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

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 CD2F1 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 mM and remained ≥1 mM 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 mM by 15 min after dosing and remained between 0.8 and 1 mM 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 mM, respectively, by 45 min after dosing. Plasma butyrate concentrations in these mice remained above 0.1 mM 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 mM 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 mM, and plasma butyrate concentrations remained above 1 mM for 20–30 min. Sodium butyrate delivered orally to mice at 5 g/kg produced peak plasma butyrate concentrations of approximately 9 mM at 15 min after dosing and plasma butyrate concentrations exceeding 1 mM for 90 min after dosing. In rats the 10.3-g/kg oral dose of tributyrin produced peak plasma butyrate concentrations of approximately 3 mM by 75 min after dosing and butyrate concentrations exceeding 1 mM from 30 to 90 min after dosing. The
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 mM, 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-1F1 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 marine pathogens by marine 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.