
Abstract: Regio-specific and non-regio-specific lipases from mammals and microorganisms catalyse the hydrolysis of short, medium and long-chain fatty acid anhydrides. All the lipases tested in the present study can catalyse the hydrolysis of pure fatty acid anhydrides more efficiently than that of glycerol tributyrate. Molecular turnovers more than four times higher than that measured using glycerol tributyrate were calculated. The presence of 0.5% (by mass) anhydride in a triacylglyceride can double the initial rate of proton release during enzymatic hydrolysis. This should be taken into account when testing the chain specificity of a lipase for various synthetic substrates. Lipase inhibition was found to be associated very often with anhydride hydrolysis. The inhibition rates depended on the anhydride and the origin of the lipase. Inhibition of lipase activity is probably due to the formation of a poorly reversible acyl-lipase complex which differs from the classical fully reversible acyl-lipase complex at the catalytic centre.

Reference Type: Journal Article
Record Number: 301
Author: Tuchman, M.; Knopman, D. S.; Shih, V. E.
Year: 1990
Title: Episodic hyperammonemia in adult siblings with hyperornithinemia, hyperammonemia, and homocitrullinuria syndrome
Journal: Arch Neurol
Volume: 47
Issue: 10
Pages: 1134-7
Abstract: A 39-year-old man and his 42-year-old sister, both vegetarians, had episodic confusion for many years, but their mental function was normal between those episodes. They were recently diagnosed with hyperornithinemia, hyperammonemia, and homocitrullinuria syndrome. Hyperammonemia was documented during an episode of confusion in the male sibling but not in his sister. Both had elevated plasma ornithine, glutamine, and alanine levels and persistently low plasma lysine levels. Homocitrulline was present in their urine, and orotic aciduria and orotidinuria developed in the male sibling following ingestion of allopurinol. Studies on their cultured skin fibroblasts showed deficient metabolism of ornithine, indicating a defect in ornithine transport across the mitochondrial membrane. During therapy with citrulline and phenylbutyrate sodium, plasma ornithine levels increased in both patients, while plasma levels of glutamine and alanine decreased to normal. Since therapy started, their clinical conditions have also improved, and no recurrent neurologic dysfunction has occurred during a follow-up period of 20 months.

Reference Type: Journal Article
Record Number: 299

Author: Brusilow, S. W.
Year: 1991
Title: Phenylacetylglutamine may replace urea as a vehicle for waste nitrogen excretion
Journal: Pediatr Res
Volume: 29
Issue: 2
Pages: 147-50
Abstract: Phenylacetylglutamine (PAG), the amino acid acetylation product of phenylacetate (or phenylbutyrate after beta-oxidation) was evaluated as a waste nitrogen product in patients with inborn errors of urea synthesis. A boy with carbamyl phosphate synthetase deficiency receiving a low nitrogen intake excreted 80-90% of administered phenylacetate or phenylbutyrate as PAG. The amount of PAG nitrogen excreted varied from 38-44% of his dietary nitrogen, similar to the relationship between urea nitrogen and dietary nitrogen found in normal subjects receiving low dietary nitrogen. With few exceptions, neither phenylacetate nor phenylbutyrate accumulated in plasma. Treatment with relatively high dose phenylacetate or phenylbutyrate (0.5-0.6 g/kg/d) resulted in normal daytime levels of glutamine. These data suggest that PAG may replace urea as a waste nitrogen product when phenylbutyrate is administered at a dose that yields PAG nitrogen excretion equal to 40-44% of a low nitrogen intake.

Reference Type: Journal Article
Record Number: 295
Author: Besancon, X.; Smet, C.; Chabalier, C.; Rivemale, M.; Reverbel, J. P.; Ratomahenina, R.; Galzy, P.
Year: 1992
Title: Study of surface yeast flora of Roquefort cheese
Journal: Int J Food Microbiol
Volume: 17
Issue: 1
Pages: 9-18
Abstract: The change in yeast flora on the surface of two batches of Roquefort cheese was monitored over a period of 6 months. 401 isolates were determined and their technological properties were investigated. The main species isolated were: Debaryomyces hansenii and its non sporulating form Candida famata, Kluyveromyces lactis and its non sporulating form Candida sphaerica and Candida species. The species Debaryomyces hansenii inoculated on the surface of the cheese in one of the batches just before the salting phase was abundant throughout the ripening phases but never exceeded 50% of the yeast count. About 80% of the isolates of each species were resistant to 15% (w/v) of sodium chloride. Most of the species were able to assimilate lactose and lactic acid. 50-90% of the isolates of each species were able to hydrolyze rapeseed oil and glycerol tributyrate. Ten isolates among 401 hydrolyzed gelatin. Most of them were able to assimilate cadaverine, histamine, putrescine and tyramine.

Reference Type: Journal Article
Record Number: 296

Author: Dover, G. J.; Brusilow, S.; Samid, D.
Year: 1992
Title: Increased fetal hemoglobin in patients receiving sodium 4-phenylbutyrate
Journal: N Engl J Med
Volume: 327
Issue: 8
Pages: 569-70

Reference Type: Journal Article
Record Number: 298
Author: Elder, D. J.; Morgan, P.; Kelly, D. J.
Year: 1992
Title: Anaerobic degradation of trans-cinnamate and omega-phenylalkane carboxylic acids by the photosynthetic bacterium Rhodopseudomonas palustris: evidence for a beta-oxidation mechanism
Journal: Arch Microbiol
Volume: 157
Issue: 2
Pages: 148-54
Abstract: The mechanism responsible for the initial steps in the anaerobic degradation of trans-cinnamate and omega-phenylalkane carboxylates by the purple non-sulphur photosynthetic bacterium Rhodopseudomonas palustris was investigated. Phenylacetate did not support growth and there was a marked CO2 dependence for growth on acids with greater side-chain lengths. Here, CO2 was presumably acting as a redox sink for the disposal of excess reducing equivalents. Growth on benzoate did not require the addition of exogenous CO2. Aromatic acids with an odd number of side-chain carbon atoms (3-phenylpropionate, 5-phenylvalerate, 7-phenylheptanoate) gave greater apparent molar growth yields than those with an even number of side-chain carbon atoms (4-phenylbutyrate, 6-phenylhexanoate, 8-phenyloctanoate). HPLC analysis revealed that phenylacetate accumulated and persisted in the culture medium during growth on these latter compounds. Cinnamate and benzoate transiently accumulated in the culture medium during growth on 3-phenylpropionate, and benzoate alone accumulated transiently during the course of trans-cinnamate degradation. The transient accumulation of 4-phenyl-2-butenoic acid occurred during growth on 4-phenylbutyrate, and phenylacetate accumulated to a 1:1 molar stoichiometry with the initial 4-phenylbutyrate concentration. It is proposed that the initial steps in the anaerobic degradation of trans-cinnamate and the group of acids from 3-phenylpropionate to 8-phenyloctanoate involves beta-oxidation of the side-chain.

Reference Type: Journal Article
Record Number: 294
Author: Heeremans, J. L.; Kraaijenga, J. J.; Los, P.; Kluft, C.; Crommelin, D. J.
Year: 1992
Title: Development of a procedure for coupling the homing device glu-plasminogen to liposomes
Journal: Biochim Biophys Acta
Volume: 1117

Issue: 3
Pages: 258-64
Abstract: The aim of this study was to find a suitable way of coupling the homing-device glu-plasminogen to the outside of liposomes. The described procedure is based on the reaction of thiol-groups introduced in the protein with thiol-reactive groups of the liposome. Details on the thiolation of proteins with the reagent succinimidyl-S-acetylthioacetate (SATA) were studied for a model-protein, amylase. Increasing the incubation-ratio SATA: amylase resulted in a gradually growing number of introduced thiol-groups, until a maximum of about 5 mol SH per mol amylase was reached. The enzymatic activity of the derivatized protein was even higher than that of native amylase. The thiol-introduction was then applied to glu-plasminogen itself. After activation with SATA, the protein was incubated with liposomes containing the thiol-reactive anchor maleimido-4-(p-phenylbutyrate)-phosphatidylethanolamine (MPB-PE). Under the chosen conditions, incubation of 0.5-2.5 mg/ml protein with 6.0-7.5 mumol/ml phospholipid for 30-120 min resulted in coupling-ratios of 20 to 94 micrograms glu-plasminogen per mumol phospholipid. This corresponds with about 140 to 660 protein molecules per liposome. SATA-derivatization of glu-plasminogen brought about a loss of its enzymatic activity induced by streptokinase. This activity of liposomally coupled plasminogen was about 52 to 74% of the activity of native glu-plasminogen (depending on the coupling-ratio). Although this may seem a significant loss of activity, it was shown that the capacity of liposomal glu-plasminogen to bind to its target, fibrin, was not reduced but several fold higher under the used conditions than that of the free protein. Therefore, the described method for thiol-introduction is an effective way to thiolate amylase without loss of activity, and to bind the homing-device glu-plasminogen to liposomes without substantially interfering with its fibrin-binding/homing capacity.

Reference Type: Journal Article
Record Number: 297
Author: Wright, S. E.; Huang, L.
Year: 1992
Title: Bilayer stabilization of phosphatidylethanolamine by N-biotinylphosphatidylethanolamine
Journal: Biochim Biophys Acta
Volume: 1103
Issue: 1
Pages: 172-8
Abstract: We have examined the ability of biotinylated phosphatidylethanolamine and similar lipids to stabilize the bilayer phase of polymorphic dioleoylphosphatidylethanolamine (DOPE). Sonicated lipid mixtures were characterized in terms of their aggregation state, size and ability to encapsulate and retain the fluorescent dye, calcein. Titration of DOPE with N-biotinyl-PE indicated that stable liposomes could be produced by sonication of DOPE based dispersions containing N-biotinyl-PE at concentrations greater than 8 mol%. These liposomes were relatively small, could efficiently encapsulate calcein, and showed minimal leakage upon prolonged storage at 4 degrees C. Maleimido-4-(p-phenylbutyrate)-PE (MPB-PE) was equally effective at stabilizing the bilayer phase of DOPE whereas N-dinitrophenyl-PE and N-(dinitrophenyl-caproyl)-PE were relatively poor stabilizers, requiring at least 15 mol% for stabilization at pH 7.4. Differential scanning

calorimetry of dielaidoylphosphatidylethanolamine (DEPE)/N-biotinyl-PE mixtures indicated that stabilizer concentrations as low as 2 mol% could abolish the L alpha/HII phase transition of DEPE.

Reference Type: Journal Article
Record Number: 292
Author: Brusilow, S. W.; Finkelstien, J.
Year: 1993
Title: Restoration of nitrogen homeostasis in a man with ornithine transcarbamylase deficiency
Journal: Metabolism
Volume: 42
Issue: 10
Pages: 1336-9
Abstract: We evaluated the hypothesis that sodium phenylbutyrate-induced phenylacetylglutamine biosynthesis in a man with partial ornithine transcarbamylase (OTC) deficiency has a dual effect; it provides an additional vehicle for waste nitrogen excretion, and in the process it suppresses the patient's residual urea N synthesis, which then may be available for N homeostasis if the need arises. A 38-year-old man was studied over three periods. Period I was a control period during which he received a fixed caloric and N intake plus L-citrulline. Phenylbutyrate was added in period II and was maintained during period III, during which his N intake was increased. Plasma levels of ammonium and glutamine and net urea N synthesis were measured in each period; phenylacetylglutamine N synthesis was measured in periods II and III. These studies demonstrated that phenylbutyrate administration led to a 73% decrease in net de novo urea N synthesis during period II, which subsequently increased threefold in period III in response to the increased N intake. Phenylacetylglutamine N synthesis was 2.27 g/d, similar to his estimated maximum net urea N synthesis of 2.65 g/d. During periods II and III, his plasma levels of ammonium and glutamine improved as compared with period I when they were abnormally high. We conclude that sodium phenylbutyrate treatment of patients with urea cycle disorders who have significant residual enzyme activity results in both an improvement in waste N excretion and improved N homeostasis as a result of the generation of a reserve urea N synthetic capacity. This therapeutic approach may be useful in other nitrogen accumulation decreases, eg, portal-systemic encephalopathy.

Reference Type: Journal Article
Record Number: 293
Author: Fibach, E.; Prasanna, P.; Rodgers, G. P.; Samid, D.
Year: 1993
Title: Enhanced fetal hemoglobin production by phenylacetate and 4-phenylbutyrate in erythroid precursors derived from normal donors and patients with sickle cell anemia and beta-thalassemia
Journal: Blood
Volume: 82
Issue: 7
Pages: 2203-9

Abstract: In both sickle cell (SS) anemia and beta-thalassemia (beta-thal), an increase in fetal hemoglobin (HbF) ameliorates the clinical symptoms of the underlying disease. Several pharmacologic agents have been used to elevate HbF levels in adults; however, concerns regarding adverse effects of the prevailing drugs raise an urgent need for other agents capable of stimulating HbF production. We show here that sodium phenylacetate (NaPA) and its precursor, sodium 4-phenylbutyrate (NaPB), can enhance HbF production in cultured erythroid progenitor derived from normal donors and patients with SS anemia or beta-thal, when used at pharmacologic concentrations. Treatment resulted in (1) reduced cell proliferation, (2) elevated hemoglobin (Hb) content per cell (mean cellular Hb [MCH]), and (3) an increased proportion of HbF produced, associated with elevated levels of gamma-globin mRNA. Moreover, the active phenyl-fatty acids, with NaPA as a prototype, potentiated HbF induction by other drugs of clinical interest, including hydroxyurea (HU), sodium butyrate, and 5-azacytidine (5AzaC). Efficacy could be further enhanced by introducing chlorine substituents at the phenyl ring to increase drug lipophilicity. Our findings indicate that NaPA and NaPB, both already proven safe and effective in treatment of children with urea cycle disorders, might benefit also patients with severe hemoglobinopathies. The two-phase liquid culture procedure used in this study should prove valuable in further studies exploring the mechanisms of HbF induction by these agents, and might provide an assay to predict patient response in the clinical setting.

Reference Type: Journal Article
Record Number: 289
Author: Dover, G. J.; Brusilow, S.; Charache, S.
Year: 1994
Title: Induction of fetal hemoglobin production in subjects with sickle cell anemia by oral sodium phenylbutyrate
Journal: Blood
Volume: 84
Issue: 1
Pages: 339-43
Abstract: Intravenous arginine butyrate has been shown to increase fetal hemoglobin (HbF) in sickle cell and thalassemia patients. Recently, we observed that sodium 4-phenylbutyrate, a drug administered orally to treat urea cycle disorders, increases HbF production in nonanemic children and adults. We treated six subjects with sickle cell disease over a period of 14 to 179 days. All subjects received their initial therapy of 9 to 13 g/m2/day as 0.5-g tablets of sodium 4-phenylbutyrate as inpatients. All subjects showed a rapid increase in the percentage of F-reticulocytes (pretreatment, 1% to 20%; posttreatment, 10% to 44%). Four subjects were treated only 11 to 25 days as inpatients. Two of these four subjects failed to respond to the outpatient component because of their inability to maintain an intake of 30 to 40 tablets per day. One subject (C) developed a rash at day 10 and discontinued treatment at day 14. Another subject (B) was transfused for a painful crisis on day 25. Subject A, treated for 179 days, has an increased percentage of F cells, from 54% to 77%, and increased HbF levels, from 10.6% to 18%. Subject F, treated for 154 days, has an increased percentage of F cells, from 59% to 73%, and an increased percentage of HbF, from 10.4% to 16%. All subjects showed some increase in weight. Subject A developed mild transient ankle

edema. Myelotoxicity was not seen in any treated patient. Oral administration of sodium 4-phenylbutyrate rapidly increases F-cell production in sickle cell disease.

Reference Type: Journal Article
Record Number: 290
Author: Figg, W. D.; Walls, R. G.; Cooper, M. R.; Thibault, A.; Sartor, O.; McCall, N. A.; Myers, C. E.; Samid, D.
Year: 1994
Title: In vitro antitumor effect of hydroxyurea on hormone-refractory prostate cancer cells and its potentiation by phenylbutyrate
Journal: Anticancer Drugs
Volume: 5
Issue: 3
Pages: 336-42
Abstract: Previous clinical trials have suggested that hydroxyurea may possess some activity against prostate cancer. The in vitro antiproliferative activity of hydroxyurea was evaluated in three hormone-refractory prostate cancer cell lines, PC-3, DU-145 and PC-3M. Fifty-percent inhibition of growth in all three cell lines required prolonged (120 h) exposure to hydroxyurea at a concentration of approximately 100 microM. Using pharmacokinetic data obtained during the course of a clinical trial of hydroxyurea, we simulated a dosing regimen that would sustain plasma drug concentrations above 100 microM for 120 h (1 g loading dose, followed by 500 mg every 6 h for 5 days in a 70 kg man). Since this dosing regimen is likely to generate an unacceptable degree of myelosuppression, in vitro combination studies were conducted with hydroxyurea and phenylbutyrate, a new differentiating agent with no myelosuppressive effects. These studies resulted in a reduction of the hydroxyurea concentration necessary for 50% growth inhibition (50 microM of hydroxyurea plus 0.5 mM of phenylbutyrate). A regimen designed to achieve that hydroxyurea concentration (400 mg loading dose, followed by 200 mg every 6 h for 5 days) should be clinically achievable. Based on these results, this combination deserves further evaluation in patients with stage D prostate cancer.

Reference Type: Journal Article
Record Number: 288
Author: Liu, L.; Shack, S.; Stetler-Stevenson, W. G.; Hudgins, W. R.; Samid, D.
Year: 1994
Title: Differentiation of cultured human melanoma cells induced by the aromatic fatty acids phenylacetate and phenylbutyrate
Journal: J Invest Dermatol
Volume: 103
Issue: 3
Pages: 335-40
Abstract: The increasing incidence of melanoma and the poor responsiveness of disseminated disease to conventional treatments call for the development of new therapeutic approaches. Phenylacetate, a nontoxic differentiation inducer, can suppress the growth of other neuroectodermal tumors, i.e., gliomas, in laboratory models and in humans. This finding led us to explore the efficacy of phenylacetate and related aromatic fatty acids in melanoma. Phenylacetate and phenylbutyrate were

found to a) induce selective cytostasis and maturation of cultured human melanoma cells, b) modulate the expression of genes implicated in tumor metastasis (type IV collagenase and tissue inhibitor of metalloproteinases-2) and immunogenicity (HLA class I); and c) enhance the efficacy of other agents of clinical interest, including retinoids, interferon-alpha, suramin, and 5-aza-2'-deoxycytidine. Reflecting on the phenotypic heterogeneity of melanoma, the degree of biologic alterations induced by phenylacetate/phenylbutyrate varied significantly among the tumor cell lines tested. Although losing invasive capacity and tumorigenicity in athymic mice, poorly differentiated cells exhibited only a marginal change in morphology, remained amelanotic, and resumed growth after treatment was discontinued. By contrast, treatment of melanoma cells that were in a more advanced stage of maturation resulted in profound alterations in cell growth, morphology, and pigmentation consistent with terminal differentiation. The in vitro antitumor activity was observed with nontoxic, pharmacologic concentrations of phenylacetate and phenylbutyrate, suggesting potential clinical use of these drugs in the treatment of melanomas.

Reference Type: Journal Article
Record Number: 291
Author: Watson, A. M.; Chambers, H.; Chambers, J. E.
Year: 1994
Title: An investigation of activities and paraoxon sensitivities of hepatic aliesterases in beta-naphthoflavone-treated rats
Journal: Toxicol Lett
Volume: 71
Issue: 3
Pages: 217-25
Abstract: Aliesterases (carboxylesterases, EC 3.1.1.1) are serine esterases which may protect acetylcholinesterase during organophosphorus insecticide intoxication by providing alternative phosphorylation sites. Levels of hepatic aliesterase activity were investigated after the intraperitoneal administration of beta-naphthoflavone (BNF) to female rats using nine 4-nitrophenyl esters as substrates (including straight and branched chain aliphatic and aromatic esters) and 1-naphthyl acetate. In addition, the in vitro sensitivities of aliesterases to inhibition by paraoxon, the active metabolite of the common insecticide parathion, were studied. Hepatic aliesterases from BNF-treated rats displayed lower activities than those from the controls with all substrates except 4-nitrophenyl phenylbutyrate and isovalerate. The aliesterases from BNF-treated rats were more sensitive to paraoxon inhibition with 4-nitrophenyl phenylbutyrate, valerate, and butyrate. Esterases hydrolyzing 4-nitrophenyl butyrate, valerate, and branched chain esters were most sensitive to paraoxon inhibition while those hydrolyzing 4-nitrophenyl hexanoate and aromatic esters were least sensitive. The results suggested that BNF-induced changes in hepatic aliesterases could alter responses to organophosphates.

Reference Type: Journal Article
Record Number: 285
Author: Carlson, P.; Eerola, E.; Kontiainen, S.
Year: 1995

Title: Additional tests to differentiate Arcanobacterium haemolyticum and Actinomyces pyogenes
Journal: Zentralbl Bakteriol
Volume: 282
Issue: 3
Pages: 232-6
Abstract: A commercially available biochemical test panel, commercially available diagnostic tablets and gas-liquid chromatography (GLC) of cellular fatty acids were used to find out whether Arcanobacterium haemolyticum and Actinomyces pyogenes could be further differentiated from each other. Xylitol and alpha-methyl-D-glucoside fermentation, Voges-Proskauer reaction and tributyrate hydrolysis were found to be useful additional tests which differentiated Arc. haemolyticum and A. pyogenes. GLC analysis revealed major differences in the cellular 16:0, 18:2(9,12) and 18:1(9) fatty acid composition of the two species. Especially the Voges-Proskauer test available as diagnostic tablets can be easily performed in clinical microbiology laboratories, in addition to the tests now used to differentiate Arc. haemolyticum from A. pyogenes.

Reference Type: Journal Article
Record Number: 287
Author: Collins, A. F.; Pearson, H. A.; Giardina, P.; McDonagh, K. T.; Brusilow, S. W.; Dover, G. J.
Year: 1995
Title: Oral sodium phenylbutyrate therapy in homozygous beta thalassemia: a clinical trial
Journal: Blood
Volume: 85
Issue: 1
Pages: 43-9
Abstract: Butyrate analogues have been shown to increase fetal hemoglobin (HbF) production in vitro and in vivo. Sodium phenylbutyrate (SPB), an oral agent used to treat individuals with urea-cycle disorders, has been shown to increase HbF in nonanemic individuals and in individuals with sickle cell disease. We have treated eleven patients with homozygous beta thalassemia (three transfusion dependent) and one sickle-beta-thalassemia patient with 20 g/d (forty 500-mg tablets) of SPB for 41 to 460 days. All patients showed an increase in the percent of F reticulocytes associated with treatment, but only four patients responded by increasing their Hb levels by greater than 1 g/dL (mean increase, 2.1 g/dL; range, 1.2 to 2.8 g/dL). None of the transfusion-dependent thalassemia subjects responded. Increase in Hb was associated with an increase in red blood cell number (mean increase, 0.62 x 10(12)/L), and mean corpuscular volume (mean increase, 6 fL). Changes in percent HbF, absolute HbF levels, or alpha- to non-alpha-globin ratios as measured by levels of mRNA and globin protein in peripheral blood did not correlate with response to treatment. Response to treatment was not associated with the type of beta-globin mutation, but baseline erythropoietin levels of greater than 120 mU/mL was seen in all responders and only two of eight nonresponders to SPB. Compliance with treatment was greater than 90% as measured by pill counts. Side effects of the drug included weight gain and/or edema caused by increase salt load in 2/12, transient epigastric discomfort in 7/12, and abnormal body odor in 3/12 subjects. Two splenectomized patients who were not on prophylactic antibiotics developed sepsis

while on treatment. We conclude that SPB increases Hb in some patients with thalassemia, but the precise mechanism of action is unknown.

Reference Type: Journal Article
Record Number: 283
Author: Lea, M. A.; Tulsyan, N.
Year: 1995
Title: Discordant effects of butyrate analogues on erythroleukemia cell proliferation, differentiation and histone deacetylase
Journal: Anticancer Res
Volume: 15
Issue: 3
Pages: 879-83
Abstract: Actions of butyrate and structural analogues on histone deacetylase activity were compared with effects on proliferation and differentiation of erythroleukemia cells. Proliferation was inhibited by 5 mM tert- butylacetate, phenylacetate, phenylbutyrate, 3-bromopropionate and ethyl butyrate without induction of hemoglobin synthesis. n - Butyramide was a stronger inhibitor of cell proliferation and a more effective inducer of hemoglobin synthesis than isobutyramide. The data from combination studies were compatible with butyramide and isobutyramide being weaker agonists that competed for a common site with butyrate. Butyramide and isobutyramide were weaker inhibitors of histone deacetylase than 4-phenylbutyrate and phenylacetate, which in turn were less effective than 3-bromopropionate and butyrate. Butyrate and analogues had similar inhibitory effects on histone deacetylase activity in nuclei from mouse DS19 cells and human K562 cells. Effects on histone deacetylase did not show a consistent correlation with inhibition of cell proliferation or induction of hemoglobin synthesis.

Reference Type: Journal Article
Record Number: 282
Author: Liu, L.; Hudgins, W. R.; Miller, A. C.; Chen, L. C.; Samid, D.
Year: 1995
Title: Transcriptional upregulation of TGF-alpha by phenylacetate and phenylbutyrate is associated with differentiation of human melanoma cells
Journal: Cytokine
Volume: 7
Issue: 5
Pages: 449-56
Abstract: The aromatic fatty acids phenylacetate (PA) and phenylbutyrate (PB) induce tumour cell differentiation in experimental models and both are currently in clinical trials. The purpose of this study was to determine the effect of these antitumour agents on the expression of transforming growth factor-alpha (TGF-alpha) in neoplastic cells. Treatment of human melanoma 1011 cultures with either PA or PB caused over 40-fold increase in TGF-alpha biosynthesis and secretion into the media. Whereas elevation in TGF-alpha mRNA steady-state levels became evident within 6-12 h and reached peak quantities the following day, the amounts of its coded protein increased gradually over a period of 5 days of treatment. Further molecular analysis revealed that regulation of TGF-alpha expression occurred at the transcriptional level.

In contrast to TGF-alpha, expression of its receptor remained below detectable levels, indicating that an autocrine loop involving this growth factor is unlikely. Interestingly, the increase in TGF-alpha production paralleled drug-induced cytostasis and differentiation defined by morphological changes and increased melanogenesis. Like PA and PB, other differentiation inducers such as all-trans-retinoic acid, dimethyl sulfoxide, and 5-aza-2'-deoxycytidine, all induced TGF-alpha expression in the melanoma cells. The close association between enhanced TGF-alpha production and melanoma cell differentiation suggests that this growth factor, often linked to mitogenesis, may play a novel role in tumour differentiation by PA and PB.

Reference Type: Journal Article
Record Number: 280
Author: Maestri, N. E.; Clissold, D. B.; Brusilow, S. W.
Year: 1995
Title: Long-term survival of patients with argininosuccinate synthetase deficiency
Journal: J Pediatr
Volume: 127
Issue: 6
Pages: 929-35
Abstract: OBJECTIVE: To monitor long-term survival and outcome of patients with neonatal-onset argininosuccinate synthetase deficiency (ASD) who were treated with specific therapeutic protocols designed to activate alternative pathways of waste nitrogen excretion. DESIGN: Patients for this study included 24 infants born before 1990 and rescued from hyperammonemic coma caused by neonatal-onset ASD; they were referred to this center for enrollment in ongoing clinical studies of sodium benzoate, sodium phenylacetate, and sodium phenylbutyrate. Collaborating physicians throughout the United States and Canada provided information on survival, intellectual development, intercurrent hyperammonemic episodes, and anthropometric and biochemical measurements. RESULTS: The cumulative survival rate was 87.5% at 5 years and 72% at 10 years of age. Survivors include 15 patients currently treated with high doses of sodium phenylbutyrate; two patients have withdrawn. Among the treated group, 11 are classified as severely to profoundly mentally retarded. The remaining four patients have IQ measurements in the borderline to mentally retarded range. All patients have had intercurrent hyperammonemic episodes; our data indicate that the frequency of the episodes has decreased with implementation of the current protocol. These patients are growth retarded, but most have height-for-weight z scores within 2 SD of the mean. Laboratory studies of plasma amino acids and of hematopoietic, renal, and hepatic function are within normal limits with the exception of slightly elevated serum aminotransferase values. CONCLUSION: Our results indicate that these drugs are safe and that the current protocol improves survival rates. However, survival is accompanied by mental retardation, growth retardation, risk of hyperammonemic episodes, and the necessity of lifetime adherence to strict medication and dietary management.

Reference Type: Journal Article
Record Number: 286
Author: Newmark, H. L.; Young, C. W.
Year: 1995

Title: Butyrate and phenylacetate as differentiating agents: practical problems and opportunities
Journal: J Cell Biochem Suppl
Volume: 22
Pages: 247-53
Abstract: Differentiating agents, including butyrate, phenylacetate and several other agents, have long been known to alter abnormal or transformed cell lines in vitro to a more normal state including phenotype and function. The effect depends on prolonged exposure to a minimum concentration of the agent. In vivo studies of butyrate and analogues have been limited, largely due to rapid in vivo metabolism. A butyrate prodrug, the triglyceride tributyrin, shows great promise in achieving effective and prolonged serum levels when given orally to mice and rats, and has been recommended for human trial. In vitro, butyrate and its mono- and triglyceride have shown potent synergy with retinoic acid, suggesting a ten-fold reduction in serum level requirements. Other butyrate prodrugs have been prepared and studied; several sugar esters of butyrate show promise. Phenylacetate, a normal mammalian metabolite, is also a potent differentiating agent, but its clinical use is limited by its objectionable odor per se and in treated subjects. Phenylbutyrate, a prodrug of phenylacetate, is more acceptable and may have greater promise. The availability of effective prodrugs of effective differentiating agents, such as tributyrin and phenylbutyrate, creates many opportunities for possible therapeutic and chemopreventive applications, especially if synergy in vivo can be demonstrated with retinoids (e.g., retinoic acid) or deltanoids (e.g., active vitamin D analogues), confirming in vitro studies. Particular disease targets would include certain leukemias, thalassemia, and sickle cell anemia.

Reference Type: Journal Article
Record Number: 284
Author: Piscitelli, S. C.; Thibault, A.; Figg, W. D.; Tompkins, A.; Headlee, D.; Lieberman, R.; Samid, D.; Myers, C. E.
Year: 1995
Title: Disposition of phenylbutyrate and its metabolites, phenylacetate and phenylacetylglutamine
Journal: J Clin Pharmacol
Volume: 35
Issue: 4
Pages: 368-73
Abstract: Phenylacetate, an inducer of tumor cytostasis and differentiation, shows promise as a relatively nontoxic antineoplastic agent. Phenylacetate, however, has an unpleasant odor that might limit patient acceptability. Phenylbutyrate, an odorless compound that also has activity in tumor models, is known to undergo rapid conversion to phenylacetate by beta-oxidation in vivo. This phase I study examined the pharmacokinetics of phenylbutyrate and characterized the disposition of the two metabolites, phenylacetate and phenylacetylglutamine. Fourteen patients with cancer (aged 51.8 +/- 13.8 years) received a 30-minute infusion of phenylbutyrate at 3 dose levels (600, 1200, and 2000 mg/m2). Serial blood samples and 24-hour urine collections were obtained. Samples were assayed by high-performance liquid chromatography. A model to simultaneously describe the pharmacokinetics of all three compounds was developed using ADAPT II. Data were modeled as molar

equivalents. The model fit the data well as shown by mean (+/- SD) coefficients of determination (r2) for phenylbutyrate, phenylacetate, and phenylacetylglutamine, which were 0.96 +/- 0.07, 0.88 +/- 0.10, and 0.92 +/- 0.06, respectively. The intrapatient coefficient of variation percentage (CV%) around the parameter estimates were small (range 7.2-33.5%). Phenylbutyrate achieved peak concentrations in the range of in vitro tumor activity (500-2000 mumol/L) and exhibited saturable elimination (Km = 34.1 +/- 18.1 micrograms/mL and Vmax = 18.1 +/- 18 mg/h/kg). Metabolism was rapid; the times to maximum concentration for phenylacetate and phenylacetylglutamine were 1 and 2 hours, respectively. The conversion of phenylbutyrate to phenylacetate was extensive (80 +/- 12.6%), but serum concentrations of phenylacetate were low owing to rapid, subsequent conversion to phenylacetylglutamine.(ABSTRACT TRUNCATED AT 250 WORDS)

Reference Type: Journal Article
Record Number: 281
Author: Prasanna, P.; Shack, S.; Wilson, V. L.; Samid, D.
Year: 1995
Title: Phenylacetate in chemoprevention: in vitro and in vivo suppression of 5-aza-2'-deoxycytidine-induced carcinogenesis
Journal: Clin Cancer Res
Volume: 1
Issue: 8
Pages: 865-71
Abstract: Differentiation inducers selected for their low cytotoxic and genotoxic potential could be of major value in chemoprevention and maintenance therapy. We focus here on phenylacetate, a naturally occurring plasma component recently shown to affect the growth and differentiation of established neoplasms in experimental models. The ability of phenylacetate to prevent carcinogenesis by the chemotherapeutic hypomethylating drug 5-aza-2'-deoxycytidine (5AzadC) was tested in vitro and in mice. Transient exposure of immortalized, but poorly tumorigenic ras-transformed 4C8 fibroblasts to 5AzadC resulted in neoplastic transformation manifested by loss of contact inhibition of growth, acquired invasiveness, and increased tumorigenicity in athymic mice. The latter was associated with elevation in ras expression and a decline in collagen biosynthesis. These profound phenotypic and molecular changes were prevented by a simultaneous treatment with phenylacetate. Protection from 5AzadC carcinogenesis by phenylacetate was: (a) highly efficient despite DNA hypomethylation by both drugs, (b) free of cytotoxic and genotoxic effects, (c) stable after treatment was discontinued, and (d) reproducible in vivo. Whereas athymic mice bearing 4C8 cells developed fibrosarcomas following a single i.p. injection with 5AzadC, tumor development was significantly inhibited by systemic treatment with nontoxic doses of phenylacetate. Phenylacetate and its precursor suitable for oral administration, phenylbutyrate, may thus represent a new class of chemopreventive agents, the efficacy and safety of which should be further evaluated.

Reference Type: Journal Article
Record Number: 272
Year: 1996

Title: Sodium phenylbutyrate for urea cycle enzyme deficiencies
Journal: Med Lett Drugs Ther
Volume: 38
Issue: 988
Pages: 105-6

Reference Type: Journal Article
Record Number: 271
Author: Boudoulas, S.; Lush, R. M.; McCall, N. A.; Samid, D.; Reed, E.; Figg, W. D.
Year: 1996
Title: Plasma protein binding of phenylacetate and phenylbutyrate, two novel antineoplastic agents
Journal: Ther Drug Monit
Volume: 18
Issue: 6
Pages: 714-20
Abstract: Phenylacetate and phenylbutyrate, two novel inducers of tumor cytostasis and differentiation, are currently in clinical trials for the treatment of cancer in adults. The purpose of our study was to evaluate the plasma protein-binding characteristics of phenylacetate and phenylbutyrate in the plasma of normal volunteers and that of patients with cancer. Drug plasma protein-binding analysis was examined using three separate devices: a micropartition system and two equilibrium dialysis systems, all of which exhibited similar results. Phenylacetate and phenylbutyrate concentrations were determined by high-performance liquid chromatography. Both drugs exhibited concentration-dependent binding. Our results showed sodium phenylacetate to have a higher free fraction than sodium phenylbutyrate at corresponding concentrations (> 0.442 +/- 0.008 and > 0.188 +/- 0.001, respectively). Plasma pH did not greatly affect protein binding of either drug. As albumin concentration decreased, an increase in free fraction of both drugs was observed, however alpha 1-acid glyco-protein showed no change in free fraction as its concentration increased. Patients with cancer with lower levels of albumin showed an increase in free fraction with both phenylacetate and phenylbutyrate. When phenylacetate and phenylbutyrate were added together in plasma, the free fraction of phenylacetate increased, whereas the phenylbutyrate free fraction slightly decreased. We conclude that phenylacetate and phenylbutyrate have high free fractions that change with varying albumin levels and when both phenylacetate and phenylbutyrate are present together in plasma.

Reference Type: Journal Article
Record Number: 279
Author: Carducci, M. A.; Nelson, J. B.; Chan-Tack, K. M.; Ayyagari, S. R.; Sweatt, W. H.; Campbell, P. A.; Nelson, W. G.; Simons, J. W.
Year: 1996
Title: Phenylbutyrate induces apoptosis in human prostate cancer and is more potent than phenylacetate
Journal: Clin Cancer Res
Volume: 2
Issue: 2

Pages: 379-87
Abstract: Phenylbutyrate (PB), a novel lead compound for prostate cancer therapy, has molecular activities distinct from its metabolite, phenylacetate (PA). Both PB and PA promote differentiation in human prostate cancer cell lines, yet little data exist comparing the cytotoxic effects of each drug. We found that PB is more potent than PA in vitro; PB is 1.5-2.5 times more active at inhibiting growth and inducing programmed cell death than PA at clinically achievable doses against each human prostate cancer line studied. PB is equipotent to sodium butyrate, which induces apoptosis and differentiation through multiple mechanisms. Exposure of prostate cancer cell lines to PB reduces their DNA synthesis, leads to fragmentation of genomic DNA, and causes 50-60% of cells to undergo apoptosis. These PB-induced effects are 2-10 times greater than those of the control or PA. The stereotypical changes of apoptosis can be seen with sodium butyrate at similar concentrations, but not with PA. Prostate cancer cell lines overexpressing P-glycoprotein or possessing heterogeneous molecular alterations, including p53 mutations, are also sensitive to the effects of PB. In vivo, Copenhagen rats treated with oral PB had delayed growth of the androgen refractory Dunning R-3327 MAT-LyLu prostate cancer subline by 30-45% in a dose-dependent manner. These results demonstrate that PB induces cytotoxicity via apoptosis in human prostate cancer, in addition to its differentiating properties.

Reference Type: Journal Article
Record Number: 274
Author: Hudgins, W. R.; Fibach, E.; Safaya, S.; Rieder, R. F.; Miller, A. C.; Samid, D.
Year: 1996
Title: Transcriptional upregulation of gamma-globin by phenylbutyrate and analogous aromatic fatty acids
Journal: Biochem Pharmacol
Volume: 52
Issue: 8
Pages: 1227-33
Abstract: Phenylbutyrate has been shown recently to induce fetal hemoglobin (HbF) production in patients with sickle cell anemia and beta thalassemia. We have now examined related aromatic fatty acids in order to define the range of active structures and identify plausible mechanisms of action. Structure-function analysis revealed that for effective stimulation of HbF in erythroid precursors: (1) the ideal length for the aliphatic side chain is four carbons; (2) oxygen or sulfur substitutions in the carboxylic chain are allowed, as evidenced by the equal or increased activity of phenoxypropionate, benzylthioglycolate, and benzyloxyacetate compared with phenylbutyrate; and (3) blocking the carboxylate group by conversion to the amide form greatly reduces potency. Molecular analysis indicated that the prototype agent, phenylbutyrate, increases HbF production through transcriptional activation of the gamma-globin gene. The latter contains a butyrate responsive promoter known to up-regulate transcription in the presence of short-chain fatty acids of three to five carbons. To determine whether stimulation of an element in this promoter by phenylbutyrate and its analogues might contribute to their mechanism of action, we used a transient expression system involving K562 erythroleukemia cells transfected with a luciferase reporter gene driven by the minimum gamma-globin promoter.

Transcriptional activation in this experimental system correlated well with the capacity of an aromatic fatty acid to increase HbF production in erythroid precursors (r = 0.94). Our studies identify potent analogues of phenylbutyrate for the treatment of beta-chain hemoglobinopathies, and suggest that stimulation of a butyrate responsive promoter may be responsible for their activity.

Reference Type: Journal Article
Record Number: 275
Author: Maestri, N. E.; Brusilow, S. W.; Clissold, D. B.; Bassett, S. S.
Year: 1996
Title: Long-term treatment of girls with ornithine transcarbamylase deficiency
Journal: N Engl J Med
Volume: 335
Issue: 12
Pages: 855-9
Abstract: BACKGROUND: Ornithine transcarbamylase is an X-linked mitochondrial enzyme that catalyzes the synthesis of citrulline from carbamoyl phosphate and ornithine. A deficiency of this enzyme leads to hyperammonemia and hyperglutaminemia. In boys the disease is often fatal when its onset occurs during the neonatal period, but it is milder when onset occurs later in childhood. Heterozygous girls may be normal or may have episodes of hyperammonemic encephalopathy and decline in cognitive function. We report here on the long-term outcome in girls with ornithine transcarbamylase deficiency enrolled in studies of treatments designed to activate new pathways of waste-nitrogen excretion. METHODS: We studied 32 girls (age, 1 to 17 years) with ornithine transcarbamylase deficiency who had had at least one episode of encephalopathy. The patients were assigned to treatment that consisted of sodium benzoate, alone or in combination with sodium phenylacetate or sodium phenylbutyrate, or sodium phenylbutyrate alone. Collaborating physicians provided clinical, metabolic, and developmental data at specified intervals. RESULTS: Patients treated according to these protocols had greater than 90 percent survival at five years and maintained appropriate weight for height. The frequency of hyperammonemic episodes decreased with increasing age and with sodium phenylacetate or sodium phenylbutyrate treatment. Although the mean IQ before treatment was in the low average range, 19 of the 23 girls in whom intelligence was tested longitudinally had stable test scores. CONCLUSIONS: Girls with symptomatic ornithine transcarbamylase deficiency who are treated with drugs that activate new pathways of waste-nitrogen excretion have fewer hyperammonemic episodes and a reduced risk of further cognitive decline.

Reference Type: Journal Article
Record Number: 277
Author: Pineau, T.; Hudgins, W. R.; Liu, L.; Chen, L. C.; Sher, T.; Gonzalez, F. J.; Samid, D.
Year: 1996
Title: Activation of a human peroxisome proliferator-activated receptor by the antitumor agent phenylacetate and its analogs
Journal: Biochem Pharmacol
Volume: 52

Issue: 4
Pages: 659-67
Abstract: The aromatic fatty acid phenylacetate and its analogs induce tumor cytostasis and differentiation in experimental models. Although the underlying mechanisms of action are not clear, effects on lipid metabolism are evident. We have now examined whether these compounds, structurally similar to the peroxisome proliferator clofibrate, affect the human peroxisome proliferator-activated receptor (hPPAR), a homolog of the rodent PPAR alpha, a transcriptional factor regulating lipid metabolism and cell growth. Gene transfer experiments showed activation of hPPAR, evident by the increased expression of the reporter gene chloramphenicol acetyltransferase linked to PPAR-response element from either the rat acyl-CoA oxidase or rabbit CYP4A6 genes. The relative potency of tested drugs in the co-transfection assay was: 4-iodophenylbutyrate > 4-chlorophenylbutyrate > clofibrate > phenylbutyrate > naphthylacetate > 2,4-D > 4-chlorophenylacetate > phenylacetate >> indoleacetate. Phenylacetylglutamine, in which the carboxylic acid is blocked, was inactive. The ability of the aromatic fatty acids to activate PPAR was confirmed in vivo, as CYP4A mRNA levels increased in hepatocytes of treated rats. Further studies using human prostate carcinoma, melanoma, and glioblastoma cell lines showed a tight correlation between drug-induced cytostasis, increased expression of the endogenous hPPAR, and receptor activation documented in the gene-transfer model. These results identify phenylacetate and its analogs as a new class of aromatic fatty acids capable of activating hPPAR, and suggest that this nuclear receptor may mediate tumor cytostasis induced by these drugs.

Reference Type: Journal Article
Record Number: 278
Author: Shack, S.; Miller, A.; Liu, L.; Prasanna, P.; Thibault, A.; Samid, D.
Year: 1996
Title: Vulnerability of multidrug-resistant tumor cells to the aromatic fatty acids phenylacetate and phenylbutyrate
Journal: Clin Cancer Res
Volume: 2
Issue: 5
Pages: 865-72
Abstract: Cytotoxic chemotherapies often give rise to multidrug resistance, which remains a major problem in cancer management. In pursuit of alternative treatments for chemoresistant tumor cells, we tested the response of multidrug-resistant (MDR) tumor cell lines to the aromatic fatty acids phenylacetate (PA) and phenylbutyrate (PB), two differentiation inducers currently in clinical trials. Both compounds induced cytostasis and maturation of multidrug-resistant breast, ovarian, and colon carcinoma cells with no significant effect on cell viability. In contrast to their poor response to doxorubicin, the MDR cells were generally more sensitive to growth arrest by PA and PB than their parental counterparts. The aromatic fatty acids, like the differentiation-inducing aliphatic fatty acid butyrate, up-regulated mdr-1 gene expression. However, while butyrate increased multidrug resistance, PA and PB potentiated the cytotoxic activity of doxorubicin against MDR cells. The latter was associated with time-dependent declines in glutathione levels and in the activity of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione S-transferase, the antioxidant enzymes implicated in cell resistance to free radical-based therapies.

Taken together, our in vitro data indicate that PA and PB, differentiation inducers of the aromatic fatty acid class, may provide an alternative approach to the treatment of MDR tumors.

Reference Type: Journal Article
Record Number: 276
Author: Wikner, B. N.; Peterson, C.
Year: 1996
Title: [Is tributyrate effective against cancer? Usual clinical studies on the discussed miracle medicine are not available]
Journal: Lakartidningen
Volume: 93
Issue: 37
Pages: 3161-2

Reference Type: Journal Article
Record Number: 273
Author: Yudkoff, M.; Daikhin, Y.; Nissim, I.; Jawad, A.; Wilson, J.; Batshaw, M.
Year: 1996
Title: In vivo nitrogen metabolism in ornithine transcarbamylase deficiency
Journal: J Clin Invest
Volume: 98
Issue: 9
Pages: 2167-73
Abstract: We developed a new technique that monitors metabolic competency in female heterozygotes for ornithine transcarbamylase deficiency (OTCD). The method uses mass spectrometry to measure conversion of (15)NH4Cl to [15N]urea and [5-(15)N]glutamine following an oral load of (15)NH4Cl. We found that heterozygotes converted significantly less NH3 nitrogen to urea, with this difference being particularly obvious for symptomatic carriers, in whom the blood [15N]urea concentration (mM) was significantly less than control values at most time points. The blood concentration of [5-(15)N]-glutamine (microM) was significantly higher in both asymptomatic and symptomatic heterozygotes than it was in the control subjects. The administration of a test dose of sodium phenylbutyrate to the control group did not affect the rate of [15N]urea formation. We conclude: (a) This test effectively monitors in vivo N metabolism and might obviate the need for liver biopsy to measure enzyme activity in OTCD; (b) Asymptomatic OTCD carriers form urea at a normal rate, indicating that ureagenesis can be competent even though enzyme activity is below normal; (c) Although ostensibly asymptomatic OTCD carriers form urea at a normal rate, their nitrogen metabolism is still abnormal, as reflected in their increased production of [5-(15)N]glutamine; and (d) This new test may be important for monitoring the efficacy of novel treatments for OTCD, e.g., liver transplantation and gene therapy.

Reference Type: Journal Article
Record Number: 267
Author: Farias, R. N.; Torres, M.; Canela, R.

Year: 1997
Title: Spectrophotometric determination of the positional specificity of nonspecific and 1,3-specific lipases
Journal: Anal Biochem
Volume: 252
Issue: 1
Pages: 186-9
Abstract: Using commercially available thiosubstrates, such as 2,3-dimercapto-1-propanol tributyrate, the regio-specificities of 1,3-specific and nonspecific lipases was confirmed. The spectrophotometric test is a simple, rapid, and convenient alternative method to those previously reported for the characterization of the positional specificities of new lipases.

Reference Type: Journal Article
Record Number: 252
Author: Gore, S. D.; Samid, D.; Weng, L. J.
Year: 1997
Title: Impact of the putative differentiating agents sodium phenylbutyrate and sodium phenylacetate on proliferation, differentiation, and apoptosis of primary neoplastic myeloid cells
Journal: Clin Cancer Res
Volume: 3
Issue: 10
Pages: 1755-62
Abstract: Sodium phenylacetate (PA) and sodium phenylbutyrate (PB) are aromatic fatty acids that can effect differentiation in a variety of cell lines at doses that may be clinically attainable. We have studied the impact of these two agents on lineage- and differentiation stage-specific antigen expression, proliferation, apoptosis, and clonogenic cell survival in primary cultures of bone marrow samples from patients with myeloid neoplasms at presentation and in remission and from normal volunteers. PB inhibited the proliferation of primary acute myeloid leukemia cells in suspension culture with an ID50 of 6.6 mM, similar to its ED50 in cell lines. At higher doses (>/=5 mM), PB also induced apoptosis. PB inhibited clonogenic leukemia cell growth with a median ID50 of less than 2 mM; however, colony-forming units-granulocyte/macrophage from patients with myelodysplasia and normal volunteers were inhibited with a similar ID50. In contrast to PB, its metabolite PA had no significant effect on either acute myeloid leukemia proliferation or apoptosis. Expression of the monocytic marker CD14 was increased in monocytic and myelomonocytic leukemias in response to PB, and to a lesser extent, PA. Surprisingly, both agents appeared to increase expression of the progenitor cell antigen CD34, as well as the DR locus of the human leukocyte antigen. These data indicate that PB, but not its metabolite PA, has significant cytostatic and differentiating activity against primary neoplastic myeloid cells at doses that may be achievable clinically.

Reference Type: Journal Article
Record Number: 269
Author: Miller, A. C.; Whittaker, T.; Thibault, A.; Samid, D.

Year: 1997
Title: Modulation of radiation response of human tumour cells by the differentiation inducers, phenylacetate and phenylbutyrate
Journal: Int J Radiat Biol
Volume: 72
Issue: 2
Pages: 211-8
Abstract: The aromatic fatty acids phenylacetate (PA) and phenylbutyrate (PB) are novel antitumour agents currently under clinical evaluation. Their ability to induce tumour differentiation in laboratory models and their low clinical toxicity profile makes them promising candidates for combination with conventional therapies. In the present studies, we characterized the interactions between these aromatic fatty acids and radiation, using as a model cell lines derived from cancers of the prostate, breast, brain and colon. Analysis of the radiation response of the tumour lines using the linear-quadratic model, demonstrated that cellular exposure to pharmacological, non-toxic concentrations of either PA or PB resulted in time-dependent and contrasting changes in radiation response. While drug pretreatment for 24 h reduced radiation sensitivity (significant alterations in both alpha and beta parameters), pre treatment for 72 h significantly increased radiosensitivity (significant alterations in alpha and beta parameters). In replicating tumour cells, these changes were accompanied by a gradual G1-phase arrest. Cytostasis alone, however, could not explain radiosensitization, as similar alterations in radiation response were documented also in non-cycling cells. Modulation of tumour radiobiology by PA and PB was tightly correlated with early rise followed by decline in intracellular glutathione levels and the activity of antioxidant enzymes such as catalase, superoxide dismutase, glutathione reductase, glutathione peroxidase and glutathione S-transferase. Although in vitro findings identify the aromatic fatty acids PA and PB as a new class of non-toxic modulators of radiation response, the antagonistic effect of these compounds on radiation response needs further examination. Our data strongly suggest that for PA or PB to have a role in clinical radiotherapy, appropriate scheduling of combination therapies must take into account their time-dependent effects in order to achieve clinical radiosensitization.

Reference Type: Journal Article
Record Number: 268
Author: Olivieri, N. F.; Rees, D. C.; Ginder, G. D.; Thein, S. L.; Brittenham, G. M.; Waye, J. S.; Weatherall, D. J.
Year: 1997
Title: Treatment of thalassaemia major with phenylbutyrate and hydroxyurea
Journal: Lancet
Volume: 350
Issue: 9076
Pages: 491-2

Reference Type: Journal Article
Record Number: 266
Author: Rubenstein, R. C.; Egan, M. E.; Zeitlin, P. L.
Year: 1997

Title: In vitro pharmacologic restoration of CFTR-mediated chloride transport with sodium 4-phenylbutyrate in cystic fibrosis epithelial cells containing delta F508-CFTR
Journal: J Clin Invest
Volume: 100
Issue: 10
Pages: 2457-65
Abstract: The most common cystic fibrosis transmembrane conductance regulator mutation, delta F508-CFTR, is a partially functional chloride channel that is retained in the endoplasmic reticulum and degraded. We hypothesize that a known transcriptional regulator, sodium 4-phenylbutyrate (4PBA), will enable a greater fraction of delta F508-CFTR to escape degradation and appear at the cell surface. Primary cultures of nasal polyp epithelia from CF patients (delta F508 homozygous or heterozygous), or the CF bronchial epithelial cell line IB3-1 (delta F508/W1282X) were exposed to 4PBA for up to 7 d in culture. 4PBA treatment at concentrations of 0.1 and 2 mM resulted in the restoration of forskolin-activated chloride secretion. Protein kinase A-activated, linear, 10 pS chloride channels appeared at the plasma membrane of IB3-1 cells at the tested concentration of 2.5 mM. Treatment of IB3-1 cells with 0.1-1 mM 4PBA and primary nasal epithelia with 5 mM 4PBA also resulted in the appearance of higher molecular mass forms of CFTR consistent with addition and modification of oligosaccharides in the Golgi apparatus, as detected by immunoblotting of whole cell lysates with anti-CFTR antisera. Immunocytochemistry in CF epithelial cells treated with 4PBA was consistent with increasing amounts of delta F508-CFTR. These data indicate that 4PBA is a promising pharmacologic agent for inducing correction of the CF phenotype in CF patients carrying the delta F508 mutation.

Reference Type: Journal Article
Record Number: 270
Author: Samid, D.; Hudgins, W. R.; Shack, S.; Liu, L.; Prasanna, P.; Myers, C. E.
Year: 1997
Title: Phenylacetate and phenylbutyrate as novel, nontoxic differentiation inducers
Journal: Adv Exp Med Biol
Volume: 400A
Pages: 501-5
Abstract: Phenylacetate and analogs represent a new class of pleiotropic growth regulators that alter tumor cell biology by affecting gene expression at both the transcriptional and post transcriptional levels. Based on these findings, NaPA and NaPB entered clinical trials at the National Cancer Institute. Ongoing phase I studies with NaPA, involving adults with prostate and brain cancer, have confirmed that therapeutic levels can be achieved with no significant toxicities, and provide preliminary evidence for benefit to patients with advanced disease (Thibault et al., submitted).

Reference Type: Journal Article
Record Number: 262
Author: Darmaun, D.; Welch, S.; Rini, A.; Sager, B. K.; Altomare, A.; Haymond, M. W.

Year: 1998
Title: Phenylbutyrate-induced glutamine depletion in humans: effect on leucine metabolism
Journal: Am J Physiol
Volume: 274
Issue: 5 Pt 1
Pages: E801-7
Abstract: The present study was designed to determine whether sodium phenylbutyrate (phi B) acutely induces a decrease in plasma glutamine in healthy humans, and, if so, will decrease estimates of whole body protein synthesis. In a first group of three healthy subjects, graded doses (0, 0.18, and 0.36 g.kg-1.day-1) of phi B were administered for 24 h before study: postabsorptive plasma glutamine concentration declined in a dose-dependent manner, achieving an approximately 25% decline for a dose of 0.36 g phi B.kg-1.day-1. A second group of six healthy adults received 5-h infusions of L-[1-14C]leucine and L-[1-13C]glutamine in the postabsorptive state on two separate days: 1) under baseline conditions and 2) after 24 h of oral treatment with phi B (0.36 g.kg-1.day-1) in a randomized order. The 24-h phenylbutyrate treatment was associated with 1) an approximately 26% decline in plasma glutamine concentration from 514 +/- 24 to 380 +/- 15 microM (means +/- SE; $P < 0.01$ with paired t-test) with no change in glutamine appearance rate or de novo synthesis; 2) no change in leucine appearance rate (Ra), an index of protein breakdown (123 +/- 7 vs. 117 +/- 5 mumol.kg-1.h-1; not significant); 3) an approximately 22% rise in leucine oxidation (Ox) from 23 +/- 2 to 28 +/- 2 mumol.kg-1.h-1 ($P < 0.01$), resulting in an approximately 11% decline in nonoxidative leucine disposal (NOLD = Ra-Ox), an index of protein synthesis, from 100 +/- 6 to 89 +/- 5 mumol.kg-1.h-1 ($P < 0.05$). The data suggest that, in healthy adults, 1) large doses of oral phenylbutyrate can be used as a "glutamine trap" to create a model of glutamine depletion; 2) a moderate decline in plasma glutamine does not enhance rates of endogenous glutamine production; and 3) a short-term depletion of plasma glutamine decrease estimates of whole body protein synthesis.

Reference Type: Journal Article
Record Number: 260
Author: Dover, G. J.
Year: 1998
Title: Hemoglobin switching protocols in thalassemia. Experience with sodium phenylbutyrate and hydroxyurea
Journal: Ann N Y Acad Sci
Volume: 850
Pages: 80-6
Abstract: Homozygous beta thalassemia affects thousands of people around the world. Current management of this condition includes regular transfusion of red cells, which leads to transfusional iron overload requiring chelation therapy: increasing hemoglobin levels while decreasing or eliminating iron overload is therefore a major therapeutic goal in the treatment of thalassemia. Bone marrow transplantation may achieve this goal, but it is not an option for most patients. This study reports on efforts to increase gamma-globin transcription and HbF production using sodium phenylbutyrate (SPB) and hydroxyurea (HU). It was found that 36% (4/11) of all patients or 50% (4/8) of non-transfused patients responded to SPB (increase in Hb

levels of 1 g/dL). A positive correlation between baseline serum erythropoietin level and likelihood of response to SPB was observed. Since HU may also increase HbF production, evaluation of combination therapy with these drugs is underway and preliminary results are reported.

Reference Type: Journal Article
Record Number: 264
Author: Engelhard, H. H.; Homer, R. J.; Duncan, H. A.; Rozental, J.
Year: 1998
Title: Inhibitory effects of phenylbutyrate on the proliferation, morphology, migration and invasiveness of malignant glioma cells
Journal: J Neurooncol
Volume: 37
Issue: 2
Pages: 97-108
Abstract: The purpose of this study was to characterize the effects of sodium 4-phenylbutyrate (phenylbutyrate) on the proliferation, morphology, migration and invasiveness of malignant glioma cells in vitro. Phenylbutyrate is a novel differentiating and cytotoxic compound used clinically with low toxicity in the treatment of beta-thalassemia, sickle cell anemia and urea cycle disorders. Preliminary clinical trials testing phenylbutyrate as an anti-cancer agent have included patients with malignant glioma. However, little information is available regarding the effects of phenylbutyrate on glioma cells, particularly with respect to the expression of genes important in the pathogenesis of glial malignancy. In experiments reported here, glioma cell lines and explant cells from a tumor patient were exposed to 2, 4 and 8 mM phenylbutyrate and compared to untreated control cells. The effect on cellular proliferation was assessed using cell counts and DNA flow cytometry. Changes in morphology were evaluated using vimentin staining. Scratch and Matrigel assays were performed to assess changes in cellular migration and invasiveness. Finally, Northern blot analysis was used to study c-myc and urokinase expression. Phenylbutyrate was found to have dose-dependent inhibitory effects on glioma cell proliferation, morphology, migration, invasiveness and c-myc and urokinase expression. Mean growth-inhibitory (IC50) phenylbutyrate concentrations ranged from 0.5 mM for T98G cells to 5.0 mM for explant cells. Phenylbutyrate treatment reduced % S phase cells, increased % G0/G1 cells, and produced morphologic changes consistent with induction of differentiation. 24 hours of treatment with 4 mM phenylbutyrate resulted in a 50% reduction in migration and invasiveness. Northern blots showed a decrease in urokinase and c-myc expression at non-cytotoxic doses. We conclude that phenylbutyrate is a promising candidate compound for treating patients with malignant glioma.

Reference Type: Journal Article
Record Number: 255
Author: Huang, Y.; Waxman, S.
Year: 1998
Title: Enhanced growth inhibition and differentiation of fluorodeoxyuridine-treated human colon carcinoma cells by phenylbutyrate
Journal: Clin Cancer Res

Volume: 4
Issue: 10
Pages: 2503-9
Abstract: The effect of phenylbutyrate (PB), a nontoxic differentiation inducer, in human colon carcinoma cell lines treated with 5-fluorodeoxyuridine (FUdR) was evaluated. Two HT-29 human colon carcinoma subclones (U4 well differentiated and U9 poorly differentiated) were equally growth inhibited by 16 h of FUdR (0.2 microM) treatment but recovered cell growth in 3-6 days after the removal of FUdR. PB as a single agent had minimal effect on cell growth, but after FUdR treatment, PB inhibited cell growth for 12 days. The inhibition of cell growth in FUdR-treated cells by PB was more sustained in U4 than U9 cells and was associated with an increased and sustained expression of p21waf1 protein, secretion of transforming growth factor beta1, mediators of p53-dependent or -independent G1 cell cycle arrest, and an increase in the alkaline phosphatase activity as well, considered a marker of differentiation in colon carcinoma cells. These effects of PB were seen only in FUdR-pretreated cells because PB alone had minimal effect on the expression of these genes. The sequential use of FUdR followed by PB in patients with colon carcinoma should be explored because two subclones of HT29, irrespective of their state of differentiation, respond to this clinically achievable regimen.

Reference Type: Journal Article
Record Number: 254
Author: Kemp, S.; Wei, H. M.; Lu, J. F.; Braiterman, L. T.; McGuinness, M. C.; Moser, A. B.; Watkins, P. A.; Smith, K. D.
Year: 1998
Title: Gene redundancy and pharmacological gene therapy: implications for X-linked adrenoleukodystrophy
Journal: Nat Med
Volume: 4
Issue: 11
Pages: 1261-8
Abstract: As more functional redundancy in mammalian cells is discovered, enhanced expression of genes involved in alternative pathways may become an effective form of gene therapy. X-linked adrenoleukodystrophy (X-ALD) is a peroxisomal disorder with impaired very-long-chain fatty acid metabolism. The X-ALD gene encodes a peroxisomal membrane protein (ALDP) that is part of a small family of related peroxisomal membrane proteins. We show that 4-phenylbutyrate treatment of cells from both X-ALD patients and X-ALD knockout mice results in decreased levels of and increased beta-oxidation of very-long-chain fatty acids; increased expression of the peroxisomal protein ALDRP; and induction of peroxisome proliferation. We also demonstrate that ALDP and ALDRP are functionally related, by ALDRP cDNA complementation of X-ALD fibroblasts. Finally, we demonstrate the in vivo efficacy of dietary 4-phenylbutyrate treatment through its production of a substantial reduction of very-long-chain fatty acid levels in the brain and adrenal glands of X-ALD mice.

Reference Type: Journal Article
Record Number: 257

Author: Lea, M. A.; Randolph, V. M.
Year: 1998
Title: Induction of reporter gene expression by inhibitors of histone deacetylase
Journal: Anticancer Res
Volume: 18
Issue: 4A
Pages: 2717-22
Abstract: The relationship between histone acetylation and induction of gene expression was studied in Ros 17/2.8 rat osteosarcoma cells transfected with the pCH110 plasmid. This plasmid is commonly used in cotransfections as a measure of transfection efficiency. Cells were incubated for 48 hours with sodium butyrate, phenylbutyrate, 3-bromopropionate or trichostatin A. There was an approximate relationship between the extent of beta-galactosidase induction and the degree of histone hyperacetylation. Trichostatin A was the most effective agent followed by sodium butyrate and then phenylbutyrate. The toxicity of 3-bromopropionate made it difficult to compare its action with the other agents. Phenylbutyrate was less effective than sodium butyrate in causing induction of gene expression and histone hyperacetylation but this action may be a factor in the growth-inhibitory and differentiating activity of phenylbutyrate which has also been attributed to glutamine depletion.

Reference Type: Journal Article
Record Number: 256
Author: List, A. F.
Year: 1998
Title: Hematopoietic stimulation by amifostine and sodium phenylbutyrate: what is the potential in MDS?
Journal: Leuk Res
Volume: 22 Suppl 1
Pages: S7-11

Reference Type: Journal Article
Record Number: 258
Author: MacMillan, M. L.; Fouladi, M.; Nisbet-Brown, E.; Waye, J. S.; Olivieri, N. F.
Year: 1998
Title: Treatment of two infants with Cooley's anemia with sodium phenylbutyrate
Journal: Ann N Y Acad Sci
Volume: 850
Pages: 452-4

Reference Type: Journal Article
Record Number: 250
Author: Melichar, B.; Ferrandina, G.; Verschraegen, C. F.; Loercher, A.; Abbruzzese, J. L.; Freedman, R. S.
Year: 1998
Title: Growth inhibitory effects of aromatic fatty acids on ovarian tumor cell lines

Journal: Clin Cancer Res
Volume: 4
Issue: 12
Pages: 3069-76
Abstract: Epithelial ovarian cancer is a major cause of cancer-related mortality in women, making the search for new treatment modalities essential. Sodium phenylacetate (NaPa), a phenylalanine derivative, has been shown to induce cytostasis and differentiation by inhibiting protein isoprenylation. Similar effects have been observed with phenylbutyrate, a phenylacetate congener. We examined in parallel the growth inhibitory activity against human ovarian carcinoma cell lines of phenylacetate, phenylbutyric acid (PB), and certain related compounds, and comparisons were made with lovastatin. On a molar basis, hydroxykynurenine and kynurenine showed the highest activity followed by PB and NaPa. Ovarian carcinoma cell lines were also sensitive to lovastatin in micromolar concentrations. Additive effects were observed when PB was combined with cisplatin or when NaPa or PB were combined with lovastatin. NaPa and PB, but not kynurenine, inhibited incorporation of [3H]mevalonate into ovarian carcinoma cells. An immune modulatory role might also be suggested for PB because it resulted in increased ovarian tumor cell expression of human leukocyte antigen class I and the cluster of differentiation molecule CD58, whereas transforming growth factor-beta2 expression was decreased. Phenylbutyrate, which is the ester form of PB, has shown acceptable pharmacological properties and clinical responses in patients with other malignancies, and might be considered for evaluation in ovarian cancer.

Reference Type: Journal Article
Record Number: 259
Author: Olivieri, N. F.; Rees, D. C.; Ginder, G. D.; Thein, S. L.; Waye, J. S.; Chang, L.; Brittenham, G. M.; Weatherall, D. J.
Year: 1998
Title: Elimination of transfusions through induction of fetal hemoglobin synthesis in Cooley's anemia
Journal: Ann N Y Acad Sci
Volume: 850
Pages: 100-9
Abstract: Pharmacological stimulation of fetal hemoglobin production is of considerable interest as an alternative approach to therapy for Cooley's anemia. While intravenous compounds have been effective in inducing short-term increases in fetal hemoglobin in a few patients, long-term elimination of transfusion requirement has not been reported. In patients with Cooley's anemia, treatment with oral sodium phenylbutyrate alone, sodium phenylbytyrate combined with hydroxyurea, and hydroxyurea alone, has augmented fetal hemoglobin production and increased total hemoglobin concentration as much as 5 g/dl over baseline eliminating transfusion requirement in two patients. Parallel declines in circulating nucleated red cell count, and concentrations of serum transform receptor and erythropoietin, are consistent with more effective erythropoiesis. Over extended periods of treatment, no induction of other fetal proteins and no adverse effects were observed. Particular disease mutations and other genetic factors may be of prime importance in determining the response to agents that induce production of fetal hemoglobin.

Reference Type: Journal Article
Record Number: 263
Author: Pelidis, M. A.; Carducci, M. A.; Simons, J. W.
Year: 1998
Title: Cytotoxic effects of sodium phenylbutyrate on human neuroblastoma cell lines
Journal: Int J Oncol
Volume: 12
Issue: 4
Pages: 889-93
Abstract: Sodium phenylbutyrate (NaPB) is used in urea cycle disorders. We screened 6 neuroblastoma cell lines for in vitro potency of NaPB as an antiproliferative agent, evaluated multiple dosing schedules, and assessed its activity in combination with clinically active agents for neuroblastoma. We determined that NaPB achieves a 30-80% growth inhibition at 5 mM. Repeated dosing and prolonged drug exposure enhanced the cytotoxic effect. NaPB had additive cytotoxic effects when administered with vincristine; however, NaPB did not affect the activity of etoposide, adriamycin, 4-hydroxycyclophosphamide or cisplatinum. These results suggest that NaPB is an active agent against neuroblastoma and could be combined with vincristine in novel chemotherapy regimens.

Reference Type: Journal Article
Record Number: 249
Author: Plecko, B.; Erwa, W.; Wermuth, B.
Year: 1998
Title: Partial N-acetylglutamate synthetase deficiency in a 13-year-old girl: diagnosis and response to treatment with N-carbamylglutamate
Journal: Eur J Pediatr
Volume: 157
Issue: 12
Pages: 996-8
Abstract: We report on a now 13-year-old girl, who presented with recurrent episodes of vomiting, psychotic behaviour and confusion during puberty until the diagnosis of partial N-acetylglutamate synthetase deficiency was established. She had suffered one prior unclear episode of acute vomiting, lethargy and somnolence at the age of 13 months, and from childhood onward had aversion to high protein food. Treatment with a protein-restricted diet and administration of phenylbutyrate as well as L-arginine were sufficient to normalize ammonia levels but glutamine concentrations remained high. Supplementation with N-carbamylglutamate rapidly improved her protein tolerance and reduced the need for co-medication. To our knowledge, so far only seven patients with N-acetylglutamate synthetase deficiency have been reported. CONCLUSION: Partial N-carbamylglutamate deficiency has to be considered in the differential diagnosis of hyperammonaemia. If proven by enzyme determination in liver tissue, the disorder should be cautiously treated with N-carbamylglutamate.

Reference Type: Journal Article
Record Number: 265

Author: Rubenstein, R. C.; Zeitlin, P. L.
Year: 1998
Title: A pilot clinical trial of oral sodium 4-phenylbutyrate (Buphenyl) in deltaF508-homozygous cystic fibrosis patients: partial restoration of nasal epithelial CFTR function
Journal: Am J Respir Crit Care Med
Volume: 157
Issue: 2
Pages: 484-90
Abstract: Sodium 4-phenylbutyrate (Buphenyl, 4PBA) is a new FDA approved drug for management of urea cycle disorders. We have previously presented data suggesting that 4PBA, at clinically achievable concentrations, induces CFTR channel function on the plasma membrane of deltaF508-expressing cystic fibrosis (CF) airway epithelial cells in vitro (Rubenstein, R. C., and P. L. Zeitlin, 1997. J. Clin. Invest. 100:2457-2463). We hypothesized that 4PBA would induce epithelial CFTR function in vivo in individuals homozygous for deltaF508-CFTR. A randomized, double-blind, placebo-controlled trial in 18 deltaF508-homozygous patients with CF was performed with the maximum approved adult dose of 4PBA, 19 grams p.o. divided t.i.d., given for 1 wk. Nasal potential difference (NPD) response patterns and sweat chloride concentrations were determined before and after study drug treatment, and 4PBA and metabolites were assayed in plasma and urine at the end of study drug treatment. Subjects in the 4PBA group demonstrated small, but statistically significant improvements of the NPD response to perfusion of an isoproterenol/amiloride/chloride-free solution; this measure reflects epithelial CFTR function and is highly discriminatory between patients with and without CF. Subjects who had received 4PBA did not demonstrate significantly reduced sweat chloride concentrations or alterations in the amiloride-sensitive NPD. Side effects due to drug therapy were minimal and comparable in the two groups. These data are consistent with 4PBA therapy inducing CFTR function in the nasal epithelia of deltaF508-homozygous CF patients.

Reference Type: Journal Article
Record Number: 261
Author: Saxon, B. R.; Rees, D.; Olivieri, N. F.
Year: 1998
Title: Regression of extramedullary haemopoiesis and augmentation of fetal haemoglobin concentration during hydroxyurea therapy in beta thalassaemia
Journal: Br J Haematol
Volume: 101
Issue: 3
Pages: 416-9
Abstract: Hydroxyurea increases fetal haemoglobin in many patients with sickle cell anaemia, but its effectiveness in thalassaemia appears to be less consistent. We describe the response to hydroxyurea in an adult male with homozygous beta thalassaemia, symptomatic paraspinal extramedullary haemopoiesis, bone pain, and progressive tissue iron loading. Prior to therapy with hydroxyurea the circulating haemoglobin (Hb) concentration was 7.0 g/dl and absolute fetal haemoglobin concentration was 5.0 g/dl. Administration of sodium phenylbutyrate had induced no increase in either parameter. Subsequent therapy with hydroxyurea was associated

with increases in total haemoglobin to 9.0 g/dl, and in fetal haemoglobin to 7.6 g/dl. Ineffective erythropoiesis was reduced and extramedullary haemopoiesis regressed during therapy.

Reference Type: Journal Article
Record Number: 253
Author: Warrell, R. P., Jr.; He, L. Z.; Richon, V.; Calleja, E.; Pandolfi, P. P.
Year: 1998
Title: Therapeutic targeting of transcription in acute promyelocytic leukemia by use of an inhibitor of histone deacetylase
Journal: J Natl Cancer Inst
Volume: 90
Issue: 21
Pages: 1621-5
Abstract: BACKGROUND: Acetylation of DNA-associated histones is linked to activation of gene transcription, whereas histone deacetylation is associated with transcriptional repression. Recent studies have shown that inhibitors of histone deacetylases can relieve transcriptional repression caused by the products of certain oncogenes. We tested whether these findings could be applied clinically to a patient with highly resistant acute promyelocytic leukemia. METHODS: A patient who had experienced multiple relapses was treated with all-trans-retinoic acid alone and in combination with sodium phenylbutyrate, an inhibitor of histone deacetylases. Immunohistochemistry and western blot analysis were used to assay for histone hyperacetylation in mononuclear cells from the patient's blood and bone marrow. Marrow mononuclear cells and reverse transcription-polymerase chain reaction (RT-PCR) analysis of messenger RNA encoded by the PML/RAR-alpha oncogene were used to assess minimal residual disease. RESULTS: The patient proved clinically resistant to treatment with all-trans-retinoic acid alone. However, 23 days after sodium phenylbutyrate was added to the treatment regimen, visible leukemic cells had been eliminated from her bone marrow, and she achieved a complete clinical and cytogenetic remission shortly thereafter. With a second treatment course, analysis for minimal residual disease by RT-PCR proved negative. Immunofluorescence and western blot analysis showed that phenylbutyrate caused a time-dependent increase in histone acetylation in blood and bone marrow mononuclear cells. CONCLUSIONS: Clinical treatment with an inhibitor of histone deacetylase induces histone hyperacetylation in target cells and may restore sensitivity to the anti-leukemic effects of all-trans-retinoic acid in acute promyelocytic leukemia. Similar therapy may prove useful in other neoplastic diseases that are associated with oncogenic repression of gene transcription due to recruitment of histone deacetylases.

Reference Type: Journal Article
Record Number: 251
Author: Yadav, R. P.; Saxena, R. K.; Gupta, R.; Davidson, S.
Year: 1998
Title: Lipase production by Aspergillus and Penicillium species
Journal: Folia Microbiol (Praha)
Volume: 43
Issue: 4

Pages: 373-8
Abstract: Forty each of aspergilli and penicillia were screened for extracellular lipase production on agar plates and in liquid medium containing olive oil as substrate. Twenty-nine aspergilli and twenty-six penicillia produced lipase. Out of these, 19 aspergilli and 22 penicillia showed activity both on Nile blue sulfate and glycerol tributyrate agar plates while only 10 aspergilli and 4 penicillia showed a positive response to glycerol tributyrate agar alone. The screening revealed 11 Aspergillus spp. and 15 Penicillium spp. as new lipase producers. Pig fat as an economic substrate for lipase production was also investigated.

Reference Type: Journal Article
Record Number: 245
Author: Bar-Ner, M.; Thibault, A.; Tsokos, M.; Magrath, I. T.; Samid, D.
Year: 1999
Title: Phenylbutyrate induces cell differentiation and modulates Epstein-Barr virus gene expression in Burkitt's lymphoma cells
Journal: Clin Cancer Res
Volume: 5
Issue: 6
Pages: 1509-16
Abstract: Although Burkitt's lymphoma (BL) is a readily treated malignancy, recurrences, as well as disease arising in immunosuppressed patients, are notoriously resistant to conventional therapeutic approaches. The EBV is associated with a significant proportion of these lymphomas that evade immune surveillance through decreased expression of both viral and cellular antigens. Increasing the immunogenicity of BL cells may, therefore, represent a potentially beneficial therapeutic maneuver. Using in vitro models of EBV-transformed lymphoblastoid as well as BL cell lines, we demonstrate increased expression of genes coding for HLA class I and EBV latent proteins by the differentiation inducer phenylbutyrate (PB). The aromatic fatty acid also caused cytostasis associated with sustained declines in c-myc expression, a direct antitumor effect that was independent of the EBV status. We conclude, therefore, that differentiation therapy of BL with PB may lead to growth arrest with increased tumor immunogenicity in vivo. The findings may have clinical relevance because the in vitro activity has been observed with PB concentrations that are well tolerated and nonimmunosuppressive in humans, a desirable feature for the different patient populations afflicted with this disease.

Reference Type: Journal Article
Record Number: 242
Author: Brunquell, P.; Tezcan, K.; DiMario, F. J., Jr.
Year: 1999
Title: Electroencephalographic findings in ornithine transcarbamylase deficiency
Journal: J Child Neurol
Volume: 14
Issue: 8
Pages: 533-6
Abstract: A 3-day-old infant presented with anorexia, irritability, hypotonia, and seizures. Blood ammonia was 2115 micromol/L and amino and organic acid analyses

were consistent with ornithine transcarbamylase deficiency. Liver biopsy confirmed only 1% enzyme activity. The patient was treated with hemodialysis. An electroencephalogram (EEG) revealed multifocal independent spike-and-sharp-wave discharges. After initial stabilization he was placed on a low-protein diet with citrulline and phenylbutyrate. Conjugating agents (arginine, sodium benzoate, and sodium phenylacetate) have been added during periods of metabolic decompensation. Although developmentally delayed, the patient has shown signs of clinical improvement and EEG activity has likewise improved with only mild background slowing and no evidence of epileptogenic activity at 4 years of age. A second infant presented at 3 days of age with a similar history, blood ammonia of 1382 micromol/L, and metabolic studies indicative of ornithine transcarbamylase deficiency. EEG showed multifocal independent ictal and interictal discharges. Electrographic abnormalities persisted despite lowering of blood ammonia with hemodialysis and conjugating agents. The patient continued to decline clinically and died on the 7th hospital day. EEG changes parallel the clinical course of ornithine transcarbamylase deficiency and may serve as an objective marker of the effectiveness of therapeutic interventions.

Reference Type: Journal Article
Record Number: 238
Author: Chiurazzi, P.; Pomponi, M. G.; Pietrobono, R.; Bakker, C. E.; Neri, G.; Oostra, B. A.
Year: 1999
Title: Synergistic effect of histone hyperacetylation and DNA demethylation in the reactivation of the FMR1 gene
Journal: Hum Mol Genet
Volume: 8
Issue: 12
Pages: 2317-23
Abstract: Most fragile X syndrome patients have expansion of a (CGG)(n)sequence with >200 repeats (full mutation) in the FMR1 gene responsible for this condition. Hypermethylation of the expanded repeat and of the FMR1 promoter is almost always present and apparently suppresses transcription, resulting in absence of the FMR1 protein. We recently showed that transcriptional reactivation of FMR1 full mutations can be achieved by inducing DNA demethylation with 5-azadeoxycytidine (5-azadC). The level of histone acetylation is another important factor in regulating gene expression; therefore, we treated lymphoblastoid cell lines of non-mosaic full mutation patients with three drugs capable of inducing histone hyperacetylation. We observed a consistent, although modest, reactivation of the FMR1 gene with 4-phenylbutyrate, sodium butyrate and trichostatin A, as shown by RT-PCR. However, we report that combining these drugs with 5-azadC results in a 2- to 5-fold increase in FMR1 mRNA levels obtained with 5-azadC alone, thus showing a marked synergistic effect of histone hyperacetylation and DNA demethylation in the reactivation of FMR1 full mutations.

Reference Type: Journal Article
Record Number: 244

Author: DiGiuseppe, J. A.; Weng, L. J.; Yu, K. H.; Fu, S.; Kastan, M. B.; Samid, D.; Gore, S. D.
Year: 1999
Title: Phenylbutyrate-induced G1 arrest and apoptosis in myeloid leukemia cells: structure-function analysis
Journal: Leukemia
Volume: 13
Issue: 8
Pages: 1243-53
Abstract: The aromatic fatty acid phenylbutyrate (PB) induces cytostasis, differentiation, and apoptosis in primary myeloid leukemic cells at clinically achievable concentrations. In the present study, we have investigated the structural and cellular basis for PB-induced cytostasis, using the ML-1 human myeloid leukemia cell line as a model system. PB induced a dose-dependent increase in cells in G1 with a corresponding decrease in cells in S-phase of the cell cycle. At comparable doses, PB induced expression of CD11b, indicating myeloid differentiation. At higher doses, the drug induced apoptosis. The antitumor activity was independent of the aromatic ring, as butyric acid (BA) was of equal or greater potency at producing these biological changes. In contrast, shortening of the fatty acid carbon chain length, as demonstrated with phenylacetate (PA), significantly diminished drug potency. Consistent with their effects on cell cycle, PB and BA, but not PA, induced the cyclin-dependent kinase inhibitor, p21(WAF1/CIP1), and led to the appearance of hypophosphorylated Rb, suggesting a role for p21(WAF1/CIP1) in PB-induced cytostasis. Therefore, it appears that the fatty acid moiety of PB, rather than its aromatic ring, is critical for its activity in myeloid leukemic cells. These data provide a potential mechanistic basis for the increased potency of PB over PA previously demonstrated in primary leukemic samples, and support the further clinical development of PB in the treatment of hematologic malignancies.

Reference Type: Journal Article
Record Number: 237
Author: Hoppe, C.; Vichinsky, E.; Lewis, B.; Foote, D.; Styles, L.
Year: 1999
Title: Hydroxyurea and sodium phenylbutyrate therapy in thalassemia intermedia
Journal: Am J Hematol
Volume: 62
Issue: 4
Pages: 221-7
Abstract: Hydroxyurea (HU) and sodium phenylbutyrate (SPB) have been shown to increase fetal hemoglobin (Hb F) levels in patients with thalassemia intermedia. The reported effects of these agents in increasing total Hb, however, have been inconsistent and there have been no studies on the combination of these medications. We describe the clinical response, as determined by increases in total Hb and decreased transfusion needs, in five patients with thalassemia intermedia treated with HU alone or in combination with SPB. All of the patients responded with increased levels of Hb F, but the responses in total Hb varied. Of the five patients, two had a marked response in total Hb in excess of 3 g/dl, two responded modestly with an increase in total Hb of 1-2 g/dl, and one did not respond. Prolonged responses were achieved with low doses of HU (3-10 mg/kg/day) and higher doses were associated

with mild reversible hematologic or hepatic toxicity and no further increases in Hb. Sodium phenylbutyrate was added to treatment with HU in two patients, but failed to produce an increase in total Hb despite increasing Hb F levels. Of the four patients who responded to HU with an increase in total Hb, all reported symptomatic improvement and three have not required further transfusions. We conclude that low-dose HU therapy in patients with thalassemia intermedia may increase total Hb levels sufficiently to eliminate the need for transfusions. We, therefore, recommend a trial of HU for thalassemia intermedia patients in whom chronic transfusion therapy is being contemplated.

Reference Type: Journal Article
Record Number: 240
Author: Illek, B.; Zhang, L.; Lewis, N. C.; Moss, R. B.; Dong, J. Y.; Fischer, H.
Year: 1999
Title: Defective function of the cystic fibrosis-causing missense mutation G551D is recovered by genistein
Journal: Am J Physiol
Volume: 277
Issue: 4 Pt 1
Pages: C833-9
Abstract: The patch-clamp technique was used to investigate the effects of the isoflavone genistein on disease-causing mutations (G551D and DeltaF508) of the cystic fibrosis transmembrane conductance regulator (CFTR). In HeLa cells recombinantly expressing the trafficking-competent G551D-CFTR, the forskolin-stimulated Cl currents were small, and average open probability of G551D-CFTR was P(o) = 0.047 +/- 0.019. Addition of genistein activated Cl currents approximately 10-fold, and the P(o) of G551D-CFTR increased to 0.49 +/- 0.12, which is a P(o) similar to wild-type CFTR. In cystic fibrosis (CF) epithelial cells homozygous for the trafficking-impaired DeltaF508 mutation, forskolin and genistein activated Cl currents only after 4-phenylbutyrate treatment. These data suggested that genistein activated CFTR mutants that were present in the cell membrane. Therefore, we tested the effects of genistein in CF patients with the G551D mutation in nasal potential difference (PD) measurements in vivo. The perfusion of the nasal mucosa of G551D CF patients with isoproterenol had no effect; however, genistein stimulated Cl-dependent nasal PD by, on average, -2.4 +/- 0.6 mV, which corresponds to 16.9% of the responses (to beta-adrenergic stimulation) found in healthy subjects.

Reference Type: Journal Article
Record Number: 241
Author: Lea, M. A.; Randolph, V. M.; Hodge, S. K.
Year: 1999
Title: Induction of histone acetylation and growth regulation in eryrthroleukemia cells by 4-phenylbutyrate and structural analogs
Journal: Anticancer Res
Volume: 19
Issue: 3A
Pages: 1971-6

Abstract: The objective of this investigation was to study the relationship between histone acetylation and growth inhibition by 4-phenylbutyrate and structural analogs. Inhibition of growth of DS19 mouse erythroleukemia cells and K562 human leukemic cells by 4-phenylbutyrate did not appear to be mediated by glutamine depletion. Vanadate blocked differentiation of DS19 cells but did not affect the hyperacetylation of histones. 2-phenylbutyrate was a more effective inhibitor of cell proliferation than 3-phenylbutyrate but was less effective as an inducer of histone acetylation. 4-Phenylbutyrate was a more effective inhibitor of histone deacetylase and inducer of histone acetylation than the structural analogs examined including 2- and 3-phenylbutyrate, cinnamate, methoxycinnamate, 2-phenoxybutyrate and phenoxyacetate.

Reference Type: Journal Article
Record Number: 239
Author: Loffing, J.; Moyer, B. D.; Reynolds, D.; Stanton, B. A.
Year: 1999
Title: PBA increases CFTR expression but at high doses inhibits Cl(-) secretion in Calu-3 airway epithelial cells
Journal: Am J Physiol
Volume: 277
Issue: 4 Pt 1
Pages: L700-8
Abstract: Sodium 4-phenylbutyrate (PBA), a short-chain fatty acid, has been approved to treat patients with urea cycle enzyme deficiencies and is being evaluated in the management of sickle cell disease, thalassemia, cancer, and cystic fibrosis (CF). Because relatively little is known about the effects of PBA on the expression and function of the wild-type CF transmembrane conductance regulator (wt CFTR), the goal of this study was to examine the effects of PBA and related compounds on wt CFTR-mediated Cl(-) secretion. To this end, we studied Calu-3 cells, a human airway cell line that expresses endogenous wt CFTR and has a serous cell phenotype. We report that chronic treatment of Calu-3 cells with a high concentration (5 mM) of PBA, sodium butyrate, or sodium valproate but not of sodium acetate reduced basal and 8-(4-chlorophenylthio)-cAMP-stimulated Cl(-) secretion. Paradoxically, PBA enhanced CFTR protein expression 6- to 10-fold and increased the intensity of CFTR staining in the apical plasma membrane. PBA also increased protein expression of Na(+)-K(+)-ATPase. PBA reduced CFTR Cl(-) currents across the apical membrane but had no effect on Na(+)-K(+)-ATPase activity in the basolateral membrane. Thus a high concentration of PBA (5 mM) reduces Cl(-) secretion by inhibiting CFTR Cl(-) currents across the apical membrane. In contrast, lower therapeutic concentrations of PBA (0.05-2 mM) had no effect on cAMP-stimulated Cl(-) secretion across Calu-3 cells. We conclude that PBA concentrations in the therapeutic range are unlikely to have a negative effect on Cl(-) secretion. However, concentrations >5 mM might reduce transepithelial Cl(-) secretion by serous cells in submucosal glands in individuals expressing wt CFTR.

Reference Type: Journal Article
Record Number: 248

Author: Melchior, S. W.; Brown, L. G.; Figg, W. D.; Quinn, J. E.; Santucci, R. A.; Brunner, J.; Thuroff, J. W.; Lange, P. H.; Vessella, R. L.
Year: 1999
Title: Effects of phenylbutyrate on proliferation and apoptosis in human prostate cancer cells in vitro and in vivo
Journal: Int J Oncol
Volume: 14
Issue: 3
Pages: 501-8
Abstract: Phenylbutyrate (PB) is a potent differentiating agent and currently under investigation for the treatment of prostate cancer (CaP) and other malignancies. We have studied the impact of PB in vitro and in vivo on differentiation, proliferation and apoptosis in the LNCaP and LuCaP 23.1 prostate cancer xenograft models. In vitro we found that i) PB increased PSA secretion/cell, ii) inhibited cell proliferation in a time- and dose-dependent manner resulting in a cell cycle arrest in G1-phase and iii) induced apoptosis at concentrations of 2.5 mM after 3 days of treatment. In PB treated animals tumor growth stabilized or regressed. Combination of castration and PB treatment had a synergistic antiproliferative effect. The growth-inhibitory and differentiating properties and a low toxicity profile of PB provide rationale for further clinical studies in patients with CaP.

Reference Type: Journal Article
Record Number: 236
Author: Raymond, G. V.
Year: 1999
Title: Peroxisomal disorders
Journal: Curr Opin Pediatr
Volume: 11
Issue: 6
Pages: 572-6
Abstract: Peroxisomes, subcellular organelles found in nearly all eukaryotic cells, are involved in numerous biochemical functions within the cell. There has been an increasing understanding of the genetic mechanism of the diseases of the single peroxisomal enzyme abnormalities as well as defects of peroxisome biogenesis. Peroxisome assembly disorders including Zellweger syndrome and rhizomelic chondrodysplasia punctata are caused by genetic defects in PEX genes and the altering of their proteins, peroxins, which are necessary for the importation of targeted proteins into the peroxisomes. Therapies for peroxisomal disorders have been unsatisfactory to date, but there has been interest in docosahexaenoic acid in assembly disorders and phenylbutyrate and lovastatin in adrenoleukodystrophy (ALD). Whether any of these therapies will result in clinical improvement awaits additional study.

Reference Type: Journal Article
Record Number: 235
Author: Saudubray, J. M.; Touati, G.; Delonlay, P.; Jouvet, P.; Narcy, C.; Laurent, J.; Rabier, D.; Kamoun, P.; Jan, D.; Revillon, Y.
Year: 1999
Title: Liver transplantation in urea cycle disorders

Journal: Eur J Pediatr
Volume: 158 Suppl 2
Pages: S55-9
Abstract: We report here our experience in the long-term management of 28 patients with citrullinaemia, 13 patients with carbamoyl phosphate synthase deficiency and 15 patients with argininosuccinic aciduria. In addition, we report a national French survey of 119 patients with ornithine transcarbamylase (OTC) deficiency enzymatically characterized in our laboratory. We also include in this report four personal patients (two with OTC and two with citrullinaemia) who were liver transplanted, and one OTC patient from the National French survey. Although this retrospective series is not really representative of the modern treatment combining low protein diet and arginine, sodium benzoate and sodium phenylbutyrate, it is obvious that the long-term outcome of all urea cycle disorders remains very guarded. We highlight the severity of the neonatal forms of such disorders, and mostly for OTC-deficient males. According to this evidence, our policy is not to treat such severely affected patients in the neonatal period who die anyway spontaneously within 2 to 3 days. At the present time, we only have three patients with neonatal citrullinaemia, aged 1, 6 and 10 years respectively, who are still doing well. One of them has been successfully liver transplanted at 5 years. Another transplanted patient died in the post-surgical phase. We emphasize the unexpected severity of argininosuccinic aciduria in which there is no one patient doing well. This is a rather surprising finding as this disorder is easy to manage and rarely presents with recurrent attacks of hyperammonaemia when it is treated by arginine supplementation. This consideration would suggest to extend the indication of orthotopic liver transplantation in this disorder. Finally, the most difficult indication is in the late onset symptomatic female OTC group. In this last group, despite a significant residual activity due to heterozygote status, even with a variable lyonisation, only seven girls are still mentally and neurologically normal. Interestingly, three of these seven were liver-transplanted before the constitution of irreversible neurological damage. These three girls and their family declare their well-being, their feeling to be cured and enjoy their normal life.

Reference Type: Journal Article
Record Number: 247
Author: Schwarz, S.; Schwab, S.; Hoffmann, G. F.
Year: 1999
Title: [Enzyme defects of the urea cycle in differential acute encephalopathy diagnosis in adulthood. Diagnosis and current therapy concepts]
Journal: Nervenarzt
Volume: 70
Issue: 2
Pages: 111-8
Abstract: Six enzyme defects of the urea cycle have been described. Ornithine transcarbamylase deficiency is the most frequent of these diseases. The cumulative frequency is 1:8000. Most patients become symptomatic in childhood, but onset of symptoms may occur later in childhood or even adulthood. The patients present with recurrent episodes of an unspecific acute encephalopathy, seizures and clouding of consciousness to a variable degree. Focal neurological signs such as hemiparesis, aphasia or ataxia may also occur. These episodes may be triggered by infection,

protein overload or drugs. Diagnostic are increased blood ammonia levels. Characteristic patterns of plasma amino acids and the determination of orotic acid in the urine mostly discriminate the individual disorders. Further diagnostic steps include the allopurinol challenge test, liver or skin biopsy for measurement of enzyme activity and molecular genetic studies. Treatment requires restriction of protein intake, supplementation of arginine and activation of alternative pathways of nitrogen excretion with benzoate or phenylbutyrate. Untreated, the acute episode may be lethal. Long-term treatment improves the clinical outcome considerably. Urea cycle defects should be included in the differential diagnosis of any encephalopathy or coma of unclear origin, and blood ammonia should be determined early in the evaluation of such patients.

Reference Type: Journal Article
Record Number: 246
Author: Wang, J.; Saunthararajah, Y.; Redner, R. L.; Liu, J. M.
Year: 1999
Title: Inhibitors of histone deacetylase relieve ETO-mediated repression and induce differentiation of AML1-ETO leukemia cells
Journal: Cancer Res
Volume: 59
Issue: 12
Pages: 2766-9
Abstract: The (8;21) translocation, found in 12% of acute myeloid leukemia (AML), creates the chimeric fusion product, AML1-ETO. Previously, we demonstrated that the ETO moiety recruits a transcription repression complex that includes the histone deacetylase (HDAC1) enzyme. Here, we used inhibitors of HDAC1 to study the pathophysiology of AML1-ETO. Both the potent inhibitor, trichostatin (TSA), and the well-known but less specific inhibitor, phenylbutyrate (PB), could partially reverse ETO-mediated transcriptional repression. PB was also able to induce partial differentiation of the AML1-ETO cell line, Kasumi-1. With the intention of developing a clinically useful protocol, we combined PB with a number of other agents that induced differentiation and apoptosis of Kasumi-1 cells. In summary, transcriptional repression mediated by AML1-ETO appears to play a mechanistic role in the t(8;21) AML, and relief of repression using agents such as PB (alone or in combination) may prove to be therapeutically useful.

Reference Type: Journal Article
Record Number: 243
Author: Yu, K. H.; Weng, L. J.; Fu, S.; Piantadosi, S.; Gore, S. D.
Year: 1999
Title: Augmentation of phenylbutyrate-induced differentiation of myeloid leukemia cells using all-trans retinoic acid
Journal: Leukemia
Volume: 13
Issue: 8
Pages: 1258-65
Abstract: Despite preliminary evidence of clinical activity of the putative differentiating agent sodium phenylbutyrate (PB) in the treatment of myeloid

neoplasms, it has proven difficult to maintain therapeutic levels of PB above 0.5 mM, well below the ED50 of 1-2 mM. We have studied the impact of combining PB with all-trans retinoic acid (ATRA) on the ML-1 myeloid leukemia cell line. ATRA augmented PB-induced differentiation, cell-cycle arrest, and apoptosis. ATRA augmented PB induction of the myelomonocytic marker CD11b at all doses of ATRA tested (0.0025-1 microM). Although ATRA did not significantly affect the ED50 of PB, the combination of ATRA (1 microM) and PB (0.5 mM) augmented PB-induced CD11b expression eight-fold. Compared to PB alone, this combination of ATRA and PB induced greater cell cycle arrest (S-phase 14% vs 38%; G0/G1-phase cells 72% vs 52%) and greater apoptosis (24% vs 16% by TUNEL assay). Treatment with ATRA (0.5 microM) in combination with PB (0.5 mM) led to significantly greater inhibition of colony formation (4.8% vs 48% inhibition). ATRA combined synergistically with PB to augment CD11b expression and inhibit colony formation. This combination also showed significant interaction in terms of S-phase inhibition. However, this interaction varied as a function of ATRA concentration: antagonistic at low concentrations of ATRA, synergistic at higher concentrations of ATRA. These data suggest that retinoids may significantly augment the cytostatic and differentiating activity of PB, leading to increased potency of the latter drug at clinically achievable doses.

Reference Type: Journal Article
Record Number: 225
Author: Amada, K.; Haruki, M.; Imanaka, T.; Morikawa, M.; Kanaya, S.
Year: 2000
Title: Overproduction in Escherichia coli, purification and characterization of a family I.3 lipase from Pseudomonas sp. MIS38
Journal: Biochim Biophys Acta
Volume: 1478
Issue: 2
Pages: 201-10
Abstract: Determination of the nucleotide sequence of the gene encoding a lipase from Pseudomonas sp. MIS38 (PML) revealed that PML is a member of the lipase family I.3 and is composed of 617 amino acid residues with a calculated molecular weight of 64510. Recombinant PML (rPML) was overproduced in Escherichia coli in an insoluble form, solubilized in the presence of 8 M urea, purified in a urea-denatured form and refolded by removing urea in the presence of the Ca(2+) ion. Gel filtration chromatography suggests that this refolded protein is monomeric. rPML showed relatively broad substrate specificities and hydrolyzed glyceryl tributyrate and olive oil with comparable efficiencies. rPML was active only in the form of a holo-enzyme, in which at least 12 Ca(2+) ions bound. These Ca(2+) ions bound too tightly to be removed from the protein upon dialysis, but were removed from it upon EDTA treatment. The resultant apo-enzyme was fully active in the presence of 10 mM CaCl(2), but was inactive in the absence of the Ca(2+) ion. PML has a GXSXG motif, which is conserved in lipases/esterases and generally contains the active-site serine. The mutation of Ser(207) within this motif to Ala completely inactivated PML, suggesting that Ser(207) is the active-site serine of PML.

Reference Type: Journal Article

Record Number: 223
Author: Andersson, C.; Roomans, G. M.
Year: 2000
Title: Activation of deltaF508 CFTR in a cystic fibrosis respiratory epithelial cell line by 4-phenylbutyrate, genistein and CPX
Journal: Eur Respir J
Volume: 15
Issue: 5
Pages: 937-41
Abstract: The cellular basis of cystic fibrosis (CF) is a defect in a cyclic adenosine monophosphate (cAMP)-activated chloride channel (CF transmembrane conductance regulator) in epithelial cells that leads to decreased chloride ion transport and impaired water transport across the cell membrane. This study investigated whether it was possible to activate the defective chloride channel in cystic fibrosis respiratory epithelial cells with 4-phenylbutyrate (4PBA), genistein and 8-cyclopentyl-1,3-dipropylxanthine (CPX). The CF bronchial epithelial cell line CFBE41o-, which expresses the deltaF508 mutation, was treated with these agents and loss of Cl-, indicating Cl- efflux, measured by X-ray microanalysis. 8-bromo-cAMP alone did not induce Cl- efflux in CFBE41o- cells, but after incubation with 4PBA a significant efflux of Cl- occurred. Stimulation of cells with a combination of genistein and cAMP also induced Cl- efflux, whereas a combination of pretreatment with 4PBA and a combined stimulation with genistein and cAMP induced an even larger Cl- efflux. Cl- efflux could also be stimulated by CPX, but this effect was not enhanced by 4PBA pretreatment. The deltaF508 mutation leads to impaired processing of the cystic fibrosis transmembrane conductance regulator. The increased efflux of chloride after 4-phenylbutyrate treatment can be explained by the fact that 4-phenylbutyrate allows the deltaF508 cystic fibrosis transmembrane conductance regulator to escape degradation and to be transported to the cell surface. Genistein and 8-cyclopentyl-1,3-dipropylxanthine act by stimulating chloride ion efflux by increasing the probability of the cystic fibrosis transmembrane conductance regulator being open. The combination of 4-phenylbutyrate and genistein may be useful in a potential pharmacological therapy for cystic fibrosis patients with the deltaF508 mutation.

Reference Type: Journal Article
Record Number: 215
Author: Bogdanovic, M. D.; Kidd, D.; Briddon, A.; Duncan, J. S.; Land, J. M.
Year: 2000
Title: Late onset heterozygous ornithine transcarbamylase deficiency mimicking complex partial status epilepticus
Journal: J Neurol Neurosurg Psychiatry
Volume: 69
Issue: 6
Pages: 813-5
Abstract: A 57 year old woman with post-traumatic complex partial seizures was admitted because of recurrent episodes of altered mental state over the preceding 4 years, each lasting up to 5 days. There was a history of dietary protein intolerance since childhood and two of her daughters had died in the neonatal period from unexplained encephalopathies. In hospital she developed fluctuating confusion, amnesia, and sudden episodes of unresponsiveness. An EEG was consistent with

complex partial status epilepticus but there was no response to benzodiazepines. Nasogastric feeding and sodium valproate were given and shortly afterwards she lapsed into a deep coma. Blood ammonia and urinary orotate were raised, and genetic testing confirmed that she was a carrier of a mutation in exon 3 of the ornithine transcarbamylase gene (C to T at position 92). Treatment with protein restriction, carnitine, and sodium phenylbutyrate led to a full recovery over a period of 3 months. To our knowledge this is the oldest age of onset yet described in a manifesting carrier. She is the fifth patient with heterozygous ornithine transcarbamylase deficiency reported to have had a severe reaction to sodium valproate. Hyperammonaemic encephalopathy should be considered in patients of any age who experience fluctuating confusion.

Reference Type: Journal Article
Record Number: 234
Author: Bradbury, N. A.
Year: 2000
Title: Focus on "Sodium 4-phenylbutyrate downregulates Hsc70: implications for intracellular trafficking of DeltaF508-CFTR"
Journal: Am J Physiol Cell Physiol
Volume: 278
Issue: 2
Pages: C257-8

Reference Type: Journal Article
Record Number: 218
Author: Cheson, B. D.; Zwiebel, J. A.; Dancey, J.; Murgo, A.
Year: 2000
Title: Novel therapeutic agents for the treatment of myelodysplastic syndromes
Journal: Semin Oncol
Volume: 27
Issue: 5
Pages: 560-77
Abstract: Few chemotherapy agents have demonstrated activity in patients with myelodysplastic syndromes (MDS) and supportive management remains the standard of care. An increasing number of new drugs in development are being directed at specific molecular or biological targets of these diseases. Topotecan, a topoisomerase I inhibitor, has shown single-agent activity and is now being combined with other agents, including cytarabine. The aminothiol amifostine induces responses in about 30% of patients; however, its role is still being clarified. Agents that inhibit histone deacetylase and target DNA hypermethylation, thus permitting derepression of normal genes, include 5-azacytidine, decitabine, phenylbutyrate, and depsipeptide. Arsenic trioxide has demonstrated impressive activity in acute promyelocytic leukemia and preclinical data suggest the potential for activity in MDS. UCN-01 is a novel agent that inhibits protein kinase C and other protein kinases important for progression through the G1 and G2 phases of the cell cycle. Dolastatin-10 has extremely potent in vitro activity against a variety of tumor cell lines. Since its dose-limiting toxicities include myelosuppression, it is being studied in acute myelogenous leukemia (AML) and MDS. Ras may play a role in MDS, and activation of this gene and its signaling

pathways may require farnesylation. Several farnesyl transferase inhibitors are now available for study in patients with MDS. An increasing body of data suggests a possible role for angiogenesis in MDS, and several antiangiogenesis agents are in clinical trials, including thalidomide, SU5416, and anti-vascular endothelial growth factor (VEGF) antibodies. Development of new drugs and regimens will be facilitated by recently developed standardized response criteria. Future clinical trials should focus on rational combinations of these agents and others with the goal of curing patients with MDS.

Reference Type: Journal Article
Record Number: 226
Author: Chung, Y. L.; Lee, Y. H.; Yen, S. H.; Chi, K. H.
Year: 2000
Title: A novel approach for nasopharyngeal carcinoma treatment uses phenylbutyrate as a protein kinase C modulator: implications for radiosensitization and EBV-targeted therapy
Journal: Clin Cancer Res
Volume: 6
Issue: 4
Pages: 1452-8
Abstract: Sodium phenylbutyrate (NaPB) represent a new non-toxic class of compounds with antiproliferative activities to different tumors and has been shown to modulate many gene expressions by inhibiting histone deacetylation and DNA methylation as the major mechanism. Butyrate and other protein kinase C (PKC) activators have been reported to be able to activate virus enzymes. The present work investigates whether NaPB has an antiproliferative effect or modulatory effects on EBV-associated nasopharyngeal carcinoma (NPC) and whether EBV thymidine kinase gene can be activated to make cells susceptible to ganciclovir (GCV) therapy. NaPB treatment displayed a dose- and time-dependent antiproliferative effect on the NPC cell line CNE2. Cell cycle analysis revealed an inhibitory effect of NaPB on G1-S-phase progression. Shortly after NaPB treatment, we found that PKC activity was activated rapidly but also decreased rapidly. Down-regulation of PKC-alpha and translocation of PKC-alpha from the cytosol to membrane were seen by Western blot. The decrease in PKC activity by NaPB corresponds to an enhanced response to radiation on CEN2 cells. Moreover, NaPB up-regulated EBV thymidine kinase activity to render EBV-associated Daudi cells susceptible to killing by GCV. Based on the observations of NaPB as a PKC modulator, the combination of NaPB, GCV, and radiation may provide a potential novel approach for treatment of EBV-associated NPC.

Reference Type: Journal Article
Record Number: 212
Author: Davis, T.; Kennedy, C.; Chiew, Y. E.; Clarke, C. L.; deFazio, A.
Year: 2000
Title: Histone deacetylase inhibitors decrease proliferation and modulate cell cycle gene expression in normal mammary epithelial cells
Journal: Clin Cancer Res
Volume: 6

Issue: 11
Pages: 4334-42
Abstract: Full-term pregnancy early in reproductive life is protective against breast cancer in women. The protective effects of parity have variously been attributed to the differentiation that accompanies pregnancy and lactation, alterations in ovarian hormone receptor levels, and altered sensitivity to ovarian hormones. Butyrate, a short-chain fatty acid, induces differentiation in breast cancer cell lines and decreases hormone receptor expression. Butyrate also inhibits proliferation in breast cancer cell lines and modulates expression of key cell cycle-regulatory proteins including cyclin D1. Given these properties, butyrate could be considered a promising agent for breast cancer prevention. Therefore, this study aimed to determine the effects of butyrate on normal human breast epithelial cells and to compare the effects of two stable butyrate derivatives with more favorable pharmacological properties: phenylacetate and its p.o. active precursor phenylbutyrate. Treatment with each agent resulted in concentration-dependent growth inhibition in a normal breast epithelial cell line and two breast cancer cell lines (MCF-7 and MDA-MB-231). Phenylbutyrate and butyrate inhibited proliferation to a similar extent, but phenylacetate was less effective in all of the cell lines. All three of the agents induced differentiation (accumulation of lipid droplets) in normal as well as in breast cancer cells and caused a decrease in estrogen receptor (ER) mRNA in MCF-7 cells. The butyrates decreased expression of cyclin D1, increased expression of p21(Waf1/Cip1), and hypophosphorylated pRB in the normal mammary epithelial cells. The effects on cyclin D1 expression correlated with the effects on cell proliferation, which suggests that modulation of cyclin D1 expression may underpin the antiproliferative effects of butyrates. We have shown that butyrate and butyrate-like agents are able to decrease proliferation and induce differentiation in normal breast cells as well as in malignant breast cells (ER-positive and ER-negative) and, as such, may be considered as candidate chemopreventative agents for women at high risk of developing breast cancer.

Reference Type: Journal Article
Record Number: 214
Author: Gore, S. D.; Carducci, M. A.
Year: 2000
Title: Modifying histones to tame cancer: clinical development of sodium phenylbutyrate and other histone deacetylase inhibitors
Journal: Expert Opin Investig Drugs
Volume: 9
Issue: 12
Pages: 2923-34
Abstract: Compounds that inhibit histone deacetylase may enable the re-expression of silenced regulatory genes in neoplastic cells, reversing the malignant phenotype. Although several molecules that inhibit histone deacetylase are undergoing preclinical development, butyric acid derivatives have undergone clinical investigation for several years, initially for non-malignant indications and more recently for the treatment of cancer. Of the butyric acid derivatives, sodium phenylbutyrate has undergone the most extensive systematic investigation. Administration of phenylbutyrate by iv. and oral routes is well-tolerated clinically at concentrations which effect acetylation of histones in vitro. Higher doses lead to reversible CNS depression. The studies presented to date have been Phase I studies and do not enable

assessment of efficacy. However, current development of phenylbutyrate is proceeding in combination with other agents based on rational biologically-based in vitro studies. The parallel development of combination therapy including phenylbutyrate and early clinical development of other, more potent histone deacetylase inhibitors will hopefully lead to feasible, clinically tolerable strategies for altering the malignant phenotype of cancer cells.

Reference Type: Journal Article
Record Number: 188
Author: Gorin, N. C.; Estey, E.; Jones, R. J.; Levitsky, H. I.; Borrello, I.; Slavin, S.
Year: 2000
Title: New Developments in the Therapy of Acute Myelocytic Leukemia
Journal: Hematology Am Soc Hematol Educ Program
Pages: 69-89
Abstract: Current conventional treatment for patients with acute myelogenous leukemia results in a high percentage of clinical responses in most patients. However, a high percentage of patients still remain refractory to primary therapy or relapse later. This review examines the search for new agents and new modes of therapy. In Section I, Dr. Estey discusses new agents directed at various targets, such as CD33, angiogenesis, inappropriately methylated (suppressor) genes, cell cycle checkpoints, proteosomes, multidrug resistance (MDR) gene, mitochondrial apoptotic pathway. He also reviews preliminary results of phase I trials with the nucleoside analog troxacitabine and liposomal anthracyclin and suggests new strategies for trials of new agents. In Section II, Dr. Jones revisits differentiation therapy and presents results of preclinical and clinical studies that demonstrate that a variety of clinically applicable cell cycle inhibitors (interferon, phenylbutyrate, vitamin D, retinoids, bryostatin-1) preferentially augments growth factor-mediated induction of myeloid leukemia terminal differentiation, as well as blocks growth factors' effects on leukemia proliferation. The combination of cell cycle inhibition plus myeloid growth factors may offer a potential treatment for resistant myeloid leukemias. In Section III, Drs. Levitsky and Borrello address the question of tumor vaccination in AML and shows that, although tumor rejection antigens in AML have not been formally identified to date, a growing number of attractive candidates are ripe for testing with defined antigen-specific vaccine strategies. Interestingly, the ability to drive leukemic blasts to differentiate into competent antigen presenting cells such as dendritic cells may be exploited in the creation of cellular vaccines. Ultimately, the successful development of active immunotherapy for AML will require integration with dose-intensive chemotherapy, necessitating a more complete understanding of host immune reconstitution. In Section IV, Dr. Slavin reviews the concept of delivering non-myeloablative stem cell transplantation (NST) and delayed lymphocyte infusion (DLI) to increase tolerance in particular in high risk and older patients, and take advantage of the graft-versus-leukemia (GVL) effect. All these approaches hold promise in reducing morbidity and mortality and differ from the older concepts aiming at delivering the highest possible doses of chemotherapy and/or total body irradiation to reach maximum leukemia cell kill, whatever the toxicity to the patient.

Reference Type: Journal Article
Record Number: 213

Author: Halicka, H. D.; Murakami, T.; Papageorgio, C. N.; Mittelman, A.; Mikulski, S. M.; Shogen, K.; Darzynkiewicz, Z.
Year: 2000
Title: Induction of differentiation of leukaemic (HL-60) or prostate cancer (LNCaP, JCA-1) cells potentiates apoptosis triggered by onconase
Journal: Cell Prolif
Volume: 33
Issue: 6
Pages: 407-17
Abstract: Onconase (Onc) is a ribonuclease from amphibian oocytes that is cytostatic and cytotoxic to many tumour lines. It shows in vivo antitumour activity in mouse tumour models and is currently in Phase III clinical trials. The present study was designed to test whether cytotoxic effects of ONC can be modulated by differentiating agents. Human leukaemic HL-60 and prostate cancer LNCaP and JCA-1 cells were treated with Onc in the absence and presence of several inducers of differentiation and frequency of apoptosis was assessed using three different cytometric methods and confirmed by analysis of cell morphology. A moderate degree of apoptosis observed after 48-72 h incubation of HL-60 cells in the presence of 0.42 microM Onc alone was markedly potentiated by administration of retinoic acid (all trans), sodium butyrate or dimethylsulfoxide at concentrations known to induce differentiation but be minimally cytotoxic. Likewise, the frequency of apoptosis of LNCaP and JCA-1 cells treated with Onc was increased in the cultures to which phenylbutyrate was added. Although cell treatment with Onc alone, with each of the differentiating agents alone or with Onc in combination with the differentiating agents led to an increase in the proportion of G1 cells, no specific cell cycle phase preference in induction of apoptosis was observed. The data suggest that cells undergoing differentiation are particularly vulnerable to Onc; a combination of Onc and differentiating agents should be considered for further in vivo tests to assess its possible usefulness in the clinic.

Reference Type: Journal Article
Record Number: 222
Author: Huang, Y.; Horvath, C. M.; Waxman, S.
Year: 2000
Title: Regrowth of 5-fluorouracil-treated human colon cancer cells is prevented by the combination of interferon gamma, indomethacin, and phenylbutyrate
Journal: Cancer Res
Volume: 60
Issue: 12
Pages: 3200-6
Abstract: We previously reported that phenylbutyrate (PB), a differentiation agent, retarded the regrowth of fluoropyrimidine-treated HT29 cells to a greater extent in a well-differentiated subclone as compared with a poorly differentiated subclone (Y. Huang and S. Waxman, Clin. Cancer Res., 4: 2503-2509, 1998). To extend these results and to overcome the known heterogeneity of human colon carcinoma (HCC) cells, the effect of cytostatic agents reported to inhibit HCC growth [IFN-alpha and IFN-gamma, indomethacin, and PB alone or in combination] on clonogenicity and HCCs recovery from 5-fluorouracil (FUra) treatment was studied in eight different HCCs. IFN-alpha proved to be ineffective in all eight HCCs, whereas IFN-gamma

induced marked growth inhibition in four HCCs that expressed wild-type K-ras. Despite large differences in HCC response to the other individual agents, strong growth inhibition was observed when PB was added in combination with indomethacin. The inhibition was even more pronounced when IFN-gamma was included in the regimen. Most importantly, after treatment with the combination of three agents, the clonogenic potential was severely inhibited (92-100%) in the IFN-gamma-sensitive cell lines, whereas in the IFN-gamma-insensitive cell lines, comparable loss of clonogenecity was obtained when the cells were pretreated with FUra. As known and described in detail, the three cytostatic agents inhibit different processes necessary for cell growth, thus requiring the cells to repair multiple pathways to restore growth. The induction of STAT1 DNA binding activity by IFN-gamma and p21WAF1 by PB, alone or in combination, correlated with growth inhibition and loss of clonogenicity. The finding that the readily reversible growth inhibition and decrease in clonogenicity of FUra-treated HCC are prolonged by subsequent treatment with the three cytostatic agents in all HCCs may be of clinical importance because FUra continues to be the most widely used cytotoxic agent in the treatment of colon carcinoma.

Reference Type: Journal Article
Record Number: 217
Author: Maslak, P.; Scheinberg, D.
Year: 2000
Title: Targeted therapies for the myeloid leukaemias
Journal: Expert Opin Investig Drugs
Volume: 9
Issue: 6
Pages: 1197-205
Abstract: Although the standard approach to myeloid leukaemias remains chemotherapy, the agents currently available rarely result in cure. Recent advances in understanding the biology of these disorders have lead to the development of targeted treatment strategies. In acute promyelocytic leukaemia (APL), all-trans retinoic acid (ATRA), sodium phenylbutyrate and arsenic trioxide are agents which either induce differentiation or apoptosis and have been used to successfully achieve remission. The tyrosine kinase inhibitor, STI-571, antisense oligonucleotides, and bcr-abl vaccines are strategies which focus on the oncogenic events in chronic myelogenous leukaemia (CML). Two anti-CD33 monoclonal antibody conjugates, Y90-HuM195 and CMA-676, have been used in acute myelogenous leukaemia (AML) and have shown some efficacy. Although the preliminary results with these targeted therapies are promising, further studies are needed to establish them as effective, less toxic alternatives to the current standard of care.

Reference Type: Journal Article
Record Number: 219
Author: McGrath-Morrow, S. A.; Stahl, J. L.
Year: 2000
Title: G(1) Phase growth arrest and induction of p21(Waf1/Cip1/Sdi1) in IB3-1 cells treated with 4-sodium phenylbutyrate
Journal: J Pharmacol Exp Ther

Volume: 294
Issue: 3
Pages: 941-7
Abstract: 4-Sodium phenylbutyrate (4-PBA) has been used for many years in the treatment of urea cycle defects and has recently been studied as a chemotherapeutic agent for certain malignancies. 4-PBA has been shown to cause growth arrest, cellular differentiation, and apoptosis in certain malignant cells. Recently, it was shown that IB3-1 cells (a cystic fibrosis cell line, Delta508/W128X) treated with 4-PBA demonstrated a partial correction of the cystic fibrosis chloride channel defect. We were interested in evaluating the effect of 4-PBA on cell growth and cell cycle regulation in IB3-1 cells treated with 2 to 10 mM concentrations. We found that cells treated with 2 mM concentrations of 4-PBA for 96 h underwent a significant decrease in cell growth ($P < .007$). Using flow cytometry, we were able to demonstrate that growth arrest occurred at the G(1) phase of the cell cycle. This was detected as early as 24 h in IB3-1 cells treated with 5 mM 4-PBA ($P < .03$). Furthermore, the percentage of IB3-1 cells with less than a 2N DNA content increased with higher concentrations of 4-PBA, although this was not associated with an increase in apoptosis. Finally, p21(Waf1/Cip1/Sdi1) protein levels were induced in IB3-1 cells receiving 2 and 5 mM concentrations of 4-PBA as early as 24 h of exposure, suggesting that G(1) phase growth arrest in IB3-1 cells treated with 4-PBA is regulated through the p21(Waf1/Cip1/Sdi1) pathway.

Reference Type: Journal Article
Record Number: 216
Author: McGuinness, M. C.; Wei, H.; Smith, K. D.
Year: 2000
Title: Therapeutic developments in peroxisome biogenesis disorders
Journal: Expert Opin Investig Drugs
Volume: 9
Issue: 9
Pages: 1985-92
Abstract: Clinically, peroxisome biogenesis disorders (PBDs) are a group of lethal diseases with a continuum of severity of clinical symptoms ranging from the most severe form, Zellweger syndrome, to the milder forms, infantile Refsum disease and rhizomelic chondrodysplasia punctata. PBDs are characterised by a number of biochemical abnormalities including impaired degradation of peroxide, very long chain fatty acids, pipecolic acid, phytanic acid and xenobiotics and impaired synthesis of plasmalogens, bile acids, cholesterol and docosahexaenoic acid. Treatment of PBD patients as a group is problematic since a number of patients, especially those with Zellweger syndrome, have significant neocortical alterations in the brain at birth so that full recovery would be impossible even with postnatal therapy. To date, treatment of PBD patients has generally involved only supportive care and symptomatic therapy. However, the fact that some of the milder PBD patients live into the second decade has prompted research into possible treatments for these patients. A number of experimental therapies have been evaluated to determine whether or not correction of biochemical abnormalities through dietary supplementation and/or modification is of clinical benefit to PBD patients. Another approach has been pharmacological induction of peroxisomes in PBD patients to improve overall peroxisomal biochemical function. Well known rodent peroxisomal proliferators were found not to

induce human peroxisomes. Recently, our laboratory demonstrated that sodium 4-phenylbutyrate induces peroxisome proliferation and improves biochemical function (very long chain fatty acid beta-oxidation rates and very long chain fatty acid and plasmalogens levels) in fibroblast cell lines from patients with milder PBD phenotypes. Dietary supplementation and/or modification and pharmacological induction of peroxisomes as treatment strategies for PBD patients will be the subject of this review.

Reference Type: Journal Article
Record Number: 187
Author: Mokrzycki, K.
Year: 2000
Title: [Anti-atherosclerotic efficacy of quercetin and sodium phenylbutyrate in rabbits]
Journal: Ann Acad Med Stetin
Volume: 46
Pages: 189-200
Abstract: The aim of the study was to evaluate the antiatherosclerotic efficacy of a natural bioflavonoid--quercetin and sodium phenylbutyrate (tributyrate) in rabbits. Fifty male mixed-breed rabbits were randomly assigned to 5 equal groups: I--control; II--fat-rich diet (FRD); III--FRD and sodium phenylbutyrate; IV--FRD and quercetin; V--FRD, quercetin and sodium phenylbutyrate. The whole study lasted 12 weeks and the following tests were performed: 1) biochemical analysis of cholesterol--total cholesterol (TCh), low density lipoprotein cholesterol (LDL-Ch), and high density lipoprotein cholesterol (HDL-Ch)) and triglycerides (TG); 2) pathomorphologic (microscopic and macroscopic) evaluation of aorta and coronary arteries. A significant reduction in total cholesterol and LDL-cholesterol was observed in animals given sodium phenylbutyrate. The hypolipemic effect of quercetin was limited, with a significant decrease in LDL-Ch and increase in HDL-Ch. Quercetin and sodium phenylbutyrate administered together were least effective, insignificantly lowering TCh, LDL-Ch, and TG and increasing HDL-Ch (Tab. 1). Macroscopic and microscopic evaluation of aorta revealed that the area covered by atherosclerotic plaques was smallest and the atherosclerotic changes thinnest in animals on FRD and quercetin (Tab. 2, Fig. 1-2). There was no significant reduction in aortic plaque area in the groups III and V in comparison with group II. Coronary arteries displayed more advanced atherosclerotic changes than aorta and were more resistant to the administered substances (Fig. 3-4). The following conclusions were drawn: 1) Sodium phenylbutyrate and quercetin have an antiatherosclerotic activity in rabbits. 2) Combination of hypolipemic drugs does not always give the expected prophylactic and therapeutic effect. 3) Severity of atherosclerotic changes and the effect of quercetin or phenylbutyrate were not identical in the aorta and coronary arteries. 4) Antiatherosclerotic properties of sodium phenylbutyrate and quercetin are worth further clinical investigations.

Reference Type: Journal Article
Record Number: 230
Author: Ng, A. Y.; Bales, W.; Veltri, R. W.
Year: 2000

Title: Phenylbutyrate-induced apoptosis and differential expression of Bcl-2, Bax, p53 and Fas in human prostate cancer cell lines
Journal: Anal Quant Cytol Histol
Volume: 22
Issue: 1
Pages: 45-54
Abstract: OBJECTIVE: To assess the mechanisms of action of phenylbutyrate (PB), an investigational chemotherapeutic agent for prostate cancer (PCa), in apoptosis induction in PCa cell lines in vitro. STUDY DESIGN: We analyzed the differential expression of different apoptosis modulators, Bcl-2, Bax, p53 and Fas, for their potential role in PB-induced apoptosis using relative quantitative flow cytometry (FCM). Both androgen-dependent (LNCaP) and androgen-independent (C-4-2, PC-3-PF and DU145) human PCa cell lines were examined. RESULTS: PB induced apoptosis in PCa cell lines in a dose-dependent manner. Fifty percent apoptosis could be induced by 5-10 mM PB. Bcl-2 was down-regulated 30-75% and the Bax:Bcl-2 ratio elevated in apoptotic PCa cell lines regardless of their androgen dependency or p53 status. FCM revealed a heterogeneous stimulatory effect on the expression of Bax and Bcl-2 in PC3-PF cells at 0.5-2.5 mM PB. In a p53-positive cell line (DU145), p53 was repressed by 70% and Fas elevated sixfold with 10 mM PB. CONCLUSION: Our data show that PB-induced PCa apoptosis is associated with the relative repression of Bcl-2 and with up-regulation of Bax and Fas proteins and that this PB-induced apoptosis is independent of p53 and androgen-dependency status of PCa cell lines.

Reference Type: Journal Article
Record Number: 221
Author: Nieder, C.; Nestle, U.
Year: 2000
Title: A review of current and future treatment strategies for malignant astrocytomas in adults
Journal: Strahlenther Onkol
Volume: 176
Issue: 6
Pages: 251-8
Abstract: BACKGROUND: For more than 20 years, after establishing the role of postoperative radiotherapy for malignant astrocytomas, no definitive improvement in survival rates could be observed, despite advances in established treatment modalities such as radiotherapy and chemotherapy. This review discusses available laboratory and clinical data as well as recent advances in our knowledge about prognostic factors (Table 1) and their implications for the design of future clinical trials. RESULTS: Elucidation of the biology of malignant astrocytomas allowed for development of rational new approaches, such as gene therapy and immunotherapy, which could interfere with established treatment regimens or being used independently. Possible strategies include the restoration of defective cancer-inhibitory genes, cell transduction or transfection with antisense DNA corresponding to genes coding for growth factors and their receptors, or with the so-called suicide genes. Several antiangiogenic approaches such as administration of thalidomide, protamine, or monoclonal antibodies against vascular endothelial growth factor have been developed, too. Further treatment possibilities include modulation of drug resistance, e.g. by P-glycoprotein antagonists or O6-alkyl-guanine-DNA-transferase inhibitors,

inhibition of matrix metalloproteinases, inhibition of protein kinase C, and administration of agents such as phenylbutyrate or valproic acid that showed promising antiproliferative effects in vitro. CONCLUSIONS: Several rational new approaches are now entering clinical trials (Table 2). In the light of limited survival after standard treatment it is recommended that patients should be offered participation in such trials.

Reference Type: Journal Article
Record Number: 231
Author: Redonnet-Vernhet, I.; Rouanet, F.; Pedespan, J. M.; Hocke, C.; Parrot, F.
Year: 2000
Title: A successful pregnancy in a heterozygote for OTC deficiency treated with sodium phenylbutyrate
Journal: Neurology
Volume: 54
Issue: 4
Pages: 1008

Reference Type: Journal Article
Record Number: 199
Author: Reynolds, S.; Cederberg, H.; Chakrabarty, S.
Year: 2000
Title: Inhibitory effect of 1-O (2 methoxy) hexadecyl glycerol and phenylbutyrate on the malignant properties of human prostate cancer cells
Journal: Clin Exp Metastasis
Volume: 18
Issue: 4
Pages: 309-12
Abstract: The ability of the naturally occurring ether lipid, 1-O (2 methoxy) hexadecyl glycerol (MHG), and phenylbutyrate (BP) to inhibit cellular proliferation, anchorage-independent growth and cellular invasion in the human prostate cancer LnCap and DU145 cells was determined. Both MHG and PB inhibited the malignant properties of these prostate cancer cells. The concentrations required to achieve similar inhibitory effect, however, were significantly different for these two agents. MHG inhibited cell growth with equal potency in these cell lines with an IC-50 value of 93 microM for LnCap, and 97 microM for DU145. The IC-50 values for PB were 1.3 mM and 7.3 mM, respectively, for LnCap and DU145 cells. Both MHG and PB (IC-50 concentrations) inhibited the anchorage-independent growth and cellular invasion in these cells. Over 50% inhibition of anchorage-independent growth was achieved for both LnCap and DU145 cells by PB, while a lesser degree of inhibition was achieved with MHG. Both MHG- and PB-treated cells showed a reduced propensity to invade matrigels. Invasion of PB-treated LnCap and DU145 cells was reduced, respectively, by approximate 41 and 30% when compared to untreated control cells, while invasion of MHG-treated LnCap and DU145 cells was reduced to a lesser extent. Because differentiation-inducing agents may possess chemopreventive properties, the use of naturally occurring MHG and nontoxic PB in the chemoprevention of malignant diseases warrants further investigation.

Reference Type: Journal Article
Record Number: 233
Author: Rubenstein, R. C.; Zeitlin, P. L.
Year: 2000
Title: Sodium 4-phenylbutyrate downregulates Hsc70: implications for intracellular trafficking of DeltaF508-CFTR
Journal: Am J Physiol Cell Physiol
Volume: 278
Issue: 2
Pages: C259-67
Abstract: The most common mutation of the cystic fibrosis transmembrane conductance regulator (CFTR), DeltaF508, is a trafficking mutant that has prolonged associations with molecular chaperones and is rapidly degraded, at least in part by the ubiquitin-proteasome system. Sodium 4-phenylbutyrate (4PBA) improves DeltaF508-CFTR trafficking and function in vitro in cystic fibrosis epithelial cells and in vivo. To further understand the mechanism of action of 4PBA, we tested the hypothesis that 4PBA modulates the targeting of DeltaF508-CFTR for ubiquitination and degradation by reducing the expression of Hsc70 in cystic fibrosis epithelial cells. IB3-1 cells (genotype DeltaF508/W1282X) that were treated with 0.05-5 mM 4PBA for 2 days in culture demonstrated a dose-dependent reduction in Hsc70 protein immunoreactivity and mRNA levels. Immunoprecipitation with Hsc70-specific antiserum demonstrated that Hsc70 and CFTR associated under control conditions and that treatment with 4PBA reduced these complexes. Levels of immunoreactive Hsp40, Hdj2, Hsp70, Hsp90, and calnexin were unaffected by 4PBA treatment. These data suggest that 4PBA may improve DeltaF508-CFTR trafficking by allowing a greater proportion of mutant CFTR to escape association with Hsc70.

Reference Type: Journal Article
Record Number: 227
Author: Samid, D.; Wells, M.; Greene, M. E.; Shen, W.; Palmer, C. N.; Thibault, A.
Year: 2000
Title: Peroxisome proliferator-activated receptor gamma as a novel target in cancer therapy: binding and activation by an aromatic fatty acid with clinical antitumor activity
Journal: Clin Cancer Res
Volume: 6
Issue: 3
Pages: 933-41
Abstract: Aromatic fatty acids, of which phenylacetate is a prototype, constitute a class of low toxicity drugs with demonstrated antitumor activity in experimental models and in humans. Using in vitro models, we show here a tight correlation between tumor growth arrest by phenylacetate and activation of peroxisome proliferator-activated receptor gamma (PPARgamma), a member of the nuclear receptor superfamily. In support are the following observations: (a) the efficacy of phenylacetate as a cytostatic agent was correlated with pre-treatment levels of PPARgamma, as documented using established tumor lines and forced expression models; (b) in responsive tumor cells, PPARgamma expression was up-regulated within 2-9 h of treatment preceding increases in p21waf1, a marker of cell cycle

arrest; (c) inhibition of mitogen-activated protein kinase, a negative regulator of PPARgamma, enhanced drug activity; and (d) phenylacetate interacted directly with the ligand-binding site of PPARgamma and activated its transcriptional function. The ability to bind and activate PPARgamma was common to biologically active analogues of phenylacetate and corresponded to their potency as antitumor agents (phenylacetate < phenylbutyrate < p-chloro-phenylacetate < p-iodo-phenylbutyrate), whereas an inactive derivative, phenylacetylglutamine, had no effect on PPARgamma. These findings point to PPARgamma as a novel target in cancer therapy and provide the first identification of ligands that have selective antitumor activity in patients.

Reference Type: Journal Article
Record Number: 207
Author: Sowa, Y.; Sakai, T.
Year: 2000
Title: Butyrate as a model for "gene-regulating chemoprevention and chemotherapy."
Journal: Biofactors
Volume: 12
Issue: 1-4
Pages: 283-7
Abstract: Recent progress in molecular genetics has facilitated understanding of the mechanisms of carcinogenesis. However, there is not yet any effective therapy or prevention for cancer based on the molecular mechanisms of carcinogenesis. So-called "gene therapy" for cancer is expected to become a new method of treatment, but there are still several serious problems with gene therapy. As a matter of fact, it seems impossible to adopt gene therapy for prevention. We therefore tried to develop a different method of cancer prevention or therapy based on the molecular mechanisms of carcinogenesis. For instance, the tumor-suppressor gene p53 is mutated in about 50% of human malignancies. It is known that p53 stimulates the promoter activities of p21/WAF1, gadd45 and bax genes, resulting in cell cycle arrest, DNA repair and apoptosis, respectively. Therefore, chemical compounds that can stimulate these genes should compensate for the function of p53. As a model of this, we found that histone deacetylase inhibitors such as butyrate or trichostatin A dramatically stimulate the p21/WAF1 gene promoter through the Spl sites, resulting in cell cycle arrest. Interestingly, another group has recently reported that phenylbutyrate, which is also known as a histone deacetylase inhibitor, is very effective for leukemia patients. We therefore consider methods of up-regulating p21/WAF, gadd45 or bax genes should be useful for cancer therapy and termed this method "Gene-regulating chemotherapy". Theoretically, the chemicals up-regulating such genes should be also useful for chemoprevention, and we also termed it as "Gene-regulating chemoprevention". In conclusion, we propose that "Gene-regulating chemotherapy or chemoprevention" may be a promising new method for cancer therapy or prevention and histone deacetylase inhibitor is a good candidate for this method.

Reference Type: Journal Article
Record Number: 220

Author: Wang, Q. M.; Feinman, R.; Kashanchi, F.; Houghton, J. M.; Studzinski, G. P.; Harrison, L. E.
Year: 2000
Title: Changes in E2F binding after phenylbutyrate-induced differentiation of Caco-2 colon cancer cells
Journal: Clin Cancer Res
Volume: 6
Issue: 7
Pages: 2951-8
Abstract: Differentiation agents use existing cellular systems to induce neoplastic cells to regain a normal phenotype and/or to cause growth arrest and therefore may offer novel chemotherapeutic approaches to treating solid tumors. In this study, we demonstrate in Caco-2 colon cancer cells that the differentiation agent phenylbutyrate (PB) causes a decrease in viable cells, an increase in cell differentiation, and a G1-S-phase block. The mechanism of this last effect is related to a PB-induced increase in p27Kip1, leading to a decrease in the activity of cyclin-dependent kinase 2 (CDK2), a positive regulator of the G1-S-phase cell cycle transition. Consistent with the decreased CDK2 kinase activity, we also observed a decrease in the phosphorylation state of the retinoblastoma protein after PB treatment. This was associated with increased binding and consequent inactivation of E2F, a transactivator of genes that regulate the G1 to S phase cell cycle transition. These data suggest that the differentiation agent PB inhibits tumor growth by limiting the availability of active E2F, with a subsequent G1-S-phase block. Additional studies should show whether PB is a clinically effective therapeutic agent against colorectal cancer.

Reference Type: Journal Article
Record Number: 224
Author: Wargovich, M. J.; Jimenez, A.; McKee, K.; Steele, V. E.; Velasco, M.; Woods, J.; Price, R.; Gray, K.; Kelloff, G. J.
Year: 2000
Title: Efficacy of potential chemopreventive agents on rat colon aberrant crypt formation and progression
Journal: Carcinogenesis
Volume: 21
Issue: 6
Pages: 1149-55
Abstract: We assessed the effects of 78 potential chemopreventive agents in the F344 rat using two assays in which the inhibition of carcinogen-induced aberrant crypt foci (ACF) in the colon was the measure of efficacy. In both assays ACF were induced by the carcinogen azoxymethane (AOM) in F344 rats by two sequential weekly injections at a dose of 15 mg/kg. Two weeks after the last AOM injection, animals were evaluated for the number of aberrant crypts detected in methylene blue stained whole mounts of rat colon. In the initiation phase protocol agents were given during the period of AOM administration, whereas in the post-initiation assay the chemopreventive agent was introduced during the last 4 weeks of an 8 week assay, a time when ACF had progressed to multiple crypt clusters. The agents were derived from a priority listing based on reports of chemopreventive activity in the literature and/or efficacy data from in vitro models of carcinogenesis. During the initiation phase carboxyl amidoimidazole, p-chlorphenylacetate, chlorpheniramine maleate,

D609, diclofenac, etoperidone, eicosatetraynoic acid, farnesol, ferulic acid, lycopene, meclizine, methionine, phenylhexylisothiocyanate, phenylbutyrate, piroxicam, 9-cis-retinoic acid, S-allylcysteine, taurine, tetracycline and verapamil were strong inhibitors of ACF. During the post-initiation phase aspirin, calcium glucarate, ketoprofen, piroxicam, 9-cis-retinoic acid, retinol and rutin inhibited the outgrowth of ACF into multiple crypt clusters. Based on these data, certain phytochemicals, antihistamines, non-steroidal anti-inflammatory drugs and retinoids show unique preclinical promise for chemoprevention of colon cancer, with the latter two drug classes particularly effective in the post-initiation phase of carcinogenesis.

Reference Type: Journal Article
Record Number: 229
Author: Wei, H.; Kemp, S.; McGuinness, M. C.; Moser, A. B.; Smith, K. D.
Year: 2000
Title: Pharmacological induction of peroxisomes in peroxisome biogenesis disorders
Journal: Ann Neurol
Volume: 47
Issue: 3
Pages: 286-96
Abstract: Inherited aberrant peroxisome assembly results in a group of neurological diseases termed peroxisome biogenesis disorders (PBDs). PBDs include three major clinical phenotypes that represent a continuum of clinical features from the most severe form, Zellweger syndrome (ZS), through neonatal adrenoleukodystrophy (NALD) to the least severe form, infantile Refsum's disease (IRD). Somatic cell complementation studies have identified 13 PBD complementation groups, each representing a defect in a peroxisomal protein (peroxin) involved in peroxisome biogenesis. Most complementation groups include a range of clinical phenotypes. In this study, peroxisome numbers were determined in fibroblasts from 29 PBD (ZS, NALD, and IRD) patients, with various phenotypes from nine complementation groups, using antibodies against either a peroxisomal membrane protein (anti-Pex14p) or peroxisomal matrix proteins (anti-SKL). A correlation between the number of peroxisomes, determined with either antibody, and PBD phenotype was found, suggesting that induction of peroxisome number might have a favorable effect on PBD. After treatment of PBD fibroblasts with sodium 4-phenylbutyrate, a human peroxisome proliferator, there was an approximate twofold increase in peroxisome number. After 4-phenylbutyrate treatment, an increase in transcription of the adrenoleukodystrophy-related gene and the peroxin gene, PEX11alpha, was found in PBD fibroblasts. In NALD and IRD, but not ZS, fibroblasts there was an increase in very-long-chain fatty acid beta-oxidation and plasmalogen concentrations, and a decrease in very-long-chain fatty acid concentrations. These data suggest that pharmacological agents that induce peroxisome proliferation, such as 4-phenylbutyrate, may have therapeutic potential in the treatment of PBD patients with milder phenotypes (NALD and IRD).

Reference Type: Journal Article
Record Number: 232
Author: Witzig, T. E.; Timm, M.; Stenson, M.; Svingen, P. A.; Kaufmann, S. H.
Year: 2000

Title: Induction of apoptosis in malignant B cells by phenylbutyrate or phenylacetate in combination with chemotherapeutic agents
Journal: Clin Cancer Res
Volume: 6
Issue: 2
Pages: 681-92
Abstract: Phenylacetate (PA) and phenylbutyrate (PB) are aromatic fatty acids that are presently undergoing evaluation as potential antineoplastic agents. In vitro, PA and PB cause differentiation or growth inhibition of malignant cells. Clinical trials of these drugs as single agents indicate that they are not myelosuppressive; therefore, combinations with other chemotherapy agents may be possible. The goals of this study were to determine whether PA and PB (a) are cytotoxic to malignant B cells from patients with non-Hodgkin's lymphoma and B-cell chronic lymphocytic leukemia and (b) exhibit additive or synergistic induction of apoptosis when administered to myeloma cell lines in combination with conventional drugs. In the clinical specimens, cytotoxicity was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, and percent apoptosis was measured using 7-aminoactinomycin D and flow cytometry. Viability was decreased by > 50% in 7% (1/15) of non-Hodgkin's lymphoma samples treated with 5 mM PA, 27% treated with 1 mM PB, and 60% treated with 2 mM PB. Likewise, viability was decreased by > 50% in 44% (4/9) of chronic lymphocytic leukemia samples treated with 5 mM PA, 67% treated with 1 mM PB, and 100% treated with 2 mM PB. Studies in the myeloma cell lines demonstrated that PB treatment induced activation of caspases 3, 7, and 9 accompanied by cleavage of their substrates and internucleosomal DNA degradation. Combinations of PA or PB with conventional drugs (cytarabine, topotecan, doxorubicin, etoposide, chlorambucil, melphalan, fludarabine, carboplatin, and cisplatin) were examined for synergism (combination index < 1 in median effect analysis) in inducing apoptosis of both the MY5 and 8226 human myeloma cell lines. At concentrations that killed > 50% of cells, most combinations were additive; however, PB was synergistic with cytarabine, etoposide, and topotecan, with the combination index < 1 at each of the 50, 75, and 95% apoptosis levels. These observations indicate that PA and PB can induce apoptosis in malignant B cells and enhance the cytotoxicity of agents used in the treatment of these malignancies.

Reference Type: Journal Article
Record Number: 228
Author: Zeitlin, P. L.
Year: 2000
Title: Pharmacologic restoration of delta F508 CFTR-mediated chloride current
Journal: Kidney Int
Volume: 57
Issue: 3
Pages: 832-7
Abstract: Cystic fibrosis (CF) is an autosomal inherited disorder caused by over 800 different mutations in the CFTR gene. The most common mutation, delta F508, causes a trafficking arrest in the endoplasmic reticulum and the CFTR protein is degraded. Restoration of CFTR trafficking in vitro restores cAMP-mediated chloride transport at the cell surface. The hypothesis of this discussion is that the short chain fatty acids, butyrate and 4-phenylbutyrate, up-regulate mature CFTR at the plasma

membrane. Evidence that these compounds regulate CFTR production and maturation in part through effects on molecular chaperones in CF cells in culture is discussed. The oral drug, 4-phenylbutyrate, was tested in a Phase I clinical trial in CF subjects and further trials are underway. Other new therapeutic approaches directed at different classes of mutations in CFTR are also discussed. Chemical and pharmacologic agents that regulate endogenous gene expression at different steps in the biosynthetic processing pathway of a membrane glycoprotein will be needed to comprehensively treat a complex inherited disorder like cystic fibrosis.

Reference Type: Journal Article
Record Number: 210
Author: Anadiotis, G.; Ierardi-Curto, L.; Kaplan, P. B.; Berry, G. T.
Year: 2001
Title: Ornithine transcarbamylase deficiency and pancreatitis
Journal: J Pediatr
Volume: 138
Issue: 1
Pages: 123-4
Abstract: We describe a male patient with a Y202H ornithine transcarbamylase deficiency gene mutation who had pancreatitis while taking a low-protein diet, citrulline, and sodium phenylbutyrate.

Reference Type: Journal Article
Record Number: 182
Author: Andratschke, N.; Grosu, A. L.; Molls, M.; Nieder, C.
Year: 2001
Title: Perspectives in the treatment of malignant gliomas in adults
Journal: Anticancer Res
Volume: 21
Issue: 5
Pages: 3541-50
Abstract: Over the last two decades, after establishing the role of postoperative radiotherapy for malignant gliomas, no definitive improvement in survival rate could be observed, despite advances in established treatment modalities such as radiotherapy and chemotherapy. Progress in exploration of the biology of these tumours allowed for translational research projects and the development of rational new approaches, such as gene therapy and immunotherapy, that could interfere with established treatment regimens or be used independently. Possible strategies include the restoration of defective cancer-inhibitory genes, cell transduction or transfection with antisense DNA corresponding to genes coding for growth factors and their receptors, or with the so-called 'suicide genes'. Several antiangiogenic approaches such as administration of thalidomide, protamine, or monoclonal antibodies against vascular endothelial growth factor have been developed, too. Further treatment possibilities include modulation of drug resistance, e.g. by P-glycoprotein antagonists or 06-alkyl-guanine-DNA-transferase inhibitors, inhibition of matrix metalloproteinases, inhibition of protein kinase C and administration of agents such as phenylbutyrate or valproic acid that showed promising antiproliferative effects in vitro. This review discusses the available laboratory and clinical data as well as recent

advances in our knowledge about prognostic and predictive factors and their implications for the design of future clinical trials.

Reference Type: Journal Article
Record Number: 209
Author: Batshaw, M. L.; MacArthur, R. B.; Tuchman, M.
Year: 2001
Title: Alternative pathway therapy for urea cycle disorders: twenty years later
Journal: J Pediatr
Volume: 138
Issue: 1 Suppl
Pages: S46-54; discussion S54-5
Abstract: Alternative pathway therapy is currently an accepted treatment approach for inborn errors of the urea cycle. This involves the long-term use of oral sodium phenylbutyrate, arginine supplements, or both, depending on the specific enzyme deficiency, and treatment of acute hyperammonemic crises with intravenous sodium benzoate/sodium phenylacetate plus arginine. A review of 20 years of experience with this approach illustrates the strengths and limitations of this treatment. It has clearly decreased the mortality and morbidity from these disorders, but they remain unacceptably high. The medications are generally well tolerated, but severe accidental overdosage has been reported because of the infrequent use of the medication. There is also a difference in their metabolism between newborns and older children that must be addressed in determining dosage. To avoid these complications it is recommended that drug levels in blood be monitored routinely and that very specific treatment protocols and oversight be followed to avoid overdoses. Finally, it must be acknowledged that alternative pathway therapy has limited effectiveness in preventing hyperammonemia and must be combined with effective dietary management. Therefore in children with neonatal-onset disease or in those with very poor metabolic control, liver transplantation should be considered. There should also be the continued search for innovative therapies that may offer a more permanent and complete correction, such as gene therapy.

Reference Type: Journal Article
Record Number: 202
Author: Berg, S.; Serabe, B.; Aleksic, A.; Bomgaars, L.; McGuffey, L.; Dauser, R.; Durfee, J.; Nuchtern, J.; Blaney, S.
Year: 2001
Title: Pharmacokinetics and cerebrospinal fluid penetration of phenylacetate and phenylbutyrate in the nonhuman primate
Journal: Cancer Chemother Pharmacol
Volume: 47
Issue: 5
Pages: 385-90
Abstract: INTRODUCTION: Phenylbutyrate (PB) and its metabolite phenylacetate (PA) demonstrate anticancer activity in vitro through promotion of cell differentiation, induction of apoptosis through the p21 pathway, inhibition of histone deacetylase, and in the case of PB, direct cytotoxicity. We studied the pharmacokinetics, metabolism, and cerebrospinal fluid (CSF) penetration of PA and

PB after intravenous (i.v.) administration in the nonhuman primate. METHODS: Three animals received 85 mg/kg PA and 130 mg/kg PB as a 30-min infusion. Blood and CSF samples were obtained at 15, 30, 35, 45, 60 or 75 min, and at 1.5, 2.5, 3.5, 5.5, 6.5, 8.5, 10.5 and 24.5 h after the start of the infusion. Plasma was separated immediately, and plasma and CSF were frozen until HPLC analysis was performed. RESULTS: After i.v. PA administration, the plasma area under the concentration-time curve (AUC) of PA (median +/- SD) was 82 +/- 16 mg/ml.min, the CSF AUC was 24 +/- 7 mg/ml.min, clearance (Cl) was 1 +/- 0.3 ml/min per kg, and the AUCCSF:AUCplasma ratio was 28 +/- 19%. After i.v. PB administration, the plasma PB AUC was 19 +/- 3 mg/ml.min, the CSF PB AUC was 8 +/- 11 mg/ml.min, the PB Cl was 7 +/- 1 ml/min per kg, and the AUCCSF:AUCplasma ratio was 41 +/- 47%. The PA plasma AUC after i.v. PB administration was 50 +/- 9 mg/ml.min, the CSF AUC was 31 +/- 24 mg/ml.min, and the AUCCSF:AUCplasma ratio was 53 +/- 46%. CONCLUSIONS: These data indicate that PA and PB penetrate well into the CSF after i.v. administration. There may be an advantage to administration of PB over PA, since the administration of PB results in significant exposure to both active compounds. Clinical trials to evaluate the activity of PA and PB in pediatric central nervous system tumors are in progress.

Reference Type: Journal Article
Record Number: 205
Author: Burlina, A. B.; Ogier, H.; Korall, H.; Trefz, F. K.
Year: 2001
Title: Long-term treatment with sodium phenylbutyrate in ornithine transcarbamylase-deficient patients
Journal: Mol Genet Metab
Volume: 72
Issue: 4
Pages: 351-5
Abstract: Ornithine transcarbamylase deficiency is a very heterogeneous urea cycle disorder resulting in hyperammonemia with various presentations from the neonatal period through adulthood. We performed a retrospective study in nine patients (four male/five female, age at diagnosis ranging from 6 days to 14 years) to evaluate the safety and efficacy of sodium phenylbutyrate (Ammonaps) in long-term treatment. All patients were diagnosed by DNA mutational analysis and/or liver enzyme measurement. They had previously been treated with sodium benzoate (median dose 248 mg/kg/day; range 106-275) and low protein diet (median 0.84 g/kg/day) and were switched to sodium phenylbutyrate (median dose of 352 mg/kg/day) at 8.9 and 4.9 years of age (median) in males and females, respectively. We analyzed clinical and biochemical data and the median follow-up duration was 26 months. During that time, there were no hyperammonemic episodes requiring hospitalization. Median plasma ammonia and glutamine levels were 30 and 902 micromol/L, respectively. Total protein intake could be increased to 0.95 g/kg/day after 18 months. No side effects related to therapy were observed. Further prospective studies should be performed to define the optimal dosage of sodium phenylbutyrate and the requirements for protein diet at different ages.

Reference Type: Journal Article

Record Number: 203
Author: Calvaruso, G.; Carabillo, M.; Giuliano, M.; Lauricella, M.; D'Anneo, A.; Vento, R.; Tesoriere, G.
Year: 2001
Title: Sodium phenylbutyrate induces apoptosis in human retinoblastoma Y79 cells: the effect of combined treatment with the topoisomerase I-inhibitor topotecan
Journal: Int J Oncol
Volume: 18
Issue: 6
Pages: 1233-7
Abstract: Our results demonstrate that sodium phenylbutyrate, a compound with a low degree of toxicity, exerted a cytotoxic effect on human retinoblastoma Y79 cells in a time- and dose-dependent manner. Treatment of Y79 cells for 72 h with phenylbutyrate reduced cell viability by 63% at 2 mM and 90% at 4 mM. Cell death caused by phenylbutyrate exhibited the typical features of apoptosis, as shown by light and fluorescent microscopy. Western blot analysis demonstrated that exposure of Y79 cells to phenylbutyrate decreased the level of the antiapoptotic factor Bcl-2 and induced the activation of caspase-3, a key enzyme in the execution phase of apoptosis. Moreover, treatment with phenylbutyrate markedly increased the level of acetylated histone-H3. Combined treatment with phenylbutyrate and topotecan, a topoisomerase I-inhibitor, resulted in a clear synergistic effect. We suggest that the effects exerted by phenylbutyrate on Y79 cells essentially depend on modifications of gene expression consequent to histone hyperacetylation.

Reference Type: Journal Article
Record Number: 190
Author: Carducci, M. A.; Gilbert, J.; Bowling, M. K.; Noe, D.; Eisenberger, M. A.; Sinibaldi, V.; Zabelina, Y.; Chen, T. L.; Grochow, L. B.; Donehower, R. C.
Year: 2001
Title: A Phase I clinical and pharmacological evaluation of sodium phenylbutyrate on an 120-h infusion schedule
Journal: Clin Cancer Res
Volume: 7
Issue: 10
Pages: 3047-55
Abstract: PURPOSE: Sodium phenylbutyrate (PB) demonstrates potent differentiating capacity in multiple hematopoietic and solid tumor cell lines. We conducted a Phase I and pharmacokinetic study of PB by continuous infusion to characterize the maximum tolerated dose, toxicities, pharmacokinetics, and antitumor effects in patients with refractory solid tumors. PATIENTS AND METHODS: Patients were treated with a 120-h PB infusion every 21 days. The dose was escalated from 150 to 515 mg/kg/day. Pharmacokinetics were performed during and after the first infusion period using a validated high-performance liquid chromatographic assay and single compartmental pharmacokinetic model for PB and its principal metabolite, phenylacetate. RESULTS: A total of 24 patients were enrolled on study, with hormone refractory prostate cancer being the predominant tumor type. All patients were evaluable for toxicity and response. A total of 89 cycles were administered. The dose-limiting toxicity (DLT) was neuro-cortical, exemplified by excessive somnolence and confusion and accompanied by clinically significant hypokalemia,

hyponatremia, and hyperuricemia. One patient at 515 mg/kg/day and another at 345 mg/kg/day experienced this DLT. Toxicity resolved < or =12 h of discontinuing the infusion. Other toxicities were mild, including fatigue and nausea. The maximum tolerated dose was 410 mg/kg/day for 5 days. Pharmacokinetics demonstrated that plasma clearance of PB increased in a continuous fashion beginning 24 h into the infusion. In individuals whose V(max) for drug elimination was less than their drug-dosing rate, the active metabolite phenylacetate accumulated progressively. Plasma PB concentrations (at 410 mg/kg/day) remained above the targeted therapeutic threshold of 500 micromol/liter required for in vitro activity. CONCLUSION: The DLT in this Phase I study for infusional PB given for 5 days every 21 days is neuro-cortical in nature. The recommended Phase II dose is 410 mg/kg/day for 120 h.

Reference Type: Journal Article
Record Number: 200
Author: Choo-Kang, L. R.; Zeitlin, P. L.
Year: 2001
Title: Induction of HSP70 promotes DeltaF508 CFTR trafficking
Journal: Am J Physiol Lung Cell Mol Physiol
Volume: 281
Issue: 1
Pages: L58-68
Abstract: The DeltaF508 cystic fibrosis transmembrane conductance regulator (CFTR) is a temperature-sensitive trafficking mutant that is detected as an immature 160-kDa form (band B) in gel electrophoresis. The goal of this study was to test the hypothesis that HSP70, a member of the 70-kDa heat shock protein family, promotes DeltaF508 CFTR processing to the mature 180-kDa form (band C). Both pharmacological and genetic techniques were used to induce HSP70. IB3-1 cells were treated with sodium 4-phenylbutyrate (4PBA) to promote maturation of DeltaF508 CFTR to band C. A dose-dependent increase in band C and total cellular HSP70 was observed. Under these conditions, HSP70-CFTR complexes were increased and 70-kDa heat shock cognate protein-CFTR complexes were decreased. Increased DeltaF508 CFTR maturation was also seen after transfection with an HSP70 expression plasmid and exposure to glutamine, an inducer of HSP70. With immunofluorescence techniques, the increased appearance of CFTR band C correlated with CFTR distribution beyond the perinuclear regions. These data suggest that induction of HSP70 promotes DeltaF508 CFTR maturation and trafficking.

Reference Type: Journal Article
Record Number: 198
Author: Clarke, K. O.; Feinman, R.; Harrison, L. E.
Year: 2001
Title: Tributyrin, an oral butyrate analogue, induces apoptosis through the activation of caspase-3
Journal: Cancer Lett
Volume: 171
Issue: 1
Pages: 57-65

Abstract: The purpose of this study was to investigate the anti-proliferative and pro-apoptotic effects of the butyrate analogues, tributyrin (TB) and phenylbutyrate (PB), in a colon cancer model. We demonstrate that HT-29 colon cancer cells exposed to PB and TB result in growth inhibition associated with an induction of apoptosis mediated through the activation of caspase-3 activity. A block in the G1/S cell cycle traverse associated with a decrease in CDK2 (cyclin dependent kinase) protein levels and retinoblastoma protein hypophosphorylation was also noted after PB and TB exposure. Importantly, TB proved to be the most potent agent in its ability to induce these phenotypic changes, and potentially may represent a novel therapy for patients with advanced colorectal cancer.

Reference Type: Journal Article
Record Number: 189
Author: Cosentini, E.; Haberl, I.; Pertschy, P.; Teleky, B.; Mallinger, R.; Baumgartner, G.; Wenzl, E.; Hamilton, G.
Year: 2001
Title: The differentiation inducers phenylacetate and phenylbutyrate modulate camptothecin sensitivity in colon carcinoma cells in vitro by intracellular acidification
Journal: Int J Oncol
Volume: 19
Issue: 5
Pages: 1069-74
Abstract: Aromatic fatty acids such as phenylbutyrate (PB) and its metabolite phenylacetate (PA) induce growth arrest, differentiation and apoptosis in solid tumor cells. Despite their antiproliferative action they were reported to exhibit a synergistic effect in combination with cytotoxic drugs like topotecan, and others. Since the activity of the camptothecines (CPTs) depends on local pH conditions, we investigated, whether PB/PA modulate CPT effects indirectly by affecting intracellular pH in SW620 and SW480 colon cancer cells. The results for the colon carcinoma cells show an antagonistic interaction for the combination of CPT and 0.25-5 mM PA in viability assays, resulting in an approximately 3-fold increase in IC50 (control: 20+/-7 nM). A synergistic effect with significantly increased numbers of late apoptotic/necrotic cancer cells (difference +21+/-4%) and 1.4-fold sensitization were detected upon inclusion of 2.5 mM PA during a 4-h CPT (10 micro;M) loading phase. In response to 0.25-1 mM PA/PB the cells exhibit a reversible decrease of pHi (0.1-0.31 pH units) in HEPES- or bicarbonate-buffered media. Dose-dependent acidification and pHi-recovery occurred following addition of PA and PB after an acid load and inhibition of the Na+/H+-antiporter and bicarbonate exchangers, pointing to a possible intracellular mechanism of cytoplasmic acidification. It is concluded that the synergistic modulation of CPT toxicity by short-term PA/PB treatment in colon carcinoma cells is caused by changes in intracellular pH, possibly affecting quantity and localization of the active closed lactone form of this drug.

Reference Type: Journal Article
Record Number: 197
Author: Gilbert, J.; Baker, S. D.; Bowling, M. K.; Grochow, L.; Figg, W. D.; Zabelina, Y.; Donehower, R. C.; Carducci, M. A.

Year: 2001
Title: A phase I dose escalation and bioavailability study of oral sodium phenylbutyrate in patients with refractory solid tumor malignancies
Journal: Clin Cancer Res
Volume: 7
Issue: 8
Pages: 2292-300
Abstract: PURPOSE: Phenylbutyrate (PB) is an aromatic fatty acid with multiple mechanisms of action including histone deacetylase inhibition. Preclinically, PB demonstrates both cytotoxic and differentiating effects at a concentration of 0.5 mM. We conducted a Phase I trial of p.o. PB patients with refractory solid tumor malignancies to evaluate toxicity, pharmacokinetic parameters, and feasibility of p.o. administration. EXPERIMENTAL DESIGN: Twenty-eight patients with refractory solid tumor malignancies were enrolled on this dose-escalation to maximally tolerated dose trial. Five dose levels of PB were studied: 9 g/day (n = 4), 18 g/day (n = 4), 27 g/day (n = 4), 36 g/day (n = 12), and 45 g/day (n = 4). Pharmacokinetic studies were performed and included an p.o. bioavailability determination. Compliance data were also collected. RESULTS: The recommended Phase II dose is 27 g/day. Overall the drug was well tolerated with the most common toxicities being grade 1-2 dyspepsia and fatigue. Nonoverlapping dose-limiting toxicities of nausea/vomiting and hypocalcemia were seen at 36 g/day. The p.o. bioavailability of PB was 78% for all dose levels, and the biologically active concentration of 0.5 mM was achieved at all dose levels. Compliance was excellent with 93.5% of all possible doses taken. No partial remission or complete remission was seen, but 7 patients had stable disease for more than 6 months while on the drug. CONCLUSIONS: PB (p.o.) is well tolerated and achieves the concentration in vivo that has been shown to have biological activity in vitro. PB may have a role as a cytostatic agent and should be additionally explored in combination with cytotoxics and other novel drugs.

Reference Type: Journal Article
Record Number: 192
Author: Goh, M.; Chen, F.; Paulsen, M. T.; Yeager, A. M.; Dyer, E. S.; Ljungman, M.
Year: 2001
Title: Phenylbutyrate attenuates the expression of Bcl-X(L), DNA-PK, caveolin-1, and VEGF in prostate cancer cells
Journal: Neoplasia
Volume: 3
Issue: 4
Pages: 331-8
Abstract: Phenylbutyrate (PB) is a histone deacetylase inhibitor that has been shown to induce differentiation and apoptosis in various cancer cell lines. Although these effects are most likely due to modulation of gene expression, the specific genes and gene products responsible for the effects of PB are not well characterized. In this study, we used cDNA expression arrays and Western blot to assess the effect that PB has on the expression of various cancer and apoptosis-regulatory gene products. We show that PB attenuates the expression of the apoptosis antagonist Bcl-X(L), the double-strand break repair protein DNA-dependent protein kinase, the prostate progression marker caveolin-1, and the pro-angiogenic vascular endothelial growth

factor. Furthermore, PB was found to act in synergy with ionizing radiation to induce apoptosis in prostate cancer cells. Taken together, our results point to the possibility that PB may be an effective anti-prostate cancer agent when used in combination with radiation or chemotherapy and for the inhibition of cancer progression.

Reference Type: Journal Article
Record Number: 196
Author: Gore, S. D.; Weng, L. J.; Zhai, S.; Figg, W. D.; Donehower, R. C.; Dover, G. J.; Grever, M.; Griffin, C. A.; Grochow, L. B.; Rowinsky, E. K.; Zabalena, Y.; Hawkins, A. L.; Burks, K.; Miller, C. B.
Year: 2001
Title: Impact of the putative differentiating agent sodium phenylbutyrate on myelodysplastic syndromes and acute myeloid leukemia
Journal: Clin Cancer Res
Volume: 7
Issue: 8
Pages: 2330-9
Abstract: Sodium phenylbutyrate (PB) is an aromatic fatty acid with cytostatic and differentiating activity against malignant myeloid cells (ID(50), 1-2 mM). Higher doses induce apoptosis. Patients with myelodysplasia (n = 11) and acute myeloid leukemia (n = 16) were treated with PB as a 7-day continuous infusion repeated every 28 days in a Phase I dose escalation study. The maximum tolerated dose was 375 mg/kg/day; higher doses led to dose-limiting reversible neurocortical toxicity. At the maximum tolerated dose, PB was extremely well tolerated, with no significant toxicities; median steady-state plasma concentration at this dose was 0.29 +/- 0.16 mM. Although no patients achieved complete or partial remission, four patients achieved hematological improvement (neutrophils in three, platelet transfusion-independence in one). Other patients developed transient increases in neutrophils or platelets and decrements in circulating blasts. Monitoring of the percentage of clonal cells using centromere fluorescence in situ hybridization over the course of PB administration showed that hematopoiesis remained clonal. Hematological response was often associated with increases in both colony-forming units-granulocyte-macrophage and leukemic colony-forming units. PB administration was also associated with increases in fetal erythrocytes. These data document the safety of continuous infusion PB and provide preliminary evidence of clinical activity in patients with myeloid malignancies.

Reference Type: Journal Article
Record Number: 193
Author: Jung, M.
Year: 2001
Title: Inhibitors of histone deacetylase as new anticancer agents
Journal: Curr Med Chem
Volume: 8
Issue: 12
Pages: 1505-11
Abstract: Inhibitors of histone deacetylase (HDAC) are an emerging class of anticancer agents. They induce hyperacetylation in chromatin usually resulting in

activation of certain genes. They induce terminal cell differentiation and/or apoptosis in cancer cells. Histone deacetylase activity is recruited by co-repressor proteins to certain regions of the chromatin and aberrant histone acetylation caused by that recruitment is responsible for the pathogenesis of certain cancers on a molecular level. Inhibitors of HDAC have been identified in natural sources and also synthetic inhibitors are available. The best studied inhibitor is trichostatin A, a hydroxamic acid that exerts its activity by complexation of a zinc ion that is supposed to mediate the acetamide cleavage at the catalytic site. There are several synthetic hydroxamic acids that bear resemblance to trichostatin. Another class of potent inhibitors are naturally occurring and synthetic cyclotetrapeptides that all contain an unusual amino acid with an epoxyketone, ketone or hydroxamic acid function in the side chain. Phenylacetate, phenylbutyrate, butyrate and similar short chain fatty acids are also weak inhibitors. Further inhibitors from natural sources are the epoxide depudecin and depsipeptide FR 901228. The benzamide MS-275 belongs to a new class of synthetic HDAC inhibitors and displays oral activity in animal models. First clinical studies have shown that histone hyperacetylation can be achieved safely in humans and that treatment of cancer is possible. Thus, inhibitors of HDAC are one of the most promising class of new anticancer agents. New screening assays are useful tools that will facilitate identification of further inhibitors.

Reference Type: Journal Article
Record Number: 157
Author: Lim, M.; Zeitlin, P. L.
Year: 2001
Title: Therapeutic strategies to correct malfunction of CFTR
Journal: Paediatr Respir Rev
Volume: 2
Issue: 2
Pages: 159-64
Abstract: Cystic fibrosis (CF) is a systemic autosomal recessive inherited disorder that results from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Although the gene was cloned 11 years ago, there still is no definitive treatment to correct the functional deficit. Current treatment strategies focus on pancreatic enzyme replacement and control of pulmonary inflammation and infection. This review examines novel strategies still in preclinical development or phase 1 clinical trials. Gene therapy is an evolving area of study that offers the potential for a cure for cystic fibrosis. CF lung disease is a significant barrier to effective gene delivery and transfer, but new vectors show promise in overcoming these limitations. There are also new pharmacological therapies aimed at correcting defects in CFTR processing and function. These are tailored to the specific class of mutation but may offer therapeutic benefit to many patients. They include phenylbutyrate, flavonoids, aminoglycosides and xanthines.

Reference Type: Journal Article
Record Number: 208
Author: Linsdell, P.
Year: 2001

Title: Direct block of the cystic fibrosis transmembrane conductance regulator Cl(-) channel by butyrate and phenylbutyrate
Journal: Eur J Pharmacol
Volume: 411
Issue: 3
Pages: 255-60
Abstract: Chloride permeation through the cystic fibrosis transmembrane conductance regulator (CFTR) Cl(-) channel is inhibited by a broad range of intracellular organic anions. Here it is shown, using patch clamp recording from CFTR-transfected mammalian cell lines, that the fatty acids butyrate and 4-phenylbutyrate cause a voltage-dependent block of CFTR Cl(-) currents when applied to the cytoplasmic face of membrane patches, with apparent K(d)s (at 0 mV) of 29.6 mM for butyrate and 6.6 mM for 4-phenylbutyrate. At the single channel level, both these fatty acids caused an apparent reduction in CFTR current amplitude, suggesting a kinetically fast blocking mechanism. The concentration-dependence of block suggests that CFTR-mediated Cl(-) currents in vivo may be affected by both 4-phenylbutyrate used in the treatment of various diseases, including cystic fibrosis, and by butyrate produced endogenously within the colonic lumen.

Reference Type: Journal Article
Record Number: 195
Author: Lombard, S.; Helmy, M. E.; Pieroni, G.
Year: 2001
Title: Lipolytic activity of ricin from Ricinus sanguineus and Ricinus communis on neutral lipids
Journal: Biochem J
Volume: 358
Issue: Pt 3
Pages: 773-81
Abstract: The present study was carried out with a view of determining ricin lipolytic activity on neutral lipids in emulsion and in a membrane-like model. Using 2,3-dimercapto-1-propanol tributyrate (BAL-TC(4)) as substrate, the lipolytic activity of ricin was found to be proportional to ricin and substrate concentrations, with an apparent K(m) (K(m,app)) of 2.4 mM, a k(cat) of 200 min(-1) and a specific activity of 1.0 unit/mg of protein. This work was extended to p-nitrophenyl (pNP) fatty acid esters containing two to twelve carbon atoms. Maximum lipolytic activity was registered on pNP decanoate (pNPC(10)), with a K(m,app) of 3.5 mM, a k(cat) of 173 min(-1) and a specific activity of 3.5 units/mg of protein. Ricin lipolytic activity is pH and galactose dependent, with a maximum at pH 7.0 in the presence of 0.2 M galactose. Using the monolayer technique with dicaprin as substrate, ricin showed a lipolytic activity proportional to the ricin concentration at 20 mN/m, which is dependent on the surface pressure of the lipid monolayer and is detectable up to 30 mN/m, a surface pressure that is of the same order of magnitude as that of natural cell membranes. The methods based on pNPC(10) and BAL-TC(4) hydrolysis are simple and reproducible; thus they can be used for routine studies of ricin lipolytic activity. Ricin from Ricinus communis and R. sanguineus were treated with diethyl p-nitrophenylphosphate, an irreversible serine esterase inhibitor, and their lipolytic activities on BAL-TC(4) and pNPC(10), and cytotoxic activity, were concurrently recorded. A reduction in lipolytic activity was accompanied by a decrease in

cytotoxicity on Caco2 cells. These data support the idea that the lipolytic activity associated with ricin is relevant to a lipase whose activity is pH and galactose dependent, sensitive to diethyl p-nitrophenylphosphate, and that a lipolytic step may be involved in the process of cell poisoning by ricin. Both colorimetric tests used in this study are sensitive enough to be helpful in the detection of possible lipolytic activities associated with other cytotoxins or lectins.

Reference Type: Journal Article
Record Number: 191
Author: McGuinness, M. C.; Zhang, H. P.; Smith, K. D.
Year: 2001
Title: Evaluation of pharmacological induction of fatty acid beta-oxidation in X-linked adrenoleukodystrophy
Journal: Mol Genet Metab
Volume: 74
Issue: 1-2
Pages: 256-63
Abstract: X-linked adrenoleukodystrophy (X-ALD) is an inherited neurometabolic disorder associated with elevated levels of saturated unbranched very-long-chain fatty acids (VLCFA; C > 22:0) in plasma and tissues, and reduced VLCFA beta-oxidation in fibroblasts, white blood cells, and amniocytes from X-ALD patients. The X-ALD gene (ABCD1) at Xq28 encodes the adrenoleukodystrophy protein (ALDP) that is related to the peroxisomal ATP-binding cassette (ABCD) transmembrane half-transporter proteins. The function of ALDP is unknown and its role in VLCFA accumulation unresolved. Previously, our laboratory has shown that sodium 4-phenylbutyrate (4PBA) treatment of X-ALD fibroblasts results in increased peroxisomal VLCFA beta-oxidation activity and increased expression of the X-ALD-related protein, ALDRP, encoded by the ABCD2 gene. In this study, the effect of various pharmacological agents on VLCFA beta-oxidation in ALD mouse fibroblasts is tested. 4PBA, styrylacetate and benzyloxyacetate (structurally related to 4PBA), and trichostatin A (functionally related to 4PBA) increase both VLCFA (peroxisomal) and long-chain fatty acid [LCFA (peroxisomal and mitochondrial)] beta-oxidation. Isobutyrate, zaprinast, hydroxyurea, and 5-azacytidine had no effect on VLCFA or LCFA beta-oxidation. Lovastatin had no effect on fatty acid beta-oxidation under normal tissue culture conditions but did result in an increase in both VLCFA and LCFA beta-oxidation when ALD mouse fibroblasts were cultured in the absence of cholesterol. The effect of trichostatin A on peroxisomal VLCFA beta-oxidation is shown to be independent of an increase in ALDRP expression, suggesting that correction of the biochemical abnormality in X-ALD is not dependent on pharmacological induction of a redundant gene (ABCD2). These studies contribute to a better understanding of the role of ALDP in VLCFA accumulation and may lead to the development of more effective pharmacological therapies.

Reference Type: Journal Article
Record Number: 206
Author: Pili, R.; Kruszewski, M. P.; Hager, B. W.; Lantz, J.; Carducci, M. A.
Year: 2001

Title: Combination of phenylbutyrate and 13-cis retinoic acid inhibits prostate tumor growth and angiogenesis
Journal: Cancer Res
Volume: 61
Issue: 4
Pages: 1477-85
Abstract: Differentiation-inducing agents, such as retinoids and short-chain fatty acids, have an inhibitory effect on tumor cell proliferation and tumor growth in preclinical studies. Clinical trials involving these compounds as single agents have been suboptimal in terms of clinical benefit. Our study evaluated the combination of phenylbutyrate (PB) and 13-cis retinoic acid (CRA) as a differentiation and antiangiogenesis strategy for prostate cancer. On the basis of previous evidence, common signal transduction pathways and possible modulation of retinoid receptors and retinoid response elements by PB could be responsible for such activities. We assessed the effect of the combination of PB and CRA on human and rodent prostate carcinoma cell lines. The combination of PB and CRA inhibited cell proliferation and increased apoptosis in vitro in an additive fashion as compared with single agents ($P < 0.014$). Prostate tumor cells treated with both PB and CRA revealed an increased expression of a subtype of retinoic acid receptor (retinoic acid receptor-beta), suggesting a molecular mechanism for the biological additive effect. The combination of PB and CRA also inhibited prostate tumor growth in vivo (up to 82-92%) as compared with single agents ($P < 0.025$). Histological examination of tumor xenografts revealed decreased in vivo tumor cell proliferation, an increased apoptosis rate, and a reduced microvessel density in the animals treated with combined drugs, suggesting an antiangiogenesis effect of this combination. Thus, endothelial cell treatment with both PB and CRA resulted in reduced in vitro cell proliferation. In vivo testing using the Matrigel angiogenesis assay showed an additive inhibitory effect in the animals treated with a combination of PB + CRA ($P < 0.004$ versus single agents). In summary, this study showed an additive inhibitory effect of combination of differentiation agents PB and CRA on prostate tumor growth through a direct effect on both tumor and endothelial cells.

Reference Type: Journal Article
Record Number: 211
Author: Roomans, G. M.
Year: 2001
Title: Pharmacological treatment of the ion transport defect in cystic fibrosis
Journal: Expert Opin Investig Drugs
Volume: 10
Issue: 1
Pages: 1-19
Abstract: Cystic fibrosis (CF) is a lethal monogenetic disease characterised by impaired water and ion transport over epithelia. The lung pathology is fatal and causes death in 95% of CF patients. The genetic basis of the disease is a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-regulated chloride channel. The most common mutation, DeltaF508, results in a protein that cannot properly be folded in the endoplasmic reticulum, is destroyed and hence does not reach the apical cell membrane. This paper will discuss those pharmacological approaches that are directed at correcting the defect in ion transport. At present, no

clinically effective drug is available, although research has defined areas in which progress might be made. These are the following: (1) the drug 4-phenylbutyrate (4PBA) increases the expression of DeltaF508-CFTR in the cell membrane, probably by breaking the association between DeltaF508-CFTR and a chaperone; (2) a number of xanthines, in particular 8-cyclopentyl-1, 3-dipropylxanthine (CPX), are effective in activating CFTR, presumably by direct binding and also possibly by correcting the trafficking defect; (3) the isoflavone genistein can activate both wild-type and mutant CFTR, probably through direct binding to the channel; (4) purinergic agonists (ATP and UTP) can stimulate chloride secretion via a Ca(2+)-dependent chloride channel and in this way compensate for the defect in CFTR, but stable analogues will be required before this type of treatment has clinical significance; (5) treatment with inhaled amiloride may correct the excessive absorption of Na(+) ions and water by airway epithelial cells that appears connected to the defect in CFTR; although clinical tests have not been very successful so far, amiloride analogues with a longer half-life may give better results. The role of CFTR in bicarbonate secretion has not yet been established with certainty, but correction of the defect in bicarbonate secretion may be important in clinical treatment of the disease. Currently, major efforts are directed at developing a pharmacological treatment of the ion transport defect in CF, but much basic research remains to be done, in particular, with regard to the mechanism by which defective CFTR is removed in the endoplasmic reticulum by the ubiquitin-proteasome pathway, which is a central pathway in protein production and of significance for several other diseases apart from CF.

Reference Type: Journal Article
Record Number: 201
Author: Rubenstein, R. C.; Lyons, B. M.
Year: 2001
Title: Sodium 4-phenylbutyrate downregulates HSC70 expression by facilitating mRNA degradation
Journal: Am J Physiol Lung Cell Mol Physiol
Volume: 281
Issue: 1
Pages: L43-51
Abstract: Intracellular trafficking of the DeltaF508 cystic fibrosis transmembrane conductance regulator (CFTR) is repaired by sodium 4-phenylbutyrate (4PBA) by an undetermined mechanism. 4PBA downregulates protein and mRNA expression of the heat shock cognate protein HSC70 (the constitutively expressed member of the 70-kDa heat shock protein family) by approximately 40-50% and decreases formation of a HSC70-DeltaF508 CFTR complex that may be important in the intracellular degradation of DeltaF508 CFTR. We examined the potential mechanisms by which 4PBA decreases HSC70 mRNA and protein expression. In IB3-1 cells, 1 mM 4PBA did not alter the activity of the Chinese hamster ovary HSC70 promoter or of a human HSC70 promoter fragment in luciferase reporter assays nor did it alter HSC70 mRNA synthesis in nuclear runoff assays. In contrast, preincubation with 4PBA increased the rate of HSC70 mRNA degradation by approximately 40%. The initial rate of 35S-HSC70 protein synthesis in 4PBA-treated IB3-1 cells was reduced by approximately 40%, consistent with the steady-state mRNA level, whereas its rate of degradation was unaltered by 4PBA. 4PBA also reduced the steady-state accumulation of (35)S-HSC70 by approximately 40%. These data suggest that 4PBA decreases the

expression of HSC70 mRNA and protein by inducing cellular adaptations that result in the decreased stability of HSC70 mRNA.

Reference Type: Journal Article
Record Number: 194
Author: Tonelli, M. R.; Aitken, M. L.
Year: 2001
Title: New and emerging therapies for pulmonary complications of cystic fibrosis
Journal: Drugs
Volume: 61
Issue: 10
Pages: 1379-85
Abstract: In the decade since the gene for cystic fibrosis (CF) was discovered, research into potential therapeutic interventions has progressed on a number of different fronts. The vast majority of morbidity and mortality in CF results from inflammation and infection of the airways. Direct delivery of antibacterials to the airway secretions via a nebuliser is an attractive therapeutic option, and a novel formulation of tobramycin designed for such a purpose has been demonstrated to improve spirometry and decrease the need for intravenous antibacterials. In addition, early clinical trials are studying the effects of small peptides with antibiotic properties (defensins) delivered directly to the airways. Inflammation, whether secondary to infection or an independent feature of CF, leads to progressive bronchiectasis. Anti-inflammatories such as prednisone and possibly ibuprofen have been shown to decrease the rate of respiratory decline in patients with CF but have tolerability profiles that limit clinical usefulness. Macrolides also have anti-inflammatory properties and clinical trials are now ongoing to assess the efficacy of these agents in CF. Multiple agents, including uridine triphosphate (UTP), genistein, phenylbutyrate and CPX (cyclopentyl dipropylxanthine), have been demonstrated in cell culture to at least partially correct the primary defect of ion transport related to mutations in the cystic fibrosis transmembrane conductance regulator (CFTR). No agent of this class has yet demonstrated clinical effectiveness, but several are in preclinical and early clinical trials. Finally, gene therapy that allows for the incorporation and expression of wild-type CFTR in respiratory epithelial cells would be definitive therapy for CF. However, multiple barriers to delivery and expression need to be overcome. With research proceeding on these multiple fronts, new therapies for pulmonary complications promise to continue to increase the life expectancy of individuals with CF.

Reference Type: Journal Article
Record Number: 204
Author: Walczak, J.; Wood, H.; Wilding, G.; Williams, T., Jr.; Bishop, C. W.; Carducci, M.
Year: 2001
Title: Prostate cancer prevention strategies using antiproliferative or differentiating agents
Journal: Urology
Volume: 57
Issue: 4 Suppl 1

Pages: 81-5
Abstract: Differentiation or antiproliferative therapies have been most effective in the treatment of promyelocytic leukemia and are being investigated for the treatment of solid tumors including prostate cancer (PCa). Research suggests that these agents may induce terminal differentiation (arrest in G(0)), induce differentiation to a mature cell with cellular functions and a growth pattern similar to nonmalignant cells, or trigger apoptosis. This review focuses on classes of agents under laboratory and clinical evaluation as antiproliferative or differentiating agents: polyamine inhibitors, vitamin D and its analogs, metabolites of vitamin A, the short-chain fatty acid, phenylbutyrate, and nonsteroidal anti-inflammatory agents. Because differentiation therapies offer a reduced toxicity profile and have potential for preventing or slowing cancer progression, they may offer an alternative to chemotherapy for men with advanced PCa, or may be useful as low-toxicity agents given chronically for chemoprevention in men at high risk for PCa. Clinical trials are needed to define the role of these agents in primary and secondary prevention.

Reference Type: Journal Article
Record Number: 166
Author: Baker, M. J.; Brem, S.; Daniels, S.; Sherman, B.; Phuphanich, S.
Year: 2002
Title: Complete response of a recurrent, multicentric malignant glioma in a patient treated with phenylbutyrate
Journal: J Neurooncol
Volume: 59
Issue: 3
Pages: 239-42
Abstract: Sodium phenylbutyrate is a biological-response modifier that acts as a dose-dependent inhibitor of glioma cell proliferation, migration, and invasiveness in vitro, possibly by inhibition of urokinase and c-myc pathways. Despite its biological activity in vitro, there have not been any prior reports of efficacy in the treatment of human malignant gliomas. We report a 44-year-old female with a recurrent, multicentric, malignant glioma who experienced a durable remission lasting more than four years. The patient initially presented with seizures caused by a biopsy-proven anaplastic astrocytoma of the frontal lobe. The patient was treated with radiation therapy and Procarbazine-CCNU-Vincristine (PCV). However, the tumor progressed and extended to the corpus callosum with midline shift, refractory to four cycles of continuous 72-h infusion of BCNU/Cisplatinum. Additional enhancing lesions appeared in the left frontal and left temporal lobes. The patient was started on sodium phenylbutyrate, 18 g daily in three divided oral doses, and reduced to 9 g/day and eventually to 4.5 g/day to eliminate mild, reversible side effects. Four years later, the patient has a KPS functional score of 100%. Phenylbutyrate is a well-tolerated, oral agent that shows potential for the treatment of malignant gliomas. Further studies should be considered to identify a subset of patients that have tumors sensitive to phenylbutyrate, either as a single agent or in combination with radiation therapy or other chemotherapeutic agents.

Reference Type: Journal Article
Record Number: 163

Author: Boivin, A. J.; Momparler, L. F.; Hurtubise, A.; Momparler, R. L.
Year: 2002
Title: Antineoplastic action of 5-aza-2'-deoxycytidine and phenylbutyrate on human lung carcinoma cells
Journal: Anticancer Drugs
Volume: 13
Issue: 8
Pages: 869-74
Abstract: Current chemotherapy of advanced non-small cell lung cancer produces only a modest increase in survival time. New approaches are needed to improve its effectiveness. During tumorigenesis, silencing of tumor suppressor genes can occur by aberrant methylation. The DNA methylation inhibitor, 5-aza-2'-deoxycytidine (5-AZA-CdR), can reactivate the expression of these genes. Nucleosomes containing unacetylated positively charged histones bind tightly to DNA producing a compact configuration, which inhibits transcription. Phenylbutyrate (PB), an inhibitor of histone deacetylase (HDAC), increases histone acetylation, neutralizing its positive charge and resulting in DNA with a more open structure, which favors transcription. It has been reported that 5-AZA-CdR in combination with HDAC inhibitor can increase the expression of silent tumor suppressor genes. The objective of our study was to determine if these agents, in combination, produce an enhancement of their antitumor activity. We evaluated the antineoplastic activity of 5-AZA-CdR and PB alone or in combination on human A549 and Calu-6 lung carcinoma cell lines by inhibition of DNA synthesis and clonogenic assays. 5-AZA-CdR and PB in combination produced a greater inhibition of DNA synthesis than either agent alone. Also, in a clonogenic assay the combination of these drugs showed a significant synergistic antitumor effect. These results provide a rationale to investigate the combination of 5-AZA-CdR and PB in patients with advanced lung cancer.

Reference Type: Journal Article
Record Number: 175
Author: Chang, K. T.; Min, K. T.
Year: 2002
Title: Regulation of lifespan by histone deacetylase
Journal: Ageing Res Rev
Volume: 1
Issue: 3
Pages: 313-26
Abstract: Aging is a universal biological phenomenon in eukaryotes, but why and how we age still remain mysterious. It would be of great biological interest and practical importance if we could uncover the molecular mechanism of aging, and find a way to delay the aging process while maintaining physical and mental strengths of youth. Histone deacetylases (HDACs) such as SIR2 and RPD3 are known to be involved in the extension of lifespan in yeast and Caenorhabditis elegans. An inhibitor of HDACs, phenylbutyrate, also can significantly increase the lifespan of Drosophila, without diminution of locomotor vigor, resistance to stress, or reproductive ability. Treatment for a limited period, either early or late in adult life, is also effective. Alteration in the pattern of gene expression, including induction or repression of numerous genes involved in longevity by changing the level and the pattern of histone acetylation may be an important factor in determining the longevity of animals.

Reference Type: Journal Article
Record Number: 178
Author: Chang, T. H.; Szabo, E.
Year: 2002
Title: Enhanced growth inhibition by combination differentiation therapy with ligands of peroxisome proliferator-activated receptor-gamma and inhibitors of histone deacetylase in adenocarcinoma of the lung
Journal: Clin Cancer Res
Volume: 8
Issue: 4
Pages: 1206-12
Abstract: PURPOSE: Histone deacetylase (HDAC) inhibitors and ligands of the peroxisome proliferator-activated receptor gamma (PPARgamma) have been shown previously to induce growth arrest and differentiation in a variety of cancer cell lines. The purpose of this study was to determine whether HDAC inhibitors function similarly in non-small cell lung cancer (NSCLC) and whether combination treatment with HDAC inhibitors and PPARgamma ligands is more efficacious than either agent alone. EXPERIMENTAL DESIGN AND RESULTS: Nanomolar concentrations of trichostatin A induced growth arrest in five of seven NSCLC cell lines, whereas sodium phenylbutyrate (PB) was markedly less potent. In adenocarcinomas, trichostatin A up-regulated general differentiation markers (gelsolin, Mad, and p21/WAF1) and down-regulated markers of the type II pneumocyte progenitor cell lineage (MUC1 and SP-A), indicative of a more mature phenotype. PB had a similar effect. Simultaneous treatment with a PPARgamma ligand and PB enhanced the growth inhibition in adenocarcinomas but not in nonadenocarcinomas. Growth arrest was accompanied by markedly decreased cyclin D1 expression but not enhanced differentiation. CONCLUSIONS: The present study demonstrates potent growth-inhibitory and differentiation-inducing activity of HDAC inhibitors in NSCLC and suggests that combination differentiation therapy should be explored further for the treatment of lung adenocarcinomas.

Reference Type: Journal Article
Record Number: 177
Author: Clarke, K. O.; Ludeman, S. M.; Springer, J. B.; Colvin, O. M.; Lea, M. A.; Harrison, L. E.
Year: 2002
Title: Exposure to a deuterated analogue of phenylbutyrate retards S-phase progression in HT-29 colon cancer cells
Journal: J Pharm Sci
Volume: 91
Issue: 4
Pages: 1054-64
Abstract: Differentiation agents that induce neoplastic cells to regain a normal phenotype and/or cause growth arrest without significantly affecting normal cells represent an attractive option for cancer treatment. Analogues of short chain fatty acids, such as phenylbutyrate (PB), have been studied as clinically relevant agents. In an attempt to improve its pharmacokinetic profile, structural modifications of PB and

other fatty acids have been studied. We hypothesize that strategic isotopic modification of PB would result in a longer half-life and thus translate into a more potent differentiation agent for clinical use. Using a colon cancer model, we demonstrated that 2,2,3,3-tetradeuterated PB (D4PB) significantly increased induction of apoptosis and inhibition of cell proliferation as compared with PB and butyrate. Difference in potency could not be explained by the effect of D4PB on the expression of specific regulatory proteins of the apoptotic cascade or from the inhibitory effect of D4PB on histone deacetylase activity. Interestingly, exposure of HT-29 colon cancer cells to D4PB resulted in a slowing of S transit, in contrast to butyrate and PB, which induced a G2/M cell cycle block. This difference in cell cycle effect may explain the differences seen in the potency of the phenotypic changes seen with treatment with D4PB. Further studies are needed to elucidate the mechanisms underlying effects of D4PB on the cell cycle.

Reference Type: Journal Article
Record Number: 170
Author: Comte, B.; Kasumov, T.; Pierce, B. A.; Puchowicz, M. A.; Scott, M. E.; Dahms, W.; Kerr, D.; Nissim, I.; Brunengraber, H.
Year: 2002
Title: Identification of phenylbutyrylglutamine, a new metabolite of phenylbutyrate metabolism in humans
Journal: J Mass Spectrom
Volume: 37
Issue: 6
Pages: 581-90
Abstract: Phenylbutyrate is used in humans for treating inborn errors of ureagenesis, certain forms of cancer, cystic fibrosis and thalassemia. The known metabolism of phenylbutyrate leads to phenylacetylglutamine, which is excreted in urine. We have identified phenylbutyrylglutamine as a new metabolite of phenylbutyrate in human plasma and urine. We describe the synthesis of phenylbutyrylglutamine and its assay by gas chromatography/mass spectrometry as a tert-butyldimethylsilyl or methyl derivative, using standards of [(2)H(5)]phenylbutyrylglutamine and phenylpropionylglutamine. After administration of phenylbutyrate to normal humans, the cumulative urinary excretion of phenylacetate, phenylbutyrate, phenylacetylglutamine and phenylbutyrylglutamine amounts to about half of the dose of phenylbutyrate. Thus, additional metabolites of phenylbutyrate are yet to be identified.

Reference Type: Journal Article
Record Number: 167
Author: Culig, Z.; Klocker, H.; Bartsch, G.; Hobisch, A.
Year: 2002
Title: Androgen receptors in prostate cancer
Journal: Endocr Relat Cancer
Volume: 9
Issue: 3
Pages: 155-70

Abstract: The androgen receptor (AR), a transcription factor that mediates the action of androgens in target tissues, is expressed in nearly all prostate cancers. Carcinoma of the prostate is the most frequently diagnosed neoplasm in men in industrialized countries. Palliative treatment for non-organ-confined prostate cancer aims to down-regulate the concentration of circulating androgen or to block the transcription activation function of the AR. AR function during endocrine therapy was studied in tumor cells LNCaP subjected to long-term steroid depletion; newly generated sublines could be stimulated by lower concentrations of androgen than parental cells and showed up-regulation of AR expression and activity as well as resistance to apoptosis. Androgenic hormones regulate the expression of key cell cycle regulators, cyclin-dependent kinase 2 and 4, and that of the cell cycle inhibitor p27. Inhibition of AR expression could be achieved by potential chemopreventive agents flufenamic acid, resveratrol, quercetin, polyunsaturated fatty acids and interleukin-1beta, and by the application of AR antisense oligonucleotides. In the clinical situation, AR gene amplification and point mutations were reported in patients with metastatic disease. These mutations generate receptors which could be activated by other steroid hormones and non-steroidal antiandrogens. In the absence of androgen, the AR could be activated by various growth-promoting (growth factors, epidermal growth factor receptor-related oncogene HER-2/neu) and pleiotropic (protein kinase A activators, interleukin-6) compounds as well as by inducers of differentiation (phenylbutyrate). AR function is modulated by a number of coactivators and corepressors. The three coactivators, TIF-2, SRC-1 and RAC3, are up-regulated in relapsed prostate cancer. New experimental therapies for prostate cancer are aimed to down-regulate AR expression and to overcome difficulties which occur because of the acquisition of agonistic properties of commonly used antiandrogens.

Reference Type: Journal Article
Record Number: 159
Author: Du, H. L.; Qi, Y.; Shi, Y. J.; Bu, D. F.; Wu, S. L.
Year: 2002
Title: [Apoptosis and re-expression of p16 gene in the myeloma cell line U266 induced by synergy of histone deacetylase inhibitor and demethylating agent]
Journal: Ai Zheng
Volume: 21
Issue: 10
Pages: 1057-61
Abstract: BACKGROUND & OBJECTIVE: Histone deacetylation is associated with transcriptional activation controlled by DNA methylation. It is important to investigate changes of tumor cells treated with agent through two kinds of mechanisms. This study was designed to investigate the synergic effect of histone deacetylase inhibitor, sodium phenylbutyrate(SPB), and demethylating agent, 5-Aza-2'-deoxycytidine(5-Aza-CdR), on cell growth and explore the possibility of re-expression of the hypermethylated and silenced p16 gene in the myeloma cell line U266. METHODS: The cell cycle was analyzed by flow cytometry. Apoptosis was observed by transmission electron microscopy, DNA ladder, fluorescence-activated cell sorter (FACS). The expression level of p16 was detected by RT-PCR and Western blot analysis. RESULTS: The apoptotic rates of U266 cells induced by 5-Aza-CdR(1 mumol/L), SPB(1 mmol/L) alone and combination of 5-Aza-CdR and SPB were 15.09%, 89.19%, and 85.18%, respectively. The G1 phase was arrested and

sub-G1 phase(50%) was induced by combination of 5-Aza-CdR and SPB. There was no G1 phase arrested when SPB or 5-Aza-CdR was used alone. The proportion of cells in G2 phase was increased with SPB alone. SPB was not able to induce the expression of p16. The expression level of p16 was induced with 5-Aza-CdR. The expression level of both mRNA and protein of p16 was increased significantly by synergy of SPB and 5-Aza-CdR. CONCLUSIONS: p16 gene in U266 cell line could be reactivated markedly with synergy of 5-Aza-CdR and SPB with cell cycle arresting in G1 phase. Meanwhile, the cell cycle phase occurring apoptosis that induced by combination of 5-Aza-CdR with SPB is different from that induced by each alone.

Reference Type: Journal Article
Record Number: 168
Author: Dyer, E. S.; Paulsen, M. T.; Markwart, S. M.; Goh, M.; Livant, D. L.; Ljungman, M.
Year: 2002
Title: Phenylbutyrate inhibits the invasive properties of prostate and breast cancer cell lines in the sea urchin embryo basement membrane invasion assay
Journal: Int J Cancer
Volume: 101
Issue: 5
Pages: 496-9
Abstract: Histone deacetylase inhibitors, such as phenylbutyrate, are currently undergoing clinical trials as potential anticancer agents. Phenylbutyrate can induce cell differentiation and apoptosis in a number of cancer cell types and can act in synergy with ionizing radiation and chemotherapy to induce apoptosis. We used the sea urchin embryo basement membrane invasion assay to show that phenylbutyrate potently inhibited the invasive properties of both prostate and breast cancer cells at clinically achievable doses. This inhibition was dose-dependent and persisted for at least 24 hr after the drug was removed. These results suggest that in addition to activating apoptosis in cancer cells, phenylbutyrate may be used in prevention of metastatic disease.

Reference Type: Journal Article
Record Number: 173
Author: Farinha, C. M.; Nogueira, P.; Mendes, F.; Penque, D.; Amaral, M. D.
Year: 2002
Title: The human DnaJ homologue (Hdj)-1/heat-shock protein (Hsp) 40 co-chaperone is required for the in vivo stabilization of the cystic fibrosis transmembrane conductance regulator by Hsp70
Journal: Biochem J
Volume: 366
Issue: Pt 3
Pages: 797-806
Abstract: The CFTR (cystic fibrosis transmembrane conductance regulator) gene, defective in cystic fibrosis, codes for a polytopic apical membrane protein functioning as a chloride channel. Wild-type (wt) CFTR matures inefficiently and CFTR with a deletion of Phe-508 (F508del), the most frequent mutation, is substantially retained as a core-glycosylated intermediate in the endoplasmic reticulum (ER), probably due to

misfolding that is recognized by the cellular quality control machinery involving molecular chaperones. Here, we overexpressed the heat-shock protein (Hsp) 70 chaperone in vivo and observed no changes in degradation rate of the core-glycosylated form, nor in the efficiency of its conversion into the fully glycosylated form, for either wt- or F508del-CFTR, contrary to previous in vitro studies on the affect of heat-shock cognate (Hsc) 70 on part of the first nucleotide-binding domain of CFTR. Co-transfection of Hsp70 with its co-chaperone human DnaJ homologue (Hdj)-1/Hsp40, however, stabilizes the immature form of wt-CFTR, but not of F508del-CFTR, suggesting that these chaperones act on a wt-specific conformation. As the efficiency of conversion into the fully glycosylated form is not increased under Hsp70/Hdj-1 overexpression, the lack of these two chaperones does not seem to be critical for CFTR maturation and ER retention. The effects of 4-phenylbutyrate and deoxyspergualin, described previously to interfere with Hsp70 binding, were also tested upon CFTR degradation and processing. The sole effect observed was destabilization of F508del-CFTR.

Reference Type: Journal Article
Record Number: 181
Author: Feinman, R.; Clarke, K. O.; Harrison, L. E.
Year: 2002
Title: Phenylbutyrate-induced apoptosis is associated with inactivation of NF-kappaB IN HT-29 colon cancer cells
Journal: Cancer Chemother Pharmacol
Volume: 49
Issue: 1
Pages: 27-34
Abstract: PURPOSE: Cytotoxic chemotherapy has been used to treat patients with metastatic colorectal cancer with limited success. Therefore novel chemotherapeutic approaches are needed. Based on encouraging preclinical data, there has been an interest in developing derivatives of butyrate as clinically applicable agents. The purpose of this study was to investigate the effects of phenylbutyrate (PB), a butyrate analogue, on the cell growth and apoptosis in a colon cancer cell model. METHODS: Growth curves, flow cytometric studies, Western blotting, DNA binding assays and transient transfection experiments were performed in vitro using the colon cancer cell line HT-29 after exposure to PB. RESULTS: Exposure of HT-29 colon cancer cells to PB resulted in growth inhibition and induction of apoptosis as measured by annexin V staining. This increase in apoptosis was associated with a decrease in mitochondrial membrane potential, an increase in caspase-3 activity and a decrease in intact PARP protein levels. Since NF-kappaB plays a pivotal role in the regulation of apoptosis, we explored the effects of PB on the DNA binding and transcriptional activity of this transcription factor. After PB treatment, NF-kappaB-DNA binding was markedly decreased and specifically, this decreased DNA binding was observed in the p50:p65 heterodimer. The decreased NF-kappaB DNA binding was observed as early as 3 h after PB treatment, while no apparent changes in annexin V binding were detected until 12 h after PB treatment. Untreated HT-29 cells transfected with a kappaB-luciferase reporter plasmid demonstrated significant constitutive activity of the kappaB binding site, which was markedly decreased after treating the cells with PB. CONCLUSION: These results suggest that PB-induced apoptosis may be partly

regulated through the inactivation of NF-kappaB. PB, an oral butyrate analogue, may have therapeutic potential in colon cancer.

Reference Type: Journal Article
Record Number: 179
Author: Gore, S. D.; Weng, L. J.; Figg, W. D.; Zhai, S.; Donehower, R. C.; Dover, G.; Grever, M. R.; Griffin, C.; Grochow, L. B.; Hawkins, A.; Burks, K.; Zabelena, Y.; Miller, C. B.
Year: 2002
Title: Impact of prolonged infusions of the putative differentiating agent sodium phenylbutyrate on myelodysplastic syndromes and acute myeloid leukemia
Journal: Clin Cancer Res
Volume: 8
Issue: 4
Pages: 963-70
Abstract: The aromatic fatty acid sodium phenylbutyrate (PB) promotes cytostasis and differentiation in a wide variety of tumor types; among several molecular activities, inhibition of histone deacetylase (HDAC) may account for many of its pharmacodynamic effects. A Phase I study demonstrated promising preliminary evidence of clinical activity in acute myeloid leukemia and myelodysplastic syndrome; however, plasma concentrations achieved at the maximum tolerated dose were less than those targeted based on in vitro studies. Because prolonged exposure to suboptimal concentrations of PB in vitro led to pharmacodynamic changes similar to a more brief exposure to higher concentrations, a study of the feasibility of prolonged administration of sodium PB was performed. Selected patients with acute myeloid leukemia and myelodysplastic syndrome were treated with sodium PB as a continuous i.v. infusion via ambulatory infusion pump. Sequential cohorts were treated for 7 consecutive days out of 14 or with 21 consecutive days out of 28. Prolonged infusions were well tolerated; dose-limiting central nervous system toxicity developed in 1 of 23 patients treated. End-of-infusion plasma concentrations were maintained within a range sufficient to inhibit HDAC. Two patients on the 21/28 schedule developed hematological improvement. Prolonged infusions of PB are well tolerated making this an attractive platform for the clinical investigation of HDAC inhibition.

Reference Type: Journal Article
Record Number: 171
Author: Grzanowski, A.; Needleman, R.; Brusilow, W. S.
Year: 2002
Title: Immunosuppressant-like effects of phenylbutyrate on growth inhibition of Saccharomyces cerevisiae
Journal: Curr Genet
Volume: 41
Issue: 3
Pages: 142-9
Abstract: Phenylbutyrate (4-phenylbutyric acid; PB) and its metabolite, phenylacetate, are effective anti-neoplastic agents in tissue culture and have shown promise in clinical trials for a variety of neoplasms. PB is a drug of remarkably low toxicity that acts in vitro as a differentiating agent, causing reversion of the

transformed phenotype by an unknown mechanism. We attempted to identify the cellular target(s) for PB using Saccharomyces as a model. PB inhibits growth of yeast on rich medium at concentrations of 0.1-1.0 mM, concentrations similar to plasma concentrations observed in human trials. Yeast cells treated with 1 mM PB remain over 90% viable for 24 h. PB inhibits tryptophan uptake, and resistance to PB can be conferred by tryptophan prototrophy, by supplementing tryptophan auxotrophs with the high levels of tryptophan, by overexpression of the aromatic amino acid permeases Tat1p or Tat2p, and by disruption of TAT1. Since tryptophan auxotrophy and transport influences resistance to PB, phytosphingosine, and the immunosuppressant FK506, these drugs might affect the same pathway. We isolated and characterized a mutant resistant to 1 mM PB and identified the mutant as bul1. A chromosomal BUL1 deletion displayed all phenotypes shown by the PB-resistant mutant.

Reference Type: Journal Article
Record Number: 176
Author: Iyer, R. K.; Yoo, P. K.; Kern, R. M.; Rozengurt, N.; Tsoa, R.; O'Brien, W. E.; Yu, H.; Grody, W. W.; Cederbaum, S. D.
Year: 2002
Title: Mouse model for human arginase deficiency
Journal: Mol Cell Biol
Volume: 22
Issue: 13
Pages: 4491-8
Abstract: Deficiency of liver arginase (AI) causes hyperargininemia (OMIM 207800), a disorder characterized by progressive mental impairment, growth retardation, and spasticity and punctuated by sometimes fatal episodes of hyperammonemia. We constructed a knockout mouse strain carrying a nonfunctional AI gene by homologous recombination. Arginase AI knockout mice completely lacked liver arginase (AI) activity, exhibited severe symptoms of hyperammonemia, and died between postnatal days 10 and 14. During hyperammonemic crisis, plasma ammonia levels of these mice increased >10-fold compared to those for normal animals. Livers of AI-deficient animals showed hepatocyte abnormalities, including cell swelling and inclusions. Plasma amino acid analysis showed the mean arginine level in knockouts to be approximately fourfold greater than that for the wild type and threefold greater than that for heterozygotes; the mean proline level was approximately one-third and the ornithine level was one-half of the proline and ornithine levels, respectively, for wild-type or heterozygote mice--understandable biochemical consequences of arginase deficiency. Glutamic acid, citrulline, and histidine levels were about 1.5-fold higher than those seen in the phenotypically normal animals. Concentrations of the branched-chain amino acids valine, isoleucine, and leucine were 0.4 to 0.5 times the concentrations seen in phenotypically normal animals. In summary, the AI-deficient mouse duplicates several pathobiological aspects of the human condition and should prove to be a useful model for further study of the disease mechanism(s) and to explore treatment options, such as pharmaceutical administration of sodium phenylbutyrate and/or ornithine and development of gene therapy protocols.

Reference Type: Journal Article
Record Number: 184
Author: Kang, H. L.; Benzer, S.; Min, K. T.
Year: 2002
Title: Life extension in Drosophila by feeding a drug
Journal: Proc Natl Acad Sci U S A
Volume: 99
Issue: 2
Pages: 838-43
Abstract: We report that feeding Drosophila throughout adulthood with 4-phenylbutyrate (PBA) can significantly increase lifespan, without diminution of locomotor vigor, resistance to stress, or reproductive ability. Treatment for a limited period, either early or late in adult life, is also effective. Flies fed PBA show a global increase in histone acetylation as well as a dramatically altered pattern of gene expression, including induction or repression of numerous genes. The delay in aging may result from the altered physiological state.

Reference Type: Journal Article
Record Number: 160
Author: Kennedy, C.; Byth, K.; Clarke, C. L.; deFazio, A.
Year: 2002
Title: Cell proliferation in the normal mouse mammary gland and inhibition by phenylbutyrate
Journal: Mol Cancer Ther
Volume: 1
Issue: 12
Pages: 1025-33
Abstract: Ovarian hormones have a pivotal role in the control of proliferation in the mammary gland, and cumulative life-time exposure to ovarian hormones is known to be a determinant of breast cancer risk. We have shown previously that a p.o.-active, long-acting butyrate analogue, sodium phenylbutyrate (PB), reduced proliferation in normal and malignant human breast cells in culture and reduced expression of ovarian hormone receptors, suggesting that PB had cellular effects consistent with decreasing breast cancer risk. The aim of this study was to determine the in vivo effects of PB in the normal mammary gland on epithelial cell proliferation, estrogen receptor alpha (ER alpha) expression, and cyclin D1 expression. BALB/c mice were treated with PB, delivered by mini-osmotic pumps, for 7 days. Moderate (250 mg/kg/day) and high (500 mg/kg/day) PB treatment resulted in a decrease in proliferation in mammary epithelial cells ($P < 0.001$), determined by bromodeoxyuridine incorporation. Analysis of ER alpha immunostaining revealed a significant reduction in moderate- and high-treatment groups ($P = 0.01$ and $P = 0.02$), and expression of cyclin D1 was virtually ablated ($P < 0.001$). Histone deacetylase inhibition is a known mechanism of butyrate action, and consistent with this, PB increased levels of acetylated histone H3 in the mammary gland. In summary, PB decreased proliferation in the mammary gland in vivo at clinically achievable doses. Decreased proliferation was accompanied by changes in the levels of ER alpha and cyclin D1. These data show that PB modulates parameters thought to be involved in the carcinogenic process in the normal mammary gland, and compounds in this class may therefore be useful candidates for breast cancer chemoprevention.

Reference Type: Journal Article
Record Number: 150
Author: Kouraklis, G.; Theocharis, S.
Year: 2002
Title: Histone deacetylase inhibitors and anticancer therapy
Journal: Curr Med Chem Anticancer Agents
Volume: 2
Issue: 4
Pages: 477-84
Abstract: Recent reports have shown that pharmacological manipulation of chromatin remodeling by histone deacetylase (HDAC) inhibitors, might develop into a potent and specific strategy for the treatment of cancer. Alterations in histone acetylation may lead to changes in chromatin structure and transcriptional dysregulation of genes that are implicated in controlling either cell cycle progression or pathways regulating cell differentiation and/or apoptosis. Dimethyl sulphoxide was one of the first chemicals to be identified as an inducer of transformed cell differentiation. In the class of HDAC inhibitors, now included a short-chain fatty acids, such as 4-phenylbutyrate and valporic acid, hydroxamic acids, such as suberoylanilide hydroxamic acid (SAHA), pyroxamide, trichostatin A, oxamflatin and CHAPSs, cyclic tetrapeptides, such as trapoxin, apicidin and depsipeptide-also known as FK-228 or FR 901228, and benzamides, such as MS-275. First clinical studies have shown that histone hyperacetylation can be achieved safely in humans and that treatment of cancer with such agents seems to become possible. Thus, HDAC inhibitors remains one of the most promising class of new anticancer agents. Further studies are needed in order to delineate the optimal dosage, the duration of therapy and possibly the efficacy of other agents able to synergize with HDAC inhibitors in the fight against cancer.

Reference Type: Journal Article
Record Number: 180
Author: Legras, A.; Labarthe, F.; Maillot, F.; Garrigue, M. A.; Kouatchet, A.; Ogier de Baulny, H.
Year: 2002
Title: Late diagnosis of ornithine transcarbamylase defect in three related female patients: polymorphic presentations
Journal: Crit Care Med
Volume: 30
Issue: 1
Pages: 241-4
Abstract: OBJECTIVE: To describe three female patients of one family with different phenotypes of the same mutation of the ornithine transcarbamylase gene. X-linked inherited ornithine transcarbamylase deficiency is the most frequent urea cycle disorder. Many of the hemizygous males die during the neonatal period. Women, who are mostly healthy carriers, can also develop symptomatic hyperammonemia. DESIGN: Case study. SETTING: Intensive care unit and internal medicine unit at a university hospital. PATIENTS: The 20-yr-old female propositus was hospitalized for unexplained coma. She had a history of headaches, recurrent vomiting, specific

anorexia for high-protein foods, and an acute neurologic crisis with alleged food poisoning 8 yrs before. The present episode began with psychiatric symptoms and seizures treated by diazepam and valproate. This unexplained coma, associated with respiratory alkalosis and major brain swelling on brain computed tomography scan, revealed hyperammonemia leading to the diagnosis of ornithine transcarbamylase deficiency. Continuous venovenous hemodiafiltration and treatment with sodium benzoate and phenylbutyrate improved the situation. However, the patient had some neurologic sequelae. DNA studies have disclosed a pathogenic mutation in the ornithine transcarbamylase gene of the patient, her mother, and her sister. For the mother, the disease was overlooked despite the onset of unusual headaches and neurologic signs that mimicked a cerebral tumor 12 yrs before. The 28-yr-old sister of the propositus has always been asymptomatic, even during pregnancy. CONCLUSIONS: Diagnosis of urea cycle disorder should be considered in any patient with unexplained neurologic and psychiatric disorders with selective anorexia, even in adulthood. Unexplained coma with cerebral edema and respiratory alkalosis requires urgent measurement of ammonemia and metabolic work-up.

Reference Type: Journal Article
Record Number: 174
Author: Leonard, J. V.; Morris, A. A.
Year: 2002
Title: Urea cycle disorders
Journal: Semin Neonatol
Volume: 7
Issue: 1
Pages: 27-35
Abstract: Most patients with urea cycle disorders who present as neonates, do so with deteriorating feeding, drowsiness and tachypnoea, following a short initial period when they appear well. The plasma ammonia should be measured at the same time as the septic screen in such patients. Ammonia levels above 200 micromol/l are usually caused by inherited metabolic diseases and it is essential to make a diagnosis for genetic counselling, even if the patients die. The aim of treatment is to lower the ammonia concentrations as fast as possible. Sodium benzoate, sodium phenylbutyrate and arginine can exploit alternative pathways for the elimination of nitrogen but haemodialysis or haemofiltration should be instituted if ammonia concentrations are >500 micromol/l or if they do not fall promptly. Long-term management involves drugs, dietary protein restriction and use of an emergency regimen during illness. Severe hyperammonaemia is usually associated with irreversible neurological damage, particularly if levels have been above 800 micromol/l for >24 hours, and the option of withdrawing treatment should be discussed with the family.

Reference Type: Journal Article
Record Number: 162
Author: Li, X.; Baumgart, E.; Dong, G. X.; Morrell, J. C.; Jimenez-Sanchez, G.; Valle, D.; Smith, K. D.; Gould, S. J.
Year: 2002

Title: PEX11alpha is required for peroxisome proliferation in response to 4-phenylbutyrate but is dispensable for peroxisome proliferator-activated receptor alpha-mediated peroxisome proliferation
Journal: Mol Cell Biol
Volume: 22
Issue: 23
Pages: 8226-40
Abstract: The PEX11 peroxisomal membrane proteins promote peroxisome division in multiple eukaryotes. As part of our effort to understand the molecular and physiological functions of PEX11 proteins, we disrupted the mouse PEX11alpha gene. Overexpression of PEX11alpha is sufficient to promote peroxisome division, and a class of chemicals known as peroxisome proliferating agents (PPAs) induce the expression of PEX11alpha and promote peroxisome division. These observations led to the hypothesis that PPAs induce peroxisome abundance by enhancing PEX11alpha expression. The phenotypes of PEX11alpha(-/-) mice indicate that this hypothesis remains valid for a novel class of PPAs that act independently of peroxisome proliferator-activated receptor alpha (PPARalpha) but is not valid for the classical PPAs that act as activators of PPARalpha. Furthermore, we find that PEX11alpha(-/-) mice have normal peroxisome abundance and that cells lacking both PEX11alpha and PEX11beta, a second mammalian PEX11 gene, have no greater defect in peroxisome abundance than do cells lacking only PEX11beta. Finally, we report the identification of a third mammalian PEX11 gene, PEX11gamma, and show that it too encodes a peroxisomal protein.

Reference Type: Journal Article
Record Number: 169
Author: Matsui, W. H.; Gladstone, D. E.; Vala, M. S.; Barber, J. P.; Brodsky, R. A.; Smith, B. D.; Jones, R. J.
Year: 2002
Title: The role of growth factors in the activity of pharmacological differentiation agents
Journal: Cell Growth Differ
Volume: 13
Issue: 6
Pages: 275-83
Abstract: Bryostatin-1 inhibits acute myeloid leukemia (AML) in vitroat doses that stimulate the growth of normal hematopoietic progenitors.Although bryostatin-1 has a number of distinct biological activities, those specifically responsible for its antileukemic activity are unclear. We found that bryostatin-1 (10(-8) M) inhibited cell cycling at G(1), induced phenotypic evidence of differentiation, and limited the clonogenic growth of both AML cell lines and patient specimens. This activity was markedly enhanced by granulocyte/macrophage-colony stimulating factor, whereas growth factor-neutralizing antibodies completely inhibited both the differentiating and antileukemic activities of bryostatin-1. Cell cycle inhibition and growth factors were also required for the differentiating activities of two unrelated agents, hydroxyurea and phenylbutyrate. These data suggest that many pharmacological differentiating agents require both cell cycle arrest and lineage-specific growth factors for full activity and may explain why these agents have demonstrated only limited clinical efficacy.

Reference Type: Journal Article
Record Number: 164
Author: Pace, B. S.; White, G. L.; Dover, G. J.; Boosalis, M. S.; Faller, D. V.; Perrine, S. P.
Year: 2002
Title: Short-chain fatty acid derivatives induce fetal globin expression and erythropoiesis in vivo
Journal: Blood
Volume: 100
Issue: 13
Pages: 4640-8
Abstract: Orally bioactive compounds that induce gamma globin gene expression at tolerable doses are needed for optimal treatment of the beta-hemoglobinopathies. Short-chain fatty acids (SCFAs) of 2 to 6 carbons in length induce gamma globin expression in animal models, and butyrate, phenylbutyrate, and valproate induce gamma globin in human patients. The usefulness of these compounds, however, is limited by requirements for large doses because of their rapid metabolism and their tendency to inhibit cell proliferation, which limits the pool of erythroid progenitors in which gamma globin can be induced. Selected short-chain fatty acid derivatives (SCFADs) were recently found to induce gamma globin and to stimulate the proliferation of hematopoietic cells in vitro. These SCFADs are now evaluated in vivo in nonanemic transgenic mice containing the human beta globin gene locus and in anemic phlebotomized baboons. In mice treated with a SCFAD once daily for 5 days, gamma globin mRNA increased 2-fold, reticulocytes increased 3- to 7-fold, and hematocrit levels increased by 27%. Administration of 3 SCFADs in anemic baboons increased F-reticulocytes 2- to 15-fold over baseline and increased total hemoglobin levels by 1 to 2 g/dL per week despite ongoing significant daily phlebotomy. Pharmacokinetic studies demonstrated 90% oral bioavailability of 2 SCFADs, and targeted plasma levels were maintained for several hours after single oral doses equivalent to 10% to 20% of doses required for butyrate. These findings identify SCFADs that stimulate gamma globin gene expression and erythropoiesis in vivo, activities that are synergistically beneficial for treatment of the beta hemoglobinopathies and useful for the oral treatment of other anemias.

Reference Type: Journal Article
Record Number: 161
Author: Resar, L. M.; Segal, J. B.; Fitzpatric, L. K.; Friedmann, A.; Brusilow, S. W.; Dover, G. J.
Year: 2002
Title: Induction of fetal hemoglobin synthesis in children with sickle cell anemia on low-dose oral sodium phenylbutyrate therapy
Journal: J Pediatr Hematol Oncol
Volume: 24
Issue: 9
Pages: 737-41
Abstract: This study was designed to determine if low doses of oral sodium phenylbutyrate (SPB) induce hemoglobin F (HbF) synthesis in children with

hemoglobin SS (HbSS). We treated 8 children with HbSS over a period of 5-30 weeks. The initial dose (1.0 g/d) was increased weekly (by 1.0 g/d) until F-reticulocytes doubled. All patients showed an increase in F-reticulocytes (P = 0.002) that was dose-dependent (P = 0.001). Three of 5 patients who continued oral SPB for more than 10 weeks had substantial increases in HbF. We conclude that lower dose SPB is effective in inducing HbF synthesis in some children with HbSS. Further trials are warranted to determine the optimal treatment regimen.

Reference Type: Journal Article
Record Number: 186
Author: Schroder, C. P.; Maurer, H. R.
Year: 2002
Title: Tributyrin-induced differentiation promotes apoptosis of LS 174T colon cancer cells in vitro
Journal: Int J Oncol
Volume: 20
Issue: 1
Pages: 195-200
Abstract: Tributyrin (glyceryl tributyrate, TB) is known to induce malignant cells to differentiate followed by arrest of cell growth and death via apoptosis. We investigated the effects of TB on the distribution of cell cycle phases, differentiation as measured by alkaline phosphatase activity (ALP), and apoptosis of LS 174T colon cancer cells expressed by morphological changes, externalization of phosphatidylserine and stimulation of various caspases. TB (0.6 mM) reduced the proliferation by a 5-fold decrease of tumor cells in the S-phase and 1.3-fold increase in the G2/M-phase of cell cycle after 24 h of incubation. The ALP activity was enhanced in a dose-dependent manner up to 180-fold by 1 mM TB. Apoptosis was seen only above 0.6 mM TB (5-fold increase). Studies with caspase inhibitors revealed that TB mediated cell death was linked to up-regulation of caspases 3 and 8. Our results indicate that TB-induced differentiation promotes apoptosis in LS 174T cells and may explain the mode of action of TB finally resulting in an arrest of tumor cell growth.

Reference Type: Journal Article
Record Number: 158
Author: Shi, M. G.; Huang, Q.; Dong, J.; Sun, Z. F.; Lan, Q.
Year: 2002
Title: [Experimental study of combination therapy against human glioma xenograft by differentiation-inducing agent and cytotoxic chemotherapeutic drug]
Journal: Ai Zheng
Volume: 21
Issue: 10
Pages: 1090-4
Abstract: BACKGROUND & OBJECTIVE: Cytotoxic agent remains the main chemotherapeutic drug for glioma, although it has many limitations. It is not known whether differentiation-inducing agent can enhance antitumor efficiency of cytotoxic agent. This study was designed to investigate anti-tumor effects of differentiation-inducing agent in combination with cytotoxic chemotherapeutic drug against glioma.

METHODS: Poorly-differentiated human brain glioma xenografted nude mice were treated with carmustine(1, 3-bis-(2-chloroethyl)-1-nitrosourea, BCNU) and sodium phenylbutyrate (SPB). The therapeutic effects were determined by measuring of tumor size, pathological changes, different phases of cell cycle of tumor cell proliferation, expression of differentiation antigen, and tumor cell apoptosis. RESULTS: The therapeutic effects of SPB plus BCNU group were much better than that of SPB or BCNU group alone, which were proved by lower growth rate of the tumor, cellularity decreasing, appearance of astroid-like polyglonal cells, G0/G1 ratio increasing, upregulation of GFAP expression. CONCLUSION: Combined application of SPB and BCNU can obviously inhibit proliferation of glioma, and promote differentiation of tumor cells.

Reference Type: Journal Article
Record Number: 185
Author: Suaud, L.; Li, J.; Jiang, Q.; Rubenstein, R. C.; Kleyman, T. R.
Year: 2002
Title: Genistein restores functional interactions between Delta F508-CFTR and ENaC in Xenopus oocytes
Journal: J Biol Chem
Volume: 277
Issue: 11
Pages: 8928-33
Abstract: The cystic fibrosis transmembrane conductance regulator (CFTR), in addition to its Cl(-) channel properties, has regulatory interactions with other epithelial ion channels including the epithelial Na(+) channel (ENaC). Both the open probability and surface expression of wild type CFTR Cl(-) channels are increased significantly when CFTR is co-expressed in Xenopus oocytes with alphabetagamma-ENaC, and conversely, the activity of ENaC is inhibited following wild type CFTR activation. Using the Xenopus oocyte expression system, a lack of functional regulatory interactions between DeltaF508-CFTR and ENaC was observed following activation of DeltaF508-CFTR by forskolin and isobutylmethylxanthine (IBMX). Whole cell currents in oocytes expressing ENaC alone decreased in response to genistein but increased in response to a combination of forskolin and IBMX followed by genistein. In contrast, ENaC currents in oocytes co-expressing ENaC and DeltaF508-CFTR remained stable following stimulation with forskolin/IBMX/genistein. Furthermore, co-expression of DeltaF508-CFTR with ENaC enhanced the forskolin/IBMX/genistein-mediated activation of DeltaF508-CFTR. Our data suggest that genistein restores regulatory interactions between DeltaF508-CFTR and ENaC and that combinations of protein repair agents, such as 4-phenylbutyrate and genistein, may be necessary to restore DeltaF508-CFTR function in vivo.

Reference Type: Journal Article
Record Number: 172
Author: Zeitlin, P. L.; Diener-West, M.; Rubenstein, R. C.; Boyle, M. P.; Lee, C. K.; Brass-Ernst, L.
Year: 2002

Title: Evidence of CFTR function in cystic fibrosis after systemic administration of 4-phenylbutyrate
Journal: Mol Ther
Volume: 6
Issue: 1
Pages: 119-26
Abstract: Most individuals with cystic fibrosis (CF) carry one or two mutations that result in a maturation defect of the full-length protein. One such mutation, deltaF508, results in a mutant membrane glycoprotein that fails to progress to the apical membrane, where the wild-type protein normally functions as a cyclic AMP-regulated chloride channel. 4-Phenylbutyrate (Buphenyl), an orally bioavailable short chain fatty acid, modulates heat shock protein expression and restores maturation of the deltaF508 protein in vitro and in vivo. We performed a randomized, double-blind, placebo-controlled, dose-escalation and safety study of Buphenyl in 19 adults with CF (homozygous deltaF508) to test the hypothesis that Buphenyl would be safe, well-tolerated, and associated with an increase in chloride transport in nasal epithelia. Three dose levels (20, 30, or 40 g divided t.i.d.) of drug or placebo were given for 1 week. Serial measurements of chloride transport by nasal potential difference (NPD) testing and metabolic safety testing were performed. A maximum tolerated dose of 20 g was defined based on minimal adverse reactions, the safety profile, and a statistically significant induction of chloride transport that was maximal by day 3. This short-term phase I/II study demonstrates proof of principle that modulation of deltaF508 CFTR biosynthesis and trafficking is a viable therapeutic approach for cystic fibrosis.

Reference Type: Journal Article
Record Number: 183
Author: Zhou, D. C.; Kim, S. H.; Ding, W.; Schultz, C.; Warrell, R. P., Jr.; Gallagher, R. E.
Year: 2002
Title: Frequent mutations in the ligand-binding domain of PML-RARalpha after multiple relapses of acute promyelocytic leukemia: analysis for functional relationship to response to all-trans retinoic acid and histone deacetylase inhibitors in vitro and in vivo
Journal: Blood
Volume: 99
Issue: 4
Pages: 1356-63
Abstract: This study identified missense mutations in the ligand binding domain of the oncoprotein PML-RARalpha in 5 of 8 patients with acute promyelocytic leukemia (APL) with 2 or more relapses and 2 or more previous courses of all-trans retinoic acid (RA)-containing therapy. Four mutations were novel (Lys207Asn, Gly289Arg, Arg294Trp, and Pro407Ser), whereas one had been previously identified (Arg272Gln; normal RARalpha1 codon assignment). Five patients were treated with repeat RA plus phenylbutyrate (PB), a histone deacetylase inhibitor, and one patient experienced a prolonged clinical remission. Of the 5 RA + PB-treated patients, 4 had PML-RARalpha mutations. The Gly289Arg mutation in the clinical responder produced the most defective PML-RARalpha function in the presence of RA with or without sodium butyrate (NaB) or trichostatin A. Relapse APL cells from this patient failed to

differentiate in response to RA but partially differentiated in response to NaB alone, which was augmented by RA. In contrast, NaB alone had no differentiation effect on APL cells from another mutant case (Pro407Ser) but enhanced differentiation induced by RA. These results indicate that PML-RARalpha mutations occurred with high frequency after multiple RA treatment relapses, indicate that the functional potential of PML-RARalpha was not correlated with clinical response to RA + PB treatment, and suggest that the response to RA + PB therapy in one patient was related to the ability of PB to circumvent the blocked RA-regulated gene response pathway.

Reference Type: Journal Article
Record Number: 143
Author: Al-Hassnan, Z. N.; Boyadjiev, S. A.; Praphanphoj, V.; Hamosh, A.; Braverman, N. E.; Thomas, G. H.; Geraghty, M. T.
Year: 2003
Title: The relationship of plasma glutamine to ammonium and of glycine to acid-base balance in propionic acidaemia
Journal: J Inherit Metab Dis
Volume: 26
Issue: 1
Pages: 89-91
Abstract: Hyperammonaemia is a common and serious complication of propionic acidaemia. Treatment of hyperammonaemia with sodium phenylacetate or phenylbutyrate has not been well studied in this disorder. We reviewed the medical records of 5 patients with propionic acidaemia over a 16-year period. We collected information on events where plasma amino acids and ammonium, plasma acids and acid-base balance, or all 3 parameters were obtained simultaneously. All patients were on protein-restricted diet and carnitine throughout the period. In contrast to hyperammonaemia in patients with a urea cycle disorder, plasma glutamine levels were below the normal mean and there was no correlation between plasma ammonium and glutamine levels. The absence of positive correlation between plasma glutamine and ammonium suggests that the routine use of sodium phenylacetate or phenylbutyrate to treat hyperammonaemia in propionic acidaemia should be questioned until further studies are done. Throughout follow-up of our propionic acidaemia patients, we have observed that plasma glycine levels correlated positively with serum bicarbonate. The association of high plasma glycine with good acid-base balance might have a potential role in management and warrants further investigation.

Reference Type: Journal Article
Record Number: 140
Author: Andersson, C.; Servetnyk, Z.; Roomans, G. M.
Year: 2003
Title: Activation of CFTR by genistein in human airway epithelial cell lines
Journal: Biochem Biophys Res Commun
Volume: 308
Issue: 3
Pages: 518-22
Abstract: Cystic fibrosis (CF) is caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel expressed in

epithelial cells. The effects of genistein and 4-phenylbutyrate (PBA) on CFTR were studied in three human airway epithelial cell lines expressing wild-type or DeltaF508 CFTR: Calu-3, CFSMEo-, and CFBE41o- cells. The cells were loaded with the fluorescent dye N-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide (MQAE) and chloride efflux was studied. Forskolin and 3-isobutyl-1-methylxanthine (IBMX) induced chloride efflux in Calu-3 cells but not in CF lines. Genistein (2.5-50 microM) alone was able to induce chloride efflux in all cell lines. Genistein did not enhance the effect of forskolin and IBMX. PBA had little or no effect on genistein-induced chloride efflux. The effect of genistein seen at low concentrations makes genistein interesting for possible pharmacological treatment of CF, since it is known that similar concentrations can be obtained in plasma by a soy-rich diet.

Reference Type: Journal Article
Record Number: 133
Author: Belinsky, S. A.; Klinge, D. M.; Stidley, C. A.; Issa, J. P.; Herman, J. G.; March, T. H.; Baylin, S. B.
Year: 2003
Title: Inhibition of DNA methylation and histone deacetylation prevents murine lung cancer
Journal: Cancer Res
Volume: 63
Issue: 21
Pages: 7089-93
Abstract: Disruption of one allele for the cytosine-DNA methyltransferase 1 (DNMT1) gene in mice with a germ-line mutation in a tumor suppressor gene was shown previously to reduce tumor formation in juvenile animals. This effect is now reproduced in our studies of mature mice where this genetic DNMT1 reduction leads to a 50% decrease in tobacco carcinogen-induced lung cancer and a similar reduction in DNMT activity in type II pneumocytes that give rise to the tumors. Short-term treatment of DNMT wild-type female mice with low doses of the demethylating agent 5-aza-2'-deoxycytidine decreased the incidence of neoplasms by 30%. Importantly, when 5-aza-2'-deoxycytidine was combined with the histone deacetylase inhibitor sodium phenylbutyrate, lung tumor development was significantly reduced by >50%; no effect was seen with phenylbutyrate alone. This identical combination of inhibitors also acts synergistically to cause re-expression of densely hypermethylated and transcriptionally silenced tumor suppressor genes in human cancer cells. Thus, reduction in DNMT and histone deacetylase activities that likely block epigenetically mediated gene silencing might provide a novel clinical strategy to help prevent the leading cause of cancer death in the United States.

Reference Type: Journal Article
Record Number: 130
Author: Bugaut, M.; Fourcade, S.; Gondcaille, C.; Gueugnon, F.; Depreter, M.; Roels, F.; Netik, A.; Berger, J.; Martin, P.; Pineau, T.; Cadepond, F.; El Etr, M.; Savary, S.
Year: 2003
Title: Pharmacological induction of redundant genes for a therapy of X-ALD: phenylbutyrate and other compounds

Journal: Adv Exp Med Biol
Volume: 544
Pages: 281-91

Reference Type: Journal Article
Record Number: 142
Author: Choi, S. J.; Hwang, J. M.; Kim, S. I.
Year: 2003
Title: A colorimetric microplate assay method for high throughput analysis of lipase activity
Journal: J Biochem Mol Biol
Volume: 36
Issue: 4
Pages: 417-20
Abstract: The present work describes a colorimetric microplate assay for lipase activity based on the reaction between 5,5'-dithiobis(2-nitro benzoic acid) (DTNB) and the hydrolysis product of 2,3-dimercapto-1-propanol tributyrate (DMPTB). Reaction mixtures containing DTNB, DMPTB, and lipase were prepared in microplate wells, and the absorbance at 405nm was recorded after incubation at 37 degrees C for 30 min. A linear relationship was obtained in the range of 0.1-1 U of lipase activity by this method. The reaction conditions were also optimized for the range of 0.01-0.1 U or 1-10 U. When assaying crude tissue extracts, the reaction of DTNB with non-specific reducing agents created a major source of error. However, this error was corrected by the use of blank samples that did not contain DMPTB.

Reference Type: Journal Article
Record Number: 134
Author: Chung, Y. L.; Lee, M. Y.; Wang, A. J.; Yao, L. F.
Year: 2003
Title: A therapeutic strategy uses histone deacetylase inhibitors to modulate the expression of genes involved in the pathogenesis of rheumatoid arthritis
Journal: Mol Ther
Volume: 8
Issue: 5
Pages: 707-17
Abstract: Rheumatoid arthritis (RA) is characterized by progressive destruction of the affected joints. The pathophysiology results from genetic susceptibility and autoimmune phenomena, leading to tissue inflammation and synovial hyperplasia termed pannus, which irreversibly destroys cartilage and bone. The current treatment options, which suppress immune responses or ameliorate inflammation, do not halt the destructive process. We found that the histone deacetylase (HDAC) inhibitors (phenylbutyrate and trichostatin A) causing histone hyperacetylation to modulate multiple gene expression not only induced the expression of p21(Cip1) and p16(INK4) in synovial cells but also inhibited the expression of tumor necrosis factor-alpha in affected tissues in adjuvant arthritis, an animal model of RA. Based on the observations that joint swelling is reduced, subintimal mononuclear cell infiltration is decreased, synovial hyperplasia is inhibited, pannus formation is suppressed, and no cartilage or bone destruction is seen, the HDAC inhibitors may represent a new class

of compounds for the treatment of RA by simultaneously, coordinately, synergistically, or epigenetically modulating multiple molecular targets in the pathogenesis of RA.

Reference Type: Journal Article
Record Number: 139
Author: Chung, Y. L.; Sheu, M. L.; Yen, S. H.
Year: 2003
Title: Hepatitis C virus NS5A as a potential viral Bcl-2 homologue interacts with Bax and inhibits apoptosis in hepatocellular carcinoma
Journal: Int J Cancer
Volume: 107
Issue: 1
Pages: 65-73
Abstract: Treatment of hepatocellular carcinoma (HCC) cells with butyrate can induce apoptosis irrespective of hepatitis B virus integration. No information is available, however, regarding the effect of butyrate on HCC in the presence of hepatitis C virus (HCV) because some HCV proteins can regulate cell survival. By gene transfer, we found that HCV core enhances but HCV NS5A antagonizes sodium phenylbutyrate (NaPB)-induced apoptosis in HCC cells, which is independent of p53. We then chose the p53-negative Hep3B HCC cell to investigate the mechanism of anti-apoptosis mediated by NS5A. In the NaPB-treated Hep3B cells without NS5A expression, induction of apoptosis was associated with Bax redistribution from the cytosol to the nucleus interior and subsequently, to a nuclear membrane-bound form. In the NS5A expressing Hep3B cells, NaPB treatment also triggered relocalization of both Bax and NS5A from the cytosol to the nucleus interior but Bax retained inside the nucleus and did not finally move to the nuclear membrane. Using double immunofluorescence and coimmunoprecipitation, we demonstrated that NS5A co-localizes and interacts with Bax in the nucleus. The HCV NS5A protein was further found to contain Bcl-2 homology domains (BH3, BH1 and BH2). Additional studies using deleted NS5A constructs were carried out to determine whether the BH2 domain or nuclear localization signal (NLS) in NS5A is required for interaction with Bax in the nucleus or inhibition of apoptosis. NS5A with deletion of both BH2 domain and NLS localized in the cytoplasm, dissociated with Bax, and lost anti-apoptosis activity during NaPB treatment. In contrast, NS5A with intact BH domains except NLS still bound directly to Bax in the perinuclear region or the nucleus, but showed less association with Bax in the nucleus and lower effect in apoptosis inhibition than full-length NS5A. These results suggest that HCV NS5A as a Bcl-2 homologue interacts with Bax to protect p53-negative HCC cells from NaPB-induced apoptosis.

Reference Type: Journal Article
Record Number: 138
Author: Claus, R.; Lubbert, M.
Year: 2003
Title: Epigenetic targets in hematopoietic malignancies
Journal: Oncogene
Volume: 22

Issue: 42
Pages: 6489-96
Abstract: Frequent genetic alterations in hematopoietic neoplasias (chromosomal translocations, point mutations, etc.) have provided biologic targets for the development of effective novel therapies. A rapidly increasing body of knowledge provides evidence also for multiple epigenetic alterations in these disorders, which can complement or even precede genetic aberrations. Gene inactivation ('silencing') of tumor suppressor and growth inhibitory genes (e.g. the cyclin-dependent kinase inhibitors p16, p15, p21) is frequently mediated by DNA methylation of gene promoters. The acetylation state of histones (functionally linked to the DNA methylation state by the methylcytosine binding protein 2, recruiting histone deacetylases) provides a second major epigenetic silencing mechanism. Therapeutic reversal strategies are being developed for acute leukemias, myelodysplastic syndromes and malignant lymphomas. Since the discovery of the DNA methyltransferase (Dnmt) inhibitory activity of two azanucleosides (5-azacytidine, 5-aza-2'-deoxycytidine/decitabine) even at doses with minimal nonhematologic toxicity, both have been clinically studied in several myeloid neoplasias, particularly in elderly patients unable to tolerate aggressive treatment. Further development of agents counteracting aberrant methylation is directed at more targeted approaches, for example, antisense molecules against Dnmts. Histone deacetylases (HDACs) can be inhibited by numerous compounds (sodium phenylbutyrate, valproic acid, novel compounds such as depsipeptide), which have entered the clinical arena in similar indications as Dnmt inhibitors. Impressive effects of HDAC inhibition in acute promyelocytic leukemia models (PML/RARA expression) translate the finding of HDAC recruitment by this chimeric transcription factor to its target genes. The recent discovery of recruitment by PML/RARA also of Dnmt activity to the retinoic acid receptor-beta promoter makes it an interesting candidate for Dnmt inhibitors. Studies combining a 're-expressor' strategy with inhibitors of Dnmts and HDACs are underway. Thus, resensitization to biological agents such as retinoids, colony-stimulating factors and other differentiation inducers may be envisioned.

Reference Type: Journal Article
Record Number: 153
Author: Dasgupta, S.; Zhou, Y.; Jana, M.; Banik, N. L.; Pahan, K.
Year: 2003
Title: Sodium phenylacetate inhibits adoptive transfer of experimental allergic encephalomyelitis in SJL/J mice at multiple steps
Journal: J Immunol
Volume: 170
Issue: 7
Pages: 3874-82
Abstract: Experimental allergic encephalomyelitis (EAE) is the animal model for multiple sclerosis. The present study underlines the importance of sodium phenylacetate (NaPA), a drug approved for urea cycle disorders, in inhibiting the disease process of adoptively transferred EAE in female SJL/J mice at multiple steps. Myelin basic protein (MBP)-primed T cells alone induced the expression of NO synthase (iNOS) and the activation of NF-kappaB in mouse microglial cells through cell-cell contact. However, pretreatment of MBP-primed T cells with NaPA markedly inhibited its ability to induce microglial expression of iNOS and activation of NF-

kappaB. Consistently, adoptive transfer of MBP-primed T cells, but not that of NaPA-pretreated MBP-primed T cells, induced the clinical symptoms of EAE in female SJL/J mice. Furthermore, MBP-primed T cells isolated from NaPA-treated donor mice were also less efficient than MBP-primed T cells isolated from normal donor mice in inducing iNOS in microglial cells and transferring EAE to recipient mice. Interestingly, clinical symptoms of EAE were much less in mice receiving NaPA through drinking water than those without NaPA. Similar to NaPA, sodium phenylbutyrate, a chemically synthesized precursor of NaPA, also inhibited the disease process of EAE. Histological and immunocytochemical analysis showed that NaPA inhibited EAE-induced spinal cord mononuclear cell invasion and normalized iNOS, nitrotyrosine, and p65 (the RelA subunit of NF-kappaB) expression within the spinal cord. Taken together, our results raise the possibility that NaPA or sodium phenylbutyrate taken through drinking water or milk may reduce the observed neuroinflammation and disease process in multiple sclerosis patients.

Reference Type: Journal Article
Record Number: 149
Author: Depreter, M.; Espeel, M.; Roels, F.
Year: 2003
Title: Human peroxisomal disorders
Journal: Microsc Res Tech
Volume: 61
Issue: 2
Pages: 203-23
Abstract: Peroxisomes are single membrane-bound cell organelles performing numerous metabolic functions. The present article aims to give an overview of our current knowledge about inherited peroxisomal disorders in which these organelles are lacking or one or more of their functions are impaired. They are multiorgan disorders and the nervous system is implicated in most. After a summary of the historical names and categories, each having distinct symptoms and prognosis, microscopic pathology is reviewed in detail. Data from the literature are added to experience in the authors' laboratory with 167 liver biopsy and autopsy samples from peroxisomal patients, and with a smaller number of chorion samples for prenatal diagnosis, adrenal-, kidney-, and brain samples. Various light and electron microscopic methods are used including enzyme- and immunocytochemistry, polarizing microscopy, and morphometry. Together with other laboratory investigations and clinical data, this approach continues to contribute to the diagnosis and further characterization of peroxisomal disorders, and the discovery of novel variants. When liver specimens are examined, three main groups including 9 novel variants (33 patients) are distinguished: (1) absence or (2) presence of peroxisomes, and (3) mosaic distribution of cells with and without peroxisomes (10 patients). Renal microcysts, polarizing trilamellar inclusions, and insoluble lipid in macrophages in liver, adrenal cortex, brain, and in interstitial cells of kidney are also valuable for classification. On a genetic basis, complementation of fibroblasts has classified peroxisome biogenesis disorders into 12 complementation groups. Peroxisome biogenesis genes (PEX), knock-out-mice, and induction of redundant genes are briefly reviewed, including some recent results with 4-phenylbutyrate. Finally, regulation of peroxisome expression during development and in cell cultures, and by physiological factors is discussed.

Reference Type: Journal Article
Record Number: 151
Author: Finzer, P.; Stohr, M.; Seibert, N.; Rosl, F.
Year: 2003
Title: Phenylbutyrate inhibits growth of cervical carcinoma cells independent of HPV type and copy number
Journal: J Cancer Res Clin Oncol
Volume: 129
Issue: 2
Pages: 107-13
Abstract: PURPOSE: Inhibitors of histone deacetylase, such as sodium butyrate, block proliferation of cervical carcinoma cells by inhibiting the G1 to S transition of the cell cycle. The derivative phenylbutyrate (PB), characterized by its higher pharmacological half-life, and its metabolite phenylacetate (PA) were tested for their growth-inhibitory function on cervical cancer cells differing in their HPV type, copy number, and integration sites. METHODS AND RESULTS: Using flow cytometric and Western blot analyses, we show that a 24-h incubation period with PB, but not with PA, was already sufficient to cause a dose-dependent growth arrest by increasing the G1 fraction with a concomitant drop in the S-phase. Consistent with the cell cycle block, only PB, but not PA, induced the cyclin-dependent kinase inhibitors p21(CIP1) and p27(KIP1). The inhibitory effect was not the result of a non-specific cytotoxic effect of PB, since cessation of cellular growth was already completely reversible 5 h after drug removal. CONCLUSIONS: Due to its broad growth inhibitory properties on different cervical carcinoma cells in vitro, and its low toxic profile demonstrated in preceding clinical studies, PB may serve as an effective drug in handling pre-cancerous lesions and cervical cancer in patients.

Reference Type: Journal Article
Record Number: 146
Author: Gordon, N.
Year: 2003
Title: Ornithine transcarbamylase deficiency: a urea cycle defect
Journal: Eur J Paediatr Neurol
Volume: 7
Issue: 3
Pages: 115-21
Abstract: The symptoms and signs of ornithine transcarbamylase deficiency are discussed. When the condition occurs among males in the neonatal period it is likely to be lethal. Pathological findings are non-specific. The diagnosis should be considered if coma with cerebral oedema and respiratory alkalosis occurs for no obvious reason. When hyperammonaemia is found, enzyme assay on a liver biopsy should be considered. A useful clue in an asymptomatic patient is a voluntary adoption of a vegetarian diet. Provocative tests, such as the allopurinol test can be used, but the method most frequently applied is mutation analysis. In the case of prenatal diagnosis this is possible on a chorionic villus sample.The prognosis of ornithine transcarbamylase deficiency is better for those with an onset after infancy, but morbidity from brain damage does not appear to be linked to the number of

episodes of hyperammonaemia that have occurred.The syndrome results from a deficiency of the mitochondrial enzyme ornithine transcarbamylase which catalyses the conversion of ornithine and carbamoyl phosphate to citrulline. The gene responsible for this enzyme is located on Xp21.1, and is expressed in the liver and gut. Mutations can be divided into two groups: those with neonatal onset with all enzyme activity abolished, and those with later onset with partial and varying enzyme deficiency.There can be a variety of precipitating causes, for example sodium valproate. Treatment can be given with a low protein diet, and with alternate pathway drugs such as sodium benzoate and phenylbutyrate. Liver transplant can be considered when symptoms are life-threatening, although there may be severe complications.Gene replacement therapy is the hope of the future.

Reference Type: Journal Article
Record Number: 144
Author: Hao, C. L.; Tang, K. J.; Tian, Z.; Xing, H. Y.; Wang, M.; Wang, J. X.
Year: 2003
Title: [Effect of phenylbutyrate, a histone deacetylase inhibitor, on differentiation and apoptosis of Kasumi-1 cells]
Journal: Zhonghua Xue Ye Xue Za Zhi
Volume: 24
Issue: 5
Pages: 241-4
Abstract: OBJECTIVE: To explore the blockade effect of phenylbutyrate (PB), a histone deacetylase inhibitor, on the in vitro biological function of AML1/ETO to reverse its transcription repression and induce Kasumi-1 cells to differentiate and apoptosis. METHODS: Kasumi-1 cells were treated with PB at different concentrations in suspension culture. Cell proliferation was analysed by MTT assay, morphological changes by light and electron microscopy, expression of myeloid-specific differentiation antigen and cell cycle by flow cytometry, cell apoptosis by annexin V staining, agarose gel electrophoresis and flow cytometry. RESULTS: PB treatment caused a dose-dependent inhibition of the cell proliferation. The IC(50) was about 2.3 mmol/L. PB treatment led to a progressive decline in the fraction of S-phase cells and increase in G(0)/G(1) cells. PB induced a time- and dose-dependent increase in expression of myeloid cell surface protein CD(11b) and CD(13). A dose-dependent increase in early apoptosis for 2 days treatment, late apoptosis for 3 days treatment. The DNA ladder of apoptosis was observed on agarose gel electrophoresis for 5 days treatment. Morphological features of monocytoid differentiation and apoptosis were seen on Wright-Giemsa staining smears. CONCLUSION: PB treatment could inhibit proliferation of Kasumi-1 cells, induce partial differentiation, apoptosis and accumulation of cells in G(0)/G(1) phase.

Reference Type: Journal Article
Record Number: 147
Author: Horslen, S. P.; McCowan, T. C.; Goertzen, T. C.; Warkentin, P. I.; Cai, H. B.; Strom, S. C.; Fox, I. J.
Year: 2003
Title: Isolated hepatocyte transplantation in an infant with a severe urea cycle disorder

Journal: Pediatrics
Volume: 111
Issue: 6 Pt 1
Pages: 1262-7
Abstract: OBJECTIVE: Transplantation of isolated hepatocytes in animal models has been shown to correct inborn errors of metabolism. Based on these studies and our experience with hepatocyte transplantation in a child with Crigler-Najjar syndrome, isolated hepatocyte transplantation was performed to attempt metabolic reconstitution in a male infant with severe ornithine transcarbamylase (OTC) deficiency. METHODS: An infant with an antenatal diagnosis of OTC deficiency was managed intensively to prevent hyperammonemia. Isolated hepatocytes were obtained by collagenase perfusion of donated livers not used for transplantation. Hepatocytes were infused in batches over the first 4 weeks of life via an umbilical venous catheter positioned in the portal vein. Immunosuppression consisted of tacrolimus and corticosteroids. RESULTS: Over 4 billion viable hepatocytes were transplanted during the first 3.5 weeks of life. A period of metabolic stability was achieved between days 20 and 31 during which normal protein intake was tolerated while phenylbutyrate was weaned. During this time, plasma ammonia and glutamine remained within normal limits. Hyperammonemia reappeared abruptly on day 31 of life. Protein tolerance diminished to baseline; metabolic stability was subsequently reattained only following successful liver transplantation at 6 months of age. CONCLUSIONS: Isolated hepatocyte transplantation appeared to result in temporary relief of hyperammonemia and protein intolerance attributable to OTC deficiency. The metabolic stability achieved was lost after 11 days presumably because of rejection of the transplanted cells because of insufficient immunosuppression. Future attempts at isolated hepatocyte transplantation for inborn errors of metabolism in humans should include adequate immunosuppression and a liver biopsy as a means of proving hepatocyte engraftment and function.

Reference Type: Journal Article
Record Number: 148
Author: Horsman, G. P.; Liu, A. M.; Henke, E.; Bornscheuer, U. T.; Kazlauskas, R. J.
Year: 2003
Title: Mutations in distant residues moderately increase the enantioselectivity of Pseudomonas fluorescens esterase towards methyl 3bromo-2-methylpropanoate and ethyl 3phenylbutyrate
Journal: Chemistry
Volume: 9
Issue: 9
Pages: 1933-9
Abstract: Directed evolution combined with saturation mutagenesis identified six different point mutations that each moderately increases the enantioselectivity of an esterase from Pseudomonas fluorescens (PFE) towards either of two chiral synthons. Directed evolution identified a Thr230Ile mutation that increased the enantioselectivity from 12 to 19 towards methyl (S)-3-bromo-2-methylpropanoate. Saturation mutagenesis at Thr230 identified another mutant, Thr230Pro, with higher-than-wild-type enantioselectivity (E=17). Previous directed evolution identified mutants Asp158Asn and Leu181Gln that increased the enantioselectivity from 3.5 to

5.8 and 6.6, respectively, towards ethyl (R)-3-phenylbutyrate. In this work, saturation mutagenesis identified other mutations that further increase the enantioselectivity to 12 (Asp158Leu) and 10 (Leu181Ser). A homology model of PFE indicates that all mutations lie outside the active site, 12-14 A from the substrate and suggests how the distant mutations might indirectly change the substrate-binding site. Since proteins contain many more residues far from the active site than close to the active site, random mutagenesis is strongly biased in favor of distant mutations. Directed evolution rarely screens all mutations, so it usually finds the distant mutations because they are more common, but probably not the most effective.

Reference Type: Journal Article
Record Number: 145
Author: Knarreborg, A.; Jensen, S. K.; Engberg, R. M.
Year: 2003
Title: Pancreatic lipase activity as influenced by unconjugated bile acids and pH, measured in vitro and in vivo
Journal: J Nutr Biochem
Volume: 14
Issue: 5
Pages: 259-65
Abstract: The relation between pancreatic lipase activity, unconjugated bile acids and pH was studied in vitro and in vivo. Lipase activity was assayed in vitro using automatic titration, where the fatty acids liberated from the hydrolysis of glycerol tributyrate (GTB) were measured. The lipase activity was determined at different ratios of conjugated to unconjugated bile acids (100:0, 75:25, 50:50, 25:75, 0:100) in response to pH 6.6, 6.8, 7.0 and 7.5. The in vivo study involved 96 one-day-old male broiler chickens. The chickens were assigned randomly, in pens of six animals, into two dietary treatments (8 replicate blocks), composing a non-supplemented diet (A(-)) and a diet supplemented (A(+)) with avilamycin (10 mg/kg feed) and salinomycin (40 mg/kg feed). After 35 days, the chickens were killed and content of the proximal part of the small intestine was collected and analyzed for bacterial counts, pH, bile acid concentration, and lipase activity. Evidence for a significant pH-dependent inhibition of lipase activity by unconjugated bile acids was provided in vitro and confirmed in vivo. Due to a reduction in nutrient fermentation, the pH in the small intestine of antibiotic-fed chickens was significantly higher than in chickens fed the non-supplemented diet. The high pH in the small intestine of chickens fed the A(+)diet was accompanied by a significant increase in lipase activity, and coincided with a significantly lower concentration of unconjugated bile acids and a higher ratio of conjugated to unconjugated bile acids. This study emphasizes the important influence of unconjugated bile acids on lipase activity at physiological pH-values.

Reference Type: Journal Article
Record Number: 141
Author: Koga, Y.; Kato, K.; Nakano, H.; Yamane, T.
Year: 2003
Title: Inverting enantioselectivity of Burkholderia cepacia KWI-56 lipase by combinatorial mutation and high-throughput screening using single-molecule PCR and in vitro expression

Journal: J Mol Biol
Volume: 331
Issue: 3
Pages: 585-92
Abstract: The enantioselectivity of lipase from Burkhorderia cepacia KWI-56 has been inverted using a novel in vitro technique for construction and screening of a protein library by single-molecule DNA amplification by PCR followed by in vitro coupled transcription/translation system termed single-molecule-PCR-linked in vitro expression (SIMPLEX). Four amino acid residues (L17, F119, L167, and L266) in the hydrophobic substrate-binding pocket of the lipase were selected for mutation based on a structural model of a substrate-enzyme complex, and a combinatorial mutation library was constructed by SIMPLEX and screened for (R) and (S)-configurations of p-nitrophenyl 3-phenylbutyrate. Some combinations of amino acid substitutions in the four positions of the lipase were found as effective for changing the enantiopreference from the (S)-form substrate to the (R)-form. Two variants were expressed in the original host cells and purified to homogeneity, showing completely reversed enantioselectivity for the (R)-form of ethyl 3-phenylbutyrate (selectivity factor E(R)=38 or 33), whereas the wild-type lipase was (S)-selective (selectivity factor E(S)=33). Thus the semi-rational and semi-random combinatorial design of a mutant library followed by a high-throughput screening based on their enzymatic activity should be a powerful tool to engineer the enantioselectivity of enzymes.

Reference Type: Journal Article
Record Number: 135
Author: Leone, G.; Voso, M. T.; Teofili, L.; Lubbert, M.
Year: 2003
Title: Inhibitors of DNA methylation in the treatment of hematological malignancies and MDS
Journal: Clin Immunol
Volume: 109
Issue: 1
Pages: 89-102
Abstract: DNA methylation abnormalities have recently emerged as one of the most frequent molecular changes in hematopoietic neoplasms. Since methylation and transcriptional status are inversely correlated, the hypermethylation of genes involved in cell-cycle control and apoptosis could have a pathogenetic role in the development of cancer. In particular, high-risk myelodysplastic syndromes (MDS) and secondary leukemias show a high prevalence of tumor suppressor gene hypermethylation. The progression of chronic myeloproliferative diseases and of myelodysplastic syndromes, as well as that of lymphoproliferative diseases, is associated with an increased methylation rate, pointing to a role for hypermethylation of critical promoter regions in the transformation to more aggressive phenotypes. In the same line, a significantly worse prognosis has been shown for patients with hypermethylation of several genes compared to that of patients with unmethylated genes. For these reasons, the use of irreversible DNA methyltransferase inhibitors, such as 5-azacytidine and Decitabine, appears to be a promising option for the treatment of MDS and acute myeloid leukemia. In clinical trials, Azacytidine results in a significantly higher response rate, improved quality of life, reduced risk of leukemic transformation, and improved survival compared to supportive care. Similarly, Decitabine showed favorable results,

promising response rates, a good nonhematologic toxicity profile, and a trend for better survival compared to intensive chemotherapy, particularly in older patients. The synergistic effect of histone deacetylase inhibitors, including phenylbutyrate (PB), in reactivating silenced genes encouraged clinical studies on the combination of PB and demethylating agents in hematological diseases, characterized by p15 silencing. The sequential administration of a "first generation" demethylating agent and HDAC inhibitors gave preliminary evidence of a reduced methylation of target genes, as also described with Decitabine. Clinical trials are still ongoing, and preliminary data indicate for the first time that the natural history of MDS may be changed by a non-intensive treatment, characterized by an outstanding toxicity profile.

Reference Type: Journal Article
Record Number: 152
Author: Miller, W. H.; Manley, P. J.; Cousins, R. D.; Erhard, K. F.; Heerding, D. A.; Kwon, C.; Ross, S. T.; Samanen, J. M.; Takata, D. T.; Uzinskas, I. N.; Yuan, C. C.; Haltiwanger, R. C.; Gress, C. J.; Lark, M. W.; Hwang, S. M.; James, I. E.; Rieman, D. J.; Willette, R. N.; Yue, T. L.; Azzarano, L. M.; Salyers, K. L.; Smith, B. R.; Ward, K. W.; Johanson, K. O.; Huffman, W. F.
Year: 2003
Title: Phenylbutyrates as potent, orally bioavailable vitronectin receptor (integrin alphavbeta3) antagonists
Journal: Bioorg Med Chem Lett
Volume: 13
Issue: 8
Pages: 1483-6
Abstract: In our continuing efforts to identify small molecule vitronectin receptor antagonists, we have discovered a series of phenylbutyrate derivatives, exemplified by 16, which have good potency and excellent oral bioavailability (approximately 100% in rats). This new series is derived conceptually from opening of the seven-membered ring of SB-265123.

Reference Type: Journal Article
Record Number: 129
Author: Roomans, G. M.
Year: 2003
Title: Pharmacological approaches to correcting the ion transport defect in cystic fibrosis
Journal: Am J Respir Med
Volume: 2
Issue: 5
Pages: 413-31
Abstract: Cystic fibrosis (CF) is a lethal genetic disease caused by a mutation in a membrane protein, the cystic fibrosis transmembrane conductance regulator (CFTR), which mainly (but not exclusively) functions as a chloride channel. The main clinical symptoms are chronic obstructive lung disease, which is responsible for most of the morbidity and mortality associated with CF, and pancreatic insufficiency. About 1000 mutations of the gene coding for CFTR are currently known; the most common of these, present in the great majority of the patients (Delta508) results in the deletion of

a phenylalanine at position 508. In this mutation, the aberrant CFTR is not transported to the membrane but degraded in the ubiquitin-proteasome pathway. The aim of this review is to give an overview of the pharmacologic strategies currently used in attempts to overcome the ion transport defect in CF. One strategy to develop pharmacologic treatment for CF is to inhibit the breakdown of DeltaF508-CFTR by interfering with the chaperones involved in the folding of CFTR. At least in in vitro systems, this can be accomplished by sodium phenylbutyrate, or S-nitrosoglutathione (GSNO), and also by genistein or benzo[c]quinolizinium compounds. It is also possible to stimulate CFTR or its mutated forms, when present in the plasma membrane, using xanthines, genistein, and various other compounds, such as benzamidizoles and benzoxazoles, benzo[c]quinolizinium compounds or phenantrolines. Experimental results are not always unambiguous, and adverse effects have been incompletely tested. Some clinical tests have been done on sodium phenyl butyrate, GSNO and genistein, mostly in respect to other diseases, and the results demonstrate that these drugs are reasonably well tolerated. Their efficiency in the treatment of CF has not yet been demonstrated, however. An alternative strategy is to compensate for the defective chloride transport by CFTR by stimulation of other chloride channels. This can be done via purinergic receptors. A phase I study using a stable uridine triphosphate analog has recently been completed. A second alternative strategy is to attempt to maintain hydration of the airway mucus by inhibiting Na(+) uptake by the epithelial Na(+) channel using amiloride or stable analogs of amiloride. Clinical tests so far have been inconclusive. A number of other suggestions are currently being explored. The minority of patients with CF who have a stop mutation may benefit from treatment with gentamicin. The difficulties in finding a pharmacologic treatment for CF may be due to the fact that CFTR has additional functions besides chloride transport, and interfering with CFTR biosynthesis or activation implies interference with central cellular processes, which may have undesirable adverse effects.

Reference Type: Journal Article
Record Number: 155
Author: Svechnikova, I.; Gray, S. G.; Kundrotiene, J.; Ponthan, F.; Kogner, P.; Ekstrom, T. J.
Year: 2003
Title: Apoptosis and tumor remission in liver tumor xenografts by 4-phenylbutyrate
Journal: Int J Oncol
Volume: 22
Issue: 3
Pages: 579-88
Abstract: 4-phenylbutyrate (triButyrate trade mark, PB) a derivative of the short-chain fatty acid, butyrate, possesses anti-tumor activity in vitro in different tumor cell lines. Unlike most cytostatic compounds, PB possesses low toxicity. In order to evaluate possible clinical use of PB in cancer therapy, hepatocarcinoma (Hep3B) and hepatoblastoma (HepT1) cell lines, as well as xenografts derived from those in nude rats, were treated with PB in different dose (1-100 mM) and time regimens. Treatment with 10 mM of PB for 24 h (or 5 mM for 48 h) was shown to significantly inhibit Hep3B cell growth in vitro. The HepT1 cell line was more sensitive to PB treatment: already 1 mM of PB for 24 h significantly inhibited the growth of the cells. PB also resulted in regression of xenografts derived from these cell lines in vivo, when

administrated by mini-pump with an intratumor catheter, yielding 20 micro mol of PB per cm3 of tumor volume per day. TUNEL assay and caspase-3 activity measurements suggested apoptosis to be the cell death mechanism in both cell lines and xenografts. Increased histones H3 and H4 acetylation was shown in both cells and xenografts, and the inhibition of histone deacetylase is proposed as the main trigger for the anti-tumor action of PB. Concomitant induction of p21Waf1/Cip1 expression was detected by RNase protection assay and Western blotting. Reduction in expression of alpha-fetoprotein was found both in Hep3B cells and xenografts, suggesting also a differentiation effect by PB.

Reference Type: Journal Article
Record Number: 156
Author: Wang, W. J.; Mulugeta, S.; Russo, S. J.; Beers, M. F.
Year: 2003
Title: Deletion of exon 4 from human surfactant protein C results in aggresome formation and generation of a dominant negative
Journal: J Cell Sci
Volume: 116
Issue: Pt 4
Pages: 683-92
Abstract: Human surfactant protein C (hSP-C) is synthesized by the alveolar type 2 cell as a 197 amino acid integral membrane proprotein and proteolytically processed to a secreted 3.7 kDa mature form. Although the SP-C null mouse possesses a non-lethal phenotype, a heterozygous substitution of A for G in the first base of intron 4 of the human SP-C gene (c.460+1A>G) has been reported in association with familial interstitial lung disease and absence of mature protein. This mutation produces a splice deletion of exon 4 (deltaExon4) resulting in removal of a positionally conserved cysteine in the C-terminal flanking propeptide. Based on a prior study showing that an identical deletion in the rat isoform diverted mutant protein to stable aggregates, we hypothesized that expression of the deltaExon4 mutation would result in disruption of intracellular trafficking of both mutant and wild-type proSP-C. We tested this in vitro using fusion proteins of EGFP conjugated either to wild-type SP-C (EGFP/hSP-C(1-197)) or to SP-C deleted of Exon4 (EGFP/hSP-C(deltaExon4)). Fluorescence microscopy showed that EGFP/hSP-C(1-197) transfected into A549 cells was expressed in a punctuate pattern in CD63 (+) cytoplasmic vesicles, whereas EGFP/hSP-C(deltaExon4) accumulated in ubiquitinated perinuclear inclusions linked to the microtubule organizing center. A similar juxtanuclear pattern was observed following transfection of SP-C cDNA lacking only cysteine residues in the C-terminal propeptide encoded by Exon 4 (EGFP/hSP-C(C120/121G)). To evaluate whether mutant proSP-C could function as a dominant negative, EGFP/hSP-C(deltaExon4) was cotransfected with HA-tagged hSP-C(1-197) and resulted in the restriction of both forms to perinuclear compartments. Addition of Na(+) 4-phenylbutyrate, a facilitator of trafficking of other misfolded proteins, attenuated the aggregation of EGFP/hSP-C(deltaExon4). We conclude that c.460+1A>G mutation of human SP-C results in disruption of disulfide-mediated folding encoded by Exon 4 leading to diversion of unprocessed proSP-C to aggresomes. The heterotypic oligomerization of hSP-C(1-197) and hSP-C(deltaExon4) provides a molecular mechanism for the dominant-negative effect observed in vivo.

Reference Type: Journal Article
Record Number: 165
Author: Witt, O.; Monkemeyer, S.; Ronndahl, G.; Erdlenbruch, B.; Reinhardt, D.; Kanbach, K.; Pekrun, A.
Year: 2003
Title: Induction of fetal hemoglobin expression by the histone deacetylase inhibitor apicidin
Journal: Blood
Volume: 101
Issue: 5
Pages: 2001-7
Abstract: Pharmacologic stimulation of fetal hemoglobin (HbF) expression may be a promising approach for the treatment of beta-thalassemia. In this study, we have investigated the HbF-inducing activity and molecular mechanisms of specific histone deacetylase (HDAC) inhibitors in human K562 erythroleukemia cells. Apicidin was the most potent agent compared with other HDAC inhibitors (trichostatin A, MS-275, HC-toxin, suberoylanilide hydroxamic acid [SAHA]) and previously tested compounds (butyrate, phenylbutyrate, isobutyramide, hydroxyurea, 5-aza-cytidine), leading to a 10-fold stimulation of HbF expression at nanomolar to micromolar concentrations. Hyperacetylation of histones correlated with the ability of HDAC inhibitors to stimulate HbF synthesis. Furthermore, analysis of different mitogen-activated protein (MAP) kinase signaling pathways revealed that p38 signaling was activated following apicidin treatment of cells and that inhibition of this pathway abolished the HbF-inducing effect of apicidin. Additionally, activation of the Agamma-globin promoter by apicidin could be inhibited by p38 inhibitor SB203580. In summary, the novel HDAC inhibitor apicidin was found to be a potent inducer of HbF synthesis in K562 cells. The present data outline the role of histone hyperacetylation and p38 MAP kinase signaling as molecular targets for pharmacologic stimulation of HbF production in erythroid cells.

Reference Type: Journal Article
Record Number: 154
Author: Vozza, A.; Borriello, A.; Criniti, V.; Vozza, G.; Della Ragione, F.
Year: 2003
Title: New established melanoma cell lines: genetic and biochemical characterization of cell division cycle
Journal: J Eur Acad Dermatol Venereol
Volume: 17
Issue: 1
Pages: 37-41
Abstract: BACKGROUND: Cancer might be envisaged as the result of a genetic process causing the unregulated proliferation of a given cell as well as its inability to undergo differentiation and/or apoptosis. Alterations of genes regulating cell division cycle appear to play a key role in the development of human cancer. OBJECTIVE: On the bases of the above considerations, we decided to establish new cell lines from human melanoma specimens, in order to analyse the molecular alterations in primary preparations of malignant cells. RESULTS: The present paper describes two new established cell lines and their genetic and biochemical features. Both the melanoma

cell lines show inactivation of the cyclin-dependent kinase inhibitor gene, CDKN2A/p16INK4A, thus demostrating that this alteration occurs in primary human melanomas. No other alterations were observable when we investigated several different cell cycle genes including those encoding cyclins, cyclin-dependent kinases and cyclin-dependent kinase inhibitors. Analyses at protein level by means of immunoblotting confirmed the results obtained at the genetic level. Moreover, the inducibility of a pivotal cyclin-dependent kinase inhibitor gene, namely p21CIP1 gene, was obtained by treating the cells with histone deacetylase inhibitors, namely butyrate and phenylbutyrate. CONCLUSIONS: Our results suggest a primary role of cyclin-dependent kinase inhibitor genes inactivation in the origin of human melanoma and allow the proposal of new therapeutic strategies based on the transcriptional activation of p21CIP1 gene.

Reference Type: Journal Article
Record Number: 118
Year: 2004
Title: Carglumic acid: new preparation. An advance in rare urea cycle disorders
Journal: Prescrire Int
Volume: 13
Issue: 69
Pages: 3-4
Abstract: (1) Previously, phenylbutyrate sodium was the only product marketed in France for the treatment of diseases caused by enzyme deficiencies affecting the urea cycle. By a non specific action, this drug partially prevents episodes of hyperammonaemia and their potentially severe consequences. (2) Marketing authorization has now been granted, through the European centralised procedure, for carglumic acid (N-carbamyl L-glutamic acid) as replacement therapy for N-acetylglutamate synthetase deficiency, the rarest urea cycle disorder. This enzyme is crucial for the first step of the urea cycle. (3) Thirteen of the 16 patients in the clinical evaluation dossier, who were treated before the onset of permanent sequelae due to hyperammonaemia, had normal growth and psychomotor development. The optimal dose of carglumic acid is not known. (4) No serious adverse effects have been observed, but too few patients have been treated to identify possible rare adverse effects. There are no data on the effects of carglumic acid in pregnant women. (5) In practice, carglumic acid is now the reference treatment for patients with N-acetylglutamate synthetase deficiency, despite several unknowns.

Reference Type: Journal Article
Record Number: 101
Author: Ammerpohl, O.; Thormeyer, D.; Khan, Z.; Appelskog, I. B.; Gojkovic, Z.; Almqvist, P. M.; Ekstrom, T. J.
Year: 2004
Title: HDACi phenylbutyrate increases bystander killing of HSV-tk transfected glioma cells
Journal: Biochem Biophys Res Commun
Volume: 324
Issue: 1
Pages: 8-14

Abstract: Malignant glioma patients have a dismal prognosis with an urgent need of new treatment modalities. Previously developed gene therapies for brain tumors showed promising results in experimental animal models, but failed in clinical trials due to low transfection rates and insufficient expression of the transgene in tumor cells, as well as low bystander killing effects. We have previously shown that the histone deacetylase inhibitor 4-phenylbutyrate (4-PB) enhances gap junction communication between glioma cells in culture. In this study, we demonstrate an activation of recombinant HSV-tk gene expression, and a dramatic enhancement of gap junction-mediated bystander killing effect by administration of the HSV-tk prodrug ganciclovir together with 4-PB. These findings that 4-PB potentiates "suicide gene" expression as well as enhances gap junctional communication and bystander killing of tumor cells justify further testing of this paradigm as an adjunct to suicide gene therapy of malignant gliomas.

Reference Type: Journal Article
Record Number: 137
Author: Andreassi, C.; Angelozzi, C.; Tiziano, F. D.; Vitali, T.; De Vincenzi, E.; Boninsegna, A.; Villanova, M.; Bertini, E.; Pini, A.; Neri, G.; Brahe, C.
Year: 2004
Title: Phenylbutyrate increases SMN expression in vitro: relevance for treatment of spinal muscular atrophy
Journal: Eur J Hum Genet
Volume: 12
Issue: 1
Pages: 59-65
Abstract: Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease, characterized by degeneration of the anterior horn cells of the spinal cord. SMA presents with a highly variable phenotype ranging from very severe to mild (type I-III). No cure for SMA is available at present. All forms of SMA are caused by homozygous loss of the functional survival motor neuron (SMN1) gene. However, all patients have one or more copies of the SMN2 gene, nearly identical to SMN1. Both genes encode the SMN protein but the level produced by SMN2 is insufficient to protect from disease. Increasing SMN2 gene expression could be of considerable therapeutic importance. The aim of this study was to assess whether SMN2 gene expression can be increased by 4-phenylbutyrate (PBA). Fibroblast cell cultures from 16 SMA patients affected by different clinical severities were treated with PBA, and full-length SMN2 transcripts were measured by real-time PCR. In all cell cultures, except one, PBA treatment caused an increase in full-length SMN2 transcripts, ranging from 50 to 160% in type I and from 80 to 400% in type II and III cultures. PBA was found also effective in enhancing SMN protein levels and the number of SMN-containing nuclear structures (gems). These data show that SMN expression is considerably increased by PBA, and suggest that the compound, owing also to its favorable pharmacological properties, could be a good candidate for the treatment of SMA.

Reference Type: Journal Article
Record Number: 112

Author: Appelskog, I. B.; Ammerpohl, O.; Svechnikova, I. G.; Lui, W. O.; Almqvist, P. M.; Ekstrom, T. J.
Year: 2004
Title: Histone deacetylase inhibitor 4-phenylbutyrate suppresses GAPDH mRNA expression in glioma cells
Journal: Int J Oncol
Volume: 24
Issue: 6
Pages: 1419-25
Abstract: The histone deacetylase (HDAC) inhibitor 4-phenylbutyrate (4-PB) is a non-toxic compound that can induce differentiation and promote maturation of various types of malignant cells. In the present study we show that 4-PB inhibit glioma cell proliferation, induce apoptosis and decrease mRNA expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in a concentration-dependent manner. Proliferation of established rat glioma cell lines (RG2 and C6) in culture was significantly decreased after treatment with 4-PB (2-40 mM). Low concentrations of 4-PB (2-20 mM) induced cell differentiation followed by apoptosis, whereas higher concentrations of 4-PB (40 mM) induced cell necrosis. Also, low concentrations of 4-PB significantly decreased GAPDH mRNA expression in C6 and RG2 rat glioma cells, suggesting a link between decreased cell proliferation, energy consumption, and down-regulation of GAPDH gene expression. We have found that GAPDH mRNA expression is markedly increased in human glioblastoma tissues. Therefore, the novel effect of 4-PB described here may offer means to suppress growth of glioma cells by diminishing the key reaction in glycolysis as a therapeutic approach for cancer.

Reference Type: Journal Article
Record Number: 116
Author: Asklund, T.; Appelskog, I. B.; Ammerpohl, O.; Ekstrom, T. J.; Almqvist, P. M.
Year: 2004
Title: Histone deacetylase inhibitor 4-phenylbutyrate modulates glial fibrillary acidic protein and connexin 43 expression, and enhances gap-junction communication, in human glioblastoma cells
Journal: Eur J Cancer
Volume: 40
Issue: 7
Pages: 1073-81
Abstract: Human glioblastoma cell cultures were established and the expression of glial fibrillary acidic protein (GFAP) and the gap-junction protein connexin 43 (Cx43) was confirmed by Western blot. Following treatment with 4-phenylbutyrate (4-PB), increased concentrations of non-phosphorylated GFAP were seen, while phosphorylated isoforms remained intact. Immunocytochemical staining of glioblastoma cells revealed an intracellular redistribution of GFAP. In addition to cytoplasmic immunostaining, GFAP immunoreactivity was also associated with the nucleus and/or the nuclear membrane. Phosphorylated and non-phosphorylated Cx43 proteins were increased 2- to 5-fold following 4-PB treatment, and were redistributed to areas of the cell surface, participating in cell-to-cell contacts. In addition, functional gap-junction coupling was amplified, as indicated by increased fluorescent dye transfer, and elevated levels of Cx43 protein were detected in parallel with enhanced

gap-junction communication. Induced cell differentiation, with improved functional coupling of tumour cells, may be of importance for therapeutic strategies involving intercellular transport of low molecular-weight compounds.

Reference Type: Journal Article
Record Number: 121
Author: Bachmann, C.; Braissant, O.; Villard, A. M.; Boulat, O.; Henry, H.
Year: 2004
Title: Ammonia toxicity to the brain and creatine
Journal: Mol Genet Metab
Volume: 81 Suppl 1
Pages: S52-7
Abstract: Symptoms of hyperammonemia are age-dependent and some are reversible. Multiple mechanisms are involved. Hyperammonemia increases the uptake of tryptophan into the brain by activation of the L-system carrier while brain glutamine plays a still undefined role. The uptake of tryptophan by the brain is enhanced when the plasma levels of branched-chain amino acids competing with the other large neutral amino acids are low. Hyperammonemia increases the utilization of branched-chain amino acids in muscle when ketoglutarate is low, and this is further enhanced by glutamine depletion (as a result of therapy with ammonia scavengers like phenylbutyrate). Anorexia, most likely a serotoninergic symptom, might further aggravate the deficiency of indispensable amino acids (e.g., branched-chain and arginine). The role of increased glutamine production in astrocytes and the excitotoxic and metabotropic effects of increased extracellular glutamate have been extensively investigated and found to differ between models of acute and chronic hyperammonemia. Using an in vitro model of cultured embryonic rat brain cell aggregates, we studied the role of creatine in ammonia toxicity. Cultures exposed to ammonia before maturation showed impaired cholinergic axonal growth accompanied by a decrease of creatine and phosphocreatine, a finding not observed in mature cultures. By using different antibodies, we have shown that the phosphorylated form of the intermediate neurofilament protein is affected. Adding creatine to the culture medium partially prevents impairment of axonal growth and the presence of glia in the culture is a precondition for this protective effect. Adequate arginine substitution is essential in the treatment of urea cycle defects as creatine is inefficiently transported into the brain.

Reference Type: Journal Article
Record Number: 99
Author: Bhalla, K.; List, A.
Year: 2004
Title: Histone deacetylase inhibitors in myelodysplastic syndrome
Journal: Best Pract Res Clin Haematol
Volume: 17
Issue: 4
Pages: 595-611
Abstract: Histone deacetylase (HDAC) inhibitors are amongst the newer therapies being introduced in refractory, relapsed, and resistant disease. These agents are mechanism-based and their use is targeted to the diseased cell or tissue. HDACs are

key enzymes in the regulation of gene expression. They maintain a dynamic equilibrium in the acetylation state of highly conserved lysine residues on histones by which they regulate chromatin remodeling and gene expression. Changes in growth and differentiation leading to malignancy appear to occur by alterations in transcriptional control and gene silencing. Histone acetylation and DNA methylation have been implicated in these aberrant phenotypes. Inhibitors of DNA methylation such as 5-azacytidine or 5-azadeoxycytidine have been able to reverse DNA methylation patterns and have shown promise in patient studies. Similarly, HDAC inhibitors block deacetylation function, causing cell cycle arrest, differentiation, and/or apoptosis of many tumors. Several HDAC inhibitors have exhibited potent antitumor activity in human xenograft models, suggesting their usefulness as novel cancer therapeutic agents. Several are currently in phase I/II clinical trials both in hematological malignancies and in solid tumors. Agents used initially, such as phenylbutyrate, are effective in millimolar concentrations. Newer agents are being developed and these are effective at much lower concentrations and are relatively less toxic. In particular, hydroxamic acid-based polar compounds and cyclic tetrapeptides have shown activity against cancers at well-tolerated doses.

Reference Type: Journal Article
Record Number: 123
Author: Chung, Y. L.; Wang, A. J.; Yao, L. F.
Year: 2004
Title: Antitumor histone deacetylase inhibitors suppress cutaneous radiation syndrome: Implications for increasing therapeutic gain in cancer radiotherapy
Journal: Mol Cancer Ther
Volume: 3
Issue: 3
Pages: 317-25
Abstract: Radiotherapy is an effective treatment for head and neck, skin, anogenital, and breast cancers. However, radiation-induced skin morbidity limits the therapeutic benefits. A low-toxicity approach to selectively reduce skin morbidity without compromising tumor killing by radiotherapy is needed. We found that the antitumor agents known as histone deacetylase (HDAC) inhibitors (phenylbutyrate, trichostatin A, and valproic acid) could suppress cutaneous radiation syndrome. The effects of HDAC inhibitors in promoting the healing of wounds caused by radiation and in decreasing later skin fibrosis and tumorigenesis were correlated with suppression of the aberrant expression of radiation-induced transforming growth factor beta and tumor necrosis factor alpha. Our findings implicate that the inhibition of HDAC may provide a novel strategy to increase the therapeutic gain in cancer radiotherapy by not only inhibiting tumor growth but also protecting normal tissues.

Reference Type: Journal Article
Record Number: 115
Author: de Jong, J. C.; Willems, P. H.; Goossens, M.; Vandewalle, A.; van den Heuvel, L. P.; Knoers, N. V.; Bindels, R. J.
Year: 2004

Title: Effects of chemical chaperones on partially retarded NaCl cotransporter mutants associated with Gitelman's syndrome in a mouse cortical collecting duct cell line
Journal: Nephrol Dial Transplant
Volume: 19
Issue: 5
Pages: 1069-76
Abstract: BACKGROUND: Epithelial cells lining the distal convoluted tubule express the thiazide-sensitive Na-Cl cotransporter (NCC) that is responsible for the reabsorption of 5-10% of the filtered load of Na(+) and Cl(-). Mutations in NCC cause the autosomal recessive renal disorder Gitelman's syndrome (GS). GS mutations give rise to mutant transporters that are either fully (class I) or partially (class II) retarded. Recent evidence indicates that class II mutations do not alter the intrinsic transport activity of NCC. These findings suggest that in GS caused by class II NCC mutations, pharmacological chaperones may be useful in treatment. METHODS: Initial attempts using 4-phenylbutyrate and glycerol to increase Na(+) uptake in Xenopus laevis oocytes expressing the class II mutant L215P were unsuccessful. To study the effect of the chaperones in a more physiological setting, we next expressed hNCC in the polarized epithelial cell line of distal tubular origin, mpkCCD. RESULTS: mpkCCD cells readily expressed the class II mutant R955Q, but not the class I mutant G741R. Wild-type hNCC was predominantly present in the approximately 120-1403 kD complex glycosylated form. In contrast, the R955Q mutant was predominantly present in a lower molecular weight form of approximately 100 kD. Pretreatment of R955Q expressing cells with 4-phenylbutyrate (5 mM, 16 h), but not thapsigargin (1 microM, 90 min), dimethyl sulfoxide (1%, 16 h) or glycerol (4%, 16 h), increased the expression of the complex glycosylated form and in parallel the number of hNCC positive cells. CONCLUSIONS: Taken together, the data indicate that 4-phenylbutyrate is a promising candidate for rescuing partially retarded but otherwise functional class II GS mutants.

Reference Type: Journal Article
Record Number: 104
Author: de Ruijter, A. J.; Kemp, S.; Kramer, G.; Meinsma, R. J.; Kaufmann, J. O.; Caron, H. N.; van Kuilenburg, A. B.
Year: 2004
Title: The novel histone deacetylase inhibitor BL1521 inhibits proliferation and induces apoptosis in neuroblastoma cells
Journal: Biochem Pharmacol
Volume: 68
Issue: 7
Pages: 1279-88
Abstract: Neuroblastoma is a childhood cancer arising from the sympathetic nervous system. Disseminated neuroblastoma has a poor prognosis despite intensive multimodality treatment. Histone deacetylases (HDACs) were recently discovered as a potential target for pharmacological gene therapy in cancer. HDACs have an important function in regulating DNA packaging in chromatin, thereby affecting the transcription of genes. In this paper, we tested the efficacy of a newly developed histone deacetylase inhibitor, BL1521, on neuroblastoma in vitro by investigating the changes in: acetylation of histone H3, in situ HDAC activity, p21(WAF1/CIP1) and

MYCN expression, metabolic activity, proliferation, morphology and the amount of apoptosis present. BL1521 inhibited the in situ HDAC activity of a panel of neuroblastoma cell lines by at least 85%. Western analysis showed an increase of histone H3 acetylation in neuroblastoma cells after incubation with BL1521. Northern analysis showed an increase in the expression of p21(WAF1/CIP1) and a decrease in the expression of MYCN in neuroblastoma cells after incubation with BL1521. Proliferation as well as the metabolic activity of neuroblastoma cells decreased significantly in response to treatment with BL1521, regardless of the MYCN status of the cells. BL1521 induced poly-(ADP-ribose) polymerase cleavage in a time- and dose-dependent manner, indicating the induction of apoptosis. Furthermore, when compared to the HDAC inhibitors Trichostatin A and 4-phenylbutyrate, BL1521 has an intermediate efficacy. Our results show that BL1521 is a potent inhibitor of HDAC and that HDACs are an attractive target for selective chemotherapy in neuroblastoma.

Reference Type: Journal Article
Record Number: 90
Author: Emionite, L.; Galmozzi, F.; Grattarola, M.; Boccardo, F.; Vergani, L.; Toma, S.
Year: 2004
Title: Histone deacetylase inhibitors enhance retinoid response in human breast cancer cell lines
Journal: Anticancer Res
Volume: 24
Issue: 6
Pages: 4019-24
Abstract: Solid tumors develop resistance to retinoids during carcinogenesis. One of the strategies to overcome this resistance may include the combination of these molecules with other differentiating, cytotoxic or chromatin-remodelling agents. We analysed the anti-proliferative activity of two histone-deacetylase inhibitors (HDACIs), Trichostatin A (TSA) and sodium phenylbutyrate (PB), alone or combined with retinoids, all-trans retinoic acid (ATRA) and Ro 41-5253, on two human breast cancer cell lines: the hormone-dependent MCF-7 and the hormone-independent MDA-MB-231. These lines responded differently to retinoids: MCF-7 were sensitive, whilst MDA-MB-231 were rather resistant. When the retinoids were combined with HDACIs, these molecules potentiated the retinoid activity on growth inhibition, especially for the association Ro 41-5253 and TSA. By FACS analysis, we observed that the anti-proliferative effects were only partially due to pro-apopotic mechanisms, suggesting a cell-cycle block. The efficacy of the retinoids/HDACIs combinations could represent a new strategy in breast cancer chemotherapy, allowing inhibition of both ER + and ER- cell populations.

Reference Type: Journal Article
Record Number: 94
Author: Gardian, G.; Yang, L.; Cleren, C.; Calingasan, N. Y.; Klivenyi, P.; Beal, M. F.
Year: 2004
Title: Neuroprotective effects of phenylbutyrate against MPTP neurotoxicity
Journal: Neuromolecular Med

Volume: 5
Issue: 3
Pages: 235-41
Abstract: There is increasing evidence that administration of histone deacetylase (HDAC) inhibitors can exert neuroprotective effects by a variety of mechanisms. Phenylbutyrate is a well-known HDAC inhibitor, which increases gene transcription of a number of genes, and also exerts neuroprotective effects. These include several antioxidant enzymes, chaperones, and genes involved in cell survival. We examined whether administration of phenylbutyrate could exert significant neuroprotective effects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which has been used to model Parkinson's disease. Administration of phenylbutyrate significantly attenuated MPTP-induced depletion of striatal dopamine and loss of tyrosine hydroxylase-positive neurons in the substantia nigra. These findings provide further evidence that administration of phenylbutyrate may be a useful approach for the treatment of neurodegenerative diseases.

Reference Type: Journal Article
Record Number: 113
Author: Gottlicher, M.
Year: 2004
Title: Valproic acid: an old drug newly discovered as inhibitor of histone deacetylases
Journal: Ann Hematol
Volume: 83 Suppl 1
Pages: S91-2
Abstract: Fusion proteins encoded by several types of chromosomal translocations in promyelocytic leukemia can serve as aberrant transcriptional repressors relying on recruitment of histonedeacetylases (HDACs) into DNA-associated multi-protein complexes. Thus, inappropriate modulation of chromatin structure by HDACs and subsequently repression of gene expression that is critical for myeloid differentiation appear to be major factors in the development of the disease. They identify inhibitors of HDACs as prime candidates for novel anti leukemic drugs. Over the last years several candidate compounds have been introduced into clinical trials and have successfully been used in compassionate use protocols. Amongst them phenylbutyrate served as the first example to establish proof of principle. Novel drugs such as suberoylanilide hydroxamic acid (SAHA) are developed for example by modifications of the microbial HDAC inhibitory compound trichostatin A with a hydroxamic acid as the key structural element. The branched chain carboxylic acid valproic acid (VPA) that is in use as antiepileptic drug over decades was also discovered to inhibit HDACs and preferentially class I HDACs. HDAC inhibition is likely to mediate the teratogenic side effects of VPA but not the antiepileptic activity. In contrast to other HDAC inhibitors VPA also induces proteasomal degradation of HDAC2. None of the currently available compounds may be the optimum HDAC inhibitory drug but each of them may serve to answer urgent questions concerning the concept of HDAC inhibition in the treatment of malignant diseases. Prominent questions are i) whether and by which mechanisms HDAC inhibition can be expected to affect a malignant disease not only in the early stage but also at later stages that have acquired additional genetic defects, ii) which forms of cancer in addition to myelocytic leukemia respond to HDAC inhibition, iii) by which markers those

susceptible forms could be identified and iv) which individual HDACs are the most critical isoenzymes to address in treatment of malignant diseases.

Reference Type: Journal Article
Record Number: 93
Author: Hao, C. L.; Lin, D.; Wang, L. H.; Xing, H. Y.; Wang, M.; Wang, J. X.
Year: 2004
Title: [Combination of phenylbutyrate and 5-Aza-2'deoxycytidine inhibits human Kasumi-1 xenograft tumor growth in nude mice.]
Journal: Zhonghua Xue Ye Xue Za Zhi
Volume: 25
Issue: 11
Pages: 658-61
Abstract: OBJECTIVE: To investigate the tumor suppression efficacy of histone deacetylase inhibitor, phenylbutyrate (PB), in combination with DNA methylation inhibitor 5-Aza-2-deoxycytidine (5-Aza-CdR) in the treatment of Kasumi-1 xenograft tumor in nude mice and its mechanism. METHODS: The nude mice model of Kasumi-1 xenograft tumor was established by subcutaneous inoculation. Latency of tumor formation, the ability of Kasumi-1 cells pre treated with PB to form the xenograft tumor, and the tumor suppression activity of PB and 5-Aza-CdR by intraperitoneal injection in xenografted mice model were detected. Cell differentiation and cell cycle parameters of the tumor cells were analyzed by flow cytometry analysis, apoptosis by TUNEL in situ hybridization, and tumor microvessel density (MVD) by immunohistochemistry study. RESULTS: The latency of tumor formation in mice with or without previous lienectomy was 17 approximately 23 and 40 approximately 50 days, respectively. Tumor cells xenografted could not be found in other tissues than in inoculation area, and still harbored the specific t(8;21) and AML1-ETO fusion gene. When the xenografted mice models treated with PB, 5-Aza-CdR, or both, the tumor growth inhibition rates were 49.07%, 25.69% and 87.46% ($P < 0.05$), the apoptosis indexes (AI) of tumor cells were (2.25 +/- 0.85)%, (1.32 +/- 0.68)%, and (5.41 +/- 1.56)% ($P < 0.05$), and the microvessel densities (MVD) were 21.69 +/- 6.25, 28.34 +/- 4.24 and 9.48 +/- 3.21 ($P < 0.01$), respectively. All the data above were significantly different from that in control ($P < 0.05$). The expression of CD11b and CD13 antigen of the tumor cells was increased in xenografted mice model treated with PB when compared with the control \[(12.08 +/- 1.02)% and (54.91 +/- 2.72)%\], respectively ($P < 0.01$), and tumor cells showed a cell cycle arrest with increased G(0)/G(1)-phase cells and decreased S-phase cells. CONCLUSION: PB inhibited the growth of Kasumi-1 xenograft tumor by inducing tumor cell apoptosis and differentiation, and suppressing its angiogenesis in vivo. 5-Aza-CdR could significantly enhance the antitumor activity of PB.

Reference Type: Journal Article
Record Number: 132
Author: Kasumov, T.; Brunengraber, L. L.; Comte, B.; Puchowicz, M. A.; Jobbins, K.; Thomas, K.; David, F.; Kinman, R.; Wehrli, S.; Dahms, W.; Kerr, D.; Nissim, I.; Brunengraber, H.
Year: 2004
Title: New secondary metabolites of phenylbutyrate in humans and rats

Journal: Drug Metab Dispos
Volume: 32
Issue: 1
Pages: 10-9
Abstract: Phenylbutyrate is used to treat inborn errors of ureagenesis, malignancies, cystic fibrosis, and thalassemia. High-dose phenylbutyrate therapy results in toxicity, the mechanism of which is unexplained. The known metabolites of phenylbutyrate are phenylacetate, phenylacetylglutamine, and phenylbutyrylglutamine. These are excreted in urine, accounting for a variable fraction of the dose. We identified new metabolites of phenylbutyrate in urine of normal humans and in perfused rat livers. These metabolites result from interference between the metabolism of phenylbutyrate and that of carbohydrates and lipids. The new metabolites fall into two categories, glucuronides and phenylbutyrate beta-oxidation side products. Two questions are raised by these data. First, is the nitrogen-excreting potential of phenylbutyrate diminished by ingestion of carbohydrates or lipids? Second, does competition between the metabolism of phenylbutyrate, carbohydrates, and lipids alter the profile of phenylbutyrate metabolites? Finally, we synthesized glycerol esters of phenylbutyrate. These are partially bioavailable in rats and could be used to administer large doses of phenylbutyrate in a sodium-free, noncaustic form.

Reference Type: Journal Article
Record Number: 122
Author: Lea, M. A.; Shareef, A.; Sura, M.; desBordes, C.
Year: 2004
Title: Induction of histone acetylation and inhibition of growth by phenyl alkanoic acids and structurally related molecules
Journal: Cancer Chemother Pharmacol
Volume: 54
Issue: 1
Pages: 57-63
Abstract: PURPOSE: A structure-activity study was undertaken to determine the influence of side chain length of phenyl alkanoic acids and the degree of unsaturation of phenyl alkenoic acids on the induction of histone acetylation and inhibition of cancer cell proliferation. MATERIALS AND METHODS: Studies on cell proliferation were performed with DS19 mouse erythroleukemic cells, PC-3 human prostate cancer cells and Caco-2 human colon cancer cells. Actions on histone deacetylase and the induction of histone acetylation were compared for 4-phenylbutyrate and structurally related molecules. RESULTS: Increasing inhibition of cell proliferation by phenyl alkanoic acids together with a decrease in cells in S phase and an increase in apoptotic cells was observed with increased chain length between four and ten carbons. Introduction of double bonds into the side chain was associated with increased growth inhibition. In contrast, 4-phenylbutyrate was a more potent inhibitor of histone deacetylase and inducer of histone acetylation than the other phenyl alkanoic acids examined. CONCLUSIONS: In comparison with the action of 4-phenylbutyrate, actions other than inhibition of histone deacetylase appear to be more important for growth inhibition by longer chain phenyl alkanoic and phenyl alkenoic acids.

Reference Type: Journal Article
Record Number: 97
Author: Lea, M. A.; Sura, M.; Desbordes, C.
Year: 2004
Title: Inhibition of cell proliferation by potential peroxisome proliferator-activated receptor (PPAR) gamma agonists and antagonists
Journal: Anticancer Res
Volume: 24
Issue: 5A
Pages: 2765-71
Abstract: This study was initiated to determine if potential PPAR gamma antagonists could block the inhibition of cell proliferation caused by 4-phenylbutyrate. The action of 4-phenylbutyrate differed from other PPAR gamma ligands examined in that it induces histone acetylation. Proliferation of DS19 mouse erythroleukemia cells was inhibited by PPAR gamma agonists (4-phenylbutyrate, rosiglitazone, ciglitazone and GW1929) and by potential PPAR gamma antagonists: BADGE (Biphenol A diglycidyl ether), GW9662, PD068235 and diclofenac. Combined incubations tended to exhibit additive inhibitory effects. Potential PPAR gamma agonists and antagonists inhibited the incorporation of thymidine into DNA of human prostate (PC3), colon (Caco-2) and breast (T47D) cancer cells but also affected NIH3T3 cells that have little or no expression of PPAR gamma. Lipid accumulation in T47D cells was seen after incubation with 4-phenylbutyrate and both potential PPAR gamma agonists and antagonists. The extent to which the effects of 4-phenylbutyrate on cell proliferation are mediated through PPAR gamma or induction of histone acetylation remains an open question. We conclude that potential PPAR gamma antagonists may fail to reverse the growth inhibitory effect of PPAR gamma ligands and may themselves act as growth inhibitory agents.

Reference Type: Journal Article
Record Number: 117
Author: Lemaire, M.; Momparler, L. F.; Farinha, N. J.; Bernstein, M.; Momparler, R. L.
Year: 2004
Title: Enhancement of antineoplastic action of 5-aza-2'-deoxycytidine by phenylbutyrate on L1210 leukemic cells
Journal: Leuk Lymphoma
Volume: 45
Issue: 1
Pages: 147-54
Abstract: Epigenetic changes, such as aberrant DNA methylation that silences tumor suppressor genes (TSGs), can play an important role in the development of leukemia. The DNA methylation inhibitor, 5-aza-2'-deoxycytidine (5-AZA-CdR), can reactivate these silent TSGs and is an interesting agent to investigate for therapy of leukemia. It has been reported that the effectiveness of 5-AZA-CdR to reactivate TSG can be enhanced by inhibitors of histone deacetylase (HDIs). HDIs can convert a compact chromatin structure to an open configuration that facilitates gene expression. An interesting HDI is phenylbutyrate (PB), which has shown some clinical activity for the therapy of leukemia. In this report we have investigated the antineoplastic activity of 5-AZA-CdR and PB alone and in combination on murine L1210 lymphoid

leukemic cells. The in vitro treatment of 5-AZA-CdR and PB in combination produced a greater inhibition of growth, DNA synthesis, and also a greater reduction on colony formation on both L1210 and human HL-60 leukemic cells as compared to either drug alone. The combination also produced a synergistic activation of the TSG, p15CDN2B, in the L1210 cells. In mice with L1210 leukemia the combination showed enhanced antineoplastic activity. We also observed an enhancement of the antineoplastic activity of this combination in mice with L1210 leukemia. These data provide a rationale to investigate 5-AZA-CdR and PB in patients with advanced leukemia.

Reference Type: Journal Article
Record Number: 126
Author: Li, X. N.; Parikh, S.; Shu, Q.; Jung, H. L.; Chow, C. W.; Perlaky, L.; Leung, H. C.; Su, J.; Blaney, S.; Lau, C. C.
Year: 2004
Title: Phenylbutyrate and phenylacetate induce differentiation and inhibit proliferation of human medulloblastoma cells
Journal: Clin Cancer Res
Volume: 10
Issue: 3
Pages: 1150-9
Abstract: PURPOSE: Phenylbutyrate (PB) and phenylacetate (PA) have antiproliferative and differentiation-inducing effects in malignant tumors, and had been evaluated in Phase I/II clinical trials. This study was undertaken to evaluate their antitumor activities in medulloblastomas. EXPERIMENTAL DESIGN: The biological effects of PB and PA, ranging from 0.1 mM to 3 mM, on two medulloblastoma cell lines (DAOY and D283-MED) were examined using various long-term in vitro and in vivo assays for morphology, proliferation, differentiation, anchorage-independent growth, apoptosis, and tumorigenicity. RESULTS: PB and PA can both induce morphological changes and suppress proliferation in a time- and dose-dependent manner. These effects were more pronounced with PB and became irreversible in D283-MED cells after continuous exposure to 3 mM PB for 28 days. Both PB and PA were able to increase expression of glial marker glial fibriliary acidic protein and neuronal marker synaptophysin in two cell lines. For anchorage-independent growth, PB showed a more significant suppression than PA in D283-MED cells. PB caused more pronounced cell cycle arrest and remarkably reduced tumorigenicity in D283-MED cells than in DAOY cells. Apoptosis was readily induced in D283-MED cells with either low dose of PB or short-term treatment. In contrast, much higher concentrations of PB or longer treatment were required to achieve similar effect with DAOY cells. PB induced increased histones H3 acetylation in both cell lines, but histone H4 acetylation was only observed in D283-MED cells. CONCLUSIONS: PB, through induction of hyperacetylation of histone H3 and H4, is a much more potent antitumor agent than PA. 283-MED cells are more responsive to PB than DAOY cells, which may be dependent on their original state of differentiation as well as the changes of histone H4 acetylation status.

Reference Type: Journal Article
Record Number: 109

Author: Lim, M.; McKenzie, K.; Floyd, A. D.; Kwon, E.; Zeitlin, P. L.
Year: 2004
Title: Modulation of deltaF508 cystic fibrosis transmembrane regulator trafficking and function with 4-phenylbutyrate and flavonoids
Journal: Am J Respir Cell Mol Biol
Volume: 31
Issue: 3
Pages: 351-7
Abstract: Over 70% of patients with cystic fibrosis have the DeltaF508 mutation. This protein is a partially functional chloride (Cl-) channel that is prematurely degraded in the endoplasmic reticulum. Specific members of the flavonoid class of compounds have been shown to increase Cl- conductance of wild-type and DeltaF508 cystic fibrosis transmembrane regulator (CFTR). Although flavonoid effects on CFTR processing are unknown, evidence of effects on heat shock proteins, specifically those that have been shown to interact with CFTR, led us to believe that there would be an effect on CFTR processing through modulation of CFTR-chaperone interactions. We sought to determine (i) the effect of apigenin, genistein, kaempferol, and quercetin on CFTR processing in IB3-1 cells (F508/W1282X) and (ii) whether sequential treatment with 4-phenylbutyrate (4-PBA) to increase CFTR processing and flavonoid to directly stimulate CFTR would increase Cl- conductance. Our results show no significant effect on CFTR processing as measured by immunoblotting with 1 microM or 5 microM of apigenin, genistein, kaempferol, or quercetin. However, despite no effect on CFTR processing as determined by immunoblot, immunofluorescence demonstrated a favorable change in the intracellular distribution of CFTR with 24 h treatments of apigenin, kaempferol, and genistein. Furthermore, we observed an increase in Cl- conductance as measured by Cl- efflux in cells that were treated for 24 h with 4-PBA and then assayed with forskolin and 1 microM or 5 microM genistein, and also with cells treated for 24 h with either 4-PBA, 5 microM apigenin, or 1 microM quercetin. Thus, a combination of chronic treatment with 4-PBA or select flavonoids, followed by acute flavonoid exposure, may be beneficial in cystic fibrosis.

Reference Type: Journal Article
Record Number: 124
Author: Linz, U.
Year: 2004
Title: Complete response of a recurrent, multicentric malignant glioma in a patient treated with phenylbutyrate
Journal: J Neurooncol
Volume: 66
Issue: 1-2
Pages: 251; author reply 251

Reference Type: Journal Article
Record Number: 100
Author: Liu, M.; Brusilow, W. S.; Needleman, R.
Year: 2004
Title: Activity of the yeast Tat2p tryptophan permease is sensitive to the anti-tumor agent 4-phenylbutyrate

Journal: Curr Genet
Volume: 46
Issue: 5
Pages: 256-68
Abstract: 4-Phenylbutyrate (PB) induces differentiation and is being intensively studied as a treatment for brain, prostate, breast, and hematopoietic cancer. While many different primary targets for PB have been proposed, the mechanism by which it causes cellular differentiation remains unknown. To identify the primary cellular target, we investigated its effects on Saccharomyces cerevisiae and showed that it inhibits tryptophan transport. We show here that PB and sorbic acid induce an ubiquitin-dependent turnover of the tryptophan permease Tat2p. However, the inhibition of transport is not a consequence of the loss of Tat2p, since it also occurs when turnover is prevented by deleting the Tat2p ubiquitination sites. When we tested the effects of PB and other growth inhibitory agents on the growth of amino acid auxotrophs, we found that several auxotrophs are hypersensitive to a number of chemically unrelated agents, including PB and some, but not all, weak acids; and this sensitivity is due to the inhibition of amino acid transport. For the inhibitory weak acids, inhibition is not confined to aromatic amino acid auxotrophs, nor is it a general weak acid stress response, since the degree of inhibition is independent of weak acid hydrophobicity and p Ka. Our results show that diverse agents affect the activity of the Tat2p permease rather than its stability and suggest the hypothesis that the anti-neoplastic action of PB is due to a decrease in the activity of surface receptors or other membrane proteins needed to maintain the transformed state.

Reference Type: Journal Article
Record Number: 108
Author: Liu, X. L.; Done, S. C.; Yan, K.; Kilpelainen, P.; Pikkarainen, T.; Tryggvason, K.
Year: 2004
Title: Defective trafficking of nephrin missense mutants rescued by a chemical chaperone
Journal: J Am Soc Nephrol
Volume: 15
Issue: 7
Pages: 1731-8
Abstract: The nephrin gene (NPHS1) is mutated in congenital nephrotic syndrome of the Finnish type. Most mutations found in non-Finnish patients are missense mutations. The most common consequence of missense mutations in congenital nephrotic syndrome is a defect in intracellular transport and retention of the mutant proteins in the endoplasmic reticulum (ER), possibly as a result of misfolding and unfavored conformation. Because sodium 4-phenylbutyrate has been shown to function as a chemical chaperone and to correct the cellular trafficking of several mislocalized or misfolded mutant plasma membrane proteins, the effects of this compound on the missense mutants identified in patients with congenital nephrotic syndrome of the Finnish type were investigated. This study was performed using human embryonic kidney 293 cells stably expressing wild-type or missense nephrin mutants trapped in the ER. Immunofluorescence microscopy and cell surface biotinylation showed that treatment with sodium 4-phenylbutyrate rescued several of the missense mutants from the ER to the cell surface. All of the rescued mutants were

found to be able to interact with Neph1. Furthermore, their tyrosine phosphorylation was rapidly induced by clustering with anti-nephrin antibodies, suggesting that the rescued mutants may be functionally intact.

Reference Type: Journal Article
Record Number: 131
Author: Lu, Q.; Yang, Y. T.; Chen, C. S.; Davis, M.; Byrd, J. C.; Etherton, M. R.; Umar, A.; Chen, C. S.
Year: 2004
Title: Zn2+-chelating motif-tethered short-chain fatty acids as a novel class of histone deacetylase inhibitors
Journal: J Med Chem
Volume: 47
Issue: 2
Pages: 467-74
Abstract: Among various classes of histone deacetylase (HDAC) inhibitors, short-chain fatty acids exhibit the least potency, with IC(50) in the millimolar range. We rationalized that this weak potency was, in part, attributable to their inability to access the zinc cation in the HDAC active-site pocket, which is pivotal to the deacetylation catalysis. We thus explored the structural optimization of valproate, butyrate, phenylacetate, and phenylbutyrate by coupling them with Zn(2+)-chelating motifs (hydroxamic acid and o-phenylenediamine) through aromatic omega-amino acid linkers. This strategy has led to a novel class of Zn(2+)-chelating, motif-tethered, short-chain fatty acids that exhibited varying degrees of HDAC inhibitory potency. One hydroxamate-tethered phenylbutyrate compound, N-hydroxy-4-(4-phenylbutyrylamino)benzamide (HTPB), displayed nanomolar potency in inhibiting HDAC activity. Exposure of several cancer cell lines to HTPB at the submicromolar level showed reduced cell proliferation accompanied by histone hyperacetylation and elevated p21(WAF/CIP1) expression, which are hallmark features associated with intracellular HDAC inhibition.

Reference Type: Journal Article
Record Number: 127
Author: Mally, P.; Mishra, R.; Gandhi, S.; Decastro, M. H.; Nankova, B. B.; Lagamma, E. F.
Year: 2004
Title: Stereospecific regulation of tyrosine hydroxylase and proenkephalin genes by short-chain fatty acids in rat PC12 cells
Journal: Pediatr Res
Volume: 55
Issue: 5
Pages: 847-54
Abstract: Circulating short-chain fatty acids (SCFAs) are primarily derived from bacterial fermentation of carbohydrates in the colon where they function as physiologic modulators of epithelial cell maturation. Butyrate has been shown to induce tyrosine hydroxylase, the rate-limiting enzyme of catecholamine synthesis, and enkephalin neuropeptide gene transcription, suggesting a role in perinatal sympathoadrenal stress-adaptation. We sought to determine whether there were SCFA

structural requirements for this effect. Nine biologically relevant SCFAs and butyrate derivatives were tested in an in vitro model (PC12, rat pheochromocytoma cells) for their ability to regulate neurotransmitter-related gene expression. Our results revealed that among all the studied SCFAs, only propionate and butyrate increased tyrosine hydroxylase and proenkephalin mRNA levels. The functional activity was selective to the carbon atom chain length and associated with the presence of an ethyl moiety in the carbon atom backbone chain. Modifications or absence of this domain affected the gene induction response, suggesting a receptor-mediated mechanism(s). Moreover, propionate, butyrate, and the drug 4-phenylbutyrate were each shown to regulate transmitter genes via at least three independent mechanisms: histone hyperacetylation, cAMP signaling, or peroxisome proliferator-activated receptor gamma-mediated pathways. Thus, the biologic impact of SCFAs on catecholaminergic and opioid systems depend on the activation of SCFA-specific, dose-specific, and gene-specific molecular mechanisms. We speculate that 1) circulating levels of SCFAs may influence sympathoadrenal transmitter biosynthesis and hence whole animal stress-adaptive responsiveness after birth, and 2) the adverse effects of antibiotics on delayed acquisition of postnatal gut flora may affect this apparent evolutionary advantage of gut colonization.

Reference Type: Journal Article
Record Number: 111
Author: Marks, P. A.; Richon, V. M.; Kelly, W. K.; Chiao, J. H.; Miller, T.
Year: 2004
Title: Histone deacetylase inhibitors: development as cancer therapy
Journal: Novartis Found Symp
Volume: 259
Pages: 269-81; discussion 281-8
Abstract: Histone deacetylase (HDAC) inhibitors represent a new class of targeted anticancer agents. A number of structural classes of HDAC inhibitors have been developed of which several are in clinical trials, including phenylbutyrate (PB) and related compounds; the hydroxamic acids, suberoylanilide hydroxamic acid (SAHA) and depsipeptide (FK-228); and the benzamides, MS-275 and C1-994. This review will focus on our studies with the hydroxamic acid HDAC inhibitors, of which SAHA is the lead agent. X-ray crystallographic studies with a HDAC homologue (HDLP) demonstrated that the hydroxamic acid group, most of the aliphatic chain and part of the phenyl amino group of SAHA inserts into the pocket-like catalytic site of the enzyme, at the base of which is a zinc molecule. SAHA inhibits the activity of class I and II HDACs and is selective in altering gene expression. SAHA is synergistic in its anticancer activity with radiation, kinase inhibitors, cytotoxic agents and differentiating agents. In phase I clinical trial with orally administered SAHA the agent caused accumulation of acetylated histones in peripheral mononuclear cells and tumour cells, has excellent bioavailability and has shown antitumour activity in patients with haematologic and solid tumours.

Reference Type: Journal Article
Record Number: 128

Author: Mercuri, E.; Bertini, E.; Messina, S.; Pelliccioni, M.; D'Amico, A.; Colitto, F.; Mirabella, M.; Tiziano, F. D.; Vitali, T.; Angelozzi, C.; Kinali, M.; Main, M.; Brahe, C.
Year: 2004
Title: Pilot trial of phenylbutyrate in spinal muscular atrophy
Journal: Neuromuscul Disord
Volume: 14
Issue: 2
Pages: 130-5
Abstract: The aim of this study was to evaluate tolerability and efficacy of phenylbutyrate (PB) in patients with spinal muscular atrophy (SMA). Ten patients with SMA type II confirmed by DNA studies (age range 2.6-12.7 years, mean age 6.01) were started on oral PB (triButyrate) in powder or tablets. The dosage was 500 mg/kg per day (maximum dose 19 g/d), divided in five doses (every 4 h, skipping one night-dose) using an intermittent schedule (7 days on and 7 days off). Measures of efficacy were the change in motor function from baseline to 3 and 9 weeks, by means of the Hammersmith functional motor scale. In children older than 5 years, muscle strength, assessed by myometry, and forced vital capacity were also measured. We found a significant increase in the scores of the Hammersmith functional scale between the baseline and both 3-weeks ($P < 0.012$) and 9-weeks assessments ($P < 0.004$). Our results indicate that PB might be beneficial to SMA patients without producing any major side effect. Larger prospective randomised, double-blind, placebo controlled trials are needed to confirm these preliminary findings.

Reference Type: Journal Article
Record Number: 106
Author: Mo, H.; Elson, C. E.
Year: 2004
Title: Studies of the isoprenoid-mediated inhibition of mevalonate synthesis applied to cancer chemotherapy and chemoprevention
Journal: Exp Biol Med (Maywood)
Volume: 229
Issue: 7
Pages: 567-85
Abstract: Pools of farnesyl diphosphate and other phosphorylated products of the mevalonate pathway are essential to the post-translational processing and physiological function of small G proteins, nuclear lamins, and growth factor receptors. Inhibitors of enzyme activities providing those pools, namely, 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase and mevalonic acid-pyrophosphate decarboxylase, and of activities requiring substrates from the pools, the prenyl protein transferases, have potential for development as novel chemotherapeutic agents. Their potentials as suggested by the clinical responses recorded in Phase I and II investigations of inhibitors of HMG CoA reductase (the statins), of mevalonic acid-pyrophosphate decarboxylase (sodium phenylacetate and sodium phenylbutyrate), and of farnesyl protein transferase (R115777, SCH66336, BMS-214662, Tipifarnib, L-778,123, and, prematurely, perillyl alcohol) are dimmed by dose-limiting toxicities. These nondiscriminant growth-suppressive agents induce G1 arrest and initiate apoptosis and differentiation, effects attributed to modulation of cell signaling pathways either by modulating gene expression, suppressing the post-

translational processing of signaling proteins and growth factor receptors, or altering diacylglycerol signaling. Diverse isoprenoids and the HMG CoA reductase inhibitor, lovastatin, modulate cell growth, induce cell cycle arrest, initiate apoptosis, and suppress cellular signaling activities. Perillyl alcohol, the isoprenoid of greatest clinical interest, initially was considered to inhibit farnesyl protein transferase; follow-up studies revealed that perillyl alcohol suppresses the synthesis of small G proteins and HMG CoA reductase. In sterologenic tissues, sterol feedback control, mediated by sterol regulatory element binding proteins (SREBPs) 1a and 2, exerts the primary regulation on HMG CoA reductase activity at the transcriptional level. Secondary regulation, a nonsterol isoprenoid-mediated fine-tuning of reductase activity, occurs at the levels of reductase translation and degradation. HMG CoA reductase activity in tumors is elevated and resistant to sterol feedback regulation, possibly as a consequence of aberrant SREBP activities. Nonetheless, tumor reductase remains sensitive to isoprenoid-mediated post-transcriptional downregulation. Farnesol, an acyclic sesquiterpene, and farnesyl homologs, gamma-tocotrienol and various farnesyl derivatives, inhibit reductase synthesis and accelerate reductase degradation. Cyclic monoterpenes, d-limonene, menthol and perillyl alcohol and beta-ionone, a carotenoid fragment, lower reductase mass; perillyl alcohol and d-limonene lower reductase mass by modulating translational efficiency. The elevated reductase expression and greater demand for nonsterol products to maintain growth amplify the susceptibility of tumor reductase to isoprenoids, therein rendering tumor cells more responsive than normal cells to isoprenoid-mediated growth suppression. Blends of lovastatin, a potent nondiscriminant inhibitor of HMG CoA reductase, and gamma-tocotrienol, a potent isoprenoid shown to post-transcription-ally attenuate reductase activity with specificity for tumors, synergistically affect the growth of human DU145 and LNCaP prostate carcinoma cells and pending extensive preclinical evaluation, potentially offer a novel chemotherapeutic strategy free of the dose-limiting toxicity associated with high-dose lovastatin and other nondiscriminant mevalonate pathway inhibitors.

Reference Type: Journal Article
Record Number: 107
Author: Qi, X.; Hosoi, T.; Okuma, Y.; Kaneko, M.; Nomura, Y.
Year: 2004
Title: Sodium 4-phenylbutyrate protects against cerebral ischemic injury
Journal: Mol Pharmacol
Volume: 66
Issue: 4
Pages: 899-908
Abstract: Sodium 4-phenylbutyrate (4-PBA) is a low molecular weight fatty acid that has been used for treatment of urea cycle disorders in children, sickle cell disease, and thalassemia. It has been demonstrated recently that 4-PBA can act as a chemical chaperone by reducing the load of mutant or mislocated proteins retained in the endoplasmic reticulum (ER) under conditions associated with cystic fibrosis and liver injury. In the present study, we evaluated the neuroprotective effect of 4-PBA on cerebral ischemic injury. Pre- or post-treatment with 4-PBA at therapeutic doses attenuated infarction volume, hemispheric swelling, and apoptosis and improved neurological status in a mouse model of hypoxia-ischemia. Moreover, 4-PBA suppressed ER-mediated apoptosis by inhibiting eukaryotic initiation factor 2alpha

phosphorylation, CCAAT/enhancer-binding protein homologous protein induction, and caspase-12 activation. In neuroblastoma neuro2a cells, 4-PBA reduced caspase-12 activation, DNA fragmentation, and cell death induced by hypoxia/reoxygenation. It protected against ER stress-induced but not mitochondria-mediated cell death. Additionally, 4-PBA inhibited the expression of inducible nitric-oxide synthase and tumor necrosis factor-alpha in primary cultured glial cells under hypoxia/reoxygenation. These results indicate that 4-PBA could protect against cerebral ischemia through inhibition of ER stress-mediated apoptosis and inflammation. Therefore, the multiple actions of 4-PBA may provide a strong effect in treatment of cerebral ischemia, and its use as a chemical chaperone would provide a novel approach for the treatment of stroke.

Reference Type: Journal Article
Record Number: 125
Author: Rishikof, D. C.; Ricupero, D. A.; Liu, H.; Goldstein, R. H.
Year: 2004
Title: Phenylbutyrate decreases type I collagen production in human lung fibroblasts
Journal: J Cell Biochem
Volume: 91
Issue: 4
Pages: 740-8
Abstract: Fibrotic lung diseases are characterized by excess extracellular matrix production, in particular type I collagen. Phenylbutyrate (PB) is a non-toxic pharmacological compound that functions as a weak histone deacetylase inhibitor. In hepatic stellate cells, the synthesis of type I collagen expression is decreased by inhibiting histone acetylation. Our studies examined the regulation of type I collagen by PB in human lung fibroblasts. We found that PB decreases basal and transforming growth factor-beta-stimulated alpha1(I) collagen mRNA and protein levels. Northern blot analyses demonstrated that PB decreases steady-state alpha1(I) collagen mRNA levels by 78% without significantly changing the stability of the mRNA transcript. PB stimulates cAMP production and increases the acetylation of histone H4, but does not affect the activity of two transforming growth factor-beta (TGF-beta)-responsive luciferase reporter constructs. These data suggest that PB regulates type I collagen expression in human lung fibroblasts by mechanisms that include cAMP production and histone acetylation. PB may have therapeutic use in fibrotic lung diseases.

Reference Type: Journal Article
Record Number: 114
Author: Sachs, M. D.; Ramamurthy, M.; Poel, H.; Wickham, T. J.; Lamfers, M.; Gerritsen, W.; Chowdhury, W.; Li, Y.; Schoenberg, M. P.; Rodriguez, R.
Year: 2004
Title: Histone deacetylase inhibitors upregulate expression of the coxsackie adenovirus receptor (CAR) preferentially in bladder cancer cells
Journal: Cancer Gene Ther
Volume: 11
Issue: 7
Pages: 477-86

Abstract: Studies on bladder cancer cell lines have shown that low adenoviral (Ad) infectivity is associated with low-level coxsackie adenovirus receptor (CAR) expression. Recently, we and others demonstrated a tumor stage- and grade-dependent downregulation of CAR expression in a large series of clinical bladder cancer specimens. Here, we demonstrate adenoviral gene transfer can be markedly enhanced in bladder cancer cells by upregulation of CAR through the use of certain differentiating agents, including the histone deacetylase inhibitors (HDACI) trichostatin A and sodium phenylbutyrate. CAR upregulation to supraphysiologic levels was demonstrated by quantitative rt-PCR, Western blotting, flow cytometry and adenoviral gene transfer. Normal urothelial cells and CAR-positive papilloma cells (RT4) failed to demonstrate upregulation under the same conditions. Upregulation was cell cycle dependent, associated with increased adenoviral gene transfer and persisted for at least 7 days after a single treatment. Such upregulation, however, appears to be tumor cell specific, as other CAR-negative cell lines failed to demonstrate enhanced adenoviral gene transfer with the same treatments. These results provide a rational basis for combining HDACI therapy with gene therapy as a method of augmenting activity in bladder cancer, but this strategy may not be universally applicable to other cell types.

Reference Type: Journal Article
Record Number: 120
Author: Scaglia, F.; Carter, S.; O'Brien, W. E.; Lee, B.
Year: 2004
Title: Effect of alternative pathway therapy on branched chain amino acid metabolism in urea cycle disorder patients
Journal: Mol Genet Metab
Volume: 81 Suppl 1
Pages: S79-85
Abstract: Urea cycle disorders (UCDs) are a group of inborn errors of hepatic metabolism caused by the loss of enzymatic activities that mediate the transfer of nitrogen from ammonia to urea. These disorders often result in life-threatening hyperammonemia and hyperglutaminemia. A combination of sodium phenylbutyrate and sodium phenylacetate/benzoate is used in the clinical management of children with urea cycle defects as a glutamine trap, diverting nitrogen from urea synthesis to alternatives routes of excretion. We have observed that patients treated with these compounds have selective branched chain amino acid (BCAA) deficiency despite adequate dietary protein intake. However, the direct effect of alternative therapy on the steady state levels of plasma branched chain amino acids has not been well characterized. We have measured steady state plasma branched chain and other essential non-branched chain amino acids in control subjects, untreated ornithine transcarbamylase deficiency females and treated null activity urea cycle disorder patients in the fed steady state during the course of stable isotope studies. Steady-state leucine levels were noted to be significantly lower in treated urea cycle disorder patients when compared to either untreated ornithine transcarbamylase deficiency females or control subjects ($P<0.0001$). This effect was reproduced in control subjects who had depressed leucine levels when treated with sodium phenylacetate/benzoate ($P<0.0001$). Our studies suggest that this therapeutic modality has a substantial impact on the metabolism of branched chain amino acids in urea cycle disorder patients. These findings suggest that better titration of protein restriction could be achieved

with branched chain amino acid supplementation in patients with UCDs who are on alternative route therapy.

Reference Type: Journal Article
Record Number: 105
Author: Song, Y.; Sonawane, N. D.; Salinas, D.; Qian, L.; Pedemonte, N.; Galietta, L. J.; Verkman, A. S.
Year: 2004
Title: Evidence against the rescue of defective DeltaF508-CFTR cellular processing by curcumin in cell culture and mouse models
Journal: J Biol Chem
Volume: 279
Issue: 39
Pages: 40629-33
Abstract: Curcumin, the yellow colored component of the spice turmeric, has been reported to rescue defective DeltaF508-cystic fibrosis transmembrane conductance regulator (CFTR) cellular processing in homozygous mutant mice, restoring nasal potential differences and improving survival (Egan, M. E., Pearson, M., Weiner, S. A., Rajendran, V., Rubin, D., Glockner-Pagel, J., Canny, S., Du, K., Lukacs, G. L., and Caplan, M. J. (2004) Science 304, 600-602). Because of the implied potential use of curcumin or similar compounds in the therapy of cystic fibrosis caused by the DeltaF508 mutation, we tried to reproduce and extend the pre-clinical data of Egan et al. Fluorometric measurements of iodide influx in Fischer rat thyroid cells expressing DeltaF508-CFTR showed no effect of curcumin (1-40 microm) when added for up to 24 h prior to assay in cells grown at 37 degrees C. Controls, including 27 degrees C rescue and 4 mm phenylbutyrate at 37 degrees C, were strongly positive. Also, curcumin did not increase short circuit current in primary cultures of a human airway epithelium homozygous for DeltaF508-CFTR with a 27 degrees C rescue-positive control. Nasal potential differences in mice were measured in response to topical perfusion with serial solutions containing amiloride, low Cl-, and forskolin. Robust low Cl- and forskolin-induced hyperpolarization of 22 +/- 3 mV was found in wild type mice, with 2.1 +/- 0.4 mV hyperpolarization in DeltaF508 homozygous mutant mice. No significant increase in Cl-/forskolin hyperpolarization was seen in any of the 22 DeltaF508 mice studied using different curcumin preparations and administration regimens, including that used by Egan et al. Assay of serum curcumin by ethyl acetate extraction followed by liquid chromatography/mass spectrometry indicated a maximum serum concentration of 60 nm, well below that of 5-15 microm, where cellular effects by sarcoplasmic/endoplasmic reticulum calcium pump inhibition are proposed to occur. Our results do not support further evaluation of curcumin for cystic fibrosis therapy.

Reference Type: Journal Article
Record Number: 103
Author: Tang, X. X.; Robinson, M. E.; Riceberg, J. S.; Kim, D. Y.; Kung, B.; Titus, T. B.; Hayashi, S.; Flake, A. W.; Carpentieri, D.; Ikegaki, N.
Year: 2004
Title: Favorable neuroblastoma genes and molecular therapeutics of neuroblastoma
Journal: Clin Cancer Res

Volume: 10
Issue: 17
Pages: 5837-44
Abstract: PURPOSE AND EXPERIMENTAL DESIGN: Neuroblastoma (NB) is a common pediatric solid tumor that exhibits a striking clinical bipolarity: favorable and unfavorable. Favorable NB genes (EPHB6, EFNB2, EFNB3, NTRK1, and CD44) are genes whose high-level expression predicts favorable NB outcome, and forced expression of these genes inhibits growth of unfavorable NB cells. In this study, we investigated whether favorable NB gene expression could be augmented in unfavorable NB cells by chemical compounds and whether an increased expression of these genes was associated with suppression of NB growth and metastasis. RESULTS: We found that inhibitors of DNA methylation [5-aza-2'-deoxycytidine (5AdC)], histone deacetylase (HDAC) [4-phenylbutyrate (4PB)], and proteasome (MG262) enhanced the expression of favorable NB genes in NB cell lines and inhibited the growth of these cells in vitro ($P < 0.0005$). The growth-inhibitory effects of 5AdC and 4PB in vitro were in part due to caspase-dependent cell death and inhibition of DNA synthesis. Administration of 5AdC and/or 4PB also suppressed growth of subcutaneous NB xenografts in nude mice ($P < 0.001$), which was accompanied by enhanced favorable NB gene expression and an increase in apoptosis. Moreover, 4PB suppressed bone marrow and liver metastases of NB cells in severe combined immunodeficient/Beige mice ($P = 0.007$ and $P = 0.008$, respectively). The growth-suppressive activity of HDAC inhibitors on NB was further confirmed by the efficacy of trichostatin A, a potent and specific HDAC inhibitor. CONCLUSIONS: Collectively, these observations further emphasize the link between the elevated favorable NB gene expression and a benign phenotype of NB.

Reference Type: Journal Article
Record Number: 110
Author: Teckman, J. H.
Year: 2004
Title: Lack of effect of oral 4-phenylbutyrate on serum alpha-1-antitrypsin in patients with alpha-1-antitrypsin deficiency: a preliminary study
Journal: J Pediatr Gastroenterol Nutr
Volume: 39
Issue: 1
Pages: 34-7
Abstract: OBJECTIVE: In homozygotes with ZZ genotype alpha-1-antitrypsin (alpha1AT) deficiency, mutant alpha1ATZ protein (alpha1ATZ) accumulates in hepatocytes, rather than being secreted into the blood. Homozygous individuals experience emphysema as a result of reduced levels of circulating alpha1AT in the lung with which to inhibit connective tissue breakdown. Homozygotes may also experience liver disease from the accumulation of alpha1ATZ within hepatocytes, which causes liver damage. A previous study indicated that the compound 4-phenylbutyrate (4-PBA) mediated a significant increase in release of alpha1ATZ from cells in tissue culture and in a mouse model of alpha1AT deficiency. The authors hypothesized that 4-PBA could be used to treat both the liver and lung disease of humans with alpha1AT deficiency. METHODS: In this preliminary, open label study the authors evaluated the effect of 14 days of oral 4-PBA therapy on alpha1AT blood levels in 10 patients with alpha1AT deficiency. RESULTS: There was no significant

increase in alpha1AT blood level associated with 4-PBA administration. Symptomatic and metabolic side effects were significant. CONCLUSION: 4-PBA did not increase alpha1AT blood levels in humans with alpha1AT deficiency in this preliminary trial.

Reference Type: Journal Article
Record Number: 119
Author: Wilcken, B.
Year: 2004
Title: Problems in the management of urea cycle disorders
Journal: Mol Genet Metab
Volume: 81 Suppl 1
Pages: S86-91
Abstract: Several recent reviews describe the management of urea cycle disorders. There is much agreement on diet, alternative pathway therapy, maintenance of arginine and ornithine levels in acute and chronic management, sick-day regimens, and some aspects of monitoring. However, differences remain in several areas, and physicians at most treatment centers have relatively little experience, because these disorders are rare. Early suspicion of the diagnosis of a urea cycle disorder, and prompt referral to a tertiary center is vital. Drug treatment using chronic administration of sodium benzoate has been abandoned by some centers, but the acceptability of phenylbutyrate is an issue for many patients. Using citrulline chronically is not always successful in recommended doses, and may result in an arginine level too low for maximum control. Appetite and nutrition problems are common. One major concern is the early identification and management of chronic catabolism, theoretically easy, but hard in practice. Biochemical measurement problems complicate monitoring, and there are disagreements about the optimum way of identifying OTC carriers. It is not always clear whom to treat. Within a kindred with an early-onset phenotype, an asymptomatic newborn girl may need treatment for some undetermined time, but target values for monitoring are not clear. In late-onset phenotypes, management of asymptomatic males identified by family screening is also difficult. Most centers do not have sufficient cases to solve these conundrums, some of which require further multicenter study. This paper examines the recommendations of a consensus conference on management, outlines some remaining problems, and incorporates in the text the points raised in open discussion during a session of a symposium held in Sydney in 2003 entitled "New Developments in Urea Cycle Disorders."

Reference Type: Journal Article
Record Number: 136
Author: Wright, J. M.; Zeitlin, P. L.; Cebotaru, L.; Guggino, S. E.; Guggino, W. B.
Year: 2004
Title: Gene expression profile analysis of 4-phenylbutyrate treatment of IB3-1 bronchial epithelial cell line demonstrates a major influence on heat-shock proteins
Journal: Physiol Genomics
Volume: 16
Issue: 2
Pages: 204-11

Abstract: Most individuals with cystic fibrosis (CF) carry one or two mutations that result in a maturation defect of the full-length CFTR protein. The DeltaF508 mutation results in a mutant protein that is degraded by the proteosome instead of progressing to the apical membrane where it functions as a cAMP-regulated chloride channel. 4-Phenylbutyrate (PBA) modulates heat-shock protein expression and promotes trafficking of DeltaF508, thus permitting maturation and membrane insertion. The goal of this study was to gain insight into the genetic mechanism of PBA action through a large-scale analysis of gene expression. The Affymetrix genome-spanning U133 microarray set was used to compare mRNA expression levels in untreated IB3-1 cell line cultures with cultures treated with 1 mM PBA for 12 and 24 h. The most notable changes in mRNA levels were transient elevations in heat-shock proteins. The majority of genes downregulated throughout the application period were functionally associated with control of gene expression. Another set of genes increased in expression starting at 24 h, suggesting these are downstream effects of altered gene expression initiated by PBA. More than one-third of the genes in this late expressing set were identified as having potential significance in understanding the pathology of CF. Our results demonstrate the usefulness of gene expression profile analysis in understanding the consequences of PBA treatment and provide insights in how this drug exerts its effect on the trafficking of CFTR.

Reference Type: Journal Article
Record Number: 102
Author: Zhang, X.; Wei, L.; Yang, Y.; Yu, Q.
Year: 2004
Title: Sodium 4-phenylbutyrate induces apoptosis of human lung carcinoma cells through activating JNK pathway
Journal: J Cell Biochem
Volume: 93
Issue: 4
Pages: 819-29
Abstract: Sodium 4-phenylbutyrate (PB) has been used in the therapy of urea cycle defects for many years. Recently, it has been shown to cause cellular differentiation, growth arrest, and apoptosis in certain malignancies. We have analyzed the effects of PB on human lung carcinoma cells. PB has distinct patterns of effects on different lung carcinoma cells, inducing apoptosis in NCI-H460 and NCI-H1792 cells, causing G1 arrest in A549 and SK-LU-1 cells, but having no effect on a non-transformed bronchial epithelial cell line HBE4-E6/E7. We investigated the role of MAP kinase family members, extracellular signal-regulated kinase (ERK), JNK, and p38 mitogen-activated protein kinase (MAPK), as well as other important cell survival signaling molecules in PB-induced apoptosis. We observed activation of JNK and ERK by PB in the lung cancer cells. JNK was activated only in the two apoptotic cells, whereas ERK was activated in both the apoptotic and the growth-arrested cells, demonstrating a correlation between apoptosis and activation of JNK in response to PB. Both JNK inhibitor and JNK RNA interference (RNAi) inhibited PB-induced apoptosis, whereas MEK inhibitor did not, supporting that apoptosis induced by PB is through activation of JNK. De novo protein synthesis is required for the PB-induced JNK activation and induction of apoptosis. However, the production of known upstream activators of JNK, namely Fas/Fas ligand, tumor necrosis factor (TNF)-alpha, TNF-beta, and TRAIL, are not altered by PB treatment. Therefore, PB activates JNK through an

unidentified and cell type-specific mechanism. Understanding of this mechanism is of therapeutic value in treating cancer patients with PB.

Reference Type: Journal Article
Record Number: 64
Author: Bachmann, C.
Year: 2005
Title: Long-term outcome of urea cycle disorders
Journal: Acta Gastroenterol Belg
Volume: 68
Issue: 4
Pages: 466-8
Abstract: Evaluation of long-term outcome of patients with urea cycle diseases (UCD) is needed for medical decisions and counselling. Own data comparing outcome of UCD patients with the old treatment limited to protein restriction (i.e. close to the natural history) with that of patients on the modern conservative treatment have shown that gains in survival occur at the cost of more mentally retarded surviving patients. We discuss the possible bias in long-term outcome studies of those rare inheritable disorders where non-predictable environmental factors leading to catabolic crises have a crucial impact on prognosis. A combination of peak or initial ammonia value combined with the duration of coma is discussed as a criterion for prognosis of handicap. The neglect of dietary compensation of branched chain amino acid deficiency worsened by phenylbutyrate treatment in some published protocols could well be an additional cause of the non satisfactory long-term results of conservative treatment which--in our view--mainly aim at bridging optimally the period of late neonatal presentation until liver transplantation in patients with CPS and OTC deficiency (except for mild forms).

Reference Type: Journal Article
Record Number: 74
Author: Borovecki, F.; Lovrecic, L.; Zhou, J.; Jeong, H.; Then, F.; Rosas, H. D.; Hersch, S. M.; Hogarth, P.; Bouzou, B.; Jensen, R. V.; Krainc, D.
Year: 2005
Title: Genome-wide expression profiling of human blood reveals biomarkers for Huntington's disease
Journal: Proc Natl Acad Sci U S A
Volume: 102
Issue: 31
Pages: 11023-8
Abstract: Huntington's disease (HD) is an autosomal dominant disorder caused by an expansion of glutamine repeats in ubiquitously distributed huntingtin protein. Recent studies have shown that mutant huntingtin interferes with the function of widely expressed transcription factors, suggesting that gene expression may be altered in a variety of tissues in HD, including peripheral blood. Affymetrix and Amersham Biosciences oligonucleotide microarrays were used to analyze global gene expression in blood samples of HD patients and matched controls. We identified 322 mRNAs that showed significantly altered expression in HD blood samples, compared with controls ($P < 0.0005$), on two different microarray platforms. A subset of up-regulated

mRNAs selected from this group was able to distinguish controls, presymptomatic individuals carrying the HD mutation, and symptomatic HD patients. In addition, early presymptomatic subjects showed gene expression profiles similar to those of controls, whereas late presymptomatic subjects showed altered expression that resembled that of symptomatic HD patients. These elevated mRNAs were significantly reduced in HD patients involved in a dose-finding study of the histone deacetylase inhibitor sodium phenylbutyrate. Furthermore, expression of the marker genes was significantly up-regulated in postmortem HD caudate, suggesting that alterations in blood mRNAs may reflect disease mechanisms observed in HD brain. In conclusion, we identified changes in blood mRNAs that clearly distinguish HD patients from controls. These alterations in mRNA expression correlate with disease progression and response to experimental treatment. Such markers may provide clues to the state of HD and may be of predictive value in clinical trials.

Reference Type: Journal Article
Record Number: 96
Author: Brahe, C.; Vitali, T.; Tiziano, F. D.; Angelozzi, C.; Pinto, A. M.; Borgo, F.; Moscato, U.; Bertini, E.; Mercuri, E.; Neri, G.
Year: 2005
Title: Phenylbutyrate increases SMN gene expression in spinal muscular atrophy patients
Journal: Eur J Hum Genet
Volume: 13
Issue: 2
Pages: 256-9
Abstract: Spinal muscular atrophy (SMA) is caused by insufficient levels of survival motor neuron (SMN) protein. Recently, we found that sodium 4-phenylbutyrate (PB), a well-tolerated FDA approved drug, enhances SMN gene expression in vitro. We provide here the first evidence that oral administration of PB (triButyrate significantly increases SMN expression in leukocytes of SMA patients. This finding provides a strong rationale to further investigate the effects of PB as also supported by preliminary clinical data.

Reference Type: Journal Article
Record Number: 95
Author: Burzynski, S. R.
Year: 2005
Title: Aging: gene silencing or gene activation?
Journal: Med Hypotheses
Volume: 64
Issue: 1
Pages: 201-8
Abstract: According to the author's theory of gene silencing, the key process in aging involves reduced expression of a number of genes. Silencing of genes has a complex mechanism, which involves methylation of DNA, histone modification and chromatin remodeling. In addition to deacetylation of the histones and methylation of DNA, recently described RNAi mechanism could initiate formation of silenced chromatin. Hypermethylation of the promoter will silence the gene. Genome-wide

hypomethylation will induce genomic instability, amplification of oncogenes and also silencing of the genes through RNAi mechanism. Studies by different groups, conducted in yeast, worms, flies and mice, confirmed substantial changes in gene expression in aging. Among them, the most important was silencing of tumor suppressors and other genes involved in the control of cell cycle, apoptosis, detoxification, and cholesterol metabolism. There was also increased expression of the smaller group of oncogenes and other genes which are associated with typical diseases of old age. Caloric restriction normalizes expression of a substantial percentage of these genes. Animal studies confirmed importance of caloric restriction, which decreases signaling through the IGF-1/AKT pathway and expression of gene p53. These studies, however, cannot be directly applied to human aging. It is proposed that age management therapy should attempt to normalize gene expression in the older population to the level typical for young adults. This would require activation of silenced genes and normalization of overexpressed genes. Caloric restriction and exercise are helpful in decreasing the activity of important oncogenes and activation of silenced tumor suppressors, and may have a positive impact, not only on aging, but also on prevention of cancer. Dietary supplements containing phytochemicals should normalize increased expression of oncogenes. Examples are: genistein and EGCG, which effect signaling through the IGF-1/AKT pathway and resveratrol and limonen, which do so through the RAS pathway. A group of amino acid derivatives and organic acids of animal and human origin should activate silenced tumor suppressor genes (Aminocare A10, Aminocare Extra). Among them 3-phenylacetylamino-2, 6-piperidinedione intercalates specifically with DNA and protects sequences of tumor suppressor genes, which are vulnerable to the effects of carcinogens. Phenylacetate activates p53 and p21 through inhibition of methyltransferase and farnesylation of the RAS protein. Phenylbutyrate activates tumor suppressor genes through inhibition of histone deacetylation. Phenylacetylglutamine decreases genomic instability and expression of oncogenes and promotes apoptosis. The application of DNA microarray techniques to human studies should provide more information about differences in gene expression in different age groups and help design more effective age management regimens.

Reference Type: Journal Article
Record Number: 71
Author: Choi, S. W.; Ryu, O. H.; Choi, S. J.; Song, I. S.; Bleyer, A. J.; Hart, T. C.
Year: 2005
Title: Mutant tamm-horsfall glycoprotein accumulation in endoplasmic reticulum induces apoptosis reversed by colchicine and sodium 4-phenylbutyrate
Journal: J Am Soc Nephrol
Volume: 16
Issue: 10
Pages: 3006-14
Abstract: As a consequence of uromodulin gene mutations, individuals develop precocious hyperuricemia, gout, and progressive renal failure. In vitro studies suggest that pathologic accumulation of uromodulin/Tamm-Horsfall glycoprotein (THP) occurs in the endoplasmic reticulum (ER), but the pathophysiology of renal damage is unclear. It was hypothesized that programmed cell death triggered by accumulation of misfolded THP in the ER causes progressive renal disease. Stably transfected human embryonic kidney 293 cells and immortalized thick ascending limb of Henle's loop

cells with wild-type and mutated uromodulin cDNA were evaluated to test this hypothesis. Immunocytochemistry, ELISA, and deglycosylation studies indicated that accumulation of mutant THP occurred in the ER. FACS analyses showed a significant increase in early apoptosis signal in human embryonic kidney 293 and thick ascending limb of Henle's loop cells that were transfected with mutant uromodulin constructs. Colchicine and sodium 4-phenylbutyrate treatment increased secretion of THP from the ER to the cell membrane and into the culture media and significantly improved cell viability. These findings indicate that intracellular accumulation of THP facilitates apoptosis and that this may provide the pathologic mechanism responsible for the progressive renal damage associated with uromodulin gene mutations. Colchicine and sodium 4-phenylbutyrate reverse these processes and could potentially be beneficial in ameliorating the progressive renal damage in uromodulin-associated kidney diseases.

Reference Type: Journal Article
Record Number: 81
Author: Fu, S. H.; Chen, S. T.; Hsu, B. R.
Year: 2005
Title: Attenuation of primary nonfunction for syngeneic islet graft using sodium 4-phenylbutyrate
Journal: Transplant Proc
Volume: 37
Issue: 4
Pages: 1830-1
Abstract: Sodium 4-phenylbutyrate (4-SPB), an aromatic derivative of butyric acid, was examined to elucidate its effect on islet engraftment in a syngeneic transplantation model using C57BL/6 mice. Diabetic mice that received subrenal implantation of 150 islets on day 0 and oral administration of twice daily 4-SPB (500 mg/kg body weight) on days -2 through 28 displayed a significantly shorter duration of posttransplantation temporary hyperglycemia than diabetic mice that received islets in isotonic sodium chloride solution (NaCl), namely 16 +/- 2 (n = 12) vs 23 +/- 2 days (n = 7; P < .05). Four weeks after transplantation, the insulin content (IC) of grafts from mice treated with islets and 4-SPB was substantially higher than that of grafts from mice treated with islets and NaCl, namely 2.59 +/- 0.37 (n = 8) vs 1.36 +/- 0.36 mug (n = 13; P < .01). The IC of pancreatic remnants showed no significant difference between groups after 2 and 4 weeks of incubation. In vitro studies demonstrated that the net glucose-stimulated insulin secretion (GSIS) and the ratio of net GSIS to the IC of islets cultured with 4-SPB (1 mM) did not differ significantly from those cultured with NaCl. The lipopolysaccharide-stimulated secretions of IL-1beta, IL-10, and IFNgamma from peritoneal exudate monocytes were significantly reduced by co-incubation with 4-SPB (1 mM). In conclusion, our data suggest that daily administration of 4-SPB reduces primary nonfunction and enhances islet engraftment in a syngeneic mouse transplantation model.

Reference Type: Journal Article
Record Number: 68
Author: Gao, J.; Ruan, X.; Pan, X.; Xu, F.; Lei, D.; Liu, D.
Year: 2005

Title: [The effect of sodium phenylbutyrate to agents used in induction chemotherapy on laryngeal carcinoma cells Hep-2 in vitro]
Journal: Lin Chuang Er Bi Yan Hou Ke Za Zhi
Volume: 19
Issue: 15
Pages: 680-2
Abstract: OBJECTIVE: To study the effect of sodium phenylbutyrate when it combined with agents used in induction chemotherapy on laryngeal carcinoma cells Hep-2 in vitro. METHOD: MTT were used to examine the growth inhibition of Hep-2 cells treated by the combination of PB with 5-FU or CDDP in vitro. RESULT: When 5-FU or CDDP combined with PB respectively, there was significantly difference between every two dose groups of the two agents or every dose group and control group ($P < 0.05$). When the dosage of 5-FU or CDDP was definition,there was significantly difference between every two dose groups of PB ($P < 0.05$). CONCLUSION: PB could enhance the cytotoxic effects of agents used in induction chemotherapy on laryngeal carcinoma cells Hep-2 in vitro, which showed the possibility in reinforcement the treatment effect and reduction the occurence of the complication and toxic reaction of induction chemotherapy on laryngeal carcinoma.

Reference Type: Journal Article
Record Number: 98
Author: Gardian, G.; Browne, S. E.; Choi, D. K.; Klivenyi, P.; Gregorio, J.; Kubilus, J. K.; Ryu, H.; Langley, B.; Ratan, R. R.; Ferrante, R. J.; Beal, M. F.
Year: 2005
Title: Neuroprotective effects of phenylbutyrate in the N171-82Q transgenic mouse model of Huntington's disease
Journal: J Biol Chem
Volume: 280
Issue: 1
Pages: 556-63
Abstract: Huntington's disease (HD) is caused by an expansion of exonic CAG triplet repeats in the gene encoding the huntingtin protein (Htt), however, the means by which neurodegeneration occurs remains obscure. There is evidence that mutant Htt interacts with transcription factors leading to reduced histone acetylation. We report that administration of the histone deacetylase inhibitor phenylbutyrate after onset of symptoms in a transgenic mouse model of HD significantly extends survival and attenuates both gross brain and neuronal atrophy. Administration of phenylbutyrate increased brain histone acetylation and decreased histone methylation levels as assessed by both immunocytochemistry and Western blots. Phenylbutyrate increased mRNA for components of the ubiquitin-proteosomal pathway and down-regulated caspases implicated in apoptotic cell death, and active caspase 3 immunoreactivity in the striatum. These results show that administration of phenylbutyrate, at doses that are well tolerated in man, exerts significant neuroprotective effects in a transgenic mouse model of HD, and therefore represents a very promising therapeutic approach for HD.

Reference Type: Journal Article
Record Number: 89

Author: Gondcaille, C.; Depreter, M.; Fourcade, S.; Lecca, M. R.; Leclercq, S.; Martin, P. G.; Pineau, T.; Cadepond, F.; ElEtr, M.; Bertrand, N.; Beley, A.; Duclos, S.; De Craemer, D.; Roels, F.; Savary, S.; Bugaut, M.
Year: 2005
Title: Phenylbutyrate up-regulates the adrenoleukodystrophy-related gene as a nonclassical peroxisome proliferator
Journal: J Cell Biol
Volume: 169
Issue: 1
Pages: 93-104
Abstract: X-linked adrenoleukodystrophy (X-ALD) is a demyelinating disease due to mutations in the ABCD1 (ALD) gene, encoding a peroxisomal ATP-binding cassette transporter (ALDP). Overexpression of adrenoleukodystrophy-related protein, an ALDP homologue encoded by the ABCD2 (adrenoleukodystrophy-related) gene, can compensate for ALDP deficiency. 4-Phenylbutyrate (PBA) has been shown to induce both ABCD2 expression and peroxisome proliferation in human fibroblasts. We show that peroxisome proliferation with unusual shapes and clusters occurred in liver of PBA-treated rodents in a PPARalpha-independent way. PBA activated Abcd2 in cultured glial cells, making PBA a candidate drug for therapy of X-ALD. The Abcd2 induction observed was partially PPARalpha independent in hepatocytes and totally independent in fibroblasts. We demonstrate that a GC box and a CCAAT box of the Abcd2 promoter are the key elements of the PBA-dependent Abcd2 induction, histone deacetylase (HDAC)1 being recruited by the GC box. Thus, PBA is a nonclassical peroxisome proliferator inducing pleiotropic effects, including effects at the peroxisomal level mainly through HDAC inhibition.

Reference Type: Journal Article
Record Number: 79
Author: Hao, C. L.; Tang, K. J.; Chen, S.; Xing, H. Y.; Wang, M.; Wang, J. X.
Year: 2005
Title: [5-Aza-2'-deoxycytidine enhances differentiation and apoptosis induced by phenylbutyrate in Kasumi-1 cells]
Journal: Zhonghua Zhong Liu Za Zhi
Volume: 27
Issue: 3
Pages: 148-51
Abstract: OBJECTIVE: To investigate whether phenylbutyrate (PB) combined with 5-aza-2'-deoxycytidine (5-Aza-CdR)could inhibit transcription repression and induce t(8;21) acute myelogenous leukemia (AML) Kasumi-1 cells to differentiate and undergo apoptosis. METHODS: Kasumi-1 cells were treated with PB and 5-Aza-CdR at different concentrations in suspension culture. Cellular proliferation was determined by the MTT assay, expression of myeloid-specific differentiation antigen and cell cycles were analyzed by flow cytometry. Cell apoptosis were assessed using AnnexinV/PI staining and flow cytometry. RESULTS: Treatment of Kasumi-1 cells with PB caused a dose-dependent inhibition of proliferation, with an IC(50) of 2.3 mmol/L. When combined with 5-Aza-CdR, PB resulted in a greater growth inhibition with an IC(50) of 1.95 mmol/L. Treatment of Kasumi-1 cells with PB resulted in cell cycle arrest at G(0)/G(1), while combined treatment with PB and 5-Aza-CdR led to cell cycle arrest at G(2)/M. Expression of myeloid cell differentiation antigens CD11b

and CD13 induced by PB was enhanced when Kasumi-1 cells were pretreated with low dose of 5-Aza-CdR. High, but not low, concentrations of 5-Aza-CdR could enhance early apoptosis of Kasumi-1 cells induced by PB. CONCLUSION: Phenylbuty rate, when combined with 5-Aza-CdR, inhibits AML cell in vitro proliferation and increases apoptosis in a synergistic fashion.

Reference Type: Journal Article
Record Number: 85
Author: Heckmann, M.; Wermuth, B.; Haberle, J.; Koch, H. G.; Gortner, L.; Kreuder, J. G.
Year: 2005
Title: Misleading diagnosis of partial N-acetylglutamate synthase deficiency based on enzyme measurement corrected by mutation analysis
Journal: Acta Paediatr
Volume: 94
Issue: 1
Pages: 121-4
Abstract: N-acetylglutamate synthase (NAGS) deficiency is a rare urea cycle disorder. Most of the patients present in the early neonatal period with severe hyperammonaemia and marked neurological impairment. We report on a Turkish family with an index patient, who died due to hyperammonemia, and another three siblings, who received a prophylactic treatment consisting of arginine hydrochloride, sodium benzoate and phenylbutyrate directly after birth. Enzyme measurement in a liver biopsy suggested a diagnosis of partial NAGS deficiency in all three siblings. Thereafter, N-carbamylglutamate was added to the treatment. None of the patients developed hyperammonaemia. After the human NAGS gene was identified, mutation analysis revealed that the consanguineous parents and two siblings were heterozygous for a private mutation (W484R), whereas the wild-type gene was found in the eldest sibling. Therapy was stopped without any deterioration of urea cycle function. CONCLUSION: Diagnosis of partial NAGS deficiency based on enzyme measurement may be misleading and should be completed by mutation analysis.

Reference Type: Journal Article
Record Number: 75
Author: Kato, R.; Nakano, H.; Konishi, H.; Kato, K.; Koga, Y.; Yamane, T.; Kobayashi, T.; Honda, H.
Year: 2005
Title: Novel strategy for protein exploration: high-throughput screening assisted with fuzzy neural network
Journal: J Mol Biol
Volume: 351
Issue: 3
Pages: 683-92
Abstract: To engineer proteins with desirable characteristics from a naturally occurring protein, high-throughput screening (HTS) combined with directed evolutional approach is the essential technology. However, most HTS techniques are simple positive screenings. The information obtained from the positive candidates is used only as results but rarely as clues for understanding the structural rules, which

may explain the protein activity. In here, we have attempted to establish a novel strategy for exploring functional proteins associated with computational analysis. As a model case, we explored lipases with inverted enantioselectivity for a substrate p-nitrophenyl 3-phenylbutyrate from the wild-type lipase of Burkhorderia cepacia KWI-56, which is originally selective for (S)-configuration of the substrate. Data from our previous work on (R)-enantioselective lipase screening were applied to fuzzy neural network (FNN), bioinformatic algorithm, to extract guidelines for screening and engineering processes to be followed. FNN has an advantageous feature of extracting hidden rules that lie between sequences of variants and their enzyme activity to gain high prediction accuracy. Without any prior knowledge, FNN predicted a rule indicating that "size at position L167," among four positions (L17, F119, L167, and L266) in the substrate binding core region, is the most influential factor for obtaining lipase with inverted (R)-enantioselectivity. Based on the guidelines obtained, newly engineered novel variants, which were not found in the actual screening, were experimentally proven to gain high (R)-enantioselectivity by engineering the size at position L167. We also designed and assayed two novel variants, namely FIGV (L17F, F119I, L167G, and L266V) and FFGI (L17F, L167G, and L266I), which were compatible with the guideline obtained from FNN analysis, and confirmed that these designed lipases could acquire high inverted enantioselectivity. The results have shown that with the aid of bioinformatic analysis, high-throughput screening can expand its potential for exploring vast combinatorial sequence spaces of proteins.

Reference Type: Journal Article
Record Number: 83
Author: Kerem, E.
Year: 2005
Title: Pharmacological induction of CFTR function in patients with cystic fibrosis: mutation-specific therapy
Journal: Pediatr Pulmonol
Volume: 40
Issue: 3
Pages: 183-96
Abstract: CFTR mutations cause defects of CFTR protein production and function by different molecular mechanisms. Mutations can be classified according to the mechanisms by which they disrupt CFTR function. This understanding of the different molecular mechanisms of CFTR dysfunction provides the scientific basis for the development of targeted drugs for mutation-specific therapy of cystic fibrosis (CF). Class I mutations are nonsense mutations that result in the presence of a premature stop codon that leads to the production of unstable mRNA, or the release from the ribosome of a short, truncated protein that is not functional. Aminoglycoside antibiotics can suppress premature termination codons by disrupting translational fidelity and allowing the incorporation of an amino acid, thus permitting translation to continue to the normal termination of the transcript. Class II mutations cause impairment of CFTR processing and folding in the Golgi. As a result, the mutant CFTR is retained in the endoplasmic reticulum (ER) and eventually targeted for degradation by the quality control mechanisms. Chemical and molecular chaperones such as sodium-4-phenylbutyrate can stabilize protein structure, and allow it to escape from degradation in the ER and be transported to the cell membrane. Class III mutations disrupt the function of the regulatory domain. CFTR is resistant to

phosphorylation or adenosine tri-phosphate (ATP) binding. CFTR activators such as alkylxanthines (CPX) and the flavonoid genistein can overcome affected ATP binding through direct binding to a nucleotide binding fold. In patients carrying class IV mutations, phosphorylation of CFTR results in reduced chloride transport. Increases in the overall cell surface content of these mutants might overcome the relative reduction in conductance. Alternatively, restoring native chloride pore characteristics pharmacologically might be effective. Activators of CFTR at the plasma membrane may function by promoting CFTR phosphorylation, by blocking CFTR dephosphorylation, by interacting directly with CFTR, and/or by modulation of CFTR protein-protein interactions. Class V mutations affect the splicing machinery and generate both aberrantly and correctly spliced transcripts, the levels of which vary among different patients and among different organs of the same patient. Splicing factors that promote exon inclusion or factors that promote exon skipping can promote increases of correctly spliced transcripts, depending on the molecular defect. Inconsistent results were reported regarding the required level of corrected or mutated CFTR that had to be reached in order to achieve normal function.

Reference Type: Journal Article
Record Number: 72
Author: Lu, Q.; Wang, D. S.; Chen, C. S.; Hu, Y. D.; Chen, C. S.
Year: 2005
Title: Structure-based optimization of phenylbutyrate-derived histone deacetylase inhibitors
Journal: J Med Chem
Volume: 48
Issue: 17
Pages: 5530-5
Abstract: Previously, we developed a strategy to develop a novel class of histone deacetylase (HDAC) inhibitors by tethering short-chain fatty acids with Zn(2+)-chelating motifs, which led to N-hydroxy-4-(4-phenylbutyryl-amino)benzamide (HTPB), a hydroxamate-tethered phenylbutyrate derivative with sub-micromolar potency in inhibiting HDAC activity and cancer cell proliferation. In this study, we carried out structure-based optimization of HTPB by using the framework generated by the structure of histone deacetylase-like protein (HDLP)-trichostatin A (TSA) complexes. Docking of HTPB into the HDLP binding domain suggested that the hydrophobic microenvironment encompassed by Phe-198 and Phe-200 could be exploited for structural optimization. This premise was corroborated by the greater potency of (S)-(+)-N-hydroxy-4-(3-methyl-2-phenylbutyrylamino)-benzamide [(S)-11] (IC(50) in HDAC inhibition, 16 nM), of which the isopropyl moiety was favorable in interacting with this hydrophobic motif. (S)-11 at concentrations as low as 0.1 microM was effective in causing histone hyperacetylation and p21(WAF/CIP1) overexpression and suppressing proliferation in cancer cells.

Reference Type: Journal Article
Record Number: 69
Author: Meng, M.; Jiang, J. M.; Liu, H.; In, C. Y.; Zhu, J. R.
Year: 2005

Title: Effects of sodium phenylbutyrate on differentiation and induction of the P21WAF1/CIP1 anti-oncogene in human liver carcinoma cell lines
Journal: Chin J Dig Dis
Volume: 6
Issue: 4
Pages: 189-92
Abstract: OBJECTIVES: To explore the effects of sodium phenylbutyrate on the proliferation, differentiation, cell cycle arrest and induction of the P(21WAF1/CIP1) anti-oncogene in human liver carcinoma cell lines Bel-7402 and HepG2. METHODS: Bel-7402 and HepG2 human liver carcinoma cells were treated with sodium phenylbutyrate at different concentrations. Light microscopy was used to observe morphological changes in the carcinoma cells. Effects on the cell cycle were detected by using flow cytometry. P(21WAF1/CIP1) expression was determined by both reverse transcription-polymerase chain reaction and western blotting. Statistical analysis was performed by using one-way anova and Student's t-test. RESULTS: Sodium phenylbutyrate treatment caused time- and dose-dependent growth inhibition of Bel-7402 and HepG2 cells. This treatment also caused a decline in the proportion of S-phase cells and an increase in the proportion of G(0)/G(1) cells. Sodium phenylbutyrate increased the expression of P(21WAF1/CIP1). CONCLUSIONS: Sodium phenylbutyrate inhibits the proliferation of human liver carcinoma cells Bel-7402 and HepG2, induces partial differentiation, and increases the expression of P(21WAF1/CIP1).

Reference Type: Journal Article
Record Number: 82
Author: Meynial-Denis, D.; Verdier, L.; Mignon, M.; Leclerc, J. N.; Bayle, G.; Darmaun, D.
Year: 2005
Title: Does acute glutamine depletion enhance the response of glutamine synthesis to fasting in muscle in adult and old rats?
Journal: Clin Nutr
Volume: 24
Issue: 3
Pages: 398-406
Abstract: BACKGROUND AND AIMS: In earlier studies, skeletal muscle glutamine synthetase (GS) activity was shown to be enhanced by fasting and glucocorticoids, and inhibited by exogenous glutamine (Gln) supplementation. The current study was designed to determine whether phenylbutyrate (PhiB), a Gln-chelating agent in humans, (1) could trap Gln and produce a decline in plasma Gln in rats, as it does in humans, and (2) if so, whether (Phi)B would further enhance the response of muscle GS activity to fasting in rats. METHODS: Adult (6-8 months) and aged (20-21 months) rats were fasted for 5 days and received two doses of 0.5 g(Phi)Bby orogastric route at times 0 and 4 h, and were then sacrificed at 5.5 h. Plasma Gln was measured by enzymatic methods, other amino acids were quantified by amino acid analysis. GS activity was measured in soleus (SO) and tibialis anterior (TA) muscles. RESULTS: (Phi)B treatment was associated with: (1) a 20% decline in plasma Gln concentration from 572+/-54 to 424+/-34 micromol/L ($P<0.05$) and from 476+/-49 to 360+/-80 micromol/L ($P<0.05$) in fasted adult and old rats, respectively; and (2) a preservation of GS up-regulation by fasting in TA and SO muscles in both adult and

aged rats, with TA muscle GS activities of 198+/-65 vs. 203+/-68 ((Phi)B-treated vs. vehicle-treated, NS), and 244+/-81 vs. 274+/-59 (NS) nmol/h/mg protein in adult and aged rats, respectively. CONCLUSION: These data suggest that: (1) large doses of (Phi)B deplete plasma Gln in fasted rats, regardless of age, (2) Gln depletion induced by Phi)B does not alter GS activity.

Reference Type: Journal Article
Record Number: 84
Author: Milkevitch, M.; Shim, H.; Pilatus, U.; Pickup, S.; Wehrle, J. P.; Samid, D.; Poptani, H.; Glickson, J. D.; Delikatny, E. J.
Year: 2005
Title: Increases in NMR-visible lipid and glycerophosphocholine during phenylbutyrate-induced apoptosis in human prostate cancer cells
Journal: Biochim Biophys Acta
Volume: 1734
Issue: 1
Pages: 1-12
Abstract: DU145 human prostatic carcinoma cells were treated with the differentiating agents phenylacetate (PA) and phenylbutyrate (PB) and examined in perfused cultures by diffusion-weighted 1H and 31P nuclear magnetic resonance spectroscopy (NMR). PA and PB (10 mM) induced significant (>3-fold) time-dependent increases in the level of NMR-visible lipids and total choline in 1H spectra, and glycerophosphocholine levels in the 31P spectra, with the increases being greater for PB. These effects were accompanied by significant increases in cytoplasmic lipid droplets and intracellular lipid volume fraction as observed by morphometric analysis of Oil Red O-stained cells. PB treatment caused cell cycle arrest in the G1 phase and induction of apoptosis. In contrast, PA-treated DU145 cells showed an accumulation of cells in G2/M and no evidence of apoptosis. These results demonstrate that significant differences exist in the mechanism of PA and PB activity, although both compounds cause similar, but graded alterations in lipid metabolism. The simultaneous accumulation of mobile lipid and glycerophosphocholine suggests that PB and PA induce phospholipid catabolism via a phospholipase-mediated pathway. The mobile lipid accumulation following the induction of either apoptosis and cytostasis by related differentiating agents indicate that the presence of NMR-visible lipids may not be a specific event causally resulting from the induction of apoptosis.

Reference Type: Journal Article
Record Number: 92
Author: Monneret, C.
Year: 2005
Title: Histone deacetylase inhibitors
Journal: Eur J Med Chem
Volume: 40
Issue: 1
Pages: 1-13
Abstract: Histones are small basic proteins that, by complexing wtih DNA, form the nucleosome core. Repetitive units of this nucleosome led to the chromatin in which all the human genome is packaged. Histones can be in one of the two antagonist forms,

acetylated or deacetylated, equilibrium regulated by the corresponding enzymes, histone acetylases and histones deacetylases (HDACs). Inhibition of HDACs represents a new strategy in human cancer therapy since these enzymes play a fundamental role in regulating gene expression and chromatin assembly. They are potent inducers of growth arrest, differentiation and apoptosis of tumor cells. A wide variety of HDACs of both natural and synthetic origin has been reported. Except depsispeptide FK228, natural HDACs (trichostatin (TSA), depudecin, trapoxins, apicidins) as well as sodium butyrate, phenylbutyrate and suberoyl anilide hydroxamic acid (SAHA), while effective in vivo, are inefficient due to instability and low retention. Subsequently, synthetic analogs isolated from screening libraries (oxamflatin, scriptaid) were discovered as havind a common structure with TSA and SAHA: an hydroxamic acid zinc-binding group linked via a spacer (5 or 6 CH2) to a hydrophobic group. Design of a second generation of HDACs was based upon these data affording potent HDACs such as LAQ824 and PDX101 currently under phase I clinical trials. Simultaneously, synthetic benzamide-containing HDACs were reported and two of them, MS-275 and CI-994, have reached phase II and I clinical trials, respectively.

Reference Type: Journal Article
Record Number: 77
Author: Munshi, A.; Kurland, J. F.; Nishikawa, T.; Tanaka, T.; Hobbs, M. L.; Tucker, S. L.; Ismail, S.; Stevens, C.; Meyn, R. E.
Year: 2005
Title: Histone deacetylase inhibitors radiosensitize human melanoma cells by suppressing DNA repair activity
Journal: Clin Cancer Res
Volume: 11
Issue: 13
Pages: 4912-22
Abstract: PURPOSE: Histone deacetylase (HDAC) inhibitors have emerged recently as promising anticancer agents. They arrest cells in the cell cycle and induce differentiation and cell death. The antitumor activity of HDAC inhibitors has been linked to their ability to induce gene expression through acetylation of histone and nonhistone proteins. However, it has recently been suggested that HDAC inhibitors may also enhance the activity of other cancer therapeutics, including radiotherapy. The purpose of this study was to evaluate the ability of HDAC inhibitors to radiosensitize human melanoma cells in vitro. EXPERIMENTAL DESIGN: A panel of HDAC inhibitors that included sodium butyrate (NaB), phenylbutyrate, tributyrin, and trichostatin A were tested for their ability to radiosensitize two human melanoma cell lines (A375 and MeWo) using clonogenic cell survival assays. Apoptosis and DNA repair were measured by standard assays. RESULTS: NaB induced hyperacetylation of histone H4 in the two melanoma cell lines and the normal human fibroblasts. NaB radiosensitized both the A375 and MeWo melanoma cell lines, substantially reducing the surviving fraction at 2 Gy (SF2), whereas it had no effect on the normal human fibroblasts. The other HDAC inhibitors, phenylbutyrate, tributyrin, and trichostatin A had significant radiosensitizing effects on both melanoma cell lines tested. NaB modestly enhanced radiation-induced apoptosis that did not correlate with survival but did correlate with functional impairment of DNA repair as determined based on the host cell reactivation assay. Moreover, NaB

significantly reduced the expression of the repair-related genes Ku70 and Ku86 and DNA-dependent protein kinase catalytic subunit in melanoma cells at the protein and mRNA levels. Normal human fibroblasts showed no change in DNA repair capacity or levels of DNA repair proteins following NaB treatment. We also examined gamma-H2AX phosphorylation as a marker of radiation response to NaB and observed that compared with controls, gamma-H2AX foci persisted long after ionizing exposure in the NaB-treated cells. CONCLUSIONS: HDAC inhibitors radiosensitize human tumor cells by affecting their ability to repair the DNA damage induced by ionizing radiation and that gamma-H2AX phosphorylation can be used as a predictive marker of radioresponse.

Reference Type: Journal Article
Record Number: 78
Author: Navarro-Llorens, J. M.; Patrauchan, M. A.; Stewart, G. R.; Davies, J. E.; Eltis, L. D.; Mohn, W. W.
Year: 2005
Title: Phenylacetate catabolism in Rhodococcus sp. strain RHA1: a central pathway for degradation of aromatic compounds
Journal: J Bacteriol
Volume: 187
Issue: 13
Pages: 4497-504
Abstract: In gram-negative bacteria, a pathway for aerobic degradation of phenylacetic acid (PAA) that proceeds via phenylacetyl-coenzyme A (CoA) and hydrolytic ring fission plays a central role in the degradation of a range of aromatic compounds. In contrast, the PAA pathway and its role are not well characterized in gram-positive bacteria. A cluster including 13 paa genes encoding enzymes orthologous to those of gram-negative bacteria was identified on the chromosome of Rhodococcus sp. strain RHA1. These genes were transcribed during growth on PAA, with 11 of the genes apparently in an operon yielding a single transcript. Quantitative proteomic analyses revealed that at least 146 proteins were more than twice as abundant in PAA-grown cells of RHA1 than in pyruvate-grown cells. Of these proteins, 29 were identified, including 8 encoded by the paa genes. Knockout mutagenesis indicated that paaN, encoding a putative ring-opening enzyme, was essential for growth on PAA. However, paaF, encoding phenylacetyl-CoA ligase, and paaR, encoding a putative regulator, were not essential. paaN was also essential for growth of RHA1 on phenylacetaldehyde, phenylpyruvate, 4-phenylbutyrate, 2-phenylethanol, 2-phenylethylamine, and l-phenylalanine. In contrast, growth on 3-hydroxyphenylacetate, ethylbenzene, and styrene was unaffected. These results suggest that the range of substrates degraded via the PAA pathway in RHA1 is somewhat limited relative to the range in previously characterized gram-negative bacteria.

Reference Type: Journal Article
Record Number: 88
Author: Phuphanich, S.; Baker, S. D.; Grossman, S. A.; Carson, K. A.; Gilbert, M. R.; Fisher, J. D.; Carducci, M. A.
Year: 2005

Title: Oral sodium phenylbutyrate in patients with recurrent malignant gliomas: a dose escalation and pharmacologic study
Journal: Neuro Oncol
Volume: 7
Issue: 2
Pages: 177-82
Abstract: We determined the maximum tolerated dose (MTD), toxicity profile, pharmacokinetic parameters, and preliminary efficacy data of oral sodium phenylbutyrate (PB) in patients with recurrent malignant gliomas. Twenty-three patients with supratentorial recurrent malignant gliomas were enrolled on this dose escalation trial. Four dose levels of PB were studied: 9, 18, 27, and 36 g/day. Data were collected to assess toxicity, response, survival, and pharmacokinetics. All PB doses of 9, 18, and 27 g/day were well tolerated. At 36 g/day, two of four patients developed dose-limiting grade 3 fatigue and somnolence. At the MTD of 27 g/day, one of seven patients developed reversible grade 3 somnolence. Median survival from time of study entry was 5.4 months. One patient had a complete response for five years, and no partial responses were noted, which yielded an overall response rate of 5%. Plasma concentrations of 706, 818, 1225, and 1605 muM were achieved with doses of 9, 18, 27, and 36 g/day, respectively. The mean value for PB clearance in this patient population was 22 liters/h, which is significantly higher than the 16 liters/h reported in patients with other malignancies who were not receiving P450 enzyme-inducing anticonvulsant drugs (P = 0.038). This study defines the MTD and recommended phase 2 dose of PB at 27 g/day for heavily pretreated patients with recurrent gliomas. The pharmacology of PB appears to be affected by concomitant administration of P450-inducing anticonvulsants.

Reference Type: Journal Article
Record Number: 87
Author: Pleasure, D.
Year: 2005
Title: New treatments for denervating diseases
Journal: J Child Neurol
Volume: 20
Issue: 3
Pages: 258-62
Abstract: There has been considerable recent progress in understanding mechanisms by which gene mutations cause degeneration of motoneurons and peripheral nerves. Novel therapies inspired by these insights have begun to yield promising results in mouse models of these genetic diseases. Among these have been the use of small molecules or proteins to suppress gain-of-function mutations (eg, ascorbic acid for Charcot-Marie-Tooth disease type 1A) or to restore enzyme activities that are deficient because of loss-of-function mutations (eg, treatment of Fabry's disease with recombinant alpha-galactosidase or with low-molecular-weight alpha-galactosidase chaperones and treatment of spinal muscular atrophy with phenylbutyrate). Some of these therapies are already being tested in humans. Equally exciting is the prospect that small molecules and proteins will be identified that exert potent therapeutic effects in a broad spectrum of inherited and acquired motoneuron and peripheral nerve disorders.

Reference Type: Journal Article
Record Number: 76
Author: Rasool, S.; Johri, S.; Riyaz-ul-Hassan, S.; Maqbool, Q. U.; Verma, V.; Koul, S.; Taneja, S. C.; Qazi, G. N.
Year: 2005
Title: Molecular cloning of enantioselective ester hydrolase from Bacillus pumilus DBRL-191
Journal: FEMS Microbiol Lett
Volume: 249
Issue: 1
Pages: 113-20
Abstract: A gene from Bacillus pumilus expressed under its native promoter was cloned in Escherichia coli. Recombinant B. pumilus esterase (BPE) affects the kinetic resolution of racemic mixtures such as unsubstituted and substituted 1-(phenyl)ethanols (E approximately 33-103), ethyl 3-hydroxy-3-phenylpropanoate (E approximately 45-71), trans-4-fluorophenyl-3-hydroxymethyl-N-methylpiperidine (E approximately 10-13) and ethyl 2-hydroxy-4-phenylbutyrate (E approximately 7). The enzyme is composed of a 34-amino acid signal peptide and a 181-amino acid mature protein corresponding to a molecular weight of approximately 19.2kD and pI approximately 9.4. 3-D the structural model of the enzyme built by homology modelling using the atomic coordinates from the crystal structure of B. subtilis lipase (LipA) showed a compact minimal alpha/beta hydrolase fold.

Reference Type: Journal Article
Record Number: 86
Author: Rudek, M. A.; Zhao, M.; He, P.; Hartke, C.; Gilbert, J.; Gore, S. D.; Carducci, M. A.; Baker, S. D.
Year: 2005
Title: Pharmacokinetics of 5-azacitidine administered with phenylbutyrate in patients with refractory solid tumors or hematologic malignancies
Journal: J Clin Oncol
Volume: 23
Issue: 17
Pages: 3906-11
Abstract: PURPOSE: To characterize the pharmacokinetic behavior of 5-azacitidine (5-AC), a cytidine nucleoside analog, when given with phenylbutyrate, a histone deaceytlase inhibitor. PATIENTS AND METHODS: Pharmacokinetic data were obtained from two trials involving patients with solid tumor and hematologic malignancies. 5-AC at doses ranging from 10 to 75 mg/m2/d was administered once daily as a subcutaneous injection for 5 to 21 days in combination with phenylbutyrate administered as a continuous intravenous infusion for varying dose and duration every 28 or 35 days. Serial plasma samples were collected up to 24 hours after 5-AC administration. 5-AC was quantitated using a validated liquid chromatograph/tandem mass spectrometry method. RESULTS: 5-AC was rapidly absorbed with the mean T(max) occurring at 0.47 hour. Average maximum concentration (C(max)) and area under the curve (AUC(0-infinity)) values increased in a dose-proportionate manner with increasing dose from 10 to 75 mg/m2/d; the mean +/- SD C(max) and AUC(0-infinity) at 10 mg/m2/d were 776 +/- 459 nM and 1,355 +/- 1,125 h*nM, respectively,

and at 75 mg/m2/d were 4,871 +/- 1,398 nM and 6,582 +/- 2,560 h*nM, respectively. Despite a short terminal half-life of 1.5 +/- 2.3 hours, inhibition of DNA methyl transferase activity in tumors of patients receiving 5-AC has been documented. CONCLUSION: 5-AC is rapidly absorbed and eliminated when administered subcutaneously. Sufficient 5-AC exposure is achieved to produce pharmacodynamic effects in tumors.

Reference Type: Journal Article
Record Number: 80
Author: Ryu, H.; Smith, K.; Camelo, S. I.; Carreras, I.; Lee, J.; Iglesias, A. H.; Dangond, F.; Cormier, K. A.; Cudkowicz, M. E.; Brown, R. H., Jr.; Ferrante, R. J.
Year: 2005
Title: Sodium phenylbutyrate prolongs survival and regulates expression of anti-apoptotic genes in transgenic amyotrophic lateral sclerosis mice
Journal: J Neurochem
Volume: 93
Issue: 5
Pages: 1087-98
Abstract: Multiple molecular defects trigger cell death in amyotrophic lateral sclerosis (ALS). Among these, altered transcriptional activity may perturb many cellular functions, leading to a cascade of secondary pathological effects. We showed that pharmacological treatment, using the histone deacetylase inhibitor sodium phenylbutyrate, significantly extended survival and improved both the clinical and neuropathological phenotypes in G93A transgenic ALS mice. Phenylbutyrate administration ameliorated histone hypoacetylation observed in G93A mice and induced expression of nuclear factor-kappaB (NF-kappaB) p50, the phosphorylated inhibitory subunit of NF-kappaB (pIkappaB) and beta cell lymphoma 2 (bcl-2), but reduced cytochrome c and caspase expression. Curcumin, an NF-kappaB inhibitor, and mutation of the NF-kappaB responsive element in the bcl-2 promoter, blocked butyrate-induced bcl-2 promoter activity. We provide evidence that the pharmacological induction of NF-kappaB-dependent transcription and bcl-2 gene expression is neuroprotective in ALS mice by inhibiting programmed cell death. Phenylbutyrate acts to phosphorylate IkappaB, translocating NF-kappaB p50 to the nucleus, or to directly acetylate NF-kappaB p50. NF-kappaB p50 transactivates bcl-2 gene expression. Up-regulated bcl-2 blocks cytochrome c release and subsequent caspase activation, slowing motor neuron death. These transcriptional and post-translational pathways ultimately promote motor neuron survival and ameliorate disease progression in ALS mice. Phenylbutyrate may therefore provide a novel therapeutic approach for the treatment of patients with ALS.

Reference Type: Journal Article
Record Number: 91
Author: Wang, H. E.; Wu, H. C.; Kao, S. J.; Tseng, F. W.; Wang, Y. S.; Yu, H. M.; Chou, S. L.; Yen, S. H.; Chi, K. H.
Year: 2005
Title: Modulation of 5-fluorouracil cytotoxicity through thymidylate synthase and NF-kappaB down-regulation and its application on the radiolabelled iododeoxyuridine therapy on human hepatoma cell

Journal: Biochem Pharmacol
Volume: 69
Issue: 4
Pages: 617-26
Abstract: The inhibition of thymidylate synthase (TS) by 5-fluorouracil (5-FU) was known to increase the incorporation of radiolabelled iododeoxyuridine (IdUrd) into DNA. The relatively non-toxic compounds such as thiol-containing antioxidant pyrrolidinodithiocarbamte (PDTC) or aromatic fatty acid phenylbutyrate (PB) had been reported to enhance the cytotoxic efficacy of 5-FU. We designed a novel strategy through triplet combination of PB, PDTC and 5-FU to increase the radiolabelled IdUrd uptake and investigated the underlying mechanisms. The growth inhibition and [(125)I]IdUrd-DNA incorporation by PB, PDTC, 5-FU in different combinations were tested on parent or p21(Waf1) transfected Hep3B cells. The combination of PB and PDTC was more effective in enhancing 5-FU cytotoxicity than either drug alone. The combination of PB/PDTC and 5-FU blocked cells in S-phase and resulted in 8.5-fold increase of radiolabelled IdUrd-DNA incorporation. The transfection of p21(Waf1) did not change the general pattern of enhancement. Intriguingly, the combination of PB and PDTC effectively down-regulated NF-kappaB and TS and prevented their up-regulation from 5-FU treatment than either drug alone through a p21(Waf1)-independent mechanism. Based on this strategy, the 3-drug combination offered potential for improved radiolabelled IdUrd molecular radiotherapy for hepatoma treatment.

Reference Type: Journal Article
Record Number: 73
Author: Wernig, G.; Janzen, V.; Schafer, R.; Zweyer, M.; Knauf, U.; Hoegemeier, O.; Mundegar, R. R.; Garbe, S.; Stier, S.; Franz, T.; Wernig, M.; Wernig, A.
Year: 2005
Title: The vast majority of bone-marrow-derived cells integrated into mdx muscle fibers are silent despite long-term engraftment
Journal: Proc Natl Acad Sci U S A
Volume: 102
Issue: 33
Pages: 11852-7
Abstract: Bone-marrow-derived cells can contribute nuclei to skeletal muscle fibers. However, serial sectioning of muscle in mdx mice implanted with GFP-labeled bone marrow reveals that only 20% of the donor nuclei chronically incorporated in muscle fibers show dystrophin (or GFP) expression, which is still higher than the expected frequency of "revertant" fibers, but there is no overall increase above controls over time. Obviously, the vast majority of incorporated nuclei either never or only temporarily turn on myogenic genes; also, incorporated nuclei eventually loose the activation of the beta-actin::GFP transgene. Consequently, we attempted to enhance the expression of dystrophin. In vivo application of the chromatin-modifying agents 5-azadeoxycytidine and phenylbutyrate as well as local damage by cardiotoxin injections caused a small increase in dystrophin-positive fibers without abolishing the appearance of "silent" nuclei. The results thus confirm that endogenous repair processes and epigenetic modifications on a small-scale lead to dystrophin expression from donor nuclei. Both effects, however, remain below functionally significant levels.

Reference Type: Journal Article
Record Number: 70
Author: Vilatoba, M.; Eckstein, C.; Bilbao, G.; Smyth, C. A.; Jenkins, S.; Thompson, J. A.; Eckhoff, D. E.; Contreras, J. L.
Year: 2005
Title: Sodium 4-phenylbutyrate protects against liver ischemia reperfusion injury by inhibition of endoplasmic reticulum-stress mediated apoptosis
Journal: Surgery
Volume: 138
Issue: 2
Pages: 342-51
Abstract: BACKGROUND: Evidence is emerging that the endoplasmic reticulum (ER) participates in initiation of apoptosis induced by the unfolded protein response and by aberrant Ca(++) signaling during cellular stress such as ischemia/reperfusion injury (I/R injury). ER-induced apoptosis involves the activation of caspase-12 and C/EBP homologous protein (CHOP), and the shutdown of translation initiated by phosphorylation of eIF2alpha. Sodium 4-phenylbutyrate (PBA) is a low molecular weight fatty acid that acts as a chemical chaperone reducing the load of mutant or unfolded proteins retained in the ER during cellular stress and also exerting anti-inflammatory activity. It has been used successfully for treatment of urea cycle disorders and sickle cell disease. Thus, we hypothesized that PBA may reduce ER-induced apoptosis triggered by I/R injury to the liver. METHODS: Groups of male C57BL/6 mice were subjected to warm ischemia (70% of the liver mass, 45 minutes). Serum aspartate aminotransferase was assessed 6 hours after reperfusion; apoptosis was evaluated by enzyme-linked immunosorbent assays of caspase-12 and plasma tumor necrosis factor alpha, Western blot analyses of eIF2alpha, and reverse transcriptase-polymerase chain reaction of CHOP expression. RESULTS: A dose-dependent decrease in aspartate aminotransferase was demonstrated in mice given intraperitoneal PBA (1 hour before and 12 hours after reperfusion), compared with vehicle-treated controls; this effect was associated with reduced pyknosis, parenchymal hemorrhages, and neutrophil infiltrates in PBA-treated mice, compared with controls. In a lethal model of total liver I/R injury, all vehicle-treated controls died within 3 days after reperfusion. In contrast, 50% survival (>30 days) was observed in animals given PBA. The beneficial effects of PBA were associated with a greater than 45% reduction in apoptosis, decreased ER-mediated apoptosis characterized by significant reduction in caspase-12 activation, and reduced levels of both phosphorylated eIF2alpha and CHOP. Significant reductions in plasma levels of tumor necrosis factor alpha and liver myeloperoxidase content were demonstrated after PBA treatment. CONCLUSIONS: Reduction in ER stress-induced hepatocellular injury was achieved by the administration of PBA. Targeting the ER-associated cell death pathway might offer a novel approach to reduce I/R injury to the liver.

Reference Type: Journal Article
Record Number: 58
Author: Amat di San Filippo, C.; Pasquali, M.; Longo, N.
Year: 2006

Title: Pharmacological rescue of carnitine transport in primary carnitine deficiency
Journal: Hum Mutat
Volume: 27
Issue: 6
Pages: 513-23
Abstract: Primary carnitine deficiency is a recessive disorder caused by heterogeneous mutations in the SLC22A5 gene encoding the OCTN2 carnitine transporter. Here we extend mutational analysis to eight new families with this disorder. To determine the mechanism by which missense mutations impaired carnitine transport, the OCTN2 transporter was tagged with the green fluorescent protein and expressed in CHO cells. Analysis by confocal microscopy indicated that several missense mutants (M1I, R169W, T232 M, G242 V, S280F, R282Q, W283R, A301D, W351R, R399Q, T440 M, E452 K, and T468R) matured normally to the plasma membrane. By contrast, other mutations (including R19P, DeltaF22, R83L, S280F, P398L, Y447C, and A142S/R488 H) caused significant retention of the mutant OCTN2 transporter in the cytoplasm. Failed maturation to the plasma membrane is a common mechanism in disorders affecting membrane transporters/ion channels, including cystic fibrosis. To correct this defect, we tested whether drugs reducing the efficiency of protein degradation in the endoplasmic reticulum (ER) (phenylbutyrate, curcumin) or capable of binding the OCTN2 carnitine transporter (verapamil, quinidine) could improve carnitine transport. Prolonged incubation with phenylbutyrate, quinidine, and verapamil partially stimulated carnitine transport, while curcumin was ineffective. These results indicate that OCTN2 mutations can affect carnitine transport by impairing maturation of transporters to the plasma membrane. Pharmacological therapy can be effective in partially restoring activity of mutant transporters.

Reference Type: Journal Article
Record Number: 65
Author: Cheong, N.; Madesh, M.; Gonzales, L. W.; Zhao, M.; Yu, K.; Ballard, P. L.; Shuman, H.
Year: 2006
Title: Functional and trafficking defects in ATP binding cassette A3 mutants associated with respiratory distress syndrome
Journal: J Biol Chem
Volume: 281
Issue: 14
Pages: 9791-800
Abstract: Members of the ATP binding cassette (ABC) protein superfamily actively transport a wide range of substrates across cell and intracellular membranes. Mutations in ABCA3, a member of the ABCA subfamily with unknown function, lead to fatal respiratory distress syndrome (RDS) in the newborn. Using cultured human lung cells, we found that recombinant wild-type hABCA3 localized to membranes of both lysosomes and lamellar bodies, which are the intracellular storage organelles for surfactant. In contrast, hABCA3 with mutations linked to RDS failed to target to lysosomes and remained in the endoplasmic reticulum as unprocessed forms. Treatment of those cells with the chemical chaperone sodium 4-phenylbutyrate could partially restore trafficking of mutant ABCA3 to lamellar body-like structures. Expression of recombinant ABCA3 in non-lung human embryonic kidney 293 cells

induced formation of lamellar body-like vesicles that contained lipids. Small interfering RNA knockdown of endogenous hABCA3 in differentiating human fetal lung alveolar type II cells resulted in abnormal, lamellar bodies comparable with those observed in vivo with mutant ABCA3. Silencing of ABCA3 expression also reduced vesicular uptake of surfactant lipids phosphatidylcholine, sphingomyelin, and cholesterol but not phosphatidylethanolamine. We conclude that ABCA3 is required for lysosomal loading of phosphatidylcholine and conversion of lysosomes to lamellar body-like structures.

Reference Type: Journal Article
Record Number: 56
Author: Gore, S. D.; Baylin, S.; Sugar, E.; Carraway, H.; Miller, C. B.; Carducci, M.; Grever, M.; Galm, O.; Dauses, T.; Karp, J. E.; Rudek, M. A.; Zhao, M.; Smith, B. D.; Manning, J.; Jiemjit, A.; Dover, G.; Mays, A.; Zwiebel, J.; Murgo, A.; Weng, L. J.; Herman, J. G.
Year: 2006
Title: Combined DNA methyltransferase and histone deacetylase inhibition in the treatment of myeloid neoplasms
Journal: Cancer Res
Volume: 66
Issue: 12
Pages: 6361-9
Abstract: Optimal reexpression of most genes silenced through promoter methylation requires the sequential application of DNA methyltransferase inhibitors followed by histone deacetylase inhibitors in tumor cell cultures. Patients with myelodysplastic syndrome or acute myeloid leukemia (AML) were treated with the methyltransferase inhibitor 5-azacitidine (aza-CR) followed by the histone deacetylase inhibitor sodium phenylbutyrate. Major responses associated with cytogenetic complete response developed in patients receiving prolonged dosing schedules of aza-CR. Bisulfite sequencing of the p15 promoter in marrow DNA during the first cycle of treatment showed heterogeneous allelic demethylation in three responding patients, suggesting ongoing demethylation within the tumor clone, but no demethylation in two nonresponders. Six of six responding patients with pretreatment methylation of p15 or CDH-1 promoters reversed methylation during the first cycle of therapy (methylation-specific PCR), whereas none of six nonresponders showed any demethylation. Gene demethylation correlated with the area under the aza-CR plasma concentration-time curve. Administration of both drugs was associated with induction of acetylation of histones H3 and H4. This study provides the first demonstration that molecular mechanisms responsible for responses to DNA methyltransferase/histone deacetylase inhibitor combinations may include reversal of aberrant epigenetic gene silencing. The promising percentage of major hematologic responses justifies the testing of such combinations in prospective randomized trials.

Reference Type: Journal Article
Record Number: 43
Author: Hsu, B. R.; Chen, S. T.; Fu, S. H.
Year: 2006
Title: Enhancing engraftment of islets using perioperative sodium 4-phenylbutyrate

Journal: Int Immunopharmacol
Volume: 6
Issue: 13-14
Pages: 1952-9
Abstract: Primary nonfunction (PNF) adversely impacts islet transplantation. In addition to determining whether sodium 4-phenylbutyrate (4-SPB), an anti-inflammatory agent, reduces PNF, this study investigates how 4-SPB affects PNF. Streptozotocin-induced diabetic C57BL/6 mice, that received 75 syngeneic islets underneath left subrenal space, were fed twice daily of either 4-SPB at 500 mg/kg body weight or isotonic saline (NaCl) from 2 days before through 7 days after transplantation. The graft was removed at days 3, 10 and 84 following transplantation. At 68 h following transplantation, serum levels of interleukin-1beta (IL-1beta) were 2.2+/-0.4 and 0.4+/-0.2 pmol/L (n=6, p<0.005) for NaCl and 4-SPB groups, respectively. Graft genetic expression of IL-1beta was significantly suppressed in 4-SPB group (p<0.01). At day 10, the blood glucose levels were 22.7+/-1.0 and 17.1+/-1.7 mmol/L (n=12, p<0.05) and graft insulin contents (IC) were 35.0+/-8.3 and 107.6+/-29.7 pmol (n=12, p<0.05) for NaCl and 4-SPB groups, respectively. Moreover, the 4-SPB group had a shorter temporary hyperglycemia (15+/-2, n=21 vs. 25+/-2 days, n=19, p=0.001) and a higher cumulative cure rate of diabetes (p<0.001) than the NaCl group. In-vitro studies indicated that 4-SPB did not impact the islets function. These experimental results demonstrated that perioperative administration of 4-SPB decreased serum level and graft genetic expression of IL-1beta and attenuated PNF, which enhanced islet engraftment in a syngeneic transplantation mouse model.

Reference Type: Journal Article
Record Number: 55
Author: Kerem, E.
Year: 2006
Title: Mutation specific therapy in CF
Journal: Paediatr Respir Rev
Volume: 7 Suppl 1
Pages: S166-9
Abstract: CFTR mutations cause defects of CFTR protein production and function by different molecular mechanisms. The mutations can be classified according to the mechanisms by which mutations disrupt CFTR function. This understanding of the different molecular mechanism of CFTR dysfunction provides the scientific basis for development of targeted drugs for mutation specific therapy of CF. Class I mutations are nonsense mutations that result in the presence of premature stop codon that leads to the production of unstable mRNA or the release from the ribosome of a short truncated protein that is not functional. The aminoglycoside antibiotics can suppress premature termination codons by disrupting translational fidelity and allowing the incorporation of an amino acid, thus permitting translation to continue to the normal termination of the transcript. Class II mutations cause impairment of CFTR processing and folding in the Golgi. As a result the mutant CFTR is retained in the ER and eventually targeted for degradation by the quality control mechanisms. Chemical and molecular chaperons such as Sodium-4-phenylbutyrate can stabilize protein structure, and allow it to escape from degradation in the ER and be transported to the cell membrane. Class III mutations disrupt the function of the regulatory domain. CFTR is resistant to phosphorylation or ATP binding. CFTR activators such as

alkylxanthines (CPX) and the flavonoid genistein can overcome the affected ATP binding through direct binding to a nucleotide binding fold. In patients carrying class IV mutations, phosphorylation of CFTR results in reduced chloride transport. Increases in the overall cell surface content of these mutants might overcome the relative reduction in conductance. Alternatively restoring native chloride pore characteristics pharmacologically might be effective. Activators of CFTR at the plasma membrane may function by promoting CFTR phosphorylation, by blocking CFTR dephosphorylation, by interacting directly with CFTR, and/or by modulation of CFTR protein-protein interactions. Class V mutations affect the spicing machinery and generate both aberrantly and correctly spliced transcripts, the level of which vary among different patients and among different organs of the same patient. Splicing factors that promote exon inclusion or factors that promote exon skipping can promote increase of correctly spliced transcripts, depending on the molecular defect. Inconsistent results were reported regarding the required level of corrected or mutated CFTR that has to be reached in order to achieve normal function.

Reference Type: Journal Article
Record Number: 60
Author: Kubota, K.; Niinuma, Y.; Kaneko, M.; Okuma, Y.; Sugai, M.; Omura, T.; Uesugi, M.; Uehara, T.; Hosoi, T.; Nomura, Y.
Year: 2006
Title: Suppressive effects of 4-phenylbutyrate on the aggregation of Pael receptors and endoplasmic reticulum stress
Journal: J Neurochem
Volume: 97
Issue: 5
Pages: 1259-68
Abstract: Endoplasmic reticulum (ER) stress is defined as an accumulation of unfolded proteins in the endoplasmic reticulum. 4-phenylbutyrate (4-PBA) has been demonstrated to promote the normal trafficking of the DeltaF508 cystic fibrosis transmembrane conductance regulator (CFTR) mutant from the ER to the plasma membrane and to restore activity. We have reported that 4-PBA protected against cerebral ischemic injury and ER stress-induced neuronal cell death. In this study, we revealed that 4-PBA possesses chemical chaperone activity in vitro, which prevents the aggregation of denatured alpha-lactalbumin and bovine serum albumin (BSA). Furthermore, we investigated the effects of 4-PBA on the accumulation of Parkin-associated endothelin receptor-like receptor (Pael-R) pathologically relevant to the loss of dopaminergic neurons in autosomal recessive juvenile parkinsonism (AR-JP). Interestingly, 4-PBA restored the normal expression of Pael-R protein and suppressed ER stress induced by the overexpression of Pael-R. In addition, we showed that 4-PBA attenuated the activation of ER stress-induced signal transduction pathways and subsequent neuronal cell death. Moreover, 4-PBA restored the viability of yeasts that fail to induce an ER stress response under ER stress conditions. These results suggest that 4-PBA suppresses ER stress by directly reducing the amount of misfolded protein, including Pael-R accumulated in the ER.

Reference Type: Journal Article
Record Number: 51

Author: Kulp, S. K.; Chen, C. S.; Wang, D. S.; Chen, C. Y.; Chen, C. S.
Year: 2006
Title: Antitumor effects of a novel phenylbutyrate-based histone deacetylase inhibitor, (S)-HDAC-42, in prostate cancer
Journal: Clin Cancer Res
Volume: 12
Issue: 17
Pages: 5199-206
Abstract: PURPOSE: To assess the antitumor effects of a novel phenylbutyrate-derived histone deacetylase (HDAC) inhibitor, (S)-HDAC-42, vis-a-vis suberoylanilide hydroxamic acid (SAHA) in in vitro and in vivo models of human prostate cancer. EXPERIMENTAL DESIGN: The in vitro effects of (S)-HDAC-42 and SAHA were evaluated in PC-3, DU-145, or LNCaP human prostate cancer cell lines. Cell viability, apoptosis, and indicators of HDAC inhibition were assessed. Effects on Akt and members of the Bcl-2 and inhibitor of apoptosis protein families were determined by immunoblotting. Immunocompromised mice bearing established s.c. PC-3 xenograft tumors were treated orally with (S)-HDAC-42 (50 mg/kg q.o.d. or 25 mg/kg q.d.) or SAHA (50 mg/kg q.d.) for 28 days. In vivo end points included tumor volumes and intratumoral changes in histone acetylation, phospho-Akt status, and protein levels of Bcl-xL and survivin. RESULTS: (S)-HDAC-42 was more potent than SAHA in suppressing the viability of all cell lines evaluated with submicromolar IC50 values. Relative to SAHA, (S)-HDAC-42 exhibited distinctly superior apoptogenic potency, and caused markedly greater decreases in phospho-Akt, Bcl-xL, and survivin in PC-3 cells. The growth of PC-3 tumor xenografts was suppressed by 52% and 67% after treatment with (S)-HDAC-42 at 25 and 50 mg/kg, respectively, whereas SAHA at 50 mg/kg suppressed growth by 31%. Intratumoral levels of phospho-Akt and Bcl-xL were markedly reduced in (S)-HDAC-42-treated mice, in contrast to mice treated with SAHA. CONCLUSIONS: (S)-HDAC-42 is a potent orally bioavailable inhibitor of HDAC, as well as targets regulating multiple aspects of cancer cell survival, which might have clinical value in prostate cancer chemotherapy and warrants further investigation in this regard.

Reference Type: Journal Article
Record Number: 53
Author: Leng, Y.; Chuang, D. M.
Year: 2006
Title: Endogenous alpha-synuclein is induced by valproic acid through histone deacetylase inhibition and participates in neuroprotection against glutamate-induced excitotoxicity
Journal: J Neurosci
Volume: 26
Issue: 28
Pages: 7502-12
Abstract: Emerging evidence suggests that alpha-synuclein (alpha-syn), which is traditionally thought to have a pathophysiological role in neurodegenerative diseases, can have neuroprotective effects. This study aimed to investigate whether endogenous alpha-syn in neurons can be induced by valproic acid (VPA), a mood-stabilizer, anticonvulsant and histone deacetylase (HDAC) inhibitor, and if so, whether the alpha-syn induction is neuroprotective. VPA treatment of rat cerebellar granule cells

caused a robust dose- and time-dependent increase in levels of alpha-syn protein and mRNA and in the intensity of alpha-syn immunostaining. Knockdown of VPA-induced alpha-syn overexpression with alpha-syn antisense oligonucleotides or siRNA completely blocked VPA-induced neuroprotection. alpha-Syn knockdown also exacerbated glutamate neurotoxicity, stimulated the expression of the proapoptotic gene ubiquitin-conjugating enzyme E2N, and downregulated the expression of the anti-apoptotic gene Bcl-2. Induction of alpha-syn by VPA was associated with inhibition of HDAC activity, resulting in hyperacetylation of histone H3 in the alpha-syn promoter and a marked increase in alpha-syn promoter activity. Moreover, VPA-induced alpha-syn induction and neuroprotection were mimicked by HDAC inhibitors sodium 4-phenylbutyrate and trichostatin A (TSA). alpha-syn was also induced by VPA in rat cerebral cortical neurons. Additionally, treatment of rats with VPA, sodium butyrate, or TSA markedly increased alpha-syn protein levels in the cortex and cerebellum. Together, our results demonstrate for the first time that VPA induces alpha-syn in neurons through inhibition of HDAC and that this alpha-syn induction is critically involved in neuroprotection against glutamate excitotoxicity. Clinically, VPA may represent a suitable treatment for excitotoxicity-related neurodegenerative diseases.

Reference Type: Journal Article
Record Number: 66
Author: Maslak, P.; Chanel, S.; Camacho, L. H.; Soignet, S.; Pandolfi, P. P.; Guernah, I.; Warrell, R.; Nimer, S.
Year: 2006
Title: Pilot study of combination transcriptional modulation therapy with sodium phenylbutyrate and 5-azacytidine in patients with acute myeloid leukemia or myelodysplastic syndrome
Journal: Leukemia
Volume: 20
Issue: 2
Pages: 212-7
Abstract: Epigenetic mechanisms underlying tumorigenesis have recently received much attention as potential therapeutic targets of human cancer. We designed a pilot study to target DNA methylation and histone deacetylation through the sequential administration of 5-azacytidine followed by sodium phenylbutyrate (PB) in patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). Ten evaluable patients (eight AML, two MDS) were treated with seven consecutive daily subcutaneous injections of 5-azacytidine at 75 mg/m2 followed by 5 days of sodium PB given intravenously at a dose of 200 mg/kg. Five patients (50%) were able to achieve a beneficial clinical response (partial remission or stable disease). One patient with MDS proceeded to allogeneic stem cell transplantation and is alive without evidence of disease 39 months later. The combination regimen was well tolerated with common toxicities of injection site skin reaction (90% of the patients) from 5-azacytidine, and somnolence/fatigue from the sodium PB infusion (80% of the patients). Correlative laboratory studies demonstrated the consistent reacetylation of histone H4, although no relationship with the clinical response could be demonstrated. Results from this pilot study demonstrate that a combination approach targeting different mechanisms of transcriptional modulation is clinically feasible with acceptable toxicity and measurable biologic and clinical outcomes.

Reference Type: Journal Article
Record Number: 63
Author: Nguyen, T. D.; Kim, U. S.; Perrine, S. P.
Year: 2006
Title: Novel short chain fatty acids restore chloride secretion in cystic fibrosis
Journal: Biochem Biophys Res Commun
Volume: 342
Issue: 1
Pages: 245-52
Abstract: Phenylalanine deletion at position 508 of the cystic fibrosis transmembrane conductance regulator (DeltaF508-CFTR), the most common mutation in cystic fibrosis (CF), causes a misfolded protein exhibiting partial chloride conductance and impaired trafficking to the plasma membrane. 4-Phenylbutyrate corrects defective DeltaF508-CFTR trafficking in vitro, but is not clinically efficacious. From a panel of short chain fatty acid derivatives, we showed that 2,2-dimethyl-butyrate (ST20) and alpha-methylhydrocinnamic acid (ST7), exhibiting high oral bioavailability and sustained plasma levels, correct the DeltaF508-CFTR defect. Pre-incubation (>or=6h) of CF IB3-1 airway cells with >or=1mM ST7 or ST20 restored the ability of 100microM forskolin to stimulate an (125)I(-) efflux. This efflux was fully inhibited by NPPB, DPC, or glibenclamide, suggesting mediation through CFTR. Partial inhibition by DIDS suggests possible contribution from an additional Cl(-) channel regulated by CFTR. Thus, ST7 and ST20 offer treatment potential for CF caused by the DeltaF508 mutation.

Reference Type: Journal Article
Record Number: 67
Author: Petri, S.; Kiaei, M.; Kipiani, K.; Chen, J.; Calingasan, N. Y.; Crow, J. P.; Beal, M. F.
Year: 2006
Title: Additive neuroprotective effects of a histone deacetylase inhibitor and a catalytic antioxidant in a transgenic mouse model of amyotrophic lateral sclerosis
Journal: Neurobiol Dis
Volume: 22
Issue: 1
Pages: 40-9
Abstract: ALS is a devastating neurodegenerative disorder for which no effective treatment exists. Multiple molecular mechanisms are involved in the pathogenesis. We tested the catalytic antioxidant AEOL 10150, the histone deacetylase inhibitor phenylbutyrate (PBA), and the combination of PBA and AEOL 10150 in the G93A transgenic mouse model, administered from disease onset. AEOL 10150 alone improved motor function and extended survival by 11%, PBA alone significantly improved motor function and extended survival by 13%. PBA and AEOL 10150 together increased survival by 19%. Increased histone acetylation was confirmed by Western blot. Quantitative real-time RT-PCR analysis revealed upregulation of compounds capable of protecting cells against oxidative stress and apoptosis. Markers of oxidative damage were reduced in the lumbar spinal cord as compared to vehicle administration. These results suggest that agents inhibiting apoptosis and blocking

oxidative stress show efficacy in treating mutant-SOD1-associated ALS and that a combination of agents targeting different disease mechanisms may exert additive therapeutic effects.

Reference Type: Journal Article
Record Number: 44
Author: Schniewind, B.; Heintz, K.; Kurdow, R.; Ammerpohl, O.; Trauzold, A.; Emme, D.; Dohrmann, P.; Kalthoff, H.
Year: 2006
Title: Combination phenylbutyrate/gemcitabine therapy effectively inhibits in vitro and in vivo growth of NSCLC by intrinsic apoptotic pathways
Journal: J Carcinog
Volume: 5
Pages: 25
Abstract: BACKGROUND: Standard chemotherapy protocols in NSCLC are of limited clinical benefit. Histone deacetylase (HDAC) inhibitors represent a new strategy in human cancer therapy. In this study the combination of the HDAC inhibitor phenylbutyrate (PB) and the nucleoside analogue gemcitabine (GEM) was evaluated and the mechanisms underlying increased cell death were analyzed. METHODS: Dose escalation studies evaluating the cytotoxicity of PB (0.01-100 mM), GEM (0.01-100 microg/ml) and a combination of the two were performed on two NSCLC cell lines (BEN and KNS62). Apoptotic cell death was quantified. The involvement of caspase-dependent cell death and MAP-kinase activation was analyzed. Additionally, mitochondrial damage was determined. In an orthotopic animal model the combined effect of PB and GEM on therapy was analyzed. RESULTS: Applied as a single drug both GEM and PB revealed limited potential to induce apoptosis in KNS62 and Ben cells. Combination therapy was 50-80% ($p = 0.012$) more effective than either agent alone. On the caspase level, combination therapy significantly increased cleavage of the pro-forms compared to single chemotherapy. The broad spectrum caspase-inhibitor zVAD was able to inhibit caspase cleavage completely, but reduced the frequency of apoptotic cells only by 30%. Combination therapy significantly increased changes in MTP and the release of cyto-c, AIF and Smac/Diabolo into the cytoplasm. Furthermore, the inhibitors of apoptosis c-IAP1 and c-IAP2 were downregulated and it was shown that in combination therapy JNK activation contributed significantly to induction of apoptosis. The size of the primary tumors growing orthotopically in SCID mice treated for 4 weeks with GEM and PB was significantly reduced (2.2-2.7 fold) compared to GEM therapy alone. The Ki-67 (KNS62: $p = 0.015$; Ben: $p = 0.093$) and topoisomerase IIalpha (KNS62: $p = 0.008$; Ben: $p = 0.064$) proliferation indices were clearly reduced in tumors treated by combination therapy, whereas the apoptotic index was comparably low in all groups. CONCLUSION: Therapy combining GEM and the HDAC inhibitor PB initiates a spectrum of apoptosis-inducing mitochondrial and further JNK-dependent events, thereby overcoming the therapeutic resistance of NSCLC tumor cells. In vivo, the combination therapy substantially reduced tumor cell proliferation, suggesting that the well tolerated PB is a useful supplemental therapeutic agent in NSCLC.

Reference Type: Journal Article

Record Number: 61
Author: Singh, O. V.; Vij, N.; Mogayzel, P. J., Jr.; Jozwik, C.; Pollard, H. B.; Zeitlin, P. L.
Year: 2006
Title: Pharmacoproteomics of 4-phenylbutyrate-treated IB3-1 cystic fibrosis bronchial epithelial cells
Journal: J Proteome Res
Volume: 5
Issue: 3
Pages: 562-71
Abstract: 4-Phenylbutyrate (4-PBA) is an oral butyrate derivative that has recently been approved for treatment of urea cycle disorders and is under investigation in clinical trials of cancer, hemoglobinopathies, and cystic fibrosis (CF). We hypothesized that proteome profiling of IB3-1 cystic fibrosis bronchial epithelial cells treated with 4-PBA would identify butyrate-responsive cellular chaperones, protein processing enzymes, and cell trafficking molecules associated with the amelioration of the chloride transport defect in these cells. Protein profiles were analyzed by two-dimensional gel electrophoresis and mass spectrometry. Over a pI range of 4-7 and molecular weight from 20 to 150 kDa a total of 85 differentially expressed proteins were detected. Most of the identified proteins were chaperones, catalytic enzymes, and proteins comprising structural elements, cellular defense, protein biosynthesis, trafficking activity, and ion transport. Subsets of these proteins were confirmed by immunoblot analysis. These data represent a first-draft of the pharmacoproteomics map of 4-PBA treated cystic fibrosis bronchial epithelial cells.

Reference Type: Journal Article
Record Number: 54
Author: Traynor, B. J.; Bruijn, L.; Conwit, R.; Beal, F.; O'Neill, G.; Fagan, S. C.; Cudkowicz, M. E.
Year: 2006
Title: Neuroprotective agents for clinical trials in ALS: a systematic assessment
Journal: Neurology
Volume: 67
Issue: 1
Pages: 20-7
Abstract: BACKGROUND: Riluzole is currently the only Food and Drug Administration-approved treatment for ALS, but its effect on survival is modest. OBJECTIVE: To identify potential neuroprotective agents for testing in phase III clinical trials and to outline which data need to be collected for each drug. METHODS: The authors identified 113 compounds by inviting input from academic clinicians and researchers and via literature review to identify agents that have been tested in ALS animal models and in patients with ALS. The list was initially narrowed to 24 agents based on an evaluation of scientific rationale, toxicity, and efficacy in previous animal and human studies. These 24 drugs underwent more detailed pharmacologic evaluation. RESULTS: Twenty drugs were selected as suitable for further development as treatments for patients with ALS. Talampanel and tamoxifen have completed early phase II trials and have demonstrated preliminary efficacy. Other agents (ceftriaxone, minocycline, ONO-2506, and IGF-1 polypeptide) are already in phase III trials involving large numbers of patients with ALS. Remaining

agents (AEOL 10150, arimoclomol, celastrol, coenzyme Q10, copaxone, IGF-1-viral delivery, memantine, NAALADase inhibitors, nimesulide, scriptaid, sodium phenylbutyrate, thalidomide, trehalose) require additional preclinical animal data, human toxicity and pharmacokinetic data including CNS penetration prior to proceeding to large scale phase III human testing. Further development of riluzole analogues should be considered. CONCLUSIONS: Several potential neuroprotective compounds, representing a wide range of mechanisms, are available and merit further investigation in ALS.

Reference Type: Journal Article
Record Number: 59
Author: Vij, N.; Fang, S.; Zeitlin, P. L.
Year: 2006
Title: Selective inhibition of endoplasmic reticulum-associated degradation rescues DeltaF508-cystic fibrosis transmembrane regulator and suppresses interleukin-8 levels: therapeutic implications
Journal: J Biol Chem
Volume: 281
Issue: 25
Pages: 17369-78
Abstract: Endoplasmic reticulum (ER)-associated degradation (ERAD) is the major quality control pathway of the cell. The most common disease-causing protein folding mutation, DeltaF508-cystic fibrosis transmembrane regulator (CFTR), is destroyed by ERAD to cause cystic fibrosis (CF). p97/valosin-containing protein (VCP) physically interacts with gp78/autocrine motility factor receptor to couple ubiquitination, retrotranslocation, and proteasome degradation of misfolded proteins. We show here that p97/VCP and gp78 form complexes with CFTR during translocation from the ER for degradation by the cytosolic proteasome. Interference in the VCP-CFTR complex promoted accumulation of immature CFTR in the ER and partial rescue of functional chloride channels to the cell surface. Moreover, under these conditions, interleukin-8 (IL8), the expression of which is regulated by the proteasome, was reduced. Inhibition of the proteasome with bortezomib (PS-341/Velcade) also rescued CFTR, but with less efficiency, and suppressed NFkappaB-mediated IL8 activation. The inhibition of the major stress-inducible transcription factor CHOP (CCAAT/enhancer-binding protein homologous protein)/GADD153 together with bortezomib was most effective in repressing NFkappaB-mediated IL8 activation compared with interference of VCP, MLN-273 (proteasome inhibitor), or 4-phenylbutyrate (histone deacetylase inhibitor). Immunoprecipitation of DeltaF508-CFTR from primary CF bronchial epithelial cells confirmed the interaction with VCP and associated chaperones in CF. We conclude that VCP is an integral component of ERAD and cellular stress pathways induced by the unfolded protein response and may be central to the efficacy of CF drugs that target the ubiquitin-proteasome pathway.

Reference Type: Journal Article
Record Number: 57
Author: Vila-Carriles, W. H.; Kovacs, G. G.; Jovov, B.; Zhou, Z. H.; Pahwa, A. K.; Colby, G.; Esimai, O.; Gillespie, G. Y.; Mapstone, T. B.; Markert, J. M.; Fuller, C. M.; Bubien, J. K.; Benos, D. J.

Year: 2006
Title: Surface expression of ASIC2 inhibits the amiloride-sensitive current and migration of glioma cells
Journal: J Biol Chem
Volume: 281
Issue: 28
Pages: 19220-32
Abstract: Gliomas are primary brain tumors with a complex biology characterized by antigenic and genomic heterogeneity and a propensity for invasion into normal brain tissue. High grade glioma cells possess a voltage-independent, amiloride-inhibitable, inward Na+ current. This current does not exist in normal astrocytes or low grade tumor cells. Inhibition of this conductance decreases glioma growth and cell migration making it a potential therapeutic target. Our previous results have shown that the acid-sensing ion channels (ASICs), members of the epithelial Na+ channel (ENaC)/degenerin (DEG) family of ion channels are part of this current pathway. We hypothesized that one member of the ENaC/DEG family, ASIC2, is retained intracellularly and that it is the lack of functional expression of ASIC2 at the cell surface that results in hyperactivity of this conductance in high grade gliomas. In this study we show that the chemical chaperone, glycerol, and the transcriptional regulator, sodium 4-phenylbutyrate, inhibit the constitutively activated inward current and reduce cell growth and migration in glioblastoma multiforme. The results suggest that these compounds induce the movement of ASIC2 to the plasma membrane, and once there, the basally active inward current characteristic of glioma cells is abolished by inherent negative regulatory mechanisms. This in turn compromises the ability of the glioma cell to migrate and proliferate. These results support the hypothesis that the conductance pathway in high grade glioma cells is comprised of ENaC/DEG subunits and that abolishing this channel activity promotes a reversion of a high grade glioma cell to a phenotype resembling that of normal astrocytes.

Reference Type: Journal Article
Record Number: 48
Author: Wirth, B.; Brichta, L.; Hahnen, E.
Year: 2006
Title: Spinal muscular atrophy and therapeutic prospects
Journal: Prog Mol Subcell Biol
Volume: 44
Pages: 109-32
Abstract: The molecular genetic basis of spinal muscular atrophy (SMA), an autosomal recessive neuromuscular disorder, is the loss of function of the survival motor neuron gene (SMN1). The SMN2 gene, a nearly identical copy of SMN1, has been detected as a promising target for SMA therapy. Both genes are ubiquitously expressed and encode identical proteins, but markedly differ in their splicing patterns: While SMN1 produces full-length (FL)-SMN transcripts only, the majority of SMN2 transcripts lacks exon 7. Transcriptional SMN2 activation or modulation of its splicing pattern to increase FL-SMN levels is believed to be clinically beneficial and therefore a crucial challenge in SMA research. Drugs such as valproic acid, phenylbutyrate, sodium butyrate, M344 and SAHA that mainly act as histone deacetylase inhibitors can mediate both: they stimulate the SMN2 gene transcription and/or restore the splicing pattern, thereby elevating the levels of FL-SMN2 protein.

Preliminary phase II clinical trials and individual experimental curative approaches SMA patients show promising results. However, phase III double-blind placebo controlled clinical trials have to finally prove the efficacy of these drugs.

Reference Type: Journal Article
Record Number: 50
Author: Wirth, B.; Brichta, L.; Hahnen, E.
Year: 2006
Title: Spinal muscular atrophy: from gene to therapy
Journal: Semin Pediatr Neurol
Volume: 13
Issue: 2
Pages: 121-31
Abstract: The molecular basis of spinal muscular atrophy (SMA), an autosomal recessive neuromuscular disorder, is the homozygous loss of the survival motor neuron gene 1 (SMN1). A nearly identical copy of the SMN1 gene, called SMN2, modulates the disease severity. The functional difference between both genes is a translationally silent mutation that, however, disrupts an exonic splicing enhancer causing exon 7 skipping in most SMN2 transcripts. Only 10% of SMN2 transcripts encode functional full-length protein identical to SMN1. Transcriptional activation, facilitation of correct SMN2 splicing, or stabilization of the protein are considered as strategies for SMA therapy. Among various drugs, histone deacetylase inhibitors such as valproic acid (VPA) or 4-phenylbutyrate (PBA) have been shown to increase SMN2-derived RNA and protein levels. Recently, in vivo activation of the SMN gene was shown in VPA-treated SMA patients and carriers. Clinical trials are underway to investigate the effect of VPA and PBA on motor function in SMA patients.

Reference Type: Journal Article
Record Number: 62
Author: Wirth, B.; Brichta, L.; Schrank, B.; Lochmuller, H.; Blick, S.; Baasner, A.; Heller, R.
Year: 2006
Title: Mildly affected patients with spinal muscular atrophy are partially protected by an increased SMN2 copy number
Journal: Hum Genet
Volume: 119
Issue: 4
Pages: 422-8
Abstract: Spinal muscular atrophy (SMA) is a recessive neuromuscular disorder caused by loss of the SMN1 gene. The clinical distinction between SMA type I to IV reflects different age of onset and disease severity. SMN2, a nearly identical copy gene of SMN1, produces only 10% of full-length SMN RNA/protein and is an excellent target for a potential therapy. Several clinical trials with drugs that increase the SMN2 expression such as valproic acid and phenylbutyrate are in progress. Solid natural history data for SMA are crucial to enable a correlation between genotype and phenotype as well as the outcome of therapy. We provide genotypic and phenotypic data from 115 SMA patients with type IIIa (age of onset <3 years), type IIIb (age of onset >3 years) and rare type IV (onset >30 years). While 62% of type IIIa patients

carry two or three SMN2 copies, 65% of type IIIb patients carry four or five SMN2 copies. Three type IV SMA patients had four and one had six SMN2 copies. Our data support the disease-modifying role of SMN2 leading to later onset and a better prognosis. A statistically significant correlation for > or =4 SMN2 copies with SMA type IIIb or a milder phenotype suggests that SMN2 copy number can be used as a clinical prognostic indicator in SMA patients. The additional case of a foetus with homozygous SMN1 deletion and postnatal measurement of five SMN2 copies illustrates the role of genotypic information in making informed decisions on the management and therapy of such patients.

Reference Type: Journal Article
Record Number: 41
Author: Ammerpohl, O.; Trauzold, A.; Schniewind, B.; Griep, U.; Pilarsky, C.; Grutzmann, R.; Saeger, H. D.; Janssen, O.; Sipos, B.; Kloppel, G.; Kalthoff, H.
Year: 2007
Title: Complementary effects of HDAC inhibitor 4-PB on gap junction communication and cellular export mechanisms support restoration of chemosensitivity of PDAC cells
Journal: Br J Cancer
Volume: 96
Issue: 1
Pages: 73-81
Abstract: Pancreatic ductal adenocarcinoma (PDAC) is a fatal disease and one of the cancer entities with the lowest life expectancy. Beside surgical therapy, no effective therapeutic options are available yet. Here, we show that 4-phenylbutyrate (4-PB), a known and well-tolerable inhibitor of histone deacetylases (HDAC), induces up to 70% apoptosis in all cell lines tested (Panc 1, T4M-4, COLO 357, BxPc3). In contrast, it leads to cell cycle arrest in only half of the cell lines tested. This drug increases gap junction communication between adjacent T3M-4 cells in a concentration-dependent manner and efficiently inhibits cellular export mechanisms in Panc 1, T4M-4, COLO 357 and BxPc3 cells. Consequently, in combination with gemcitabine 4-PB shows an overadditive effect on induction of apoptosis in BxPc3 and T3M-4 cells (up to 4.5-fold compared to single drug treatment) with accompanied activation of Caspase 8, BH3 interacting domain death agonist (Bid) and poly (ADP-ribose) polymerase family, member 1 (PARP) cleavage. Although the inhibition of the mitogen-activated protein kinase-pathway has no influence on fulminant induction of apoptosis, the inhibition of the JNK-pathway by SP600125 completely abolishes the overadditive effect induced by the combined application of both drugs, firstly reported by this study.

Reference Type: Journal Article
Record Number: 49
Author: Camacho, L. H.; Olson, J.; Tong, W. P.; Young, C. W.; Spriggs, D. R.; Malkin, M. G.
Year: 2007
Title: Phase I dose escalation clinical trial of phenylbutyrate sodium administered twice daily to patients with advanced solid tumors
Journal: Invest New Drugs

Volume: 25
Issue: 2
Pages: 131-8
Abstract: BACKGROUND: Phenylbutyrate (PBA), and its metabolite phenylacetate (PAA), induce growth inhibition and cellular differentiation in multiple tumor models. However, despite their potential anti-cancer properties, several pharmacodynamic aspects remain unknown. METHODS: We conducted a dose escalating trial to evaluate twice-daily intravenous PBA infusions for two consecutive weeks (Monday through Friday) every month at five dose levels (60-360 mg/kg/day). Twenty-one patients with the following malignancies were treated: colon carcinoma 4, non-small cell lung carcinoma 4; anaplastic astrocytoma 3, glioblastoma multiforme 3, bladder carcinoma 2, sarcoma 2, and ovarian carcinoma, rectal hemangiopericytoma, and pancreatic carcinoma 1 each. RESULTS: Conversion of PBA to PAA and phenylacetylglutamine (PAG) was documented without catabolic saturation. Plasma content of PBA > or =1 mM was documented for only 3 h following each dose at the top two dosages. The therapy was well tolerated overall. Common adverse effects included grade 1 nausea/vomiting, fatigue, and lightheadedness. Dose limiting toxicities were short-term memory loss, sedation, confusion, nausea, and vomiting. Two patients with anaplastic astrocytoma and a patient with glioblastoma remained stable without tumor progression for 5, 7, and 4 months respectively. CONCLUSIONS: Administration of PBA in a twice-daily infusion schedule is safe. The maximum tolerated dose is 300 mg/kg/day. Study designs with more convenient treatment schedules and specific molecular correlates may help to further delineate the mechanism of action of this compound. Future studies evaluating PBA's ability to induce histone acetylation and cell differentiation alone or in combination with other anti-neoplastics are recommended.

Reference Type: Journal Article
Record Number: 20
Author: Caruthers, R. L.; Johnson, C. E.
Year: 2007
Title: Stability of extemporaneously prepared sodium phenylbutyrate oral suspensions
Journal: Am J Health Syst Pharm
Volume: 64
Issue: 14
Pages: 1513-5
Abstract: PURPOSE: In an effort to minimize barriers to compliance and adherence and to improve the accuracy of dosage measurement, sugar-containing and sugar-free sodium phenylbutyrate suspensions were formulated, and the stability of these products over a 90-day period was determined. METHODS: An oral suspension of sodium phenylbutyrate 200 mg/mL was prepared by thoroughly grinding 12 g of Sodium Phenylbutyrate Powder, USP, in a glass mortar. Thirty milliliters of Ora-Plus and 30 mL of either Ora-Sweet or Ora-Sweet SF were mixed and added to the powder to make a final volume of 60 mL. Three identical samples of each formulation were prepared and placed in 2-oz amber plastic bottles with child-resistant caps and were stored at room temperature. A 500-microL sample was withdrawn from each of the six bottles with a micropipette immediately after preparation and at 7, 14, 28, 60, and 90 days. After further dilution to an expected concentration of 100 microg/mL with

the mobile phase, the samples were assayed by high-performance liquid chromatography. Stability was defined as the retention of at least 90% of the initial concentration. RESULTS: At least 95% of the initial sodium phenylbutyrate concentration remained throughout the 90-day study period in both preparations. There were no detectable changes in color, odor, taste, and pH and no visible microbial growth in any sample. CONCLUSION: Extemporaneously compounded suspensions of sodium phenylbutyrate, 200 mg/mL, in a 1:1 mixture of Ora-Plus and Ora-Sweet or Ora-Sweet SF were stable for at least 90 days when stored in 2-oz amber plastic bottles at room temperature.

Reference Type: Journal Article
Record Number: 10
Author: Christov, K.; Grubbs, C. J.; Shilkaitis, A.; Juliana, M. M.; Lubet, R. A.
Year: 2007
Title: Short-term modulation of cell proliferation and apoptosis and preventive/therapeutic efficacy of various agents in a mammary cancer model
Journal: Clin Cancer Res
Volume: 13
Issue: 18 Pt 1
Pages: 5488-96
Abstract: PURPOSE: The methylnitrosourea (MNU)-induced mammary cancer model in rats is similar to estrogen receptor-positive breast cancer in women. In prevention studies using this model, tumor incidence and multiplicity were typically primary end points. The ability of various agents administered for a short period to modulate cell proliferation [proliferation index (PI)] and apoptosis [apoptotic index (AI)] in mammary cancers was compared with their efficacy in long-term prevention and therapy studies. EXPERIMENTAL DESIGN: Rats were injected with MNU to induce mammary cancers. For the prevention studies, agents were administered by gavage or in the diet beginning 5 days after MNU. For proliferation (PI) and apoptosis (AI) experiments, animals with a palpable mammary cancer were treated with the agents for only 4 to 7 days. PI was determined following 5-bromodeoxyuridine labeling whereas AI was determined using the terminal deoxyribonucleotidyl transferase-mediated dUTP nick end labeling assay. Therapeutic efficacy was evaluated by measuring cancer size over a 6-week period. RESULTS: Treatments with differing chemopreventive efficacy and mechanism(s) of action were examined: (a) hormonal treatments [tamoxifen, vorozole (an aromatase inhibitor), and ovariectomy]; (b) retinoid X receptor agonists (targretin, 9-cis retinoic acid, and UAB30); (c) inducers of drug-metabolizing enzymes (indole-3-carbinol, 5,6 benzoflavone, and diindoylmethane); (d) agents that alter signal transduction (R115777, a farnesyltransferase inhibitor); Iressa (an epidermal growth factor receptor inhibitor); sulindac and celecoxib (cyclooxygenase 1/2 and cyclooxygenase 2 inhibitors); and (e) diverse agents including meclizine, vitamin C, and sodium phenylbutyrate. Correlations between inhibition of PI, increase of AI, and chemopreventive efficacy were observed. Although most agents with moderate or low preventive efficacy suppressed PI, they minimally affected AI. CONCLUSIONS: The data confirmed that the short-term effects of various agents on cell proliferation and apoptosis in small mammary cancers can predict their preventive/therapeutic efficacy. Thus, these biomarkers can be used to help determine the efficacy of compounds in phase II clinical prevention trials.

Reference Type: Journal Article
Record Number: 6
Author: Claus, R.; Ruter, B.; Lubbert, M.
Year: 2007
Title: Targets of epigenetic therapy - Gene reactivation as a novel approach in MDS treatment
Journal: Cancer Treat Rev
Volume: 33 Suppl 1
Pages: S47-52
Abstract: Pathogenesis of myelodysplastic syndromes (MDS) involves epigenetic in addition to genetic aberrations. Gene silencing by epigenetic mechanisms is mainly mediated through variable states of DNA methylation in CpG islands within gene promoter regions in collaboration with post-translational covalent histone modifications, leading to consecutive chromatin remodelling. In patients with MDS, an increasing variety of epigenetically inactivated genes involved in cell growth, cell cycle control, differentiation, DNA repair, and cell death is known. Therefore therapeutic reversal of epigenetic silencing displays an effective treatment strategy for those patients. The use of nucleosidic DNA methyltransferase inhibitors (DNMT) 5-aza-2' deoxycytidine (decitabine) and 5-azacytidine in different low-dose schedules has been shown to have significant activity in the treatment of MDS. Several studies demonstrated haematologic responses including CRs and PRs in up to 50% of patients, making these compounds promising treatment options especially for older patients not eligible for standard induction therapy. Recently, both drugs have been approved by the FDA for MDS treatment. In phase I/II studies using histone deacetylase (HDAC) inhibitors like sodium phenylbutyrate and valproic acid, alone or in combination with DNMTs, activity in MDS patients could also be demonstrated. In conclusion, low-dose schedules of epigenetically active drugs like DNMT and HDAC inhibitors display promising treatment options particularly for elderly MDS patients, also because of a favourable non-haematologic toxicity profile.

Reference Type: Journal Article
Record Number: 1
Author: Cunha De Santis, G.; de Barros Tamarozzi, M.; Sousa, R. B.; Moreno, S. E.; Secco, D.; Garcia, A. B.; Lima, A. S.; Faccioli, L. H.; Falcao, R. P.; Cunha, F. Q.; Rego, E. M.
Year: 2007
Title: Adhesion molecules and Differentiation Syndrome: phenotypic and functional analysis of the effect of ATRA, As2O3, phenylbutyrate, and G-CSF in acute promyelocytic leukemia
Journal: Haematologica
Volume: 92
Issue: 12
Pages: 1615-22
Abstract: BACKGROUND AND OBJECTIVES: Differentiation Syndrome (DS) is a treatment complication which can occur in patients treated with acute promyelocytic leukemia (APL) with all transretinoic acid (ATRA) or As(2)O(3), and is characterized by enhanced leukocyte transmigration. As(2)O(3), Phenylbutyrate (PB) and G-CSF

are known to potentiate ATRA effects. Our aim was to analyze the changes in expression and function of adhesion molecules induced by ATRA, As(2)O(3), G-CSF and PB, and their association. DESIGN AND METHODS: APL blasts and NB4 cells were treated with ATRA, As(2)O(3), PB, G-CSF or their association and the expression of adhesion molecules was determined by flow cytometry. Cell adhesion was evaluated in vitro using Matrigel and for the in vivo analysis, Balb-c mice were injected with NB4 cells pre-treated with ATRA, As(2)O(3), ATRA+G-CSF or ATRA+As(2)O(3). In addition, CD54 and CD18 knock-out mice were injected with NB4 cells and concomitantly treated with ATRA. In both models, the MPO activity in the lungs was determined 6 hours after the injection of the cells. RESULTS: In NB4 and APL blasts, ATRA and As(2)O(3) increased CD54 expression, but no synergism was detected. CD11b and CD18 were also up-regulated by ATRA in primary cells. PB and G-CSF had no effect, but the latter potentiated ATRA-induced CD18 up-regulation. These changes were accompanied by increased adhesion to Matrigel and to lung microvasculature, and reversed by anti-CD54, anti-CD18 antibodies. In CD54 and CD18 knock-out mice the ATRA effect was canceled. INTERPRETATION AND CONCLUSIONS: The use of As(2)O(3), PB and G-CSF in association with ATRA should not aggravate DS in APL.

Reference Type: Journal Article
Record Number: 25
Author: Daosukho, C.; Chen, Y.; Noel, T.; Sompol, P.; Nithipongvanitch, R.; Velez, J. M.; Oberley, T. D.; St Clair, D. K.
Year: 2007
Title: Phenylbutyrate, a histone deacetylase inhibitor, protects against Adriamycin-induced cardiac injury
Journal: Free Radic Biol Med
Volume: 42
Issue: 12
Pages: 1818-25
Abstract: Cardiac injury is a major complication for oxidative-stress-generating anticancer agents exemplified by Adriamycin (ADR). Recently, several histone deacetylase inhibitors (HDACIs) including phenylbutyrate (PBA) have shown promise in the treatment of cancer with little known toxicity to normal tissues. PBA has been shown to protect against oxidative stress in normal tissues. Here, we examined whether PBA might protect heart against ADR toxicity in a mouse model. The mice were i.p. injected with ADR (20 mg/kg). PBA (400 mg/kg/day) was i.p. injected 1 day before and daily after the ADR injection for 2 days. We found that PBA significantly decreased the ADR-associated elevation of serum lactate dehydrogenase and creatine kinase activities and diminished ADR-induced ultrastructural damages of cardiac tissue by more than 70%. Importantly, PBA completely rescued ADR-caused reduction of cardiac functions exemplified by ejection fraction and fraction shortening, and increased cardiac manganese superoxide dismutase (MnSOD) protein and activity. Our results reveal a previously unrecognized role of HDACIs in protecting against ADR-induced cardiac injury and suggest that PBA may exert its cardioprotective effect, in part, by the increase of MnSOD. Thus, combining HDACIs with ADR could add a new mechanism to fight cancer while simultaneously decrease ADR-induced cardiotoxicity.

Reference Type: Journal Article
Record Number: 4
Author: Darras, B. T.; Kang, P. B.
Year: 2007
Title: Clinical trials in spinal muscular atrophy
Journal: Curr Opin Pediatr
Volume: 19
Issue: 6
Pages: 675-679
Abstract: PURPOSE OF REVIEW: Spinal muscular atrophy is a neuromuscular disorder manifesting as weakness and hypotonia across a broad spectrum of severity. Mutations in the telomeric copy of the survival motor neuron gene (SMN1) cause the autosomal recessive form. Disease severity is modified by the number of centromeric copies of the gene (SMN2) and the quantity of survival motor neuron protein. This has given rise to a number of treatment strategies. RECENT FINDINGS: Histone deacetylase inhibitors appear to increase the expression of SMN2, with an increase in survival motor neuron protein in various cell types. Clinical trials have been performed with three histone deacetylase inhibitors which are already licensed in the USA. Phenylbutyrate showed promise in a mouse model and an open-label pilot study, but was not effective in a phase 2 trial. Valproate may enhance transcription and reverse SMN2 splicing pattern, and has induced promising motor-function improvement in patients. Hydroxyurea may enhance splice function and increase the number of nuclear 'gems', small nuclear organelles in which survival motor neuron protein concentrates. SUMMARY: Discoveries regarding the genetics and pathogenesis of spinal muscular atrophy have identified potential targets for pharmacotherapy, raising hope that better treatments will eventually be developed.

Reference Type: Journal Article
Record Number: 19
Author: de Almeida, S. F.; Picarote, G.; Fleming, J. V.; Carmo-Fonseca, M.; Azevedo, J. E.; de Sousa, M.
Year: 2007
Title: Chemical chaperones reduce endoplasmic reticulum stress and prevent mutant HFE aggregate formation
Journal: J Biol Chem
Volume: 282
Issue: 38
Pages: 27905-12
Abstract: HFE C282Y, the mutant protein associated with hereditary hemochromatosis (HH), fails to acquire the correct conformation in the endoplasmic reticulum (ER) and is targeted for degradation. We have recently shown that an active unfolded protein response (UPR) is present in the cells of patients with HH. Now, by using HEK 293T cells, we demonstrate that the stability of HFE C282Y is influenced by the UPR signaling pathway that promotes its degradation. Treatment of HFE C282Y-expressing cells with tauroursodeoxycholic acid (TUDCA), a bile acid derivative with chaperone properties, or with the chemical chaperone sodium 4-phenylbutyrate (4PBA) impeded the UPR activation. However, although TUDCA led to an increased stability of the mutant protein, 4PBA contributed to a more efficient

disposal of HFE C282Y to the degradation route. Fluorescence microscopy and biochemical analysis of the subcellular localization of HFE revealed that a major portion of the C282Y mutant protein forms intracellular aggregates. Although neither TUDCA nor 4PBA restored the correct folding and intracellular trafficking of HFE C282Y, 4PBA prevented its aggregation. These data suggest that TUDCA hampers the UPR activation by acting directly on its signal transduction pathway, whereas 4PBA suppresses ER stress by chemically enhancing the ER capacity to cope with the expression of misfolded HFE, facilitating its degradation. Together, these data shed light on the molecular mechanisms involved in HFE C282Y-related HH and open new perspectives on the use of orally active chemical chaperones as a therapeutic approach for HH.

Reference Type: Journal Article
Record Number: 34
Author: Entin-Meer, M.; Rephaeli, A.; Yang, X.; Nudelman, A.; Nudelman, A.; Haas-Kogan, D. A.
Year: 2007
Title: AN-113, a novel prodrug of 4-phenylbutyrate with increased anti-neoplastic activity in glioma cell lines
Journal: Cancer Lett
Volume: 253
Issue: 2
Pages: 205-14
Abstract: Butyroyloxymethyl-4-phenylbutyrate (AN-113) is a novel HDACI that releases potent anti-neoplastic derivatives upon intracellular hydrolysis. The precursor of AN-113, 4-phenylbutyrate has shown promising results in a Phase I study of gliomas, and we hypothesized that AN-113 offers significant advantages over the parent drug. AN-113 demonstrates selective in vitro cytotoxicity against malignant cells while sparing normal astrocytes, effective at doses over 20-fold lower than 4-phenylbutyrate. Combining AN-113 and radiation results in additive therapeutic effects. Enthusiasm is lent to this approach by the ability of AN-113 to efficiently kill glioma cells, its bioavailability and potency when administered orally, its capacity to cross the blood-brain barrier, and its effectiveness in combination with radiation.

Reference Type: Journal Article
Record Number: 31
Author: Hanada, S.; Harada, M.; Kumemura, H.; Bishr Omary, M.; Koga, H.; Kawaguchi, T.; Taniguchi, E.; Yoshida, T.; Hisamoto, T.; Yanagimoto, C.; Maeyama, M.; Ueno, T.; Sata, M.
Year: 2007
Title: Oxidative stress induces the endoplasmic reticulum stress and facilitates inclusion formation in cultured cells
Journal: J Hepatol
Volume: 47
Issue: 1
Pages: 93-102
Abstract: BACKGROUND/AIMS: The precise mechanism of formation and significance of Mallory bodies (MBs) are poorly understood. The endoplasmic

reticulum (ER) is the organelle responsible for proper folding and elimination of unfolded proteins. Therefore, failure of this function increases defective proteins in the cell. METHODS: We examined the effects of oxidative stress on induction of ER stress and keratin 8 and 18 (K8/18)-containing inclusion formation in cultured human hepatoma cells and hepatocytes by immunofluorescence and immunoblot analyses. RESULTS: Generation of H(2)O(2) was detected in glucose oxidase (GO)-treated cells by 2',7'-dichlorodihydrofluorescein diacetate and co-treatment with GO and acetyl-leucyl-leucyl-norleucinal (ALLN), a proteasome inhibitor, induced formation of extensive keratin inclusions that were inhibited by pre-treatment with N-acetyl-cysteine. These inclusions shared similar features with MBs by immunofluorescence analysis. Electron microscopy showed that these structures appeared near the nuclei, surrounded by filamentous structures. GO and ALLN upregulated the expression of ER stress markers, however, 4-phenylbutyrate, a chemical chaperone, reduced formation of inclusions and expression of the ER stress markers. CONCLUSIONS: The oxidative stress coupled with limited inhibition of the proteasome induces dysfunction of the ER and results in inclusion formation in cultured cells. This suggests that ER stress plays a role in MB formation in liver disease.

Reference Type: Journal Article
Record Number: 26
Author: Hattori, Y.; Fukushima, M.; Maitani, Y.
Year: 2007
Title: Non-viral delivery of the connexin 43 gene with histone deacetylase inhibitor to human nasopharyngeal tumor cells enhances gene expression and inhibits in vivo tumor growth
Journal: Int J Oncol
Volume: 30
Issue: 6
Pages: 1427-39
Abstract: Dysregulation of connexin expression is believed to have a role in carcinogenesis, because levels of connexin are reduced in various tumors. We examined the role of connexin 43 (Cx43) alone and combined with a histone deactylase (HDAC) inhibitor in tumor growth inhibition. The transfection of Cx43 plasmid DNA (pCMV-Cx43) into human nasopharyngeal cancer KB cells using folate-linked nanoparticles induced inhibition of cell growth. Cx43 induced a tumor suppressive effect via a gap junctional intercellular communication-independent mechanism. The transfection of pCMV- Cx43 along with an HDAC inhibitor, 4-phenylbutyrate (4-PB), enhanced Cx43 expression greatly in vitro, and inhibited significantly the tumor growth of KB cells and xenografts compared with that of pCMV-Cx43 alone. 4-PB induced increased expression of genes of DNA damage checkpoints and of apoptosis via the down-regulation of anti-apoptotic bcl-2 mRNA expression and up-regulation of the activity of the apoptosis-associated enzyme caspase-3/7. Thus, the amplified Cx43 expression by an antitumor agent, an HDAC inhibitor, may have great potential as a growth inhibitor for nasopharyngeal tumors.

Reference Type: Journal Article
Record Number: 24
Author: Hayashi, H.; Sugiyama, Y.

Year: 2007
Title: 4-phenylbutyrate enhances the cell surface expression and the transport capacity of wild-type and mutated bile salt export pumps
Journal: Hepatology
Volume: 45
Issue: 6
Pages: 1506-16
Abstract: Progressive familial intrahepatic cholestasis type 2 (PFIC2) is caused by a mutation in the bile salt export pump (BSEP/ABCB11) gene. We previously reported that E297G and D482G BSEP, which are frequently found mutations in European patients, result in impaired membrane trafficking, whereas both mutants retain their transport function. The dysfunctional localization is probably attributable to the retention of BSEP in endoplasmic reticulum (ER) followed by proteasomal degradation. Because sodium 4-phenylbutyrate (4PBA) has been shown to restore the reduced cell surface expression of mutated plasma membrane proteins, in the current study, we investigated the effect of 4PBA treatment on E297G and D482G BSEP. Transcellular transport and cell surface biotinylation studies using Madin-Darby canine kidney (MDCK) II cells demonstrated that 4PBA treatment increased functional cell surface expression of wild-type (WT), E297G, and D482G BSEP. The prolonged half-life of cell surface-resident BSEP with 4PBA treatment was responsible for this result. Moreover, treatment of Sprague-Dawley rats with 4PBA resulted in an increase in BSEP expression at the canalicular membrane, which was accompanied by an increase in the biliary excretion of [(3)H]taurocholic acid (TC). CONCLUSION: 4PBA treatment with a clinically achievable concentration enhances the cell surface expression and the transport capacity of WT, E297G, and D482G BSEP in MDCK II cells, and also induces functional BSEP expression at the canalicular membrane and bile acid transport via canalicular membrane in vivo. 4PBA is a potential pharmacological agent for treating not only PFIC2 patients with E297G and D482G mutations but also other cholestatic patients, in whom the BSEP expression at the canalicular membrane is reduced.

Reference Type: Journal Article
Record Number: 15
Author: Hogarth, P.; Lovrecic, L.; Krainc, D.
Year: 2007
Title: Sodium phenylbutyrate in Huntington's disease: a dose-finding study
Journal: Mov Disord
Volume: 22
Issue: 13
Pages: 1962-4
Abstract: Transcriptional dysregulation in Huntington's disease (HD) is mediated in part by aberrant patterns of histone acetylation. We performed a dose-finding study in human HD of sodium phenylbutyrate (SPB), a histone deacetylase inhibitor that ameliorates the HD phenotype in animal models. We used a dose-escalation/de-escalation design, using prespecified toxicity criteria and standard clinical and laboratory safety measures. The maximum tolerated dose was 15 g/day. At higher doses, toxicity included vomiting, lightheadedness, confusion, and gait instability. We saw no significant laboratory or electrocardiographic abnormalities. Gene expression

changes in blood suggested an inverse dose-response. In conclusion, SPB at 12 to 15 g/day appears to be safe and well-tolerated in human HD.

Reference Type: Journal Article
Record Number: 14
Author: Hwu, W. L.; Chien, Y. H.; Tang, N. L.; Law, L. K.; Lin, C. Y.; Lee, N. C.
Year: 2007
Title: Deficiency of the carnitine transporter (OCTN2) with partial N-acetylglutamate synthase (NAGS) deficiency
Journal: J Inherit Metab Dis
Volume: 30
Issue: 5
Pages: 816
Abstract: A patient with recurrent episodes of hyperammonaemia (highest ammonia level recorded 229 micromol/L, normal 9-33) leading to altered levels of consciousness was diagnosed with partial N-acetylglutamate synthase (NAGS) deficiency (9% residual activity) at age 5 years and was treated with ammonia-conjugating agents (Ucephan 250 mg/kg per day and later sodium phenylbutyrate 200-250 mg/kg per day) for 15 years. A chronically low serum carnitine level (pretreatment plasma free carnitine 4 nmol/L, normal 37 +/- 8 nmol/L; total carnitine 8 nmol/L, normal 46 +/- 10) was assumed to be secondary and was treated with supplemental carnitine (30-50 mg/kg per day). Hypoglycaemia (blood sugar 35 mg/dl, normal 70-100), cardiomegaly, and fatty liver were also noted at diagnosis. The patient died unexpectedly at age 20 years. In retrospect, it was learned that the patient had stopped his carnitine without medical consultation several weeks prior to his death. Additional molecular investigations identified two mutations (R254X and IVS3 + 1G > A) in the patient's OCTN2 (SLC22A5) gene, consistent with a diagnosis of primary carnitine deficiency due to carnitine transporter defect. R245X is a founder mutation in Southern Chinese populations. It is unknown whether the original NAGS deficiency was primary or secondary, but molecular analysis of the NAGS gene failed to identify mutations. Urea cycle enzyme expression may be affected by fatty acid suppression of an AP-1 binding site in the promoter enhancer region of the urea cycle gene. Regardless, it is clear that the NAGS abnormality has led to delay of recognition of the OCTN2 defect, and modified the clinical course in this patient.

Reference Type: Journal Article
Record Number: 28
Author: Inden, M.; Kitamura, Y.; Takeuchi, H.; Yanagida, T.; Takata, K.; Kobayashi, Y.; Taniguchi, T.; Yoshimoto, K.; Kaneko, M.; Okuma, Y.; Taira, T.; Ariga, H.; Shimohama, S.
Year: 2007
Title: Neurodegeneration of mouse nigrostriatal dopaminergic system induced by repeated oral administration of rotenone is prevented by 4-phenylbutyrate, a chemical chaperone
Journal: J Neurochem
Volume: 101
Issue: 6
Pages: 1491-1504

Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder that is primarily characterized by the degeneration of dopaminergic neurons in the nigrostriatal pathway. Previous studies have demonstrated that chronic systemic exposure of Lewis rats to rotenone produced many features of PD, and cerebral tauopathy was also detected in the case of severe weight loss. The present study was designed to assess the neurotoxicity of rotenone after daily oral administration for 28 days at several doses in C57BL/6 mice. In addition, we examined the protective effects of 4-phenylbutyrate (4-PBA) on nigral dopamine (DA) neurons in rotenone-treated mice. 4-PBA was injected intraperitoneally daily 30 min before each oral administration of rotenone. Chronic oral administration of rotenone at high doses induced specific nigrostriatal DA neurodegeneration, motor deficits and the up-regulation of alpha-synuclein in the surviving DA neurons. In contrast to the Lewis rat model, cerebral tauopathy was not detected in this mouse model. 4-PBA inhibited rotenone-induced neuronal death and decreased the protein level of alpha-synuclein. These results suggest that this rotenone mouse model may be useful for understanding the mechanism of DA neurodegeneration in PD, and that 4-PBA has a neuroprotective effect in the treatment of PD.

Reference Type: Journal Article
Record Number: 45
Author: Iordache, C.; Duszyk, M.
Year: 2007
Title: Sodium 4-phenylbutyrate upregulates ENaC and sodium absorption in T84 cells
Journal: Exp Cell Res
Volume: 313
Issue: 2
Pages: 305-11
Abstract: Butyrate and other short-chain fatty acids (SCFA), produced by colonic bacterial flora, affect numerous epithelial cell functions. To better understand how SCFA regulate ion transport, we investigated the effects of 4-phenylbutyrate (4-PBA) on Na(+) absorption in T84 cells. Under standard cell culture conditions, the short circuit current did not display any amiloride-sensitive Na(+) absorption and was wholly representative of Cl(-) secretion. However, when T84 cells were grown in the presence of 5 mM 4-PBA, a gradual appearance of amiloride-sensitive Na(+) channel (ENaC) activity was observed that reached a plateau after 24 h. Quantitative RT-PCR and Western blot studies of ENaC subunit expression indicated that 4-PBA stimulated alpha and gamma subunits. Trichostatin A, an inhibitor of histone deacetylase, mimicked the effects of 4-PBA, suggesting that 4-PBA affects ENaC expression by inhibiting deacetylases. 4-PBA had no effect on ENaC expression in airway epithelial cells indicating tissue-specific effect. We conclude that butyrate plays an important role in regulating colonic Na(+) absorption by increasing ENaC transcription and activity.

Reference Type: Journal Article
Record Number: 40
Author: Jeng, Y. Y.; Lin, N. T.; Chang, P. H.; Huang, Y. P.; Pang, V. F.; Liu, C. H.; Lin, C. T.

Year: 2007
Title: Retinal ischemic injury rescued by sodium 4-phenylbutyrate in a rat model
Journal: Exp Eye Res
Volume: 84
Issue: 3
Pages: 486-92
Abstract: Retinal ischemia is a common cause of visual impairment for humans and animals. Herein, the neuroprotective effects of phenylbutyrate (PBA) upon retinal ischemic injury were investigated using a rat model. Retinal ganglion cells (RGCs) were retrograde labeled with the fluorescent tracer fluorogold (FG) applied to the superior collicoli of test Sprague-Dawley rats. High intraocular pressure and retinal ischemia were induced seven days subsequent to such FG labeling. A dose of either 100 or 400 mg/kg PBA was administered intraperitoneally to test rats at two time points, namely 30 min prior to the induction of retinal ischemia and 1 h subsequent to the cessation of the procedure inducing retinal ischemia. The test-rat retinas were collected seven days subsequent to the induction of retinal ischemia, and densities of surviving RGCs were estimated by counting FG-labeled RGCs within the retina. Histological analysis revealed that ischemic injury caused the loss of retinal RGCs and a net decrease in retinal thickness. For PBA-treated groups, almost 100% of the RGCs were preserved by a pre-ischemia treatment with PBA (at a dose of either 100 or 400 mg/kg), while post-ischemia treatment of RGCs with PBA did not lead to the preservation of RGCs from ischemic injury by PBA as determined by the counting of whole-mount retinas. Pre-ischemia treatment of RGCs with PBA (at a dose of either 100 or 400 mg/kg) significantly reduced the level of ischemia-associated loss of thickness of the total retina, especially the inner retina, and the inner plexiform layer of retina. Besides, PBA treatment significantly reduced the ischemia-induced loss of cells in the ganglion-cell layer of the retina. Taken together, these results suggest that PBA demonstrates a marked neuroprotective effect upon high intraocular pressure-induced retinal ischemia when the PBA is administered prior to ischemia induction.

Reference Type: Journal Article
Record Number: 29
Author: Karamertzanis, P. G.; Anandamanoharan, P. R.; Fernandes, P.; Cains, P. W.; Vickers, M.; Tocher, D. A.; Florence, A. J.; Price, S. L.
Year: 2007
Title: Toward the computational design of diastereomeric resolving agents: an experimental and computational study of 1-phenylethylammonium-2-phenylacetate derivatives
Journal: J Phys Chem B
Volume: 111
Issue: 19
Pages: 5326-36
Abstract: The crystal structures, including two new polymorphs, of three diastereomerically related salt pairs formed by (R)-1-phenylethylammonium (1) with (S&R)-2-phenylpropanoate (2), (S&R)-2-phenylbutyrate (3), and (S&R)-mandelate (4) ions were characterized by low-temperature single crystal or powder X-ray diffraction. Thermal, solubility, and solution calorimetry measurements were used to determine the relative stabilities of the salt pairs and polymorphs. These were qualitatively predicted by lattice energy calculations combining realistic models for

the dominant intermolecular electrostatic interactions and ab initio calculations for the ions' conformational energies due to the distortion of their geometries by the crystal packing forces. Crystal structure prediction studies were also performed for the highly polymorphic diastereomeric salt pair (R)-1-phenylethylammonium-(S&R)-2-phenylbutyrate (1-3) in an attempt to predict the separation efficiency without relying on experimental information. This joint experimental and computational investigation provides a stringent test for the reliability of lattice modeling approaches to explain the origins of chiral resolution via diastereomer formation (Pasteurian resolution). The further developments required for the computational screening of single-enantiomer resolving agents to achieve optimal chiral separation are discussed.

Reference Type: Journal Article
Record Number: 52
Author: Kern, R. M.; Yang, Z.; Kim, P. S.; Grody, W. W.; Iyer, R. K.; Cederbaum, S. D.
Year: 2007
Title: Arginase induction by sodium phenylbutyrate in mouse tissues and human cell lines
Journal: Mol Genet Metab
Volume: 90
Issue: 1
Pages: 37-41
Abstract: Hyperargininemia is a urea cycle disorder caused by mutations in the gene for arginase I (AI) resulting in elevated blood arginine and ammonia levels. Sodium phenylacetate and a precursor, sodium phenylbutyrate (NaPB) have been used to lower ammonia, conjugating glutamine to produce phenylacetylglutamine which is excreted in urine. The elevated arginine levels induce the second arginase (AII) in patient kidney and kidney tissue culture. It has been shown that NaPB increases expression of some target genes and we tested its effect on arginase induction. Eight 9-week old male mice fed on chow containing 7.5 g NaPB/kg rodent chow and drank water with 10 g NaPB/L, and four control mice had a normal diet. After one week all mice were sacrificed. The arginase specific activities for control and NaPB mice, respectively, were 38.2 and 59.4 U/mg in liver, 0.33 and 0.42 U/mg in kidney, and 0.29 and 1.19 U/mg in brain. Immunoprecipitation of arginase in each tissue with AI and AII antibodies showed the activity induced by NaPB is mostly AI. AII may also be induced in kidney. AI accounts for the fourfold increased activity in brain. In some cell lines, NaPB increased arginase activity up to fivefold depending on dose (1-5 mM) and exposure time (2-5 days); control and NaPB activities, respectively, are: erythroleukemia, HEL, 0.06 and 0.31 U/mg, and K562, 0.46 and 1.74 U/mg; embryonic kidney, HEK293, 1.98 and 3.58 U/mg; breast adenocarcinoma, MDA-MB-468, 1.11 and 4.06 U/mg; and prostate adenocarcinoma, PC-3, 0.55 and 3.20 U/mg. In MDA-MB-468 and HEK most, but not all, of the induced activity is AI. These studies suggest that NaPB may induce AI when used to treat urea cycle disorders. It is relatively less useful in AI deficiency, although it could have some effect in those patients with missense mutations.

Reference Type: Journal Article
Record Number: 23

Author: Khan, Z.; Akhtar, M.; Asklund, T.; Juliusson, B.; Almqvist, P. M.; Ekstrom, T. J.
Year: 2007
Title: HDAC inhibition amplifies gap junction communication in neural progenitors: potential for cell-mediated enzyme prodrug therapy
Journal: Exp Cell Res
Volume: 313
Issue: 13
Pages: 2958-67
Abstract: Enzyme prodrug therapy using neural progenitor cells (NPCs) as delivery vehicles has been applied in animal models of gliomas and relies on gap junction communication (GJC) between delivery and target cells. This study investigated the effects of histone deacetylase (HDAC) inhibitors on GJC for the purpose of facilitating transfer of therapeutic molecules from recombinant NPCs. We studied a novel immortalized midbrain cell line, NGC-407 of embryonic human origin having neural precursor characteristics, as a potential delivery vehicle. The expression of gap junction protein connexin 43 (Cx43) was analyzed by western blot and immunocytochemistry. While Cx43 levels were decreased in untreated differentiating NGC-407 cells, the HDAC inhibitor 4-phenylbutyrate (4-PB) increased Cx43 expression along with increased membranous deposition in both proliferating and differentiating cells. Simultaneously, Ser 279/282-phosphorylated form of Cx43 was declined in both culture conditions by 4-PB. The 4-PB effect in NGC-407 cells was verified by using HNSC.100 human neural progenitors and Trichostatin A. Improved functional GJC is of imperative importance for therapeutic strategies involving intercellular transport of low molecular-weight compounds. We show here an enhancement by 4-PB, of the functional GJC among NGC-407 cells, as well as between NGC-407 and human glioma cells, as indicated by increased fluorescent dye transfer.

Reference Type: Journal Article
Record Number: 17
Author: Koga, Y.; Yamane, T.; Nakano, H.
Year: 2007
Title: Creation of novel enantioselective lipases by SIMPLEX
Journal: Methods Mol Biol
Volume: 375
Pages: 165-81
Abstract: The single-molecule PCR-linked in vitro expression (SIMPLEX) technology, which can directly link a single molecule of a gene to its encoding protein, has been used to engineer enantioselectivity of lipase from Burkhorderia cepacia KWI-56. A combinatorial mutation has been introduced only to four residues in the hydrophobic substrate-binding pocket of the enzyme based on a structural model of the substrate-enzyme complex. Such focused mutation library constructed by the SIMPLEX technology has been screened for an enantiomeric substrate. Some combinations of substitutions in the four positions of the lipase have been found as effective for changing the enantio-preference from the (S)-form of p-nitrophenyl-3-phenylbutyrate to the (R)-form. Here, we describe the detail procedure to construct such an exclusively in vitro protein library and a practical screening method based on enzymatic activity.

Reference Type: Journal Article
Record Number: 11
Author: Lam, P.; Pearson, C. L.; Soroka, C. J.; Xu, S.; Mennone, A.; Boyer, J. L.
Year: 2007
Title: Levels of plasma membrane expression in progressive and benign mutations of the bile salt export pump (Bsep/Abcb11) correlate with severity of cholestatic diseases
Journal: Am J Physiol Cell Physiol
Volume: 293
Issue: 5
Pages: C1709-16
Abstract: Human BSEP (ABCB11) mutations are the molecular basis for at least three clinical forms of liver disease, progressive familial intrahepatic cholestasis type 2 (PFIC2), benign recurrent intrahepatic cholestasis type 2 (BRIC2), and intrahepatic cholestasis of pregnancy (ICP). To better understand the pathobiology of these disease phenotypes, we hypothesized that different mutations may cause significant differences in protein defects. Therefore we compared the effect of two PFIC2 mutations (D482G, E297G) with two BRIC2 mutations (A570T and R1050C) and one ICP mutation (N591S) with regard to the subcellular localization, maturation, and function of the rat Bsep protein. Bile salt transport was retained in all but the E297G mutant. Mutant proteins were expressed at reduced levels on the plasma membrane of transfected HEK293 cells compared with wild-type (WT) Bsep in the following order: WT > N591S > R1050C approximately A570T approximately E297G >> D482G. Total cell protein and surface protein expression were reduced to the same extent, suggesting that trafficking of these mutants to the plasma membrane is not impaired. All Bsep mutants accumulate in perinuclear aggresome-like structures in the presence of the proteasome inhibitor MG-132, suggesting that mutations are associated with protein instability and ubiquitin-dependent degradation. Reduced temperature, sodium butyrate, and sodium 4-phenylbutyrate enhanced the expression of the mature and cell surface D482G protein in HEK293 cells. These results suggest that the clinical phenotypes of PFIC2, BRIC2, and ICP may directly correlate with the amount of mature protein that is expressed at the cell surface and that strategies to stabilize cell surface mutant protein may be therapeutic.

Reference Type: Journal Article
Record Number: 46
Author: Le Bacquer, O.; Mauras, N.; Welch, S.; Haymond, M.; Darmaun, D.
Year: 2007
Title: Acute depletion of plasma glutamine increases leucine oxidation in prednisone-treated humans
Journal: Clin Nutr
Volume: 26
Issue: 2
Pages: 231-8
Abstract: BACKGROUND, AIMS & METHODS: To determine whether depletion in plasma glutamine worsens the catabolic response to corticosteroids, seven healthy volunteers received oral prednisone for 6 days on two separate occasions, at least 2

weeks apart, and in random order. On the sixth day of each treatment course, they received 5 h intravenous infusions of L-[1-(14)C]-leucine and L-[1-(13)C]-glutamine in the postabsorptive state (1) under baseline conditions (prednisone only day) and (2) after 24h of treatment with phenylbutyrate (prednisone+phenylbutyrate day), a glutamine chelating agent. RESULTS: Phenylbutyrate treatment was associated with (1) an approximately 15% decline in plasma glutamine concentration (627+/-39 vs. 530+/-31 micromol l(-1); P<0.05), (2) no change in leucine appearance rate, an index of protein breakdown (124+/-9 vs. 128+/-9 micromol kg(-1) h(-1); NS) nor in non-oxidative leucine disposal, an index of whole body protein synthesis (94+/-9 vs. 91+/-7 micromol kg(-1) h(-1); NS), and (3) a approximately 25% rise in leucine oxidation (30+/-1 vs. 38+/-2 micromol kg(-1) h(-1), P<0.05), despite an approximately 25% decline (P<0.05) in leucine concentration. CONCLUSIONS: In a model of mild, stress-induced protein catabolism, depletion of plasma glutamine per se may worsen branched chain amino acid and protein wasting.

Reference Type: Journal Article
Record Number: 30
Author: Leonard, J. V.; Ward Platt, M. P.; Morris, A. A.
Year: 2007
Title: Hypothesis: proposals for the management of a neonate at risk of hyperammonaemia due to a urea cycle disorder
Journal: Eur J Pediatr
Abstract: It is difficult to prevent hyperammonaemia in patients with urea cycle disorders that present in the newborn period. This is true, even if treatment is started prospectively because of an affected relative. We propose several additional measures that could be used in conjunction with conventional therapy to improve the metabolic control. Catabolism could be reduced by delivering the babies by elective caesarean section, by starting intravenous glucose immediately after delivery and, possibly, by using beta-blockers or octreotide and insulin. The effectiveness of sodium benzoate and sodium phenylbutyrate might be increased by giving phenobarbital to the mother before delivery and subsequently to the baby to induce the enzymes for conjugation. We would expect the proposed measures to reduce the risk of hyperammonaemia and to improve the outcome for these patients. They have not, however, previously been used in this context, so families would need to be counselled carefully and controlled studies should be undertaken.

Reference Type: Journal Article
Record Number: 12
Author: Lopez, C. A.; Feng, F. Y.; Herman, J. M.; Nyati, M. K.; Lawrence, T. S.; Ljungman, M.
Year: 2007
Title: Phenylbutyrate sensitizes human glioblastoma cells lacking wild-type p53 function to ionizing radiation
Journal: Int J Radiat Oncol Biol Phys
Volume: 69
Issue: 1
Pages: 214-20

Abstract: PURPOSE: Histone deacetylase (HDAC) inhibitors induce growth arrest, differentiation, and apoptosis in cancer cells. Phenylbutyrate (PB) is a HDAC inhibitor used clinically for treatment of urea cycle disorders. Because of its low cytotoxicity, cerebrospinal fluid penetration, and high oral bioavailability, we investigated PB as a potential radiation sensitizer in human glioblastoma cell lines. METHODS AND MATERIALS: Four glioblastoma cell lines were selected for this study. Phenylbutyrate was used at a concentration of 2 mM, which is achievable in humans. Western blots were used to assess levels of acetylated histone H3 in tumor cells after treatment with PB. Flow cytometry was used for cell cycle analysis. Clonogenic assays were performed to assess the effect of PB on radiation sensitivity. We used shRNA against p53 to study the role of p53 in radiosensitization. RESULTS: Treatment with PB alone resulted in hyperacetylation of histones, confirmed by Western blot analysis. The PB alone resulted in cytostatic effects in three cell lines. There was no evidence of G(1) arrest, increase in sub-G(1) fraction or p21 protein induction. Clonogenic assays showed radiosensitization in two lines harboring p53 mutations, with enhancement ratios (+/- SE) of 1.5 (+/- 0.2) and 1.3 (+/- 0.1), respectively. There was no radiopotentiating effect in two cell lines with wild-type p53, but knockdown of wild-type p53 resulted in radiosensitization by PB. CONCLUSIONS: Phenylbutyrate can produce p21-independent cytostasis, and enhances radiation sensitivity in p53 mutant human glioblastoma cells in vitro. This suggests the potential application of combined PB and radiotherapy in glioblastoma harboring mutant p53.

Reference Type: Journal Article
Record Number: 16
Author: Lu, Y. S.; Kashida, Y.; Kulp, S. K.; Wang, Y. C.; Wang, D.; Hung, J. H.; Tang, M.; Lin, Z. Z.; Chen, T. J.; Cheng, A. L.; Chen, C. S.
Year: 2007
Title: Efficacy of a novel histone deacetylase inhibitor in murine models of hepatocellular carcinoma
Journal: Hepatology
Volume: 46
Issue: 4
Pages: 1119-30
Abstract: Hepatocellular carcinoma (HCC) is a leading cause of cancer death worldwide, yet effective therapeutic options for advanced HCC are limited. This study was aimed at assessing the antitumor effect of a novel phenylbutyrate-derived histone deacetylase (HDAC) inhibitor, OSU-HDAC42, vis-a-vis suberoylanilide hydroxamic acid (SAHA), in in vitro and in vivo models of human HCC. OSU-HDAC42 was several times more potent than SAHA in suppressing the viability of PLC5, Huh7, and Hep3B cells with submicromolar median inhibitory concentration (IC(50)) values. With respect to SAHA, OSU-HDAC42 exhibited greater apoptogenic potency, which was associated with reduced levels of the apoptotic regulators phosphorylated Akt B-cell lymphoma-xL, survivin, cellular inhibitor of apoptosis protein 1, and cellular inhibitor of apoptosis protein 2. The in vivo efficacy of OSU-HDAC42 versus SAHA was assessed in orthotopic and subcutaneous xenograft tumor models in athymic nude mice. Daily oral treatments with OSU-HDAC42 and SAHA, both at 25 mg/kg, suppressed the growth of orthotopic PLC5 tumor xenografts by 91% and 66%, respectively, and of established subcutaneous PLC5 tumor xenografts

by 85% and 56%, respectively. This differential tumor suppression correlated with the modulation of intratumoral biomarkers associated with HDAC inhibition and apoptosis regulation. Moreover, the oral administration of OSU-HDAC42 at 50 mg/kg every other day markedly suppressed ectopic tumor growth in mice bearing large tumor burdens (500 mm(3)) at the start of treatment. CONCLUSION: OSU-HDAC42 is a potent, orally bioavailable inhibitor of HDAC with a broad spectrum of antitumor activity that includes targets regulating multiple aspects of cancer cell survival. These results suggest that OSU-HDAC42 has clinical value in therapeutic strategies for HCC.

Reference Type: Journal Article
Record Number: 3
Author: Maillot, F.; Crenn, P.
Year: 2007
Title: [Urea cycle disorders in adult patients]
Journal: Rev Neurol (Paris)
Volume: 163
Issue: 10
Pages: 897-903
Abstract: INTRODUCTION: Urea cycle disorders (UCD) usually present after 24 h to 48 h of life with failure to thrive, lethargy and coma leading to death, but milder forms may occur from infancy to adulthood. STATE OF THE ART: Survival of children with UCD has significantly improved and the need for transitional care to adulthood has emerged. Adult onset UCD present with chronic or acute neurological, psychiatric and digestive symptoms associated with protein avoidance. Ornithine transcarbamylase (OTC) deficiency, which is inherited as an X-linked disorder, is the most well-described UCD in adults. Acute decompensations associate the triad of encephalopathy, respiratory alkalosis and hyperammonemia. Acute encephalopathy is characterized by brain edema, which is life-threatening without treatment. Specific urea cycle enzyme deficiency can be suspected in the presence of abnormal plasma amino acids concentrations and urinary excretion of orotic acid. A measurement enzyme activity in appropriate tissue, or DNA analysis if available, is required for diagnosis. Treatment requires restriction of dietary protein intake and the use of alternative pathways of waste nitrogen excretion with sodium benzoate and sodium phenylbutyrate. Patients with acute forms may need hemodialysis or hemodiafiltration. Therapeutic goals for OTC deficiency are to maintain plasma ammonia<80 micromol/L, plasma glutamine<1,000 micromol/L, argininemia 80-150 micromol/L and branched chain amino acids within the normal range, in order to prevent episodes of potentially lethal acute hyperammonemia. CONCLUSION: Potentially fatal acute hyperammonemia may occur in male or female patients at any age. Ammonia should be measured promptly in case of acute neurological and psychiatric symptoms or coma.

Reference Type: Journal Article
Record Number: 47
Author: Mercuri, E.; Bertini, E.; Messina, S.; Solari, A.; D'Amico, A.; Angelozzi, C.; Battini, R.; Berardinelli, A.; Boffi, P.; Bruno, C.; Cini, C.; Colitto, F.; Kinali, M.;

Minetti, C.; Mongini, T.; Morandi, L.; Neri, G.; Orcesi, S.; Pane, M.; Pelliccioni, M.; Pini, A.; Tiziano, F. D.; Villanova, M.; Vita, G.; Brahe, C.
Year: 2007
Title: Randomized, double-blind, placebo-controlled trial of phenylbutyrate in spinal muscular atrophy
Journal: Neurology
Volume: 68
Issue: 1
Pages: 51-5
Abstract: OBJECTIVE: To assess the efficacy of phenylbutyrate (PB) in patients with spinal muscular atrophy in a randomized, double-blind, placebo-controlled trial involving 10 Italian centers. METHODS: One hundred seven children were assigned to receive PB (500 mg/kg/day) or matching placebo on an intermittent regimen (7 days on/7 days off) for 13 weeks. The Hammersmith functional motor scale (primary outcome measure), myometry, and forced vital capacity were assessed at baseline and at weeks 5 and 13. RESULTS: Between January and September 2004, 107 patients aged 30 to 154 months were enrolled. PB was well tolerated, with only one child withdrawing because of adverse events. Mean improvement in functional score was 0.60 in the PB arm and 0.73 in placebo arm ($p = 0.70$). Changes in the secondary endpoints were also similar in the two study arms. CONCLUSIONS: Phenylbutyrate was not effective at the regimen, schedule, and duration used in this study.

Reference Type: Journal Article
Record Number: 13
Author: Milkevitch, M.; Jeitner, T. M.; Beardsley, N. J.; Delikatny, E. J.
Year: 2007
Title: Lovastatin enhances phenylbutyrate-induced MR-visible glycerophosphocholine but not apoptosis in DU145 prostate cells
Journal: Biochim Biophys Acta
Volume: 1771
Issue: 9
Pages: 1166-76
Abstract: In this study the effects of lovastatin on DU145 prostate cancer cells treated with phenylbutyrate (PB) was investigated in order to determine the NMR-detectable metabolic changes resulting from the cooperative activity of these two agents. DU145 cells were perfused with PB in the presence or absence of 10 microM of the HMG-CoA reductase inhibitor lovastatin, and the results monitored by 31P and diffusion-weighted 1H NMR spectroscopy. Lovastatin had additive effects on the PB-induced NMR-visible total choline in 1H spectra, and glycerophosphocholine in 31P spectra but no significant effect on NMR-visible lipid. Moreover, lovastatin had no effect on the ability of PB to either promote the formation of oil red O-detectable lipid droplets or arrest the cell cycle. The most remarkable observations from these studies were that lovastatin enhanced the increase in glycerophosphocholine while reversing late markers of apoptosis and the loss of NTP caused by PB. These results identify a branch point separating the neutral lipid production and the apoptotic cell death caused by the actions of differentiating agents.

Reference Type: Journal Article

Record Number: 42
Author: Park, J. S.; Lee, E. J.; Lee, J. C.; Kim, W. K.; Kim, H. S.
Year: 2007
Title: Anti-inflammatory effects of short chain fatty acids in IFN-gamma-stimulated RAW 264.7 murine macrophage cells: involvement of NF-kappaB and ERK signaling pathways
Journal: Int Immunopharmacol
Volume: 7
Issue: 1
Pages: 70-7
Abstract: The overactivation of macrophages causes abnormal cell death and chronic inflammatory diseases. Therefore, the modulation of macrophage-mediated cytotoxicity is expected to become a new therapeutic strategy for various inflammatory diseases. In this study, three types of short chain fatty acids (sodium butyrate (NaB), sodium phenylbutyrate (NaPB), sodium phenylacetate (NaPA)) were found to have anti-inflammatory effects in IFN-gamma-stimulated RAW 264.7 cells. They inhibited the expression of iNOS, TNF-alpha, and IL-6 induced by IFN-gamma, while they enhanced the expression of the anti-inflammatory cytokine, IL-10. Their potency as anti-inflammatory agents was in the order of NaB>NaPB>NaPA. Further mechanistic studies revealed these three agents to repress the DNA binding and transcriptional activities of NF-kappaB, which is an important modulator of inflammation. In addition, these agents repressed the IFN-gamma-induced ERK1/2 phosphorylation without affecting the Jak/STAT activities. The potency of NF-kappaB and ERK inhibition was also in the order of NaB>NaPB>NaPA. The results suggest that the NF-kappaB and ERK signaling pathways are at least in part involved in the anti-inflammatory activities of these SCFAs. Considering that SCFAs are normally present in the body and have few side effects, they might be promising agents for the prevention and/or treatment of various inflammatory diseases.

Reference Type: Journal Article
Record Number: 5
Author: Phillips, J. A.; Griffin, B. E.
Year: 2007
Title: Pilot study of sodium phenylbutyrate as adjuvant in cyclophosphamide-resistant endemic Burkitt's lymphoma
Journal: Trans R Soc Trop Med Hyg
Volume: 101
Issue: 12
Pages: 1265-9
Abstract: Burkitt's lymphoma (BL) accounts for the majority of childhood malignancies seen in sub-Saharan Africa. In Malawi, cyclophosphamide (CPM), the mainstay of treatment for endemic BL, is effective in around 50% of cases. Evidence exists in support of an association between activation of replication of Epstein-Barr virus (EBV) in the tumour and response to this chemotheraupeutic agent. Phenylbutyrate (PB), approved for treatment of inborn errors of the urea cycle with minimal toxicity in children, induces EBV replication and cell lysis in BL-derived cell cultures. It has also shown some success as adjuvant in treatment of chronic leukaemia and lymphoma. We tested in African BL patients with CPM-resistant tumours, and thus unlikely to survive, the hypothesis that PB can reverse this

resistance. A study of five patients showed PB before CPM to induce shrinkage of CPM-resistant tumours in two of them. Findings suggested that for this effect PB pre-treatment should be given for a week before CPM treatment. A larger study is indicated.

Reference Type: Journal Article
Record Number: 35
Author: Picard, V.; Bergeron, A.; Larue, H.; Fradet, Y.
Year: 2007
Title: MAGE-A9 mRNA and protein expression in bladder cancer
Journal: Int J Cancer
Volume: 120
Issue: 10
Pages: 2170-7
Abstract: In a previous analysis, we showed that MAGE-As were the most frequently expressed cancer-testis antigens in human bladder tumours. Here, we further characterized by RT-PCR the expression of this family of genes by analyzing specifically MAGE-A3, -A4, -A8 and -A9 mRNAs in 46 bladder tumours and 10 normal urothelia. We found that they were expressed in 30, 33, 56 and 54% of tumours, respectively. Although MAGE-A8 was the most frequent, its expression was low and was also found in most normal urothelia. The other MAGE-A mRNAs were all tumour-specific but MAGE-A9 mRNA was expressed at a higher level and was two times more frequent in superficial than in invasive tumours. To study the expression of the protein, we produced 2 MAGE-A9-specific monoclonal antibodies (mAbs) presenting no cross-reactivity with other MAGE-A proteins. MAb 14A11, was used to analyse the expression of the antigen in testis and tumour samples by immunohistochemistry. In testis, MAGE-A9 expression was restricted to primary spermatocytes. Most bladder tumours that expressed the MAGE-A9 transcript were positive with mAb 14A11. Staining was heterogeneous but half of the tumours showed over 75% positive cells. Finally, we showed that treatment of bladder cancer cells with the methylation inhibitor, 5-aza-2'-deoxycytidine, alone or in combination with the histone deacetylase inhibitors MS-275 and 4-phenylbutyrate could strongly induce the expression of MAGE-A9. These results show that MAGE-A9 is frequently expressed in superficial bladder cancer and could be a relevant target for immunotherapy or chemoimmunotherapy because its expression can be induced by chemotherapeutic drugs.

Reference Type: Journal Article
Record Number: 8
Author: Pruliere-Escabasse, V.; Planes, C.; Escudier, E.; Fanen, P.; Coste, A.; Clerici, C.
Year: 2007
Title: Modulation of epithelial sodium channel trafficking and function by sodium 4-phenylbutyrate in human nasal epithelial cells
Journal: J Biol Chem
Volume: 282
Issue: 47
Pages: 34048-57

Abstract: Sodium 4-phenylbutyrate (4-PBA) has been shown to correct the cellular trafficking of several mutant or nonmutant plasma membrane proteins such as cystic fibrosis transmembrane conductance regulator through the expression of 70-kDa heat shock proteins. The objective of the study was to determine whether 4-PBA may influence the functional expression of epithelial sodium channels (ENaC) in human nasal epithelial cells (HNEC). Using primary cultures of HNEC, we demonstrate that 4-PBA (5 mm for 6 h) markedly stimulated amiloride-sensitive sodium channel activity and that this was related to an increased abundance of alpha-, beta-, and gamma-ENaC subunits in the apical membrane. The increase in ENaC cell surface expression (i) was due to insertion of newly ENaC subunits as determined by brefeldin A experiments and (ii) was not associated with cell surface retention of ENaC subunits because endocytosis of ENaC subunits was unchanged. In addition, we find that ENaC co-immunoprecipitated with the heat shock protein constitutively expressed Hsc70, that has been reported to modulate ENaC trafficking, and that 4-PBA decreased Hsc70 protein level. Finally, we report that in cystic fibrosis HNEC obtained from two cystic fibrosis patients, 4-PBA increased functional expression of ENaC as demonstrated by the increase in amiloride-sensitive sodium transport and in alpha-, beta-, and gamma-ENaC subunit expression in the apical membrane. Our results suggest that in HNEC, 4-PBA increases the functional expression of ENaC through the insertion of new alpha-, beta-, and gamma-ENaC subunits into the apical membrane and also suggest that 4-PBA could modify ENaC trafficking by reducing Hsc70 protein expression.

Reference Type: Journal Article
Record Number: 7
Author: Rouge, C.; Des Robert, C.; Robins, A.; Le Bacquer, O.; Volteau, C.; De La Cochetiere, M. F.; Darmaun, D.
Year: 2007
Title: Manipulation of citrulline availability in humans
Journal: Am J Physiol Gastrointest Liver Physiol
Volume: 293
Issue: 5
Pages: G1061-7
Abstract: To determine whether circulating citrulline can be manipulated in vivo in humans, and, if so, whether citrulline availability affects the levels of related amino acids, nitric oxide, urinary citrulline, and urea nitrogen, 10 healthy volunteers were studied on 3 separate days: 1) under baseline conditions; 2) after a 24-h treatment with phenylbutyrate (0.36 g.kg(-1).day(-1)), a glutamine "trapping" agent; and 3) during oral L-citrulline supplementation (0.18 g.kg(-1).day(-1)), in randomized order. Plasma, erythrocyte (RBC), and urinary citrulline concentrations were determined by gas chromatography-mass spectrometry at 3-h intervals between 1100 and 2000 on each study day. Regardless of treatment, RBC citrulline was lower than plasma citrulline, with an RBC-to-plasma ratio of 0.60 +/- 0.04, and urinary citrulline excretion accounted for <1% of the citrulline load filtered by kidney. Phenylbutyrate induced an approximately 7% drop in plasma glutamine ($P = 0.013$), and 18 +/- 14% ($P < 0.0001$) and 19 +/- 17% ($P < 0.01$) declines in plasma and urine citrulline, respectively, with no alteration in RBC citrulline. Oral L-citrulline administration was associated with 1) a rise in plasma, urine, and RBC citrulline (39 +/- 4 vs. 225 +/- 44 micromol/l, 0.9 +/- 0.3 vs. 6.2 +/- 3.8 micromol/mmol creatinine, and 23 +/- 1 vs. 52

+/- 9 micromol/l, respectively); and 2) a doubling in plasma arginine level, without altering blood urea or urinary urea nitrogen excretion, and thus enhanced nitrogen balance. We conclude that 1) depletion of glutamine, the main precursor of citrulline, depletes plasma citrulline; 2) oral citrulline can be used to enhance systemic citrulline and arginine availability, because citrulline is bioavailable and very little citrulline is lost in urine; and 3) further studies are warranted to determine the mechanisms by which citrulline may enhance nitrogen balance in vivo in humans.

Reference Type: Journal Article
Record Number: 18
Author: Strobl, J. S.; Cassell, M.; Mitchell, S. M.; Reilly, C. M.; Lindsay, D. S.
Year: 2007
Title: Scriptaid and suberoylanilide hydroxamic acid are histone deacetylase inhibitors with potent anti-Toxoplasma gondii activity in vitro
Journal: J Parasitol
Volume: 93
Issue: 3
Pages: 694-700
Abstract: Toxoplasma gondii is a well-recognized cause of disease in congenitally infected and immunocompromised individuals. Histone deacetylases (HDAC) comprise a family of enzymes that participate in the regulation of chromatin structure, gene expression, and cell signaling in eukaryotes. Toxoplasma gondii expresses a HDAC Class I enzyme homologous to human hdac3. Previous work showed that the histone deacetylase inhibitors (HDI) apicidin and valproic acid inhibit T. gondii infections in vitro. The present study compares the activity of hydroxamic-acid histone deacetylase inhibitors against the RH strain of T. gondii growing in HS68 human foreskin fibroblast cells. Nanomolar concentrations of suberoylanilide hydroxamic acid (SAHA), suberic bishydroxamic acid (SBHA), scriptaid, and trichostatin A (TSA) inhibited T. gondii tachyzoite proliferation. Scriptaid was the most potent hydroxamic acid inhibitor (IC50 = 39 nM). In comparison, the carboxylate histone deacetylase inhibitors sodium valproate, sodium butyrate, and 4-phenylbutyrate were less potent (IC50 range 1-5 mM). All of the inhibitors tested, except SBHA, completely protected the HS68 monolayers from T. gondii at concentrations 3-6 times greater than their respective IC50. In contrast, nicotinamide, an inhibitor of NADI-dependent Class III HDAC, had minimal activity against T. gondii in our in vitro assays. We conclude that the hydroxamic acid class of histone deacetylase inhibitors exhibit potent anti-T. gondii activity in vitro.

Reference Type: Journal Article
Record Number: 27
Author: Sung, M. W.; Waxman, S.
Year: 2007
Title: Combination of cytotoxic-differentiation therapy with 5-fluorouracil and phenylbutyrate in patients with advanced colorectal cancer
Journal: Anticancer Res
Volume: 27
Issue: 2
Pages: 995-1001

Abstract: BACKGROUND: Phenylbutyrate (PB), a histone deacetylase inhibitor (HDACi), has been shown in laboratory studies to potentiate growth inhibition by 5-fluorouracil (FUra) of human colon carcinoma cells. PATIENTS AND METHODS: Phase I trial of FUra (24-hour continuous intravenous infusion (CIV)) with dose escalation (2 g/m2 to 2.3 g/m2), in combination with PB (120 hour CIV at fixed dose 410 mg/kg/d x 5), repeated weekly, in patients with advanced colorectal cancer. RESULTS: Nine patients with metastatic colorectal cancer were treated, 8 of whom were evaluable for toxicity. Toxicities were dose-dependent, reversible and included somnolence, fatigue, confusion, hearing loss, triglyceridemia and hyperuricema. Three out of 4 patients who completed at least 8 weeks of treatment had stable disease (SD) lasting 12+, 25 and 54 weeks (2 out of the 3 patients with SD have had multiple prior chemotherapy regimens). CONCLUSION: Weekly infusions of FUra followed by PB were fairly well tolerated with disease stabilization in 3/4 (75%) of patients. This is the first report to demonstrate the feasibility of combining a cytotoxic agent with a HDACi as a cancer treatment.

Reference Type: Journal Article
Record Number: 39
Author: Swarts, L.; Leisegang, F.; Owen, E. P.; Henderson, H. E.
Year: 2007
Title: An OTC deficiency 'phenocopy' in association with Klinefelter syndrome
Journal: J Inherit Metab Dis
Volume: 30
Issue: 1
Pages: 101
Abstract: Late-onset urea cycle disorder in a 20-month-old boy is unusually associated with Klinefelter syndrome with a 47XXY karyotype. We record the typical clinical and biochemical findings of ornithine transcarbamylase (OTC) deficiency in a young boy with a short history of recurrent vomiting, self mutilating behaviour, lethargy, ataxia and seizures. Laboratory studies showed hyperammonaemia and orotic aciduria, with normal citrulline and other urea cycle amino acids. Unfortunately, a liver biopsy for OTC activity measurement was refused by the parents. A rapid reversal of phenotype was seen on the introduction of a low-protein diet with accompanying benzoate and phenylbutyrate administration. Linkage studies suggested the inheritance of two X chromosomes, which was confirmed by karyotype analysis. Sequencing of all exons and immediate splice site regions revealed no sequence alterations in these sections of the OTC gene. A search for skewing of X-inactivation in the liver was not possible but we did show a random pattern of X-inactivation in leukocytes. The possibility of maternal X chromosome iso-disomy in our patient was discounted by microsatellite analysis, which revealed the inheritance of two independent X chromosomes. Mutation analysis in the OTC gene has shown that approximately 20% of patients with liver biopsy confirmed OTC deficiency do not have mutations in the coding or immediate splice-site sequences of this gene. Their classification as OTC phenocopies remains speculative, awaiting clarification of the underlying DNA alteration. We report on the novel association of OTC deficiency and Klinefelter syndrome with the additional interest of a probable unusual genetic defect underlying the OTC abnormality.

Reference Type: Journal Article
Record Number: 38
Author: Svechnikova, I.; Ammerpohl, O.; Ekstrom, T. J.
Year: 2007
Title: p21waf1/Cip1 partially mediates apoptosis in hepatocellular carcinoma cells
Journal: Biochem Biophys Res Commun
Volume: 354
Issue: 2
Pages: 466-71
Abstract: p21waf1/Cip1 (p21) is a tumor suppressor gene involved in apoptosis in many cancer cell types induced by different agents. In spite of concomitant induction of p21 by many anti-cancer drugs, including inhibitors of histone deacetylases (HDACi), its pro-apoptotic action has been debated during the last several years due to a lack of direct evidence regarding the exact role of p21 in apoptosis. With the help of anti-sense p21 expression, we show here that p21 is mediating the apoptotic effects of HDACi 4-phenylbutyrate (4-PB) and Trichostatin A (TSA) on the hepatocellular hepatocarcinoma Hep3B cells. Hep3B cells were transfected by EGFP-p21 anti-sense or sense plasmids, and apoptosis induced by HDACi was assessed by TUNEL assay. The results show that the p21 anti-sense construct prevents apoptosis, induced by HDAC inhibitors in Hep3B cells. The obtained results suggest an important role for p21 in mediating the apoptotic effect of HDACi.

Reference Type: Journal Article
Record Number: 22
Author: Tanner, L. M.; Nanto-Salonen, K.; Venetoklis, J.; Kotilainen, S.; Niinikoski, H.; Huoponen, K.; Simell, O.
Year: 2007
Title: Nutrient intake in lysinuric protein intolerance
Journal: J Inherit Metab Dis
Volume: 30
Issue: 5
Pages: 716-21
Abstract: Lysinuric protein intolerance (LPI) is a rare autosomal recessive disorder characterized by defective transport of cationic amino acids. Poor intestinal absorption and increased renal loss of arginine, ornithine and lysine lead to low plasma concentrations of these amino acids and, subsequently, to impaired urea cycle function. The patients therefore have decreased nitrogen tolerance, which may lead to hyperammonaemia after ingestion of normal amounts of dietary protein. As a protective mechanism, most patients develop strong aversion to protein-rich foods early in life. Oral supplementation with citrulline, which is absorbed normally and metabolized to arginine and ornithine, improves protein tolerance to some extent, as do sodium benzoate and sodium phenylbutyrate also used by some patients. Despite effective prevention of hyperammonaemia, the patients still consume a very restricted diet, which may be deficient in energy, essential amino acids and some vitamins and minerals. To investigate the potential nutritional problems of patients with lysinuric protein intolerance, 77 three- to four-day food records of 28 Finnish LPI patients aged 1.5-61 years were analysed. The data suggest that the patients are clearly at risk for many nutritional deficiencies, which may contribute to their symptoms. Their diet is highly deficient in calcium, vitamin D, iron and zinc. Individualized nutritional

supplementation accompanied by regular monitoring of dietary intake is therefore an essential part of the treatment of LPI.

Reference Type: Journal Article
Record Number: 32
Author: Tveten, K.; Holla, O. L.; Ranheim, T.; Berge, K. E.; Leren, T. P.; Kulseth, M. A.
Year: 2007
Title: 4-Phenylbutyrate restores the functionality of a misfolded mutant low-density lipoprotein receptor
Journal: Febs J
Volume: 274
Issue: 8
Pages: 1881-93
Abstract: Familial hypercholesterolemia is an autosomal dominant disease caused by mutations in the gene encoding the low-density lipoprotein receptor. To date, more than 900 different mutations have been described. Transport-defective mutations (class 2) causing partial or complete retention of the receptor in the endoplasmic reticulum are the predominant class of mutations. In a cell culture system (Chinese hamster ovary cells), we show that chemical chaperones are able to mediate rescue of a transport-defective mutant (G544V), and that the ability to obtain rescue is mutation dependent. In particular, the low molecular mass fatty acid derivative 4-phenylbutyrate mediated a marked increase in the transport of G544V-mutant low-density lipoprotein receptor to the plasma membrane. Thirty per cent of the mutant receptor was able to escape from the endoplasmic reticulum and reach the cell surface. The rescued receptor had reduced stability, but was found to be as efficient as the wild-type low-density lipoprotein receptor in binding and internalizing low-density lipoprotein. In addition to 4-phenylbutyrate, we also studied 3-phenylpropionate and 5-phenylvalerate, and compared their effect on rescue of the G544V-mutant low-density lipoprotein receptor with their ability to increase overall gene expression caused by their histone deacetylase inhibitor activity. No correlation was found. Our results indicate that the effect of these agents was not solely mediated by their ability to induce gene expression of proteins involved in intracellular transport, but rather could be due to a direct chemical chaperone activity. These data suggest that rescue of mutant low-density lipoprotein receptor is possible and that it might be feasible to develop pharmacologic chaperones to treat familial hypercholesterolemia patients with class 2 mutations.

Reference Type: Journal Article
Record Number: 2
Author: Watanabe, N.; Lam, E.
Year: 2007
Title: BAX inhibitor-1 modulates endoplasmic reticulum stress-mediated programmed cell death in Arabidopsis
Journal: J Biol Chem
Abstract: The components and pathways that regulate programmed cell death (PCD) in plants remain poorly understood. Here we describe the impact of drug-induced endoplasmic reticulum (ER) stress on Arabidopsis seedlings and present evidence for

the role of Arabidopsis BAX inhibitor-1 (AtBI1) as a modulator of ER stress-mediated PCD. We found that treatment of Arabidopsis seedlings with tunicamycin (TM), an inhibitor of N-linked glycosylation and an inducer of ER stress by triggering accumulation of unfolded proteins in the ER, results in strong inhibition of root growth and loss of survival accompanied by typical hallmarks of PCD such as accumulation of H2O2, chromatin condensation and oligonucleosomal fragmentation of nuclear DNA. These phenotypes are alleviated by co-treatment with either of two different chemical chaperones, sodium 4-phenylbutyrate and tauroursodeoxycholic acid, both with chaperone properties that can reduce the load of misfolded protein in the ER. Expression of AtBI1 mRNA and its promoter activity are increased dramatically prior to initiation of TM-induced PCD. Compared with wild-type plants, two AtBI1 mutants (atbi1-1 and atbi1-2) exhibit hypersensitivity to TM with accelerated PCD progression. Conversely, overexpressing AtBI1 markedly reduces the sensitivity of Arabidopsis seedlings to TM. However alterations in AtBI1 gene expression levels do not cause a significant effect on the expression patterns of typical ER stress-inducible genes (AtBip2, AtPDI, AtCRT1 and AtCNX1). We propose that AtBI1 plays a pivotal role as a highly conserved survival factor during ER stress that acts in parallel to the unfolded protein response pathway.

Reference Type: Journal Article
Record Number: 36
Author: Verheul, H. M.; Qian, D. Z.; Carducci, M. A.; Pili, R.
Year: 2007
Title: Sequence-dependent antitumor effects of differentiation agents in combination with cell cycle-dependent cytotoxic drugs
Journal: Cancer Chemother Pharmacol
Volume: 60
Issue: 3
Pages: 329-39
Abstract: PURPOSE: Combination of two differentiation agents such as phenylbutyrate (PB) and 13-cis-retinoic acid (CRA) has been shown to have an additive inhibitory effect on tumor growth in preclinical studies. In this report we explored the hypotheses that these "cytostatic" agents may have a greater antitumor activity in combination with "cytotoxic" compounds and their biological effect may be sequence-dependent. METHODS: The antitumor activity of combination of PB and CRA with paclitaxel (TX) and doxorubicin (DOXO) on human prostate and colon carcinoma cell lines was assessed both in vitro and in vivo. The effect on cell cycle, apoptotic rate, cyclin expression and induction of p21 expression was also determined. RESULTS: Following treatment of tumor cells with PB + CRA + TX or DOXO, inhibition of tumor cell growth was greatly enhanced as compared to PB + CRA, TX or DOXO alone, with >90% growth inhibition. However, when the cells were pretreated with PB + CRA followed by TX or DOXO, the enhanced inhibition was abolished suggesting a protective effect to this sequence. Interestingly treatment with PB + CRA restored sensitivity to DOXO in PC-3 human prostate cancer cell line. PB + CRA induced p21 expression and cell-cycle arrest in G1 phase, while TX and DOXO induced G2/M arrest. p21 and p53-deficient colon carcinoma cell lines were more sensitive to the effect of PB + CRA and TX as single agents and in combination, as compared to the wild type cells. When p21-deficient cells were pretreated with PB + CRA followed by TX the protective effect was still observed.

Treatment of tumor cells with combination of these drugs induced cell cycle delay at multiple mitotic checkpoints before undergoing apoptosis. Tumor growth was significantly inhibited and delayed in animals treated with either TX or concomitantly with TX and PB + CRA as compared to control. Animals treated with all three agents demonstrated further growth inhibition or delay than the TX alone or PB + CRA arm. CONCLUSIONS: These results suggest a rational therapeutic approach for combination of differentiation-inducing agents with cytotoxic drugs given concomitantly, but not sequentially.

Reference Type: Journal Article
Record Number: 9
Author: Vila-Carriles, W. H.; Zhou, Z. H.; Bubien, J. K.; Fuller, C. M.; Benos, D. J.
Year: 2007
Title: Participation of the chaperone Hsc70 in the trafficking and functional expression of ASIC2 in glioma cells
Journal: J Biol Chem
Volume: 282
Issue: 47
Pages: 34381-91
Abstract: High-grade glioma cells express subunits of the ENaC/Deg superfamily, including members of ASIC subfamily. Our previous work has shown that glioma cells exhibit a basally active cation current, which is not present in low-grade tumor cells or normal astrocytes, and that can be blocked by amiloride. When ASIC2 is present within the channel complex in the plasma membrane, the channel is rendered non-functional because of inherent negative effectors that require ASIC2. We have previously shown that high-grade glioma cells functionally express this current because of the lack of ASIC2 in the plasma membrane. We now hypothesize that ASIC2 trafficking in glioma cells is regulated by a specific chaperone protein, namely Hsc70. Our results demonstrated that Hsc70 co-immunoprecipitates with ASIC2 and that it is overexpressed in glioma cells as compared with normal astrocytes. In contrast, there was no difference in the expression of calnexin, which also co-immunoprecipitates with ASIC2. In addition, glycerol and sodium 4-phenylbutyrate reduced the amount of Hsc70 expressed in glioma cells to levels found in normal astrocytes. Transfection of Hsc70 siRNA inhibited the constitutively activated amiloride-sensitive current, decreased migration, and increased ASIC2 surface expression in glioma cells. These results support an association between Hsc70 and ASIC2 that may underlie the increased retention of ASIC2 in the endoplasmic reticulum of glioma cells. The data also suggest that decreasing Hsc70 expression promotes reversion of a high-grade glioma cell to a more normal astrocytic phenotype.

Reference Type: Journal Article
Record Number: 33
Author: Yam, G. H.; Gaplovska-Kysela, K.; Zuber, C.; Roth, J.
Year: 2007
Title: Sodium 4-phenylbutyrate acts as a chemical chaperone on misfolded myocilin to rescue cells from endoplasmic reticulum stress and apoptosis
Journal: Invest Ophthalmol Vis Sci

Volume: 48
Issue: 4
Pages: 1683-90
Abstract: PURPOSE: To evaluate the effect of chemical chaperones on the trafficking of secretion-incompetent primary open-angle glaucoma-associated mutant myocilin and the possibility to rescue cells coexpressing mutant and wild-type myocilin from endoplasmic reticulum (ER) stress and apoptosis. METHODS: CHO-K1, HEK293 and human trabecular meshwork cells were transfected to express wild-type or mutant (C245Y, G364V, P370L, Y437H) myocilin-green fluorescent protein fusion protein and were treated or not with various chemical chaperones (glycerol, dimethylsulfoxide, or sodium 4-phenylbutyrate) for different time periods. The secretion, Triton X-100 solubility, and intracellular distribution of wild-type and mutant myocilin were analyzed by immunoprecipitation, Western blotting, and confocal double immunofluorescence. The effect of sodium 4-phenylbutyrate on ER stress proteins and apoptosis was examined in cells coexpressing mutant and wild-type myocilin. RESULTS: Treatment with sodium 4-phenylbutyrate, but not with glycerol or dimethylsulfoxide, reduced the amount of detergent-insoluble myocilin aggregates, diminished myocilin interaction with calreticulin, and restored the secretion of mutant myocilin. Heteromeric complexes formed by mutant and wild-type myocilin induced the ER stress-associated phosphorylated form of ER-localized eukaryotic initiation factor (eIF)-2alpha kinase and the active form of caspase 3, which resulted in an increased rate of apoptosis. Sodium 4-phenylbutyrate treatment of cells coexpressing mutant and wild-type myocilin relieved ER stress and significantly reduced the rate of apoptosis. CONCLUSIONS: These findings indicate that sodium 4-phenylbutyrate protects cells from the deleterious effects of ER-retained aggregated mutant myocilin. These data point to the possibility of a chemical chaperone treatment for myocilin-caused primary open-angle glaucoma.

Reference Type: Journal Article
Record Number: 21
Author: Yam, G. H.; Roth, J.; Zuber, C.
Year: 2007
Title: 4-Phenylbutyrate rescues trafficking incompetent mutant alpha-galactosidase A without restoring its functionality
Journal: Biochem Biophys Res Commun
Volume: 360
Issue: 2
Pages: 375-80
Abstract: Fabry disease is a lysosomal storage disorder caused by deficiency of alpha-galactosidase A. Most mutant enzyme is catalytically active but due to misfolding retained in the endoplasmic reticulum. We have tested 4-phenylbutyrate for its potential to rescue various trafficking incompetent mutant alpha-galactosidase A. Although we found that the trafficking blockade for endoplasmic reticulum-retained mutant alpha-Gal A was released, neither a mature enzyme was detectable in transgenic mice fibroblasts nor a reversal of lysosomal Gb3 storage in fibroblasts from Fabry patients could be observed. Because of lack of functionality of rescued mutant alpha-galactosidase A, 4-phenylbutyrate seems to be of limited use as a chemical chaperone for Fabry disease.

Reference Type: Journal Article
Record Number: 37
Author: Hines, P.; Dover, G. J.; Resar, L. M.
Year: 2008
Title: Pulsed-dosing with oral sodium phenylbutyrate increases hemoglobin F in a patient with sickle cell anemia
Journal: Pediatr Blood Cancer
Volume: 50
Issue: 2
Pages: 357-9
Abstract: Increasing hemoglobin F (HbF) appears to be beneficial for patients with sickle cell anemia. We previously demonstrated that daily, oral sodium phenylbutyrate (OSPB) induces HbF synthesis in pediatric and adult patients with hemoglobin SS (HbSS). The high doses and need for daily therapy, however, have limited its use. Here, we report a patient treated with pulsed-dosing of OSPB for over 3 years. This patient developed a modest, but sustained elevation in HbF over the course of therapy without side effects. Although larger studies are needed, this case demonstrates that pulsed-dosing with OSPB enhances HbF synthesis.