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Butyrate and Phenylacetate as Differentiating Agents: Practical Problems and Opportunities

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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.

Key words: Butyrate, differentiating agents, phenylacetate, phenylbutyrate, tributyrin

It has been known for some years that continuous exposure to butyric acid salts, particularly the sodium salt, acts *in vitro* on many abnormal or transformed cell lines to cause these cells to change to a more normal state, including phenotype and function [1–5]. Building upon these original observations in neoplastic cell populations, further studies have established that butyrate also produces physiologic maturational effects in non-cancerous cells, promoting erythropoiesis and differentiation of keratinocytes [6–9]. The concentration of butyrate required to achieve differentiation effects in leukemia cells and solid tumor lines is in the range of 0.3 mM or higher. In contrast, desirable changes in globin synthesis in the aberrant blood cells of sickle cell anemia and thalassemia can be obtained *in vitro* at lower concentrations of about 0.05 mM [7,8].

Clinical trials of butyrate salts in leukemia patients have generally been unsuccessful. An early attempt at using intravenous infusions of sodium butyrate at 500 mg/kg body weight per day for several days produced only a short-lived remission in a child with leukemia [10]. A larger study using the same infusion rate showed no clinical

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response; however, it also demonstrated that the infused butyrate had a very short metabolic half-life of about 6 min, resulting in peak blood levels below 0.05 mM [11]. These levels are considered ineffective to treat leukemia. Higher rates of intravenous infusion could not be considered because of the risk of toxicity from sodium overload. In contrast, a recent study used the arginine salt of butyrate to avoid sodium overload and achieved success in treating thalassemia and sickle cell anemia patients by continuous intravenous infusions of 500 mg/kg per day or higher for several days [12]. Blood levels of butyrate in these studies also did not exceed 0.05 mM, and the butyrate was apparently rapidly metabolized.

Butyrate is a normal metabolite supplied to mammals from two main sources. It is produced in the colon as a major product of bacterial fermentation of unabsorbed carbohydrate, reaching concentrations of up to 20 mM in the colon and feces of many animals and man [13]. Butyric acid is also present at low levels in many fruits and vegetables, but its richest source is from milk fat (butter, *etc.*), which contains 3–4% butyrate in a complex of glycerides or esters of glycerol [14]. Butyryl triglyceride (tributyrin, or glyceryl tributyrate) is a candidate prodrug for butyrate that could be administered orally. Tributyrin has not been assessed in tumor-bearing animals, but when used at a concentration of 5% in the feed of our laboratory mice to prevent chemically induced colon cancer, it was ineffective [15]; nevertheless, it lacked the cancer promoting effect that the sodium salt of butyrate had produced in equivalent intake [16]. Apparently, the high sodium intake acted as a cancer promoter and the butyrate was metabolized too rapidly to be an effective preventive [15].

PHARMACOKINETIC PROBLEMS OF BUTYRATE

Butyrate levels must be continuously elevated to achieve the desired differentiation effects on cancer cell lines or defective red blood cell precursors in sickle cell anemia or thalassemia patients. *In vitro*, this apparently requires about 0.05 mM for the desired red blood cell changes [7,8], and a minimum of 0.3 mM, but preferably 1–5 mM, for leukemic cells [5] and colon cancer cells [4,17]. Presumably similar butyrate concentrations would be required in plasma to be effective *in vivo*.

In the original anecdotal study in one child with previously treated acute myelogenous leukemia, 500 mg/kg per day of butyrate as the sodium salt was infused intravenously daily for 10 days, producing a transient partial remission of the disease; however, no plasma butyrate measurements were made and repeat courses of butyrate therapy were not attempted [10]. In a later careful study, Miller [11] used a similar dose and route of administration in adult leukemia patients who failed to respond clinically. Miller also noted that butyrate concentrations ≥0.5 mM were required to induce differentiation of leukemic cells *in vitro*; however, in the patient population studied, the drug infusion produced a maximum plasma butyrate concentration of only 0.05 mM. This study also established that plasma clearance of infused butyrate is extremely rapid, with a single phase half-life of 6 min. Miller *et al.* [11] attributed the lack of clinical response to the rapid clearance of butyrate. Apparently they were constrained from using a higher dose and rate of administration because of concern over possible sodium overload. A similar very short plasma half-life has also been observed in laboratory animals given butyrate ion with intravenously administered sodium butyrate [18].

Arginine, a basic amino acid, was formulated as a butyrate salt to eliminate the use of the sodium ion and safely permit higher doses and rates of infusion of the butyrate ion [18]. Perrine [12] reported successful treatment of thalassemia and sickle cell anemia patients by continuous intravenous infusions of 500 mg/kg per day, and even higher doses for several days in a few cases. However, blood levels of butyrate did not exceed 0.05 mM, and it was apparently rapidly metabolized, similar to the sodium salt [11].

TRIBUTYRIN, GLYCERYL TRIBUTYRATE

More than 95% of fatty acids are found in foods as triglycerides rather than as free fatty acids, suggesting that butyrate could be fed in this form rather than as sodium or amino acid salts. Triglycerides are relatively inert, and reduce the problem of tissue irritation and toxicity from ingesting large amounts of ionized free fatty acids. The need to ingest large quantities of damaging cations such as sodium is also avoid-

ed. Furthermore, 1 mole of tributyrin supplies 3 moles of butyrate. Our early pilot experiment examined the butyrate levels achieved following oral tributyrin administration. Sprague-Dawley rats weighing about 300 g were treated with 1 ml of tributyrin (3 ml/kg body weight) by gavage following an overnight fast. This produced a plasma butyrate level of 0.34 mM at 0.5 hr, with a subsequent decline to the basal level at 4 hr; the estimated plasma half-life of butyrate following tributyrin administration was 40 min. Studies by Yuan *et al.* [19] sponsored by the National Cancer Institute at the University of Maryland in larger groups of mice and rats have confirmed and extended these pilot data (shown in Table I).

These data in mice and rats suggest that far higher and more prolonged plasma levels of butyrate can be achieved with oral dosing than by the limited effects of intravenous sodium or arginine salts of butyrate. The oral route is also more convenient and far lower in cost, and suitable for ambulatory subjects. Our pilot study [20] and the more extensive study of Yuan *et al.* [19] in rodents measured the plasma butyrate as butyrate ion. Increased and prolonged plasma levels of butyrate after oral administration may be due to one or more causes: slow, prolonged hydrolysis and absorption of the oral bolus; absorption of partial hydrolysis products of tributyrin (mono- and dibutyrins), which are slowly hydrolyzed in blood to butyrate ion; or absorption of tributyrin itself, which may be feasible due to its comparatively low molecular weight (302 Da), low viscosity and lipophilicity. Conjugation of butyrate as any of the glyceride ester forms does not appear to reduce, and may even enhance, its pharmacodynamic activity.

Monobutyrin has been equivalent in differentiation activity with sodium butyrate in some cell lines, and only slightly less in others [21,22]. In a recent report, Chen and Breitman [23] demonstrated equivalent activity of monobutyrin with sodium butyrate in differentiation of HL-60 human myeloid leukemia cells and murine erythroleukemia cells. However, tributyrin was 4-fold more active on a molar basis, equivalent to 1.3 times more active on a molecular equivalent basis.

The dosage of tributyrin given orally to humans to achieve effective and prolonged plasma butyrate levels is as yet unknown. If it is assumed that the required human dose is about one-tenth that of the rat on a mg/kg body weight basis based on the ten-fold difference in metabolic rates of the two species, then the dosage range falls into a range of acceptability based on several previous human and animal studies with tributyrin, especially if synergy with retinoic acid occurs *in vivo*, as shown *in vitro* for leukemia cells [23].

Practical problems with tributyrin are minimal. It is high purity and commercially available at low cost. However, it has a mild, lingering odor and a bitter taste. These problems have been readily solved at the National Cancer Institute by formulating the liquid tributyrin in soft gelatin capsules; the gelatin acts as an effective odor and taste barrier, is easy to swallow, and rapidly releases the tributyrin in the stomach.

SYNERGY OF BUTYRATES WITH OTHER AGENTS

Vitamin D Metabolites

The active metabolite of vitamin D (1,25-dihydroxyvitamin D_3), along with some related synthetic analogues, have enhanced butyrate differentiation of HT-29 human colonic carcinoma cells *in vitro* [24,25].

Retinoic Acid

Chen and Breitman [23] have demonstrated a remarkable ten-fold mutual synergy of butyrate and retinoic acid on differentiation of human myeloid leukemia HL-60 cells *in vitro*. The combination readily achieved differentiation, with a butyrate level of about 0.05 M and a retinoic acid level of about 25 nM, levels which should theoretically be achieved with moderate oral doses of tributyrin and retinoic acid. However, this concept remains to be tested *in vivo*. If verified *in vivo*, a synergistic combination of retinoic acid and tributyrin could become a powerful tool for treatment and control of leukemias and possibly other tumors.

OTHER BUTYRATE DERIVATIVES

n-Butyramide, an extensively water soluble molecule, has only about 4% of sodium butyrate's activity in cell culture systems [21]. Isobutyramide has been reported to have a long

TABLE I. Plasma Pharmacokinetics (PK) of Butyrate After the Administration of TB and NaB to Mice and Rats

Dose g/kg	Route	Agent Used*	Species	Plasma Half-life (min)
10.3	po	TB	Mice	106
7.75	po	TB	Mice	55
5.2	po	TB	Mice	31
3.1	po	TB	Mice	23
1.25	iv	NaB	Mice	3.8
0.94	iv	NaB	Mice	3.8
0.62	iv	NaB	Mice	2.4
0.31	iv	NaB	Mice	1.4
10.2	po	TB	Rats	34
5.2	po	TB	Rats	46
3.6	po	TB	Rats	35
0.5	iv	NaB	Rats	4.5

* TB=tributyrin, NaB=sodium butyrate. Adapted from [19].

plasma half-life (7–10 hr) in humans after a single oral dose of 115, 150 or 300 mg/kg; these doses produced no demonstrable toxicity [26]. It appears to be a promising agent for clinical trials; however, details of effective concentrations needed in the *in vitro* model systems are lacking. Nevertheless, the authors predict that the required plasma level for stimulating fetal hemoglobin production would be 0.3 mM, which could apparently be readily achieved with two to three doses daily of 115–150 mg/kg per day [26].

A series of butyric monosaccharide esters have induced differentiation in three immortalized cell lines and two carcinoma cell lines derived from human breast tissue *in vitro*, and also induced antitumor activity in nude mice inoculated with Crocker 180TG sarcoma cells [27,28]. These esters rapidly diffuse in tissue and exhibit low toxicity in mice and rats. Two of these, the monobutyrate esters at either the 3 or 6 hydroxy position of 1,2-monoacetone glucose, are excreted 100–300 times more slowly than butyrate salts [29]. This report also studies seven butyric esters with varying degrees of hydroxyl esterification of glucose or acetone glucose, as well as pharmacokinetic studies in rabbits [29]. Using butyrate esters of sugar products appears to be very promising for development of longer acting, active butyrate-type antitumor differentiating agents.

A large series of butyrate derivatives has been developed in Israel, designed as prodrugs to release butyrate *in vivo* at a controlled rate to improve biologic activity as anticancer agents [30]. Most promising is pivayloxymethyl butyrate; its activity stems from hydrolytically released butyric acid. When tested as an antitumor agent in mice, pivalyloxymethyl butyrate increased survival and inhibited development of metastatic lung tumor foci when implanted with LL2 or 3LLD122 Lewis lung carcinoma cells [30]. This compound has displayed antitumor activity in the B16FD melanoma primary cancer model and decreased the tumor burden in mice inoculated with the highly metastatic B16F10.9 cell line [31]. Although low acute toxicity is claimed for this compound (LD_{50} = 1.36 g/kg body weight), theoretical concerns with the potential release of a mole of formaldehyde with each mole of buty-

rate released on hydrolysis has limited the clinical use of some antibiotics using similar pivalyloxymethyl substitutions (pivampicillin, pivcefalesin and amdinocillin pivoxil). However, the potential benefit in life-threatening cancer situations might largely offset the risk involved. Further studies are needed to establish the utility of this approach.

PHENYLACETATE

Phenylacetic acid, or phenylacetate, is a product of phenylalanine metabolism normally present in mammalian circulation [32]. It is also produced by the microbial flora of the large bowel and appears in feces and urine. It is excreted in the urine, largely as phenylacetylgutamine. Over the past two decades, phenylacetic acid and its prodrug, phenylbutyric acid, have been administered to children suffering from hyperammonemia due to inborn errors of urea synthesis [33, 34], and to patients with hyperammonemia resulting from chemotherapy for leukemia [35], or portal systemic encephalography [36].

Preclinical studies indicate that phenylacetate modifies the biology of various hematopoietic and solid tumors *in vitro*, including prostatic carcinoma and glioblastoma [22,37]. This effect is produced when phenylacetate is present in concentrations over 2 mM for a minimum of two weeks [22,37–39]. A recent report of a Phase I and pharmacokinetic study of intravenous phenylacetate in patients with cancer achieved stable phenylacetate serum concentrations of 200–300 mg/ml, (about 1.5–2.2 mM) for 2 weeks and also showed clinical improvement in a few of the patients [40]. The dose-limiting toxic effect of phenylacetic acid administration was reversible for central nervous system dysfunction.

A significant practical problem with clinical use of phenylacetate is its strong objectionable odor, similar to a horse stable. This odor is characteristic of the oral dosage forms and appears in patients as well. Phenylbutyrate has been an effective prodrug as a biochemical precursor of phenylacetate [41]. Phenylbutyrate is far more acceptable in terms of odor, and is considered more practical for clinical use than phenylacetate.

OPPORTUNITIES FOR BUTYRATE AND PHENYLACETATE

Many *in vitro* studies indicate potent differentiation activity and concomitant cell proliferation

TABLE II. Diseases for Possible Application of Butyrates, Phenylacetates and Related Compounds

Disease	Agent	Data	Reference
Leukemias	Butyrates	*in vitro, in vivo*	20
Leukemias	Butyrates plus Retinoic acid	*in vitro*	23
Leukemias	Phenylacetate	*in vitro*	22,35–37
Leukemias	Phenylacetate	*in vitro*	41
Breast cancer	Butyrate	*in vivo* (rat)	39
β Thalassemia	Butyrate	*in vivo* (human)	8
β Thalassemia	Phenylacetate	*in vitro*	40
β Thalassemia	Phenylacetate	*in vitro*	40
Sickle cell anemia	Phenylacetate	*in vivo* (human)	12
Neuroblastoma	Phenylacetate	*in vitro*	42
Colon cancer	Butyrate	*in vitro*	43

induction of butyrate and related compounds in cancer cell lines [1–5,22,32–39]. The current development of tributyrin as an oral ly administered agent to achieve more elevated and prolonged plasma butyrate levels, as well as evidence of strong synergy with retinoic acid [23], indicates potential for clinical, therapeutic, and possible chemopreventive applications in high-risk subjects. In similar fashion, phenylacetate and its prodrug phenylbutyrate offer equal opportunities for clinical development. Some of these opportunities are shown in Table II. The majority of these are solely based on *in vitro* data at this time, but a few studies *in vivo* in rats and humans suggest that with successful plasma levels of butyrate (from oral tributyrin), or phenylacetate (*per se*, or from phenylbutyrate), perhaps combined with retinoic acid or an active form or analogue of 1,25-dihydroxyvitamin D_3, may be very useful clinical tools.

In summary, the differentiation effects of butyrate, its derivatives and analogues, has prompted research interest, particularly for use in treating thalassemia, sickle cell anemia and certain leukemias. The aim of that research is to develop safe reliable methods of achieving effective blood levels without excessive modulation by rapid metabolism. Success could result in powerful tools for the treatment and control of several diseases.

REFERENCES

1. Leder A, Leder P: Butyric acid, a potent inducer of erythoid differentiation in cultured erythroleukemia cells. Cell 5:319–322, 1975.
2. Prasad KN, Sinha PK: Effect of sodium butyrate on mammalian cells in culture: A review. In Vitro 12: 125–132, 1976.
3. Collins SJ, Ruscetti FW, Gallagher RE, Gallo RC: Terminal differentiation of human promyelocytic leukemia cells induced by dimethylsufoxide and other polar compounds. Proc Natl Acad Sci USA, 75:2458–2462, 1978.
4. Augeron C, Laboisse CL: Emergence of permanently differentiated cell clones in a human colonic cancer cell line in culture after treatment with sodium butyrate. Cancer Res 44:3961–3969, 1984.
5. Breitman TR, He R: Combinations of retinoic acid with either sodium butyrate, dimethyl sulfoxide or hexamethylene bisacetamide synergistically induce differentiation of the human myeloid leukemia cell line HL-60. Cancer Res 50:6268–6273, 1990.
6. Perrine SP, Rudolph A, Faller DV, Roman C, Cohen RA, Chen S-J, Kan YW: Butyrate infusions in the ovine fetus delay the biologic clock for globin gene switching. Proc Natl Acad Sci USA 85:8540–8542, 1988.
7. Constantoulakis P, Knitter G, Stamatoyannopoulos G: On the induction of fetal hemoglobin by butyrates: *In vivo* and *in vitro* studies with sodium butyrate and comparison of combination treatments with 5-ZaC and AraC. Blood 74:1963–1971, 1989.
8. Perrine SP, Miller BA, Faller DV, Cohen RA, Vichinsky EP, Hurst D, Lubin BH, Papyannopoulou T: Sodium butyrate enhances fetal globin gene expression in erythroid progenitors of patients with Hb SS and β-thalassemia. Blood 74:454–459, 1989.
9. Staiano-Coico L, McMahon CK, Pagan-Charry I, LaBruna A, Piraino V, Higgins PJ: Sodium-*N*-butyrate induces cytoskeletal rearrangements and formation of cornified envelopes in cultured adult human keratinocytes. Cell Tissue Kinet 22:361–375, 1989.
10. Novogrodsky A, Dvir A, Ravid A, Shkolnik T, Stenzel KH, Rubin AL, Zaizov R: Effect of polar organic compounds on leukemic cells. Butyrate-induced partial remission of acute myelogenous leukemia in a child. Cancer 51:9–14, 1983.
11. Miller AA, Kurschel E, Osieka R, Schmidt CG: Clinical pharmacology of sodium butyrate in patients with acute leukemia. Eur J Cancer Clin Oncol 23: 1283–1287, 1987 .
12. Perrine SP, Ginder GD, Faller DV, Dover GH, Ikuta T, Witkowska HE, Cai S-P, Vichinsky EP, Olivieri NF: A short-term trial of butyrate to stimulate fetal globin gene expression in the beta globin disorders. N Engl J Med 328:81–86, 1993.
13. Cummings JH, Pomare EW, Branch WJ, Naylor CPE, MacFarlane GT: Short-chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut 28:1221–1227, 1987.
14. "Composition of Foods: Dairy and Egg Products." United States Department of Agriculture Handbook 8-1, Washington, DC, 1976.
15. Deschner EE, Ruperto JF, Lupton JR, Newmark HL: Dietary butyrate (tributyrin) does not enhance AOM-induced colon tumorigenesis. Cancer Lett 52:79–82, 1990.
16. Freeman JJ: Effects of differing concentrations of sodium butyrate on 1,2-dimethylhydrazine-induced rat intestine neoplasia. Gastroenterology 91:596–602, 1986
17. Tanaka Y, Bush KK, Klauck TM, Higgins PJ: Enhancement of butyrate-induced differentiation of HT-29 human colon carcinoma cells by 1,25-dihydroxy vitamin D_3. Biochem Pharmacol 38:3859–3865, 1989.
18. Daniel P, Brazier M, Cerutti I, Pieri F, Tardivel I, Desmet G, Baillet J, Chany C: Pharmacokinetic study of butyric acid administered *in vivo* as sodium and arginine butyrate salts. Clin Chim Acta 181:255–264, 1989.
19. Yuan Z, Eiseman J, Plasance K, Sentz D, Bigora S, Fossler M, Young D, Egorin M: Plasma pharmacokinetics (PK) of butyrate after the administration of

tributyrin and Na butyrate to mice and rats. Proc Am Assoc Cancer Res 35:429, 1994 (abstract).
20. Newmark HL, Lupton JR, Young CW: Butyrate as a differentiating agent: Pharmacokinetics analogies and current status. Cancer Lett 78:1–5, 1994.
21. Lea MA, Xiao Q, Sadhukhan A, Sharma S, Newmark HL: Butyramide and monobutyrin: Growth inhibitory and differentiating agents. Anticancer Res 13:145–150, 1993.
22. Samid D, Shack S, Sherman LT: Phenylacetate: A novel nontoxic inducer of tumor cell differentiation. Cancer Res 52:1988–1992, 1992.
23. Chen Z, Breitman T: Tributyrin: A prodrug of butyric acid for potential clinical application in differentiation therapy. Cancer Res 54:3494–3499, 1994.
24. Tanaka Y, Bush KK, Klauck TM, Higgins PJ: Enhancement of butyrate-induced differentiation of HT-29 human colon carcinoma cells by 1,25-dihydroxy vitamin D_3. Biochem Pharmacol 38:3859, 1989.
25. Tanaka Y, Bush KK, Eguchi T, Ikekawa N, Taguchi T, Kobayashi Y, Higgins PJ: Effects of 1,25-hydroxy vitamin D_3 and its analogs on butyrate-induced differentiation of HT-29 human colonic carcinoma cells and on the reversal of the differentiated phenotype. Arch Biochem Biophys 276:415, 1990.
26. Perrine SP, Faller DV: Butyrate-induced reactivation of the fetal globin genes: A molecular treatment for the β-hemoglobinopathies. Experientia 49:133–137, 1993.
27. Planchon P, Raux H, Magnien V, Ronco G, Villa P, Crepin M, Brouty-Boye D: New stable butyrate derivatives alter proliferation and differentiation in human mammary cells. Int J Cancer 48:443–449, 1991.
28. Pouillart P, Cerutti I, Ronco G, Villa P, Chany C: Butyric monosaccharide ester-induced cell differentiation and anti-tumor activity in mice. Importance of their prolonged biological effect for clinical applications in cancer therapy. Int J Cancer 49:89–95, 1991.
29. Pouillart P, Ronco G, Cerutti I, Trouvin JH, Pieri F, Villa P: Pharmacokinetic studies of *N*-butyric acid mono- and polyesters derived from monosaccharides. J Pharm Sci: 81:241–244, 1992.
30. Rephaeli A, Rabizadeh E, Aviram A, Shaklai M, Ruse M, Nudelman A: Derivatives of butyric acid as potential anti-neoplastic agents. Int J Cancer 49:66–72, 1991.
31. Nudelman A, Ruse M, Aviram A, Rabizadeh E, Shaklai M, Zimrah Y, Rephaeli A: Novel anticancer prodrugs of butyric acid. 2. J Med Chem 35:687–694, 1992.
32. Sandler M, Ruhven CRJ, Goodwin BL, Lee A, Stern GM: Phenylacetic acid in human body fluids: High correlation between plasma and cerebrospinal fluid concentration values. J Neurol Neurosurg Psychol 45:366–368, 1982.
33. Brusilow SW, Danney M, Waber LJ, Batshaw M, Burton B, Levitsky L, Roth K, McKeethren C, Ward J: Treatment of episodic hyperammonemia in children with inborn errors of urea synthesis. N Engl J Med 310:1630–1634, 1984.
34. Simell O, Sipila I, Rajanti J, Valle DL, Brusilow SW: Waste nitrogen excretion via amino acid acylation: Benzoate and phenylacetate in lysinuric protein intolerance. Pediatr Res 20:1117–1121, 1986.
35. Watson AJ, Karp JE, Walker WG, Chambers T, Risch VR, Brusilow SW: Transient idiopathic hyperammonaemia in adults. Lancet 2:1271–1274, 1985.
36. Mendenhall CL, Rouster S, Marshall L, Weesner R: A new therapy for portal systemic encephalopathy. Am J Gastroenterol 81:540–543, 1986.
37. Samid D, Yeh A, Prasanna P: Induction of erythroid differentiation and fetal hemoglobin production in human leukemic cells treated with phenylacetate. Blood 80:1576–1581, 1992.
38. Samid D, Shack S, Myers CE: Selective growth arrest and phenotypic reversion of prostate cancer cells *in vitro* by nontoxic pharmacological concentrations of phenylacetate. J Clin Invest 91:2288–2295, 1993.
39. Samid D, Ram Z, Hudgins WR, Shack S, Liu L, Walbridge S, Oldfield EH, Myers CE: Selective activity of phenylacetate against malignant gliomas: Resemblance to fetal brain damage in phenylketonuria. Cancer Res 54:891–895, 1994.
40. Thibault A, Cooper MR, Figg WD, Venzon DJ, Sartor O, Tompkins AC, Weinberger MS, Headlee DJ, McCall NA, Samid D, Myers CE: A Phase I and pharmacokinetic study of intravenous phenylacetate in patients with cancer. Cancer Res 54:1690–1694, 1994.
41. Dover GJ, Brusilow S, Samid D: Increased fetal hemoglobin in patients receiving sodium phenylbutyrate. N Engl J Med 327:569–570, 1992.
42. Yanagi S, Yamashita M, Imai S: Sodium butyrate inhibits the enhancing effect of high-fat diet on mammary tumorigenesis. Oncology 50:201–204, 1993.
43. Fibach E, Prasanna P, Rodgers GP, Samid D: Enhanced fetal hemoglobin production by phenylacetate and 4-phenylbutyrate in erythroid precursors derived from normal donors and patients with sickle cell anemia and β-thalassemia. Blood 82:2203–2209, 1993.
44. Cinatl J, Cinatl J, Mainke M, Weissflog A, Rabenau H, Kornhuber B, Doerr HW: *In vitro* differentiation of human neuroblastoma cells induced by sodium phenylacetate. Cancer Lett 70:15–24, 1993.
45. Bernard JA, Warwick G: Butyrate rapidly induces growth inhibition and differentiation in HT-29 cells. Cell Growth Differ 4:495–501, 1993.