Phase I Study of the Orally Administered Butyrate Prodrug, Tributyrin, in Patients with Solid Tumors[1]

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ABSTRACT

Butyrates have been studied as cancer differentiation agents *in vitro* and as a treatment for hemoglobinopathies. Tributyrin, a triglyceride with butyrate molecules esterified at the 1, 2, and 3 positions, induces differentiation and/or growth inhibition of a number of cell lines *in vitro*. When given p.o. to rodents, tributyrin produces substantial plasma butyrate concentrations. We treated 13 patients with escalating doses of tributyrin from 50 to 400 mg/kg/day. Doses were administered p.o. after an overnight fast, once daily for 3 weeks, followed by a 1-week rest. Intrapatient dose escalation occurred after two courses without toxicity greater than grade 2. The time course of butyrate in plasma was assessed on days 1 and 15 and after any dose escalation. Grade 3 toxicities consisted of nausea, vomiting, and myalgia. Grades 1 and 2 toxicities included diarrhea, headache, abdominal cramping, nausea, anemia, constipation, azotemia, lightheadedness, fatigue, rash, alopecia, odor, dysphoria, and clumsiness. There was no consistent increase in hemoglobin F with tributyrin treatment. Peak plasma butyrate concentrations occurred between 0.25 and 3 h after dose, increased with dose, and ranged from 0 to 0.45 mM. Peak concentrations did not increase in three patients who had dose escalation. Butyrate pharmacokinetics were not different on days 1 and 15. Because peak plasma concentrations near those effective *in vitro* (0.5–1 mM) were achieved, but butyrate disappeared from plasma by 5 h after dose, we are now pursuing dose escalation with dosing three times daily, beginning at a dose of 450 mg/kg/day.

INTRODUCTION

Most current systemic approaches to cancer treatment rely on cytotoxic and cytostatic mechanisms to eliminate malignant cells. Differentiation therapy is aimed at producing a more differentiated state, *i.e.*, a state in which the cell does not proliferate, and may even function as a mature cell (1). Differentiation therapy of cancer may also allow cancer treatment without the severe side effects that often accompany cytotoxic or cytostatic chemotherapy. Many compounds have been studied for their potential to induce differentiation of cancer. Among them are polar-planar compounds, such as *N*-methyl formamide and hexamethylene bisacetamide (1), low doses of cytotoxic drugs (1), phenylacetate (2, 3), and retinoids (4). To date, only retinoids have produced differentiation consistently at clinically tolerable doses. The excellent results of treatment of acute promyelocytic leukemia with all-*trans* retinoic acid (5, 6) have encouraged research into the activity of differentiating agents in other malignancies, including solid tumors.

Butyrates induce reversible growth inhibition or terminal differentiation in a wide variety of cell lines *in vitro* (7, 8). They have produced cell death in certain human neuroblastoma and glioma cell lines (7). These results required exposures to 0.5 to 3 mM butyrate for 4 days (7–9). Furthermore, butyrate, when combined with all-*trans* retinoic acid, has a synergistic effect on the differentiation of HL-60 leukemia cells *in vitro* (9).

The mechanisms of action by which butyrate induces differentiation are unknown. Some proposed mechanisms include: reduction in anaerobic glycolysis, with a resulting increase in cAMP concentrations and possible increased responsiveness of adenylate cyclase to membrane receptors (7), modulation of gene expression (7), increased histone acetylation with altered chromatin conformation (10), induction of apoptosis (11), and altered expression of cell surface molecules (8).

Limited human trials of butyrate have demonstrated one response in a child with acute myelogenous leukemia who was treated with a 10-day infusion of sodium butyrate (12). However, a similar trial in adults with acute myelogenous leukemia failed to demonstrate any response, although there was no severe toxicity associated with the treatment (13). In the latter study, plasma butyrate concentrations of 39–59 μM were achieved. These are less than 10% of the concentration needed to induce differentiation *in vitro*. In addition, the half-life of butyrate was approximately 6 min. Trials of continuous infusion of arginine butyrate (14) in patients with hemoglobinopathies demonstrated sustained plasma butyrate concentrations of approximately 50 μM and showed that such concentrations were

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Table 1 Patient characteristics

No. of patients	13
Men	8
Women	5
Median age (range)	56 (31–76)
Median performance status (range)	1 (0–2)
Tumor types	
Colorectal cancer	3
Small cell lung cancer	1
Renal cancer	1
Esophageal cancer	1
Squamous cancer head/neck	1
Prostate cancer	1
Gastric cancer	1
Cholangiocarcinoma	1
Adenocarcinoma, unknown primary site	1
Sarcoma	1
Melanoma	1
Previous chemotherapy and/or radiation therapy	12

Table 2 Number of courses at each dose level

Dose of tributyrin (mg/kg)	No. of patients	No. of courses
50	3	6
100	3	5
150[a]	5	9
200[a]	5	9
250[a]	3	4
300[a]	1	2
350[a]	1	2
400[a]	1	2

[a] Cohort includes patient(s) who had dose escalation (see "Materials and Methods").

able to cause dramatic increases in HbF.[3] Unfortunately, parenteral administration of sodium or arginine butyrate involves a long infusion duration, parenteral access, relatively large volumes of fluid, and significant expense. Therefore, an oral, sustained release formulation of butyrate would greatly facilitate evaluation of butyrate as a potential treatment of cancer and other diseases.

Tributyrin is a triglyceride containing three butyrate moieties esterified to glycerol. Tributyrin was found to be more than three times more potent in inducing differentiation of leukemia cells *in vitro* and did so at a slower rate and over a longer time interval than did butyrate (9). When tributyrin was administered to mice at doses of 7.75 g/kg, plasma butyrate concentrations peaked at 1 mM and remained between 0.8 and 1 mM until 60 min after dosing, without producing any mortality (15). In another study, tributyrin produced no detectable toxicity in mice treated either p.o. or i.p. with doses of 26.5 mmol/kg (8.2 g/kg; Ref. 9 and references therein). Pharmacokinetic studies in mice imply that the compound may be cleared by a saturable process, with initial slow elimination from plasma, followed by a more rapid terminal phase as concentration declines (15). The terminal half-life increased with dose (22–105 min) with a nonlinear relationship of dose to AUC (15).

We initiated a Phase I trial of p.o. administered tributyrin in cancer patients who had either not responded to or relapsed after standard treatment, or for whom no standard treatment was available. The goals of the study were: (*a*) to determine the maximum tolerable dose of tributyrin given once daily; (*b*) to record toxicities and/or responses; (*c*) to document the pharmacokinetics of butyrate in humans treated with tributyrin; (*d*) to ascertain the tributyrin dose at which plasma butyrate concentrations of 0.5–3 mM could be achieved; (*e*) to define any relationships between pharmacokinetic parameters, toxicity, and/or production of HbF; and (*f*) to develop an oral tributyrin dosing regimen that would maintain target concentrations of butyrate in plasma.

[3] The abbreviations used are: HbF, hemoglobin F; AUC, area under the plasma concentration × time curve; DLT, dose-limiting toxicity.

MATERIALS AND METHODS

Patient Eligibility. Patients were entered if they were at least 18 years of age, had a pathologically documented solid tumor refractory to standard therapy or for which standard therapy was not available, had normal hepatic (bilirubin, ≤1.5 mg/dl; aspartate aminotransferase and alanine aminotransferase, ≤1.5 times normal), hematopoietic (WBC ≥3000/μl; platelet count, ≥100,000/μl; Hb, ≥9 g/dl), and renal function (creatinine clearance, >50 ml/min/1.73 m^2, or serum creatinine, ≤1.5 mg/dl), a life expectancy of >3 months, and Eastern Cooperative Oncology Group performance status ≤2. In addition, patients signed written consent approved by the Institutional Review Board at the University of Maryland. Patients had not received radiation therapy or chemotherapy within the past 4 weeks [within the last 8 weeks for drugs with delayed toxicity, such as 1,3-bis(2-chloroethyl)-1-nitrosourea], and had recovered from all toxicity of prior treatment. Pregnant or nursing patients were not eligible, and all fertile, sexually active patients were advised to use effective birth control. Patients with unstable, serious medical or psychiatric illnesses and those with brain tumors or brain metastases were excluded.

Drug Supply. Tributyrin (NSC-661583) was supplied by the National Cancer Institute, Cancer Therapy Evaluation Program, who was responsible for its stability and purity. Drug was supplied as white, soft gelatin capsules containing 500 mg of tributyrin without additives.

Drug Treatment. Cohorts of at least three patients were studied at each dose level. Patients were entered at intervals of at least 1 week. New patients were entered at higher doses if no DLT, defined as drug-related grade ≥3 toxicity, had been observed in three patients at the previous dose. Maximum tolerable dose was defined as that dose at which more than or equal to two patients and up to six patients experienced DLT. If DLT was seen in one patient at a given dose, the cohort was expanded up to six patients. If DLT was observed in fewer than two patients in a cohort of six patients, dose escalation continued. If DLT was observed in more than or equal to two patients in a given cohort, up to three additional patients were entered in the next lower cohort. If fewer than two patients experienced DLT in this lower cohort, that dose would be declared the recommended Phase II dose. Doses were escalated in a given patient if no DLT

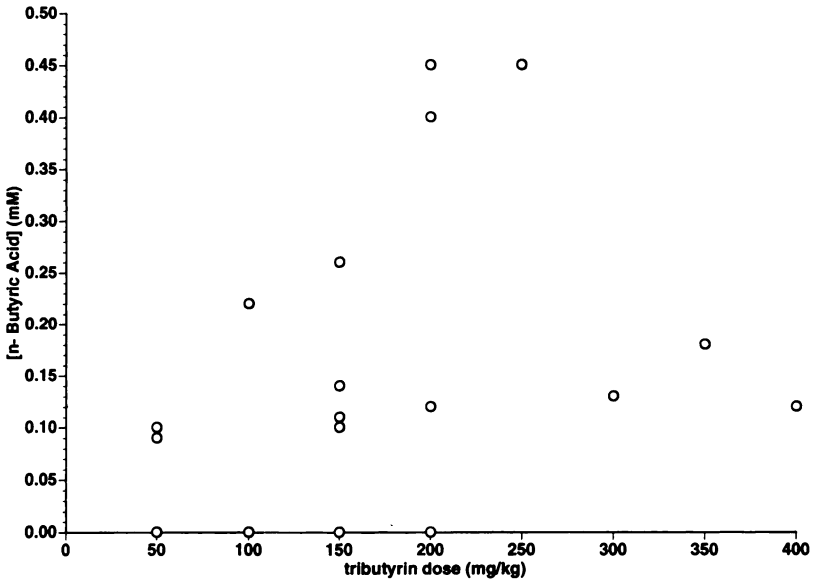

Fig. 1 Plasma butyrate concentrations (mM) day 1 of tributyrin dosing. The figure includes patients who had dose escalations.

occurred in that patient for two previous consecutive cycles. Doses were ingested with water daily between 8 and 10 a.m. and were rounded to the nearest 500 mg. When pharmacokinetics were assessed, patients did not eat solid food from the previous midnight until 4 h after dosing. Fluid intake consisted of water, and patients were encouraged to drink 1–2 liters from midnight until 4 h after dosing.

Clinical Assessment. Before treatment, all patients had a history taken (including transfusion history) and physical examination performed. Chest radiograph; electrocardiogram; any pertinent diagnostic imaging; complete blood count with differential, reticulocyte, and platelet count; Hb electrophoresis and F-reticulocytes and/or F cells; prothrombin time; partial prothrombin time; urinalysis; creatinine clearance; serum electrolytes; glucose; creatinine; blood urea nitrogen; albumin; calcium; phosphorus; magnesium; aspartate aminotransferase; alanine aminotransferase; bilirubin; total protein; and fasting lipid profile were done within 1 week before study entry. Initially, serum electrolytes, glucose, and blood urea nitrogen were performed 4 h after dosing, but after no abnormalities were detected, this assessment was done only if symptoms occurred. Toxicity was assessed according to the toxicity grading scale of the Cancer Therapy Evaluation Program, National Cancer Institute (Bethesda, MD). Pertinent history and physical examination and blood and urine studies were repeated weekly. Chest radiograph and electrocardiogram were repeated at weeks 4 and 8. Studies to assess tumor progression, if applicable, were repeated after each 2 months on treatment. For response criteria, partial response required a 50% reduction in the sum of the perpendicular diameters of all measurable lesions, without development of new lesions, lasting for at least 4 weeks. Complete response required disappearance of all measurable disease for at least 4 weeks. Progression required either a ≥25% increase in the sum of the perpendicular diameters of any measurable lesion or development of new lesions.

Compliance was assessed with patient logs. Compliance was considered to be satisfactory if 70% of planned doses were logged.

Pharmacokinetics. On days 1 and 15, as well as after any dose escalation, blood was obtained from patients before drug administration, every 30 min until 4 h after dosing, and then at 6, 8, and 24 h after dosing. Plasma was obtained from whole blood by centrifugation at 1500 × *g* for 10 min. Plasma was stored frozen at −70°C until analysis. For determination of plasma butyrate concentrations, a modification of the procedure of Boffa *et al.* (16) was used. Briefly, 300 μl of plasma were mixed for 2 min with 500 μl of 3 mM ethyl butyric acid internal standard in 70% ethanol. The mixture was chilled at 4°C for 10 min, shaken for 20 min, and centrifuged at 12,000 × *g* for 5 min at 4°C. One hundred μl of the resulting supernatant were mixed with 100 μl of 3 mM heptanoic acid in 70% ethanol and 20 μl of 10% H_3PO_4. One μl of this solution was injected into a Hewlett Packard 5890 gas chromatograph (Hewlett Packard, Palo Alto, CA), fitted with a 30-m, 0.5-mm inside diameter, HPFFA-PTA-TPA fused silical capillary column (film thickness, 1 μm), and a 1 m (0.53 inside diameter) deactivated fused silica capillary precolumn, and equipped with a Hewlett Packard 7673A autosampler. Splitless injection was used, with a purge time of 30 s and injection port temperature of 165°C. The oven temperature program was as follows: (*a*) 80°C for 30 s; (*b*) a temperature increase at a rate of 70°C/min, to 145°C; (*c*) 145°C for 5 min; and (*d*) a final temperature increase, at a rate of 5°C/min, to 185°C. After this, the column was regenerated at 200°C for 5 min. The column carrier gas was helium at 2.2 ml/min, and the make-up gas was nitrogen at 28 ml/min. Analytes were detected with a flame ionization detector set at 220°C with an air flow rate of 430 ml/min and hydrogen flow rate of 30 ml/min. The detector signal was integrated with a Hewlett Packard 3392A integrator. Under these conditions, the retention times of internal standard and butyrate were approximately 6.9 and 8 min, respectively. The

Table 3 Days 1 and 15 butyrate concentrations after once daily tributyrin dosing

Patient	Dose mg/kg	Day	Butyrate *Cmax* (mM)	Butyrate *Tmax* (h)	Butyrate AUC (mM × h)
1	50	1	0.1	0.5	
		15	0.08	0.5	
2	50	1	0.09	0.5	
3	50	1	ND[a]	ND	
4	100	1	ND	ND	
		15	ND	ND	
5	100	1	ND	ND	
		15	0.06	1.5	
6	100	1	0.22	1.5	0.89
		15	0.18	2.0	0.98
4[b]	150	1	0.11	0.5	1.04
		15	0.28	0.25	1.27
7	150	1	0.26	1.5	
		15	ND	ND	
8	150	1	0.10	3.0	
		15	ND	ND	
9	150	1	0.14	1.0	
		15	0.17	1.0	
6[b]	150	1	ND	ND	
		15	0.19	2.5	
4[b]	200	1	0.12	0.5	
		15	ND	ND	
10	200	1	0.12	1.0	
		15	0.12	1.5	
11	200	1	ND	ND	
		15	ND	ND	
12	200	1	0.45	0.5	1.52
		15	0.19	0.5	0.87
13	200	1	0.40	1.5	0.91
		15	0.21	1.5	0.86
12[b]	250	1	0.45	0.5	0.82
		15	0.42	0.5	0.98
4[b]	300	1	0.13	0.5	0.9
		15	0.21	0.5	0.87
4[b]	350	1	0.18	1.5	0.3
		15	0.27	0.5	0.3
4[b]	400	1	0.12	0.5	0.13
		15	0.08	2.0	0.17

[a] ND, not detectable.
[b] Intrapatient dose escalation.

butyrate concentration in each sample was calculated by determining the ratio of butyrate peak area to the respective internal standard area and comparing that ratio to a concomitantly performed standard curve. The assay was linear between 0.09 and 12 mM, with a coefficient of variation of 1–5%.

When sufficient $C \times T$ data were available, AUC was estimated in a noncompartmental fashion by the linear trapezoidal method. *Cmax* was taken as the highest concentration of butyrate measured in plasma, and *Tmax* was the time at which that concentration occurred.

Hematological Evaluation. The percentage of HbF was measured in hemolysates by high-performance liquid chromatography according to published methods (17). Ten ml of blood were collected in lavender-topped tubes containing citrate/EDTA. The percentage of F cells was measured using a monoclonal anti-human HbF antibody and immunofluorescence, according to published methods (17), and used the same blood specimen used for HbF measurements.

RESULTS

Patients. Thirteen patients were entered into the study. Patient characteristics are presented in Table 1. Dose escalation and patients per dose level are shown in Table 2. The maximum dose daily was 45,000 mg, which corresponds to 90 capsules. Four patients had dose escalation because minimal toxicity occurred during their first and second courses at lower doses.

Toxicity. Grade 3 nausea and vomiting were observed in two patients at the initial dose level. However, one of these patients developed a partial bowel obstruction, which probably accounted for the toxicity. Grade 3 myalgia was also seen in a patient at the 150 mg/kg dose. Other, grades 1 and 2 toxicities were observed without respect to dose and included anemia, constipation, anorexia, alopecia, azotemia, abdominal cramps, diarrhea or soft stool, headache, fatigue, nausea, flatulence, lightheadedness, dizziness, rash, odor (at the highest dose), dysphoria, and clumsiness.

Responses. There were no objective tumor responses. One patient with renal cancer remains in the study after 16 courses without progressive disease. He has had dose escalation every two courses and is presently at a dose of 400 mg/kg/day, without significant toxicity.

Pharmacokinetics. Peak plasma butyrate concentrations for day 1 are presented in Fig. 1. The median time at which peak plasma concentrations of butyrate were observed on day 1 for the 15 patients who achieved detectable butyrate concentrations was 0.5 h (range, 0.5–3.0 h). Four patients (1 at 50 mg/kg, 2 at 100 mg/kg, and 1 at 150 mg/kg) did not have detectable plasma butyrate concentrations. Because of the brief time during which butyrate was measurable (0.5–4 h) and the small number of samples, half-life could not be calculated. There was no significant difference between pharmacokinetic parameters obtained on days 1 and 15 when evaluated by the Wilcoxon rank test (Table 3). Three patients had dose escalations. The peak plasma butyrate concentration on day 1 for each dose is depicted in Fig. 2. None of the three patients studied after dose escalation had an increase in peak concentration of butyrate in plasma with increasing dose (Fig. 2; Table 3).

At doses of 200 mg/kg, peak plasma butyrate concentrations of 0.1–0.45 mM were observed. AUC was able to be calculated by the trapezoidal rule in only a few patients, most reliably at the dose of 200 mg/kg/day. At this dose, AUC was 0.91 and 1.52 mM × h on day 1 in the two patients in whom pharmacokinetics were assessed.

HbF Determinations. The percentage of F reticulocytes was determined at baseline and during treatment in five patients treated with doses of 50–150 mg/kg. In one patient, the percentage of F reticulocytes increased from 2.7% on day 1 to 14.7% on day 22. The other patients either had no detectable F reticulocytes (three patients) or had no change in the percentage of F reticulocytes with treatment (one patient). The percentage of F cells was determined at baseline and during treatment in nine patients. Values at days 1 and 15 or maximum percentage of F cells are shown in Table 4. Although there were some increases with treatment, they were not seen consistently and

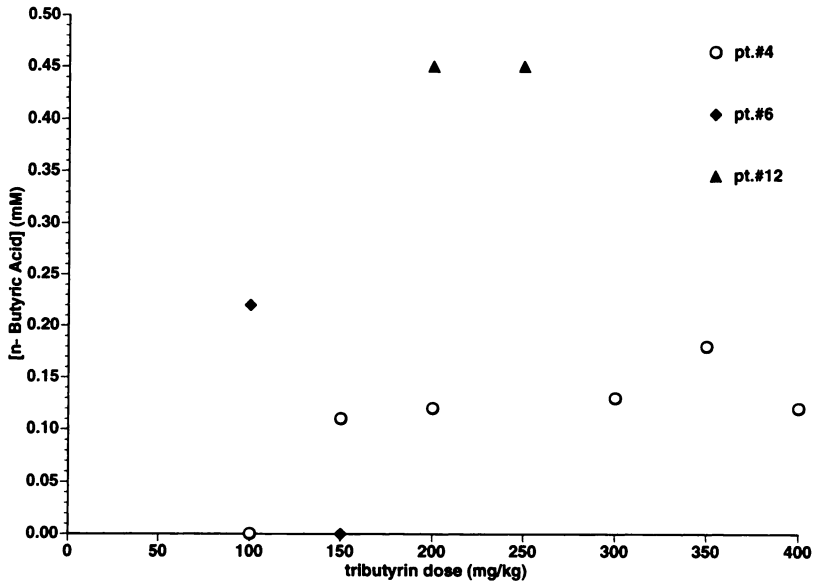

Fig. 2 Plasma butyrate concentrations (mM) in three patients after initial and escalated doses of tributyrin, day 1.

Table 4 Percentage of HbF cells by dose

Patient no.	Tributyrin dose (mg/kg)	% HbF cells	
		Baseline	Day 15
1	50	13.8	19.2
2	50	0.7	0.9
3	50	4.2	13.7
4	100	9.2	14.4
	150	–	42.1
5	100	0.9	1.1
6	100	15.1	9.1
	100	5.0	53.0
7	150	5.5	12.4
8	150	4.6	5.1
9	150	2.3	3.8

were not related to dose. Interestingly, in two patients (nos. 4 and 6), subsequent courses show an increase, when the first course did not.

DISCUSSION

We have shown that plasma butyrate concentrations approaching 0.5 mM, the minimum effective *in vitro* concentration, can be achieved in patients after oral administration of tributyrin, without severe toxicity. However, the half-life of butyrate in plasma is extremely short, and it is unlikely that once-daily administration of tributyrin will be sufficient to assess clinical activity, given that *in vitro* studies have used exposures to butyrate concentrations in the millimolar range for approximately 4 days. We have seen some modulation of the percentage of HbF cells, but, thus far, clinical conditions in this trial have not been able to duplicate the continuous exposure to butyrate concentrations of at least 50 μM, which were achieved in the trials using continuous infusion of arginine butyrate (14). In the future, we will administer tributyrin on a three-times-a-day schedule in an attempt to attain more persistent plasma concentrations of butyrate. Although DLT was not defined in this trial, the high number of capsules required daily at the higher doses and the lack of increase in *Cmax* with dose escalation in three patients precluded further daily dose escalation.

The role, if any, of differentiation agents in cancer therapy remains undefined. The promising activity of all-*trans* retinoic acid in acute leukemia (5, 6) has stimulated research into the best way to use these agents. Preclinical studies have demonstrated enhanced activity of standard cytocidal methods (chemotherapy and radiation) when used in combination with differentiation agents (18–24). In addition, combinations of differentiation agents, which likely have differing mechanisms of action, may produce tumor growth arrest or apoptosis (11, 18–24). In these efforts, it is important to pursue optimal use of oral agents such as tributyrin, because such therapy is likely to require a prolonged duration, making i.v. administration costly and less attractive. This study represents an initial Phase I clinical trial of tributyrin in which concomitant pharmacokinetic analysis defined not only a short half-life but also a dose at which it is reasonable to begin multiple daily dosing of the agent.

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