Cancer Chemother Pharmacol (1999) 43: 445–453

ORIGINAL ARTICLE

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Plasma pharmacokinetics of butyrate after intravenous administration of sodium butyrate or oral administration of tributyrin or sodium butyrate to mice and rats

Received: 27 July 1998 / Accepted: 3 November 1998

Abstract *Purpose*: To define the plasma concentrations of butyrate achieved and the profile of plasma butyrate concentrations versus time in mice and rats treated with tributyrin or sodium butyrate. *Methods*: Female CD_2F_1 mice were treated with tributyrin by oral gavage or with sodium butyrate by i.v. bolus or oral gavage. Oral tributyrin doses delivered to mice were 3.1, 5.2, 7.8, and 10.3 g/kg. Intravenous sodium butyrate doses were 0.31, 0.62, 0.94, and 1.25 g/kg. Oral sodium butyrate was given to mice at 5 g/kg. Subsequently, similar studies were performed in female Sprague-Dawley rats. Rats were given tributyrin by oral gavage at doses of 3.6, 5.2, or 10.3 g/kg or sodium butyrate i.v. at a dose of 500 mg/kg. Plasma butyrate concentrations were determined by gas chromatography. *Results*: In mice, oral dosing with tributyrin resulted in detectable plasma butyrate concentrations as early as at 5 min after treatment and produced peak plasma butyrate concentrations at between 15 and 60 min after dosing. Peak plasma butyrate concentrations increased proportionally with increasing tributyrin dose, but as the oral tributyrin dose increased there was a greater than proportional increase in the area under the curve of plasma butyrate concentrations versus time (AUC). At a tributyrin dose of 10.3 g/kg, plasma butyrate concentrations peaked at approximately 1.75 m*M* and remained ≥1 m*M* for between 10 and 60 min after dosing. However, approximately 10% of mice treated with this dose died acutely. At a tributyrin dose of 7.8 g/kg, plasma butyrate concentrations reached approximately 1 m*M* by 15 min after dosing and remained between 0.8 and 1 m*M* until 60 min after dosing. No mouse treated with this dose died acutely. Mice given tributyrin doses of 5.2 and 3.1 g/kg achieved peak plasma butyrate concentrations of approximately 0.9 and 0.5 m*M*, respectively, by 45 min after dosing. Plasma butyrate concentrations in these mice remained above 0.1 m*M* until 120 and 90 min after dosing, respectively. The four i.v. doses of sodium butyrate resulted in plasma concentration-time profiles that also indicated nonlinear pharmacokinetics and were well described by a one-compartment model with saturable elimination. Values recorded for the Michaelis-Menten constant (K_m) and the maximal velocity of the process (V_{max}) ranged between 1.02 and 5.65 m*M* and 0.60 and 1.82 mmol/min, respectively. Values noted for the volume of the central compartment (V_c) varied between 0.48 and 0.72 l/kg. At 1.25 g/kg, i.v. sodium butyrate produced peak plasma butyrate concentrations of 10.5–17.7 m*M*, and plasma butyrate concentrations remained above 1 m*M* for 20–30 min. Sodium butyrate delivered orally to mice at 5 g/kg produced peak plasma butyrate concentrations of approximately 9 m*M* at 15 min after dosing and plasma butyrate concentrations exceeding 1 m*M* for 90 min after dosing. In rats the 10.3-g/kg oral dose of tributyrin produced peak plasma butyrate concentrations of approximately 3 m*M* by 75 min after dosing and butyrate concentrations excedding 1 m*M* from 30 to 90 min after dosing. The

This work was supported by contract N01-CM27711, awarded by the National Cancer Institute

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plasma butyrate concentrations produced in rats by 5.2- and 3.6-g/kg doses were appropriately lower than those produced by the 10.3-g/kg dose, and there was no evidence of nonlinearity. The 500-mg/kg i.v. dose of sodium butyrate produced peak plasma butyrate concentrations in rats of approximately 11 m*M*, and the decline in plasma butyrate concentrations with time after dosing was consistent with saturable clearance. *Conclusion*: These studies document the ability to use oral administration of tributyrin to achieve pharmacologically relevant concentrations of butyrate in rodent plasma. They also document the nonlinear nature of butyrate clearance. These data are being used in the design of clinical trials of oral tributyrin in patients with malignancies and hemoglobinopathies.

Key words Butyrate · Tributyrin · Pharmacokinetics

Introduction

Butyrate is known to induce the differentiation of a number of animal and human leukemia and solid tumor cell lines [35]. It also selectively stimulates the δ-globin gene in fetal sheep, cultured human erythroid cells, and adult nonhuman primates [4, 26, 30–32]. As a result, butyrate has been considered an attractive candidate for the treatment of human malignancies as well as hemoglobinopathies, such as sickle cell anemia and β-thalassemia, and has actually been studied as either a sodium or an arginine salt in each of these disease states [11, 28, 29, 33, 37]. Butyrate has also been investigated as a means of modulating the response of Epstein-Barr-virus-associated lymphoma to ganciclovir [27]. However, parenteral administration of butyrate is problematic due to its very short plasma half-life [11, 28], which necessitates continuous i.v. infusion dosing, and potential complications associated with the sodium or arginine cations present in the available salt forms of butyric acid [33, 37]. Furthermore, the concentrations of butyrate produced in patients given continuous i.v. infusions of sodium or arginine butyrate are far lower than the concentrations required to produce a pharmacodynamic effect in vitro. As a result, effort has been devoted to the identification and development of analogues of butyrate [5, 8, 10, 14, 15, 18] or prodrugs of butyrate with more favorable pharmacologic properties. Ideally, such agents would, with logistically realistic dosing regimens, produce pharmacologically relevant concentrations of drug. Tributyrin is a triglyceride containing three butyrate moieties esterified to glycerol and is a component of a variety of foodstuffs [6, 23]. Moreover, tributyrin is a liquid that can be delivered orally and release butyrate when metabolized by pancreatic and, possibly, other lipases.

As a result, the animal studies described in the current manuscript were undertaken to (1) define the maximal oral doses of tributyrin tolerated by mice and rats, (2) document the plasma concentrations of butyrate associated with maximally tolerated and submaximal doses of tributyrin, (3) define the intervals over which plasma butyrate concentrations produced by oral tributyrin remained above selected threshold concentrations, and (4) compare the concentration versus time profile of butyrate produced by oral tributyrin with that produced by i.v. sodium butyrate. The data generated were to serve as a basis for an assessment of the feasibility of using oral administration of tributyrin in clinical trials and the development of a dose and dosing interval of tributyrin that would maintain desired concentrations of butyrate in human plasma for desired periods [7].

Materials and methods

Reagents

Denatured ethanol, *n*-butyric acid, 2-ethyl butyric acid, heptanoic acid, and tributyrin were purchased from Sigma Chemical Co. (St. Louis, Mo.).

Mice and rats

Specific pathogen-free adult, female CD_2F_1 mice (5–6 weeks of age) and specific pathogen-free, adult, female Sprague-Dawley rats (6–8 weeks of age) were obtained from the Animal Program administered by the Animal Genetics and Production Branch of the National Cancer Institute (Bethesda, Md.). Mice and rats were allowed to acclimate to the University of Maryland at Baltimore Animal Facility for at least 1 week before studies were initiated. To minimize exogenous infection, mice and rats were maintained in conventional cages in separate rooms and handled in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Number 85–23, 1985). Ventilation and air flow in the Animal Facility were set to 12 changes/h. Room temperatures were regulated at 72 ± 2 °F, and the rooms were on automatic 12-h light/dark cycles. Mice and rats received Purina 5001 Chow and water ad libitum except on the evening prior to dosing, when all food was removed and withheld until 4 h after dosing. On the evening prior to study, rats were anesthetized with 60 mg/kg of pentobarbital and had their jugular veins cannulated. Sentinel mice (CD-1 mice housed in cages with bedding that contained 20% bedding that had been removed from study-animal cages at cage change) were maintained in the animal rooms and assayed at monthly intervals for specific murine pathogens by murine antibody profile testing (Litton Bionetics, Charleston, S.C.). These mice remained free of specific pathogens throughout the study period, indicating that study mice and rats were also free of specific pathogens.

Tributyrin and sodium butyrate administration

Each dose of tributyrin or sodium butyrate was calculated on the basis of the fasted body weight of individual animals. Neat tributyrin was delivered to mice by oral gavage. Tributyrin doses were 3.1, 5.2, 7.75, or 10.3 g/kg. The oral dose of sodium butyrate given to mice was 5 g/kg. Oral doses were delivered by 1.5-inch, 22-gauge, curved gavage needle. Mice received i.v. sodium butyrate injected at doses of 0.31, 0.62, 0.94, or 1.25 g/kg over 30 s through a lateral tail vein.

Oral tributyrin was delivered to rats by 3-inch, 18-gauge, curved gavage needle. Oral tributyrin doses were 3.6, 5.2, and 10.3 g/kg. Intravenous sodium butyrate was delivered to rats as a bolus injection at a dose of 0.5 g/kg through a lateral tail vein.

Blood sampling

Three mice were studied at each time point. Blood was collected by cardiac puncture into heparinized syringes. Samples were obtained at 5, 10, 15, 30, 45, 60, 90, 105, 120, 150, 180, and 240 min after oral dosing with tributyrin and at 2.5, 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 210, and 240 min after i.v. dosing with sodium butyrate. Samples at 360 and 480 min were also included after oral delivery of sodium butyrate.

Three to five rats were studied at each dose level. Using each rat's indwelling venous catheter, blood was collected into heparinized syringes. Samples (250 µl) were obtained at 5, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 240, and 360 min after oral delivery of tributyrin and at 1, 3, 5, 7.5, 10, 12.5, 15, 20, 25, 30, and 40 min after i.v. delivery of sodium butyrate.

Blood samples from both species were quickly transferred to Eppendorf microcentrifuge tubes, placed into ice, and rapidly centrifuged at 13,000 *g* for 5 min to obtain plasma. Plasma and dosing solutions were stored frozen at −70 °C until analysis.

Butyrate analysis

Butyrate concentrations in plasma were determined by gas chromatography using a modification of the method of Boffa et al. [2]. In brief, 100 µl of plasma was dispensed into Eppendorf microcentrifuge tubes that contained 500 µl of a 3-m*M* solution of 2-ethyl butyric acid internal standard in 70% ethanol. The tubes were agitated briefly and then chilled in an ice bath for 10 min, after which they were shaken for 20 min on a Vortex Genie 2 (Model G-560, Scientific Industries, Inc., Bohemia, N.Y.) that was set at position 3 and housed in a cold (4 °C) room. After the shaking the tubes were centrifuged at 13,000 *g* for 15 min. Then, 100 µl of the resulting supernatant was mixed with 100 µl of 3 m*M* heptanoic acid in 70% ethanol and 20 µl of 10% H_3PO_4. This mixture was transferred with a glass Pasteur pipet into glass autosampler microvial inserts, and 1 µl was injected by autosampler into the gas chromatography system described below.

Butyrate concentrations were analyzed with a Hewlett-Packard 5890 gas chromatograph equipped with a Hewlett-Packard 7673A automatic sampler (Hewlett-Packard, Palo Alto, Calif.). Separation was accomplished on a 30-m, 0.53-mm inside diameter, HPFFA-PTA-TPA, fused-silica capillary column (film thickness 1 µm) that was preceded by a 1-m, 0.53-mm inside diameter, deactivated, fused-silica capillary precolumn. Injection was splitless with a purge time of 30 s, and the injection port was maintained at 165 °C. The carrier gas was helium, at 2.2 ml/min, and the make-up gas was nitrogen, at 28 ml/min. The oven temperature was maintained at 80 °C for 30 s and then increased at 70 °C/min to 145 °C, which was held for 5 min. After this the oven temperature was increased at 5 °C/min to 185 °C. Before being reduced to 80 °C for injection of the next sample, the oven temperature was increased to 200 °C and held there for 5 min to regenerate the column. Column effluent was monitored with a flame ionization detector maintained at 220 °C with an air flow of 430 ml/min and a hydrogen flow of 30 ml/min. The detector signal was processed with a Hewlett-Packard 3392A integrator so as to integrate the area under each peak. Under these conditions the retention times of internal standard, butyrate, and hexanoic acid were approximately 5.7, 7.5, and 10.4 min, respectively.

The butyrate concentration in each sample was calculated by determination of the ratio of the butyrate peak area to that of the corresponding internal standard peak and comparison of that ratio with a concomitantly performed standard curve prepared in the appropriate matrix. Standard curves were performed in duplicate and included butyrate concentrations between 0.05 and 15 m*M*. No decomposition was observed in solutions of butyrate prepared in mouse or rat plasma and stored overnight at −20 °C. Similarly, when samples were stored overnight at 22 °C, no decomposition was observed in extracts prepared from mouse or rat plasma containing 0.3 m*M* butyrate. There was no endogenous material in mouse or rat plasma that interfered with the determination of butyrate or internal standard. The assay was linear between 0.11 and 15 m*M* (9.9 and 1320 µg/ml). Recovery of butyrate (0.11, 0.32, and 0.64 m*M*) from mouse and rat plasma was approximately 100% and 98%, respectively, at all three concentrations. The coefficient of variability for the analysis in plasma was 1–5% with regard to both intraday analysis of any concentration on the standard curve or interday comparison of standard curves.

Pharmacokinetic analysis

Time courses of plasma concentrations of butyrate versus time were analyzed by both noncompartmental and compartmental methods. The maximal plasma butyrate concentration (C_{max}) and the time at which C_{max} occurred (T_{max}) were determined by visual inspection of the data. In studies involving oral administration of tributyrin and sodium butyrate the area under the curve, from time zero to infinity, of plasma butyrate concentrations versus time (AUC) was estimated noncompartmentally with the LaGrange function [39] as implemented by the LAGRAN computer program [36]. Similar analyses were done in studies of i.v. injection of sodium butyrate. In these studies the apparent clearance (CL_{app}) was calculated from the definition:

$$CL_{tb} = \frac{\text{Dose}}{\text{AUC}} \quad .$$

In addition, in studies of i.v. and oral sodium butyrate, concentrations of butyrate in plasma versus time were fit to a variety of compartmental models with the program ADAPT II [12] using weighted least-squares estimation. A one-compartment model with saturable elimination was judged most suitable as based on Akaike's information criteria (AIC) [1]. The model for orally delivered sodium butyrate included first-order input (k_a) from an absorptive site.

After it had been determined that the one-compartment model with saturable elimination was most suitable, the four i.v. studies in mice, which had been modeled individually, were also modeled simultaneously, with each study being treated as a separate bolus in a multiple dosing regimen and each dose being separated by a time sufficient for washout without carryover from the previous dose [16]. This time was set at 120 min. Mean values and standard deviations for individual parameters determined by a standard two-stage analysis [22] of the four individually modeled i.v. studies were then compared with the respective mean values and standard deviations produced by the simultaneous modeling.

Results

Preliminary toxicity studies

Initial efforts were directed at defining a suitably high oral dose of tributyrin for use in mice. A dose of 20.6 g/kg was lethal to four of ten mice within 24 h of drug delivery. However, only one of ten mice given 10.3 g/kg died within 24 h of drug delivery, and the remaining nine mice survived without obvious morbidity for the ensuing 7 days of observation.

Efforts were then directed at defining a suitably high i.v. dose of sodium butyrate for use in mice. A dose of 10 g/kg was lethal to two of two mice within 1 min of drug delivery. Similar results were produced by an i.v. dose of 7.5 g/kg. All five mice given a dose of 5 g/kg were dead within 24 h of dosing. All five mice given a dose of 2.5 g/kg were without a righting reflex for 30 min after dosing, and all had necrotic tails by 24 h

after dosing. All five mice given 1.25 g/kg were lethargic for 30 min after dosing but survived without obvious sequelae.

Subsequent studies defined a suitably high oral dose of sodium butyrate for delivery to mice. All five mice treated with 5 g/kg became cold and were without a righting reflex for 8 h. However, on the application of heat, all survived and were without adverse sequelae for the 7 days of observation.

In all toxicity studies, animals quickly exhibited respiratory evidence of a systemic metabolic acidosis with deep respirations similar to those observed in patients with diabetic ketoacidosis or the acidosis associated with renal failure.

Pharmacokinetics studies of oral tributyrin in rats used a maximal dose based on the ability to deliver a 2-ml volume by oral gavage and resulted in a dose of 10.3 g/kg. On the basis of the 1.25-g/kg maximal tolerated dose defined in mice, 500 mg/kg was selected as the maximal dose of sodium butyrate for i.v. delivery to rats.

Pharmacokinetics studies in mice

On the basis of the above-mentioned toxicity observations, 10.3 g/kg was selected as the highest dose to be used in studies investigating the plasma pharmacokinetics of butyrate in mice given oral tributyrin. Subsequent studies of oral tributyrin used sequential dose reductions, resulting in the delivery of doses of 7.8, 5.2, and 3.1 g/kg. These doses, which were approximately 75%, 50%, and 30% of the highest orally delivered dose, were used to evaluate the linearity of butyrate pharmacokinetics over a reasonably broad, yet relevant, range of oral tributyrin doses. After an oral tributyrin dose of 10.3 g/kg, plasma butyrate concentrations peaked at approximately 1.75 m*M* and remained above 1 m*M* for between 10 and 60 min after dosing (Fig. 1, Table 1). After an oral tributyrin dose of 7.8 g/kg, plasma butyrate concentrations reached approximately 1 m*M* by 15 min and remained between 0.8 and 1 m*M* until 60 min after dosing. Mice given tributyrin doses of 5.2 and 3.1 g/kg achieved peak plasma butyrate concentrations of approximately 0.9 and 0.5 m*M*, respectively. Plasma butyrate concentrations in these mice remained above 0.1 m*M* until 120 and 90 min after dosing, respectively. At each dose of tributyrin studied, peak plasma concentrations were achieved at between 15 and 60 min after dosing. Peak plasma butyrate concentrations increased as the tributyrin dose increased and did so in a proportional manner. The butyrate AUC also increased as the tributyrin dose increased but did so in a nonlinear fashion such that dose increases of between 5.2 and 7.8 g/kg and 7.8 and 10.3 g/kg were associated with a greater than proportional increase in the butyrate AUC (Table 1).

On the basis of the above-mentioned preliminary toxicity observations, i.v. studies of sodium butyrate used a maximal dose of 1.25 g/kg. Subsequent studies of i.v. sodium butyrate used sequential 25% decrements of this dose (0.94, 0.62, and 0.31 g/kg) to evaluate the linearity of butyrate pharmacokinetics after i.v. delivery over a relevant range of doses. The four i.v. doses of sodium butyrate resulted in plasma concentration-time profiles that also indicated nonlinear pharmacokinetics [37] and were well described by a one-compartment model with saturable elimination (Fig. 2, Table 2). In-

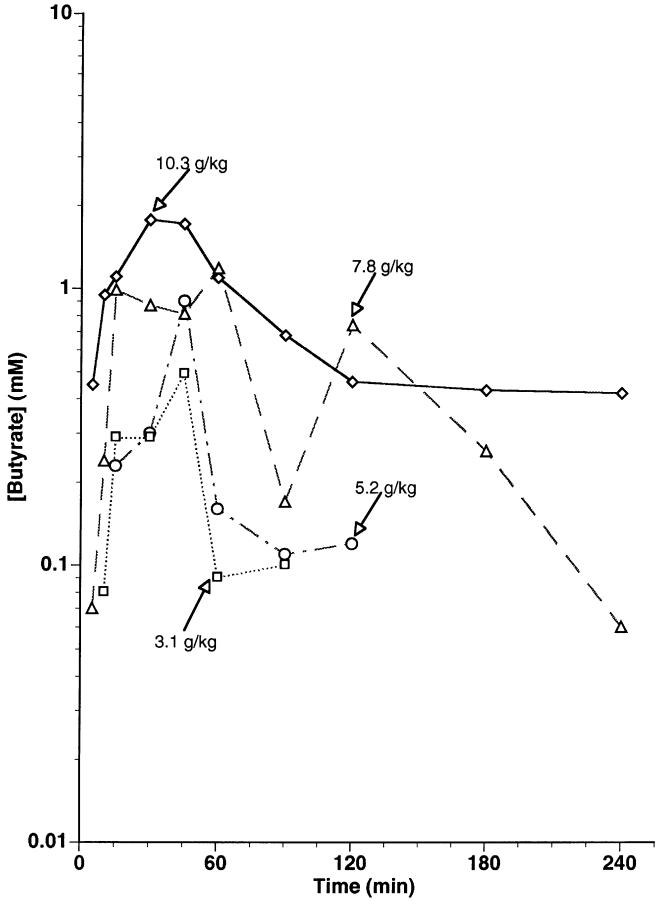

Fig. 1 Concentrations of butyrate determined in the plasma of mice given enteral tributyrin at doses of 10.3 (◇—◇), 7.8 (△- - - -△), 5.2 (○–·–·–·–○), or 3.1 (□·····□) g/kg. *Symbols* represent mean values recorded for 3 mice studied at each point

Table 1 Pharmacokinetic parameters describing butyrate concentrations achieved in mouse plasma after oral delivery of tributyrin

Dose (g/kg)	T_{max}^{a} (min)	C_{max}^{b} (m*M*)	AUC[c] (m*M* min)
10.3	30[d]	1.75[d]	236[d]
7.8	60	1.19	113
5.2	45	0.90	33
3.1	45	0.49	21

[a] Time at which maximal plasma concentration occurred
[b] Maximal plasma concentration
[c] Area under the curve of plasma butyrate versus time
[d] Values calculated on the basis of mean plasma butyrate concentrations determined in 3 mice studied at each time described in Materials and methods

travenous infusion of sodium butyrate produced peak plasma butyrate concentrations much greater than those produced by oral delivery of approximately 10-fold greater doses of tributyrin (Figs. 1, 2). However, these concentrations were not sustained as long as those produced by oral tributyrin. As a result, the butyrate AUC values produced by the highest, intermediate, and lowest doses of i.v. sodium butyrate were very similar to those associated with the highest, intermediate, and lowest doses of oral tributyrin used in this study (Tables 1, 2).

Only one dose of orally delivered sodium butyrate was studied in mice. This was 5 g/kg, which approximated the maximal tolerated dose. Oral delivery of 5 g/kg of sodium butyrate to mice produced plasma butyrate concentrations that were similar to those produced by i.v. delivery of sodium butyrate at 0.62 g/kg and were much higher than those produced by oral dosing with tributyrin (Figs. 1, 3). However, the plasma butyrate concentrations produced by oral delivery of sodium butyrate were sustained much longer than were those associated with i.v. delivery of the same agent (Figs. 2, 3). After delivery of the 5-g/kg sodium butyrate oral dose, plasma butyrate concentrations peaked at approximately 9 m*M* (Fig. 3) by 15 min after dosing and remained above 1 m*M* until 90 min after dosing. The time course of plasma butyrate concentrations resulting from a 5-g/kg oral dose of sodium butyrate was very well fit by a one-compartment model that incorporated nonlinear clearance and first-order absorption (Table 2).

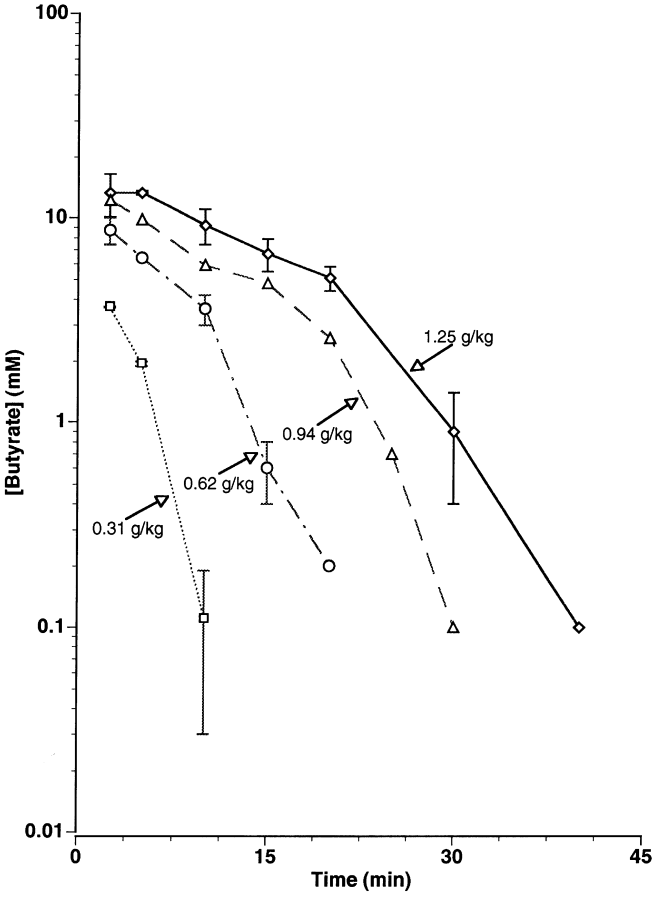

Fig. 2 Concentrations of butyrate determined in the plasma of mice given i.v. sodium butyrate doses of 1.25 (◇——◇), 0.94 (△- - -△), 0.62 (○– · – · – · –○), or 0.31 (□· · · · · □) g/kg. *Symbols* represent mean values recorded for 3 mice studied at each point. *Bars* represent SD

Pharmacokinetics studies in rats

Oral delivery of a 10.3-g/kg dose of tributyrin in rats produced peak plasma butyrate concentrations of

Table 2 Pharmacokinetic parameters describing butyrate concentrations achieved in mouse plasma after i.v. or oral delivery of sodium butyrate

Dose (g/kg)	Route	Non-compartmental modeling[a]			Compartmental modeling[a]			
		C_{max}[b] (m*M*)	AUC[c] (m*M* min)	CL_{app}[d] (l kg^{-1} min^{-1})	V[e] (l/kg)	K_m[f] (m*M*)	V_{max}[g] (mmol/min)	k_a[h] (min)
1.25	i.v.	13.3	200	0.057	0.72	2.86	0.76	NA[i]
0.94	i.v.	12.2	132	0.064	0.70	1.18	0.60	NA
0.62	i.v.	8.7	63	0.09	0.48	5.65	1.82	NA
0.31	i.v.	3.7	18	0.16	0.51	1.02	0.95	NA
0.31–1.25[j]	i.v.	NA	NA	NA	0.73	0.46	0.53	NA
5.0	p.o.	9.0	437	0.104	0.71	0.07	0.22	0.03

[a] Values calculated on the basis of mean plasma butyrate concentrations determined in 3 mice studied at each time described in Materials and methods
[b] Maximal plasma concentration
[c] Area under the curve of plasma butyrate concentrations versus time
[d] Apparent clearance
[e] Volume of the central compartment
[f] Michaelis-Menten constant
[g] Maximal velocity
[h] Absorption rate constant
[i] *NA* Not applicable
[j] Data from i.v. studies using 0.31 to 1.25-g/kg doses were co-modeled as described in Materials and methods

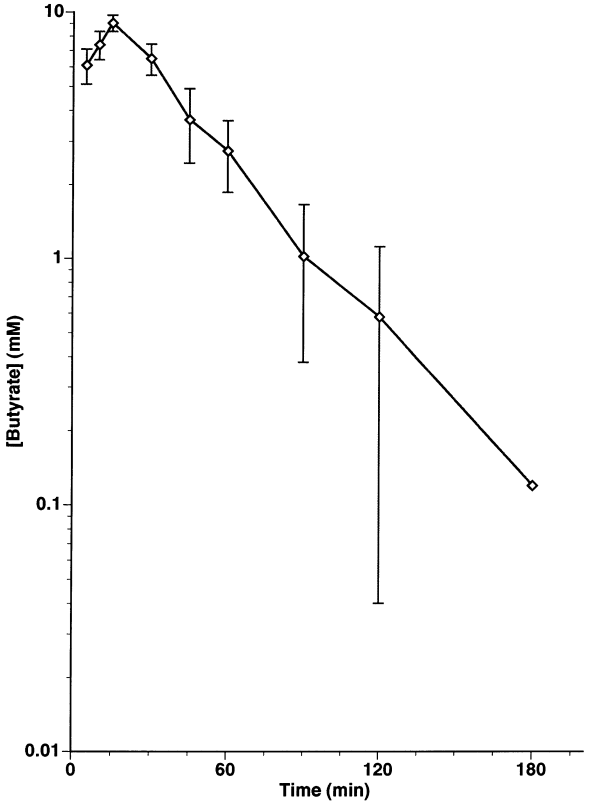

Fig. 3 Concentrations of butyrate determined in the plasma of mice given enteral sodium butyrate at a dose of 5 g/kg. *Symbols* represent mean values recorded for 3 mice studied at each point. *Bars* represent SD

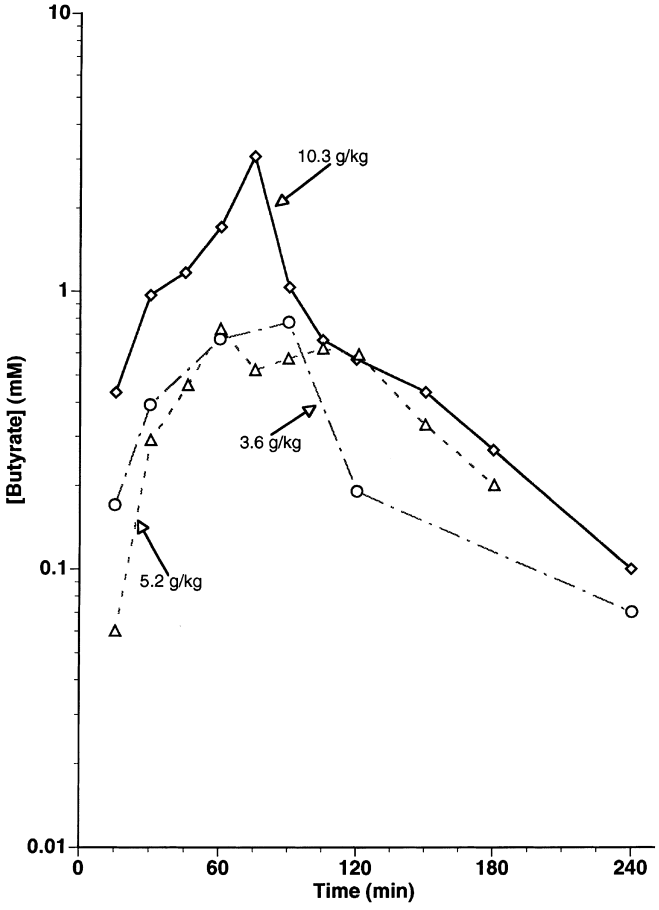

Fig. 4 Concentrations of butyrate determined in the plasma of rats given enteral tributyrin at doses of 10.3 (◇——◇), 5.2 (△- - - -△), or 3.6 (○-·- -·-○) g/kg. *Symbols* represent mean values recorded for 3–5 rats studied at each dose

approximately 3 m*M* by 75 min after dosing (Fig. 4). Plasma butyrate concentrations were approximately 0.4 m*M* by 15 min after dosing and remained above 1 m*M* for between 30 and 90 min. Subsequent studies using oral tributyrin doses of 5 and 3.6 g/kg resulted in lower plasma butyrate concentrations that peaked at 60 min after dosing and had a concentration-time profile similar to that observed with the 10.3-g/kg dose (Fig. 4). The butyrate AUC increased with increasing tributyrin doses. However, in contrast to the pattern observed in mice, the AUC increased proportionally with the dose and there was no evidence of non-linearity (Table 3).

The concentration-time profiles obtained for butyrate in the plasma of three rats given i.v. sodium butyrate at 500 mg/kg were very similar (Fig. 5). The peak plasma butyrate concentrations produced by this dose of sodium butyrate were very similar to those produced by 0.94- and 1.25-g/kg doses of sodium butyrate given i.v. to mice (Figs. 2, 5, Table 3). In rats given 500 mg/kg of sodium butyrate i.v., butyrate concentrations remained above 1 m*M* for only 15 min and by 30 min had fallen below the lower limit of quantitation of the assay used in this study. In these rats given sodium butyrate at a dose of 500 mg/kg the pattern of decline of plasma butyrate concentrations with time was consistent with a saturable clearance mechanism; however, the pattern seen in rats (Fig. 5) was much less striking than that observed in mice (Fig. 2). The AUC of butyrate in rats given 500 mg/kg of sodium butyrate i.v. was 83 m*M* min, which was lower than the butyrate AUC produced in rats by oral tributyrin doses of 5.2 and 10.3 g/kg (Table 3) and intermediate between the AUC values measured for butyrate in mice given i.v. sodium butyrate doses of 0.62 and 0.94 g/kg, respectively.

Additional pharmacokinetics studies using different doses of sodium butyrate in rats were not pursued. This decision was based on the observation that the 500-mg/kg dose was selected for rats on the basis of the maximal tolerated dose of sodium butyrate determined in mice and the observation that the peak plasma butyrate concentrations produced by this dose in rats were very similar to those measured in mice that had been given the maximal tolerated dose of sodium butyrate. Because we felt that the toxicity of sodium butyrate in mice was due to production of a systemic metabolic acidosis associated with delivery of a large acid load, we chose not to escalate the dose of sodium butyrate given i.v. to rats. In view of the dose-response information previously generated in mice and rats with oral tributyrin and in

Table 3 Pharmacokinetic parameters describing butyrate concentrations achieved in rat plasma after oral delivery of tributyrin or i.v. delivery of sodium butyrate

Dose (g/kg)	Agent	Route	Non compartmental modeling[a]				Compartmental modeling[a]		
			T_{max}^{b} (min)	C_{max}^{c} (m*M*)	AUC[d] (m*M* min)	CL_{app}^{e} (l kg^{-1} min^{-1})	V^{f} (l/kg)	K_{m}^{g} (m*M*)	V_{max}^{h} (mmol/min)
10.3	Tributyrin	p.o.	75	3.07	167	NA[i]	NA	NA	NA
5.2	Tributyrin	p.o.	60	0.73	108	NA	NA	NA	NA
3.6	Tributyrin	p.o.	60	0.72	68	NA	NA	NA	NA
0.5	Sodium butyrate	i.v.	NA[i]	11.2	83	0.54	0.45 ± 0.08[j] (0.45)	3.66 ± 2.59[j] (3.02)	1.07 ± 0.54[j] (0.98)

[a] Values calculated on the basis of mean plasma butyrate concentrations determined in 3–5 rats studied at each dose
[b] Time at which maximal plasma concentration occurred
[c] Maximal plasma concentration
[d] Area under the curve of plasma butyrate concentrations versus time
[e] Apparent clearance
[f] Volume of the central compartment
[g] Michaelis-Menten constant
[h] Maximal velocity
[i] *NA* Not applicable
[j] Values represent means ± SD of parameters calculated in 3 separate rats. *Values in parentheses* represent parameters generated when data from all 3 rats were combined and modeled as a single data set

mice with i.v. sodium butyrate, we also elected not to pursue studies in rats given lower doses of i.v. sodium butyrate.

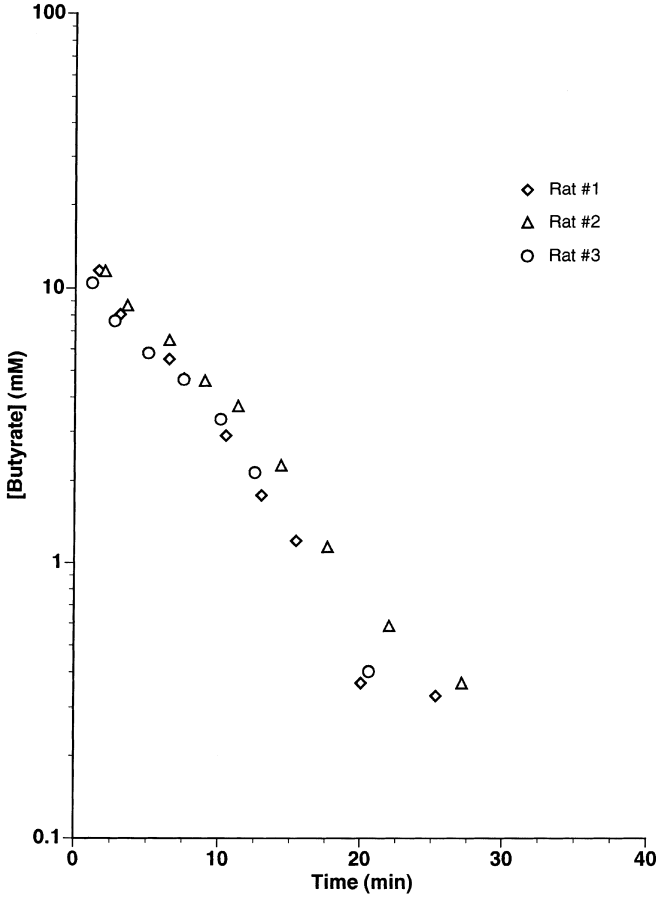

Fig. 5 Concentrations of butyrate determined in the plasma of rats given i.v. sodium butyrate at a dose of 0.5 g/kg. *Symbols* represent values recorded for 3 individual rats

Discussion

Butyrate represents an interesting potential therapy for a variety of disorders of cell growth and hemoglobin synthesis [4, 11, 26–33, 35, 37]. Unfortunately, the lack of a practical means of delivering and maintaining the butyrate concentrations felt necessary to induce cellular differentiation or stimulate the δ-globin gene has precluded widespread investigation and application of butyrate as a therapeutic agent. The current studies represent part of a preclinical and clinical [5, 7] effort to evaluate the triglyceride tributyrin as a feasible means of maintaining suitable in vivo concentrations of butyrate. The present data not only document the butyrate concentrations achievable with oral delivery of tributyrin but also characterize the saturable clearance and nonlinear pharmacokinetics of butyrate. Each of these issues has relevance to the potential clinical application of tributyrin as therapy for neoplasms or hemoglobinopathies.

Oral administration of tributyrin to both mice and rats resulted in substantial concentrations of butyrate in plasma, and at the highest deliverable dose of tributyrin these concentrations were maintained for several hours. Nevertheless, the peak plasma concentrations of butyrate produced by enteral delivery of tributyrin, although more sustained, were considerably lower than those produced by i.v. delivery of much lower doses of sodium butyrate. Furthermore, enteral delivery of sodium butyrate produced plasma butyrate concentrations that were equivalent to and as sustained as those achieved with enteral administration of tributyrin.

The saturable clearance and nonlinear pharmacokinetics of butyrate in plasma are clearly documented in the current studies. Noncompartmental calculations of butyrate AUC values produced by the delivery of differing doses of tributyrin to mice gave the first

indication of this behavior. Subsequently, i.v. delivery of four doses of sodium butyrate to mice generated a series of curves characteristic of an agent with nonlinear pharmacokinetics [20]. This nonlinear pharmacokinetic behavior was confirmed and quantified by noncompartmental and compartmental modeling of those data. Although the non linear nature of butyrate pharmacokinetics was less striking in rats, to some extent, this reflected limitations that were imposed by logistic and practical considerations on the maximal dose of trubutyrin that could be delivered to rats and our decision to curtail more extensive pharmacokinetics studies in this species.

Taken as a whole, the present data provide a basis for the pursuance of clinical studies involving oral administration of tributyrin [7]. They indicate that this therapy might produce millimolar concentrations of butyrate in the plasma of patients but that frequent dosing is likely to be required if sustained plasma butyrate concentrations are required. Clinical experience with oral tributyrin has clearly confirmed the latter point, as plasma concentrations produced by doses as high as 400 mg/kg failed to produce sustained plasma concentrations of butyrate above 0.45 m*M* [7]. To some extent, logistic considerations precluded the delivery of doses greater than this. In the phase I study, tributyrin was formulated as white, soft gelatin capsules containing 500 mg of tributyrin without additives [7]. Therefore, the delivery of a 400-mg/kg dose of tributyrin to a 70-kg patient would involve a total dose of 28 g of tributyrin, which would require the ingestion of 56 capsules. Clearly this is an unpalatable strategy for sustained daily dosing and would be totally unacceptable for schedules requiring doses of 400 mg/kg to be ingested two, three, or four times daily. It may be that an alternative formulation of tributyrin, such as an oral suspension, a custard-type preparation, or a spread, would greatly facilitate the delivery of increased doses and more frequent dosing schedules. Nonetheless, the preclinical data presented in the current manuscript raise a cautionary note regarding dose-escalation strategies in phase I clinical trials wherein the documented saturable pharmacokinetics of butyrate might result in a greater than anticipated increase in plasma butyrate concentrations as tributyrin doses are increased [25].

Perhaps butyrate therapy may be most useful in combination with other agents such as traditional cytotoxic drugs [3, 9, 13, 17, 19, 24, 38] or biologic agents such as interferon [21, 34]. Preclinical studies have described positive results for each of these strategies, which might not require as high or sustained concentrations of butyrate as have been felt necessary when butyrate is used alone. However, clinical evaluation of such strategies remains to be done.

Acknowledgement We gratefully acknowledge Ms. Rostov, Dr. Egorin's junior high school typing teacher. Without her instruction, this manuscript would never have been produced.

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