Eur J Cancer Clin Oncol, Vol. 23, No. 9, pp. 1283–1287, 1987.
Printed in Great Britain

0277–5379/87$3.00+0.00

Clinical Pharmacology of Sodium Butyrate in Patients with Acute Leukemia*

ANTONIUS A. MILLER,† ERHARD KURSCHEL, RAINHARDT OSIEKA and CARL G. SCHMIDT

Innere Universitätsklinik (Tumorforschung), West German Tumor Center, University of Essen Medical School, Hufelandstr. 55, 4300 Essen 1, F.R.G.

Abstract—*Since cancer may be regarded as a disease of differentiation and sodium butyrate induces differentiation of malignant cells* in vitro, *a study of the clinical pharmacology of sodium butyrate was undertaken. Nine patients with acute myeloid* (n = 1), *acute monocytic* (n = 1), *acute myelomonocytic* (n = 6) *and acute undifferentiated* (n = 1) *leukemia were treated. Their median age was* 52 (*range*, 27–78) *years. Six of the nine patients were pretreated with cytostatic agents. Sodium butyrate was administered i.v. at a dosage of* 500 mg/kg/day *as continuous infusion over* 10 *days. A sensitive and reproducible high-performance liquid chromatographic separation was developed after derivatization of sodium butyrate with* 2,4′*-dibromoacetophenone employing crown ether catalysis. Plasma concentrations and urinary excretion of sodium butyrate were monitored during the* 10 *days of continuous infusion and for* 2 *days thereafter. During infusion, plasma concentrations increased* 6*-fold over the endogenous butyrate level and reached* 39–59 μM. *The area under the curve of the exogenous butyrate was* 384 ± 50 μM×day (*mean* ± *S.D.*). *After the end of infusion, concentrations declined rapidly with a half-life of* 6.1 ± 1.4 *min, and reached pretreatment values within* 1 hr. *The total clearance rate was* 83 ± 12 ml/kg/min *and the volume of distribution* 738 ± 245 ml/kg. *The excreted amounts of butyrate in the urine were minimal as compared to the infused dose. Although excretion by other organs was not ruled out, it is suggested that the infused sodium butyrate was rapidly metabolized. A significant increase in peripheral blast cells was observed, whereas bone marrow cytologies before and after treatment did not reveal a signficant change in blasts. Differential counts of peripheral white blood cells did not show significant changes. No toxicity was encountered. The apparent lack of clinical efficacy may be explained by the low plasma levels of sodium butyrate due to its short half-life* in vivo. *In comparison, concentrations reported for* in vitro *studies were at least* 10 *times higher.*

INTRODUCTION

THE CONCEPT that cancer may be regarded as a disease of differentiation has recently evolved, and is supported by the observation that many transformed cells retain the potential to respond to differentiation signals [1, 2]. A variety of relatively simple chemical compounds can induce murine erythroleukemia cells as well as other transformed cell lines to differentiate with the loss of proliferative capacity and the expression of differentiated characteristics [3, 4]. Agents that induce transformed cells to undergo differentiation to non-dividing stages without oncogenic properties may be an alternative to the use of cytotoxic drugs.

Among the agents capable of inducing terminal cell division and expression of differentiated characteristics is sodium butyrate [5] which is a physiologically occurring compound. Since it has been shown that sodium butyrate induces globin gene expression and differentiation in Friend erythroleukemia cells [6], numerous *in vitro* studies of this polar, 4-carbon, fatty acid were conducted. Recently, notable effects of sodium butyrate were described in murine erythroid leukemia cells [7], Ehrlich ascites tumor cells [8], mouse neuroblastoma cells [7] HeLa cells [10], rat hepatoma cells [11], embryonic lung fibroblasts [12], as well as various human cell lines, namely, leukemia K562 cells [13], promyelocytic leukemia cells [14], hairy cells [15], colorectal cancer cells [16], retinoblastoma cells [17], pancreatic carcinoma cells [18, 19], hepatoma cells [20] and uterine cervical cancer cells [21].

Accepted 27 January 1987.
*Supported by grant PTB 03–8479–7 of the Federal Ministry for Research and Technology, Bonn, F.R.G.
†To whom requests for reprints should be addressed: Department of Medicine, Division of Hematology/Oncology, The University of Tennessee, 956 Court Ave., Memphis, Tennessee 38163, U.S.A.

Several authors have encompassed the potential clinical relevance of sodium butyrate as a differentiation inducer [4, 12, 13, 17], although it is not possible to predict which transformed cell line is blocked in a stage of differentiation susceptible to inducer-mediated effects [4]. In readily obtainable cells from patients with leukemia, it has been demonstrated that sodium butyrate effectively inhibits [^{3}H]thymidine incorporation and induces cell damage *in vitro* [22]. Furthermore, parenteral administration of butyrate to a child with acute myelogenous leukemia in relapse and refractory to conventional therapy resulted in a partial remission [22].

The present study extended the administration of sodium butyrate to nine adult patients with acute leukemia by a clinical pharmacologic investigation. The need for pharmacologic measurements in clinical trials is demonstrated.

MATERIALS AND METHODS

Materials

Sodium butyrate was purchased from Merck (Darmstadt, F.R.G.). It was dissolved in water for injection and sterilized by filtration through 0.2 μm filters (Millipore, Bedford, MA). The solution for i.v. infusion was sterile, non-pyrogenic and isotonic, contained 16.5 g/l of sodium butyrate and had a pH of 7.56. For laboratory use, 1–^{14}C-labeled sodium butyrate with a specific activity of 13.4 mCi/mmol was purchased from New England Nuclear (Boston, MA). Potassium bicarbonate was obtained from Sigma Chem. Comp. (St. Louis, MO). Dicyclohexyl-18-crown-6 and 2,4′-dibromoacetophenone were from Merck-Schuchardt (Hohenbrunn, F.R.G.). Laboratory water and organic solvents were HPL grade (LiChrosolv from Merck).

Patients and treatment

Nine patients were entered into the study after obtaining their informed consent. The diagnoses included acute myeloid (n = 1), acute monocytic (n = 1), acute myelo-monocytic (n = 6) and acute undifferentiated (n = 1) leukemias. Two male and seven female patients were treated. The median age was 52 (range, 27–78) years. Six of the nine patients were pretreated with cytostatic agents. Sodium butyrate was administered as continuous i.v. infusion over 10 days. The dosage was 500 mg/kg/day, equivalent to 30 ml/kg/day of the isotonic solution. The median daily infusion volume was 2.4 (range, 1.75–3.0) l.

Samples

Peripheral blood counts of red and white cells including blood smears were obtained daily. Bone marrow cytology was evaluated before and after treatment. Further daily analyses included hematocrit, blood clotting, glucose, serum proteins, bilirubin, liver enzymes, electrolytes, creatinine and blood urea nitrogen. Blood gases and pH were monitored twice daily. For statistical evaluation of the data, the Wilcoxon signed rank test was used (α = 0.05).

Blood samples for the pharmacological investigation were drawn from a separate i.v. catheter into heparinized tubes. Samples were processed on ice, centrifuged at 1000 ***g*** for 10 min in a cooled centrifuge and the plasma removed. Plasma concentrations of sodium butyrate were measured daily during continuous infusion as well as 2, 5, 10, 20, 30 min and 1, 2, 3, 4, 6, 9, 12, 24 and 48 hr after infusion. The excretion of sodium butyrate in urine was analyzed daily during treatment and for 2 days thereafter.

Protein binding was measured in plasma of three healthy volunteers at concentrations of 10, 50 and 100 μM of sodium butyrate. Ultrafiltration was carried out as described below.

High-performance liquid chromatography (HPLC)

Plasma and urine concentrations of sodium butyrate were measured by HPLC. Radioactive sodium butyrate was used as internal standard.

One milliliter of plasma was ultrafiltered through YMT membranes in a micropartition system (Amicon Corp., Danvers, MA) for 45 min at 2000 ***g***. To 300 μl of the ultrafiltrate, 10 μl of 1 M potassium bicarbonate were added. The sample was evaporated under a stream of nitrogen. The dry residue was dissolved in 100 μl reaction mixture and 400 μl of acetonitrile. The reaction mixture [23] consisted of 0.2 M 2,4′-dibromoacetophenone : 0.2 M dicyclohexyl-18-crown-6 20 : 1 (v : v). The reaction took place at 80°C for 30 min in a shaking water bath. After centrifugation (5 min, 1000 ***g***), 10 μl of the clear sample were directly injected onto a μBondapak C18 column (10 μm particle size, 3.9 × 300 mm) from Millipore/Waters Chromatography (Milford, MA). The mobile phase consisted of water : acetonitrile 55 : 45 (v : v), and the flow-rate was 2 ml/min.

To 1 ml of urine, 200 μl of 1 M hydrochloric acid and 3 ml of chloroform were added in a capped extraction tube. The sample was vigorously mixed for 5 min and centrifuged at 1000 ***g*** for 10 min. The organic phase was transferred to another capped tube containing 1 ml of water and 50 μl of 1 M potassium hydroxide (final pH, 7.5). After mixing (5 min) and centrifugation (10 min, 1000 ***g***), the aqueous phase was evaporated under a stream of nitrogen. The dry residue was reacted with dibromoacetophenone as above. Afterwards, 10 μl of the sample were injected onto a Resolve C18 column (5 μm particle size, 3.9 × 150 mm) from Millipore/Waters. The solvent was water :

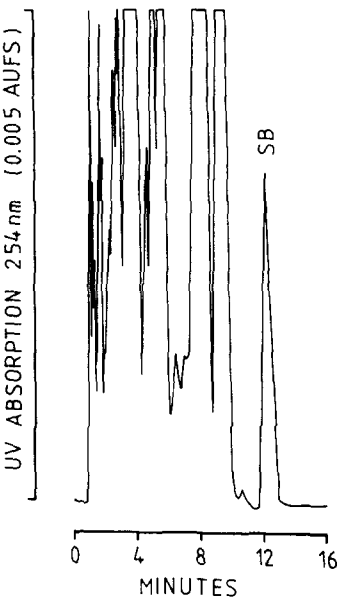

Fig. 1. HPLC chromatogram of patient plasma taken on day 2 of continuous i.v. infusion of sodium butyrate (SB, derivatized sodium butyrate; concentration, 50.4 μM).

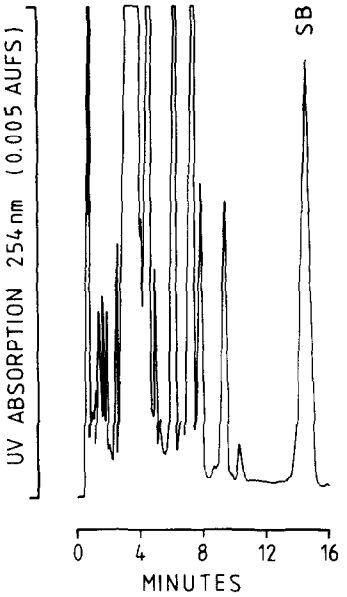

Fig. 2. HPLC chromatogram of patient urine collected on day 2 of treatment with sodium butyrate (SB, derivatized sodium butyrate; concentration 40.5 μM).

acetonitrile 65 : 35 (v : v) at a flow-rate of 2 ml/min.

The high-pressure liquid chromatograph consisted of an M6000 pump, a U6K manual injector, and an M730 integrator from Millipore/Waters. A variable UV detector (Spectroflow 773 from Kratos Inc., Westwood, NJ) set to 254 nm was used. At the retention time of sodium butyrate, the eluant (4 ml) from the HPLC column was collected into vials containing 10 ml of scintillation cocktail (Unisolve 100 from Zinsser Analytic, Frankfurt, F.R.G.), and counted for ^{14}C in an LS2800 counter from Beckman Instr. Inc. (Fullerton, CA). All samples were analyzed in duplicate.

RESULTS

Chromatography

A reported procedure [23] was adapted to derivatize butyrate in plasma and urine. Separation of the resulting bromophenacyl ester was achieved by isocratic, reversed-phase HPLC thereby allowing sensitive and reproducible measurements. The lower limits of detection were 2 and 5 μM in plasma and urine, respectively. Standard curves were linear from 2 to 200 and from 5 to 500 μM for plasma and urine, respectively. Radioactive sodium butyrate with high specific activity was a suitable internal standard. The recovery rates of the above methods were 87 and 63%, and the interassay coefficients of variation (six assays in 6 months) were 3.7 and 6.9% for plasma and urine, respectively. High performance liquid chromatograms of patient plasma and urine are shown in Figs 1 and 2.

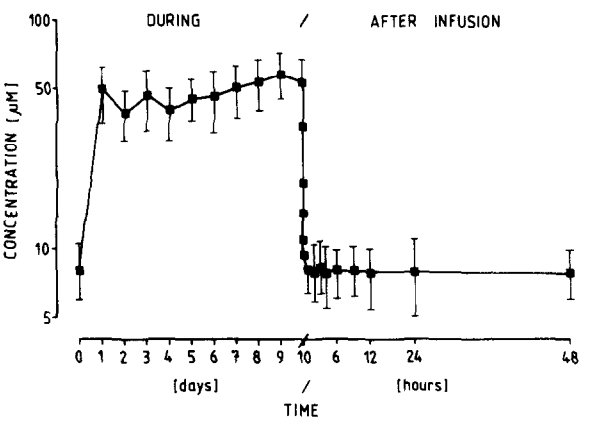

Fig. 3. Plasma concentrations (means; bars, S.D.) of sodium butyrate in nine patiens before (day 0), during (days 1–10) and after (0–48 hr) continuous i.v. infusion.

Pharmacology

In all patient samples, sodium butyrate was measurable. Protein binding accounted for 9.1% of total sodium butyrate in plasma. Figure 3 shows the total plasma concentrations observed before, during and after infusion. The pretreatment level of sodium butyrate was 8.0 ± 2.1 μM (mean ± S.D.). On the first day of treatment, the concentration rose to 48.8 ± 13.5 μM. Thereafter, a slight increase in concentrations was found until day 10.

During continuous i.v. infusion, mean concentrations ranged from 39 to 59 μM with an overall mean of 48 ± 6.1 μM. The area under the concentration vs. time curve (AUC) of the exogenous sodium butyrate was 384 ± 50 μM×day. After the end of infusion, concentrations declined rapidly with a half-life of 6.1 ± 1.4 min. The pretreatment level was reached within 1 hr after the end of

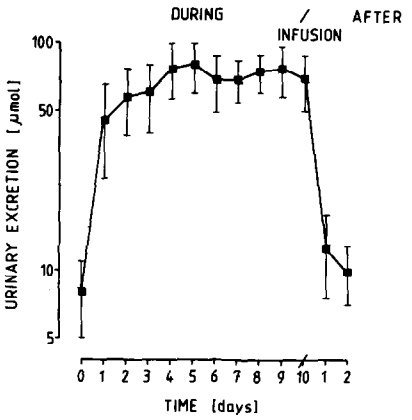

Fig. 4. Urinary excretion of butyrate in nine patients before (day 0), during (days 1–10) and after (2 days) continuous infusion (means of excreted daily amount; bars, S.D.).

infusion. The total clearance rate was calculated as 83 ± 12 ml/kg/min. The volume of distribution amounted to 738 ± 245 ml/kg. The excretion of butyrate into the urine was measured daily as shown in Fig. 4. About 0.21 ± 0.04‰ of the infused sodium butyrate were excreted.

Activity

Total leukocytes, erythrocytes, thrombocytes and hematocrit did not change significantly during treatment. Peripheral counts of promyelocytes, myelocytes, metamyelocytes, bands, polymorphonuclear neutrophils, basophils, eosinophils, monocytes and lymphocytes did not reveal significant changes. A significant ($P < 0.05$) increase in circulating blast cells was observed, whereas bone marrow cytology did not show a significant change in blasts. A slight (marginally significant, $P = 0.05$) increase in serum bilirubin and decrease in serum potassium occurred. All other laboratory tests mentioned in Materials and Methods did not reveal significant perturbations. The blood pH was not altered significantly. No side-effects, specifically no hypotension, nausea or weight loss, were encountered.

DISCUSSION

Based on the known effects of sodium butyrate *in vitro* [5–21] and the case report on 5-year-old boy with acute myelogenous leukemia [22], we have investigated the *in vivo* pharmacology by administering sodium butyrate to nine adult patients with acute leukemia. For long-term exposure, a continuous infusion regimen was chosen. The dosage (500 mg/kg/day) and the treatment duration (10 days) were the same as reported by Novogrodsky *et al.* [22].

The derivatization procedure for fatty acids reported by Durst *et al.* [23] was applied successfully to react butyrate in human samples. When a crown ether is used as catalyst, strictly anhydrous conditions are not necessary [24]. The resulting phenacyl esters absorb UV light at 254 nm [25], and can be separated by liquid chromatography (Figs 1 and 2).

During continuous infusion, plasma concentrations of sodium butyrate reached 39–59 μM. Thus, an increase of about 6-fold over the endogenous level was achieved (Fig. 3). It seems important to note, however, that the levels known to induce differentiation *in vitro* range from 500 μM to millimolar concentrations [7–22]. The AUC of the exogenous butyrate observed in the present study was small, the volume of distribution large, and the half-life after discontinuation of infusion very short. Although the excretion of butyrate into the urine rose up to 10-fold over the pretreatment value (Fig. 4), this route of elimination is negligible as compared to the total infused amount. Nevertheless, the human organism exerts a high total clearance rate for the exogenous butyrate. Although excretion by other organs was not ruled out, these observations suggest that butyrate is readily metabolized. Thus, doses of 500 mg/kg/day × 10 were not sufficient to sustain *in vivo* concentrations of sodium butyrate that have been demonstrated to induce differentiation *in vitro*.

Novogrodsky *et al.* reported on a butyrate-induced partial remission of acute myelogenous leukemia in a child [22]. The parenteral administration of butyrate resulted in an elimination of myeloblasts from the peripheral blood, an increase in mature myeloid cells, and a reduction of bone marrow myeloblasts. In the present study, the same treatment regimen was used as by Novogrodsky *et al.* No significiant clinical activity of sodium butyrate was observed in nine adult patients with acute leukemia. Peripheral blasts even increased, whereas bone marrow cytology did not show a significant change. The apparent lack of efficacy may be explained by our pharamcological results, specifically the suboptimal plasma conentrations of sodium butyrate.

In vitro effects of butyrate are dose-dependent [22]. Since no overt clinical toxicity was encountered, dose escalation seems warranted. In further clinical trials, either larger infusion volumes or hyperosmolar solutions could be used. The median daily infusion volume in the present study was 2.4 l. Therefore, the infusion of hyperosmolar solutions via a central i.v. catheter may be the better alternative. However, it appears questionable whether a 10-fold increase over present plasma levels is possible in order to reach the minimum effective concentration of *in vitro* studies (500 μM). A further option might be to develop active butyrate analogs that are not as rapidly metabolized as the endogenous compound.

Acknowledgement—The technical assistance of Gerald John is gratefully acknowledged.

REFERENCES

1. Bloch A. Induced cell differentiation in cancer therapy. *Cancer Treat Rep* 1984, **68**, 199–205.
2. Riggs AD, Jones PA. 5-Methylcytosine, gene regulation, and cancer. *Adv Cancer Res* 1983, **40**, 1–30.
3. Friend C, Scher W, Holland JG, Sato T. Hemoglobin synthesis in murine virus-induced leukemic cells *in vitro*: stimulation of erythroid differentiation by dimethyl sulfoxide. *Proc Natn Acad Sci USA* 1971, **68**, 378–382.
4. Marks PA, Rifkind RA. Differentiation modifiers. *Cancer* 1984, **54**, 2766–2769.
5. Kruh J. Effects of sodium butyrate, a new pharmacological agent, on cells in culture. *Mol Cell Biochem* 1982, **42**, 65–82.
6. Leder A, Leder P. Butyric acid, a potent inducer of erythroid differentiation in cultured erythroleukemic cells. *Cell* 1975, **5**, 319–322.
7. Leiter JME, Helliger W, Puschendorf B. Increase in histone acetylation and transitions in histone variants during Friend cell differientation. *Exp Cell Res* 1984, **155**, 222–231.
8. Multhaup I, Csordas A, Grunicke H, Pfister R, Puschendorf B. Conservation of the acetylation pattern of histones and the transcriptional activity in Ehrlich ascites tumor cells by sodium butyrate. *Arch Biochem Biophys* 1983, **222**, 497–503.
9. Schneider FH. Effects of sodium butyrate on mouse neuroblastoma cells in culture. *Biochem Pharmacol* 1976, **25**, 2309–2317.
10. Kim SH, He SQ, Kim JH. Modification of thermosensitivity of HeLa cells by sodium butyrate, dibutyryl cyclic adenosine 3′:5′-monophosphate, and retinoic acid. *Cancer Res* 1984, **44**, 697–702.
11. Cook JR, Schwartz CE, Fausel ED, Chiu JF. Effect of sodium butyrate on alpha-fetoprotein gene expression in rat hepatoma cells *in vitro*. *Cancer Res* 1985, **45**, 3215–3219.
12. De Haan JB, Gevers W, Parker MI. Effects of sodium butyrate on the synthesis and methylation of DNA in normal cells and their transformed counterparts. *Cancer Res* 1986, **46**, 713–716.
13. Andersson LF, Jokinen M, Gahmberg CG. Induction of erythroid differentiation in the human leukaemia cell line K562. *Nature* 1979, **278**, 364–365.
14. Collins SJ, Ruscetti JW, Gallagher RE, Gallo RC. Terminal differentiation of human promyelocytic leukemia cells induced by dimethyl sulfoxide and other polar compounds. *Proc Natn Acad Sci USA* 1978, **75**, 2458–2462.
15. Faille A, Turmel P, Charron DJ. Differential expression of HLA-DR and HLA-DC/DS molecules in a patient with hairy cell leukemia: restoration of HLA-DC/DS expression by (12-O-tetradecanoyl phorbol-13-acetate), 5-azacytidine, and sodium butyrate. *Blood* 1984, **64**, 33–37.
16. Chung YS, Song IS, Erickson RH, Sleisenger MH, Kim YS. Effect of growth and sodium butyrate on brush border membrane-associated hydrolases in human colorectal cancer cell lines. *Cancer Res* 1985, **45**, 2976–2982.
17. Kyritsis A, Joseph G, Chader GJ. Effects of butyrate, retinol, and retinoic acid on human Y-79 retinoblastoma cells growing in monolayer cultures. *JNCI* 1984, **73**, 649–654.
18. Bryant G, Haberern C, Rao CN, Liotta LA. Butyrate induced reduction of tumor cell laminin receptors. *Caner Res* 1986, **46**, 807–811.
19. McIntyre LJ, Kim YS. Effects of sodium butyrate and dimethylsulfoxide on human pancreatic tumor cell lines. *Eur J Cancer Clin Oncol* 1984, **20**, 265–271.
20. Nakagawa T, Nakao Y, Matsui T *et al.* Effects of sodium *n*-butyrate on alpha-fetoprotein and albumin secretion in the human hepatoma cell line PLC/PRF/5. *Br J Cancer* 1985, **51**, 357–363.
21. Nozawa S, Engvall E, Kano S, Kurihara S, Fishman WH. Sodium butyrate produces concordant expression of "early placental" alkaline phosphatase, pregnancy-specific beta 1-glycoprotein and human chorionic gonadotropin beta-subunit in a newly established uterine cervical cancer cell line (SKG-IIIa). *Int J Cancer* 1983, **32**, 267–272.
22. Novogrodsky A, Dvir A, Ravid A *et al.* Effect of polar organic compounds on leukemic cells. Butyrate-induced partial remission of acute myelogenous leukemia in a child. *Cancer* 1983, **51**, 9–14.
23. Durst HD, Milano M, Kikta EJ, Connelly SA, Grushka E. Phenacyl esters of fatty acids via crown ether catalysts for enhanced ultraviolet detection in liquid chromatography. *Anal Chem* 1975, **47**, 1797–1801.
24. Darbre A. Esterification. In: Blau K, King GS, eds. *Handbook of Derivatives for Chromatography*. London, Heyden, 1979, 52–53.
25. See ref. [24], 78–79.