[CANCER RESEARCH 54, 1690–1694, April 1, 1994]

A Phase I and Pharmacokinetic Study of Intravenous Phenylacetate in Patients with Cancer[1]

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ABSTRACT

Phenylacetate has recently been shown to suppress tumor growth and promote differentiation in experimental models. A phase I trial of phenylacetate was conducted in 17 patients with advanced solid tumors. Each patient received a single i.v. bolus dose followed by a 14-day continuous i.v. infusion of the drug. Twenty-one cycles of therapy were administered at four dose levels, achieved by increasing the rate of the continuous i.v. infusion. Phenylacetate displayed nonlinear pharmacokinetics [K_m = 105.1 ± 44.5 (SD) μg/ml, V_{max} = 24.1 ± 5.2 mg/kg/h and V_d = 19.2 ± 3.3 L]. There was also evidence for induction of drug clearance. Ninety-nine % of phenylacetate elimination was accounted for by conversion to phenylacetylglutamine, which was excreted in the urine. Continuous i.v. infusion rates resulting in serum phenylacetate concentrations exceeding K_m often resulted in rapid drug accumulation and dose-limiting toxicity, which consisted of reversible central nervous system depression, preceded by emesis. Three of nine patients with metastatic, hormone-refractory prostate cancer maintained stable prostatic specific antigen levels for more than 2 months; another had less bone pain. One of six patients with glioblastoma multiforme, whose steroid dosage has remained unchanged for the duration of therapy, has sustained functional improvement for more than 9 months. The use of adaptive control with feedback for the dosing of each patient enabled us to safely maintain stable phenylacetate concentrations up to the range of 200–300 μg/ml, which resulted in clinical improvement in some patients with advanced disease.

INTRODUCTION

Phenylacetate, a product of phenylalanine metabolism, is a small molecule (M_r 136) normally present in the mammalian circulation in low concentrations (1). It has been administered primarily to children with hyperammonemia due to inborn errors of urea synthesis (2, 3) and to patients with hyperammonemia resulting from the chemotherapy of leukemias (4) or from portal systemic encephalopathy (5). In humans, phenylacetate is conjugated with glutamine by the hepatic enzyme phenylacetyl Coenzyme A: glutamine acyltransferase to yield phenylacetylglutamine (6, 7), which is then excreted in the urine. The mobilization of glutamine-associated nitrogen is believed to be the mechanism whereby hyperammonemia is improved. More recently, phenylacetate and related compounds have received attention for their ability to induce tumor cytostasis and differentiation in laboratory models (8–11) and fetal hemoglobin synthesis in patients (12). Interest in phenylacetate as an anticancer agent was also generated by reports that antineoplaston AS2–1, a preparation which by weight is 80% phenylacetate, displayed clinical antitumor activity (13).

Preclinical studies documented that phenylacetate modifies the biology of various hematopoietic and solid tumors, including prostatic carcinoma and glioblastoma (10, 11). To achieve this effect requires that cells be exposed to phenylacetate concentrations in excess of 275 μg/ml for a minimum of two weeks. It appeared feasible to expose adults with solid tumors to similar concentrations of phenylacetate, which children with urea cycle disorders had tolerated (2, 3). Hence, a phase I trial was designed to deliver a CIVI[3] of phenylacetate over a 2-week period. We herein report the clinical and pharmacokinetic results of this study and discuss an alternative schedule of drug administration for future trials.

MATERIALS AND METHODS

Patient Population. Patients were eligible for this study if they had advanced solid tumors for which conventional therapy had been ineffective, a Karnofsky performance status greater than 60%, normal hepatic transaminases and total bilirubin, a serum creatinine less than 1.5 mg/dl, and normal leukocyte (>3,500/mm^3) and platelet counts (>150,000/mm^3). All patients signed an informed consent document that had been approved by the National Cancer Institute Clinical Research Subpanel. Seventeen patients, 16 men and 1 woman, with a median age of 57 (range: 36–75) were enrolled between January and June 1993. Disease distribution included progressive, metastatic, hormone-refractory prostate cancer (9 patients), anaplastic astrocytoma or glioblastoma multiforme (6 patients), ganglioglioma (1 patient), and malignant pleural mesothelioma (1 patient).

Drug Preparation and Administration. Sodium phenylacetate for injection was prepared from bulk sodium phenylacetate powder supplied by Elan Pharmaceutical Research Co. (Gainesville, GA). The finished injectable stock solution was manufactured by the Pharmaceutical Development Service, Pharmacy Department, Clinical Center, NIH, in vials containing sodium phenylacetate at a concentration of 500 mg/ml in sterile water for injection, USP, with sodium hydroxide and/or hydrochloric acid added to adjust the pH to approximately 8.5. Doses of sodium phenylacetate to be infused over 30 min to 2 h were prepared in 150 ml of sterile water for injection, USP. Doses of phenylacetate to be given over 24 h were prepared similarly to yield a total volume of 1000 ml and were administered using an infusion pump.

Clinical Protocol. The protocol as originally designed delivered an i.v. bolus dose of phenylacetate (150 mg/kg over 2 h) on the first day of therapy, to allow for the estimation of pharmacokinetic parameters. This was followed 24 h later by a CIVI of the drug for the next 14 days. Cycles of 2-week drug infusions were repeated every 6 weeks. The rate of drug infusion was to be increased in sequential cohorts of at least three patients, and individual patients could escalate from one dose level to the next with sequential cycles of therapy provided they had experienced no drug-related toxicity and their disease was stable or improved.

The protocol underwent several modifications over the 6-month period. (*a*) the size of the initial bolus dose was reduced from 150 to 60 mg/kg i.v. and the bolus infusion duration from 2 h to 30 min, after the first three patients were treated. This change resulted in drug concentrations optimal for estimating the pharmacokinetics of the drug (see below) within a 6-h time period. (*b*) after the nonlinear nature of the pharmacokinetics of phenylacetate was recognized (see below), the protocol was changed from a fixed dose escalation (dose levels 1 and 2, 150 and 250 mg/kg/day, respectively) to a concentration-guided escalation trial (dose levels 3 and 4, 200 and 400 μg/ml, respectively). In the latter format each patient was given an i.v. bolus dose of phenylacetate (60 mg/kg over 30 min) 1 week prior to beginning a 14-day CIVI of the drug. The patient-specific pharmacokinetic parameters estimated from the bolus dose were used to calculate an infusion rate that would maintain the serum phen-

Received 9/15/93; accepted 1/28/94.

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[1] This study was supported in part by a grant from Elan Pharmaceutical Research Co.

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[3] The abbreviations used are: CIVI, continuous i.v. infusion; CNS, central nervous

ylacetate concentration at the targeted level during the 14-day infusion. Drug concentrations were monitored according to the sampling schedule described below. Serum samples were analyzed weekly, prompting weekly reestimation of individual pharmacokinetics and dosage adjustment (adaptive control with feedback).

Sampling Schedule. With the initial 150-mg/kg i.v. bolus, blood samples were obtained through a central venous catheter at the following time points calculated from the beginning of the infusion: 0, 60, 115, 125, 135, 150, 165, 180, 240, 360, 480, and 600 min. For the 60-mg/kg bolus given over 30 min, blood sampling was performed at 0, 30, 60, 75, 90, 105, 120, 150, 180, 270, and 390 min from the beginning of the infusion. During CIVI, blood samples were obtained by venipuncture. At dose levels 1 and 2, blood samples were obtained daily during the CIVI; while at dose levels 3 and 4, blood samples were obtained on days 1, 2, 3, 8, 9, and 10 of the infusion. Twenty-four-h urine collections for the determination of phenylacetate and phenylacetylglutamine excretion were obtained on days 1, 7, and 14 of therapy. Sampling of the CSF was performed only if clinically indicated.

Analytical Method. Determination of sodium phenylacetate and phenylacetylglutamine in serum and urine by HPLC. Blood was drawn into a Vacutainer tube free of anticoagulant and was then refrigerated. It was centrifuged at 1200 × *g* for 10 min in a Sorvall RT 6000D centrifuge (DuPont Co., Wilmington, DE) at 4°C. Serum was then removed and stored in Nunc Cryotubes (Nunc Co., Denmark) at −70°C until the day of analysis.

A standard curve was generated by adding known amounts of sodium phenylacetate (Elan Pharmaceutical Research Co.) and phenylacetylglutamine (a gift from Dr. S. W. Brusilow, Johns Hopkins University, Baltimore, MD) to a commercial preparation of pooled serum (Baxter Healthcare Corporation, Deerfield, IL). The standard values spanned the expected range of serum concentrations: 0, 5, 10, 20, 50, 100, 250, 500, 750, and 1500 μg/ml.

Two hundred μl of serum were pipeted into a 1.7-ml Eppendorf tube (PGC Scientifics, Gaithersburg, MD). Protein extraction was carried out by adding 100 μl of a 10% (v/v) solution of perchloric acid (Aldrich Chemical Co., Milwaukee, WI). The tube was vortexed and then centrifuged at 4500 × *g* for 10 min. Supernatant (150 μl) was transferred to a new 1.7-ml Eppendorf tube and 25 μl of 20% $KHCO_3$ (w/v) were added to neutralize the solution. This was centrifuged at 4500 × *g* for 10 min and 125 μl of supernatant were transferred to an autosampler vial and maintained at 10°C until HPLC injection. Urine samples were processed in an identical manner after an initial 1:10 dilution with water.

The HPLC system (Gilson Medical Electronics, Middleton, WI) was composed of two pumps (305 and 306), an 805 manometric module, an 811C dynamic mixer, a 117 variable wavelength UV detector, and a 231 autosampler fitted with a 20-μl injection loop and cooled with a Grey Line model 1200 cooling device. The column was a Waters (Millipore Corporation, Milford, MA) C_{18} Nova-Pak, 3.9 x 300 mm, maintained at 60°C with a Waters temperature control module. The mobile phase solutions consisted of acetonitrile (J. T. Baker Chemical Co., Inc., Phillipsburg, NJ) and water, both acidified with phosphoric acid (0.005 M). An acetonitrile concentration gradient was used, which increased from 5% to 30% over 20 min.

Twenty μl of the neutralized supernatant were injected onto the column and eluted at 1 ml/min. The progress of the elution was followed by monitoring the UV absorbance at 208 nm. Characteristic elution times for sodium phenylacetate and phenylacetylglutamine under these conditions were 17.1 and 9.8 min, respectively.

Determination of Plasma Glutamine Concentrations by Ion-Exchange Chromatography. Glutamine concentration was measured in sodium heparin-preserved plasma (−80°C storage) following a 1:2 dilution/deproteinization with 15% 5-sulfosalicylic acid/sarcosine hydrochloride (Sigma Chemical Co., St. Louis, MO). A stock solution of L-glutamine (1000 μg/ml) (Sigma) was diluted with Li-S buffer (Beckman Instruments, Inc., Palo Alto, CA) to generate a standard curve ranging from 0.78 to 100 μg/ml. A pooled plasma sample from 250 patients was used to make a quality control solution.

Fifty-μl samples were autoinjected onto a 10-cm cation-ion exchange column integrated into a Beckman Model 6300 amino acid analyzer (Beckman). The solvent flow rate (2:1 water/ninhydrin) was maintained constant at 0.5 ml/min. Column temperature was maintained at 33°C until glutamine was eluted, at which time the column temperature was raised by 1.5°C/min to elute sarcosine, the internal standard. The column was regenerated with lithium and 440 nm following postcolumn color development with ninhydrin-RX (Beckman) at 131°C. Beckman System Gold software was used for data acquisition and data management.

Pharmacokinetic Methods. Initial estimates of V_{max} and K_m for phenylacetate were obtained by generating Lineweaver-Burk plots from concentration *versus* time curves following i.v. bolus doses. These initial parameter estimates were refined by nonlinear least squares fitting to a single compartment, open nonlinear model, using the Nelder-Mead iterative algorithm, as implemented in the Abbottbase Pharmacokinetic Systems software package (Abbott Laboratories, Abbott Park, IL; version 1.0). Each data point was weighted equally.

Statistical Methods. Student's *t* test was used to compare estimates of the pharmacokinetic parameters of phenylacetate derived from the Lineweaver-Burk plots with those obtained using nonlinear least squares regression. Serum phenylacetate concentrations observed on day 2 of the CIVI were compared with those observed on day 11, using the Wilcoxon signed rank test for paired data (14) to assess the significance of time-related changes in drug concentrations. At dose levels 3 and 4, the CIVI rate varied with time and the presence of induction of clearance was assessed by comparing a single compartment, open nonlinear model with the same model modified to incorporate two additional pharmacokinetic parameters which allowed for time-dependent changes in the maximum velocity of drug elimination (V_{max}). The performance of each model in describing a given set of dosing and concentration data was quantified by calculating the weighted sum of the errors squared following nonlinear least squares fitting. The standard deviation of the errors was modeled as a function of drug concentration multiplied by the coefficient of variation of the assay. The fitting procedure was used to maximize the likelihood of normally distributed variates, and the normality of the distribution of the standardized errors was confirmed by the method of Shapiro and Wilk (15). Confidence regions for the parameters were derived from the weighted sum of squares in the model incorporating the induction parameters, and approximate significance levels for testing between the two models were calculated using the *F* distribution (16); $P < 0.05$ was considered significant. The Spearman rank correlation method was used in an attempt to discern whether there was a relationship between the dose of phenylacetate administered and the *P* value derived from the *F* distribution for each cycle of therapy.

RESULTS

Analytical Assay. The reverse phase HPLC assay allowed both serum phenylacetate and phenylacetylglutamine concentrations to be determined simultaneously from the same sample (see Fig. 1). The lower limit of detection for both compounds in serum and urine was 5 μg/ml, based upon a signal:noise ratio of 5:1. The interassay coefficient of variation for serum concentrations was less than 6% within the range of 40–1000 μg/ml (Table 1). The lower limit of detection for glutamine was 0.5 μg/ml, with an interassay coefficient of variation that did not exceed 7%.

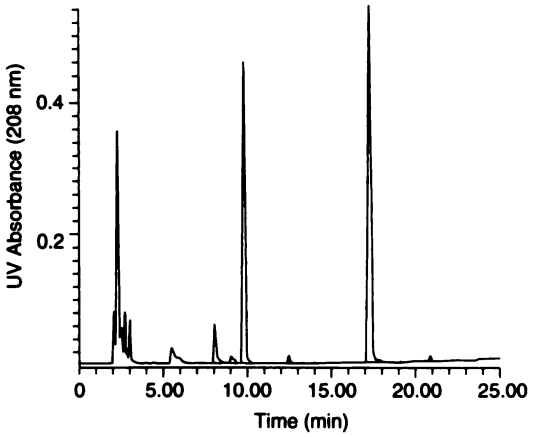

Fig. 1. Chromatogram of phenylacetate and phenylacetylglutamine. The peaks at 9.8 and 17.1 min represent phenylacetylglutamine and phenylacetate, respectively. Serum

Model Specification and Initial Parameter Estimation. Fig. 2 shows representative concentration *versus* time curves for simultaneously measured serum levels of sodium phenylacetate and phenylacetylglutamine and plasma levels of glutamine following both 150- and 60-mg/kg bolus doses of sodium phenylacetate. The decline in serum phenylacetate concentration following the 150-mg/kg bolus is linear when plotted on a nonlogarithmic scale and consistent with saturable elimination kinetics. While useful for demonstrating a zero-order process, the magnitude of the bolus was inadequate for parameter estimation insofar as most of the phenylacetate concentrations obtained over the 6-h sampling period were above K_m. In order to generate concentrations both above and below K_m, the bolus was changed to 60 mg/kg i.v. over 30 min. Visual inspection of the concentration *versus* time curves following these boluses revealed no evidence of an initial distributive phase, suggesting that a single compartment, open nonlinear model would be adequate to describe the pharmacokinetics of the drug. Initial estimates of K_m [90 ± 30 (SD) μg/ml], V_{max} (26.0 ± 10 mg/kg/h), and V_d (22.4 ± 6.8 liters) were calculated in 13 patients using the Lineweaver-Burk equation.

Table 1 *PA[a] standard curve assay variability*

PA (μg/ml)	CV (%)	PAG (μg/ml)	CV (%)
40	2.6	40	4.6
400	1.7	400	4.3
1000	3.4	1000	3.1

[a] PA, phenylacetate; PAG, phenylacetylglutamine; CV, coefficient of variation.

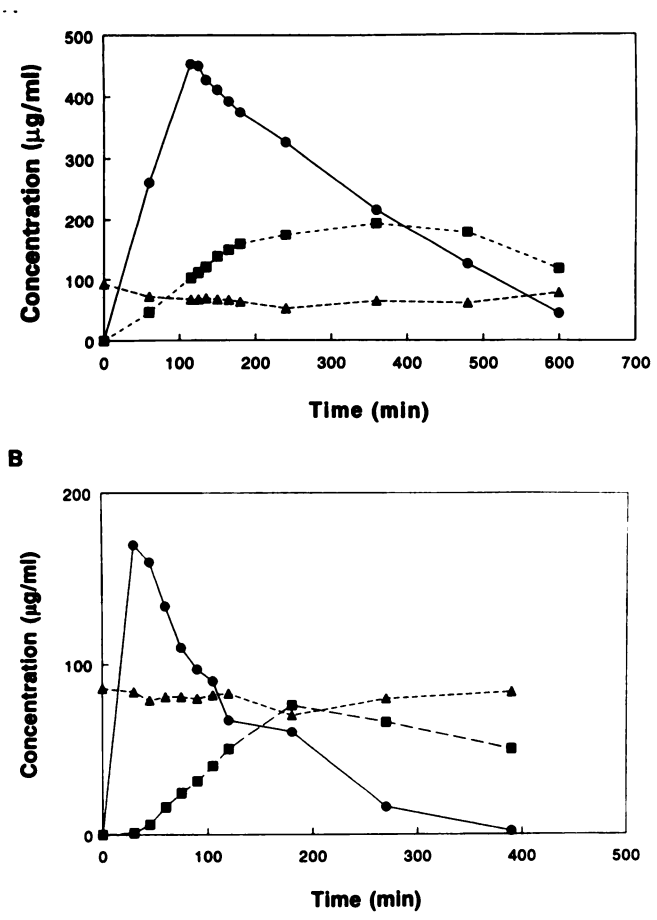

Fig. 2. Serum concentrations of phenylacetate (●) and phenylacetylglutamine (■) and plasma concentrations of glutamine (▲) following a 150-mg/kg i.v. bolus of phenylacetate

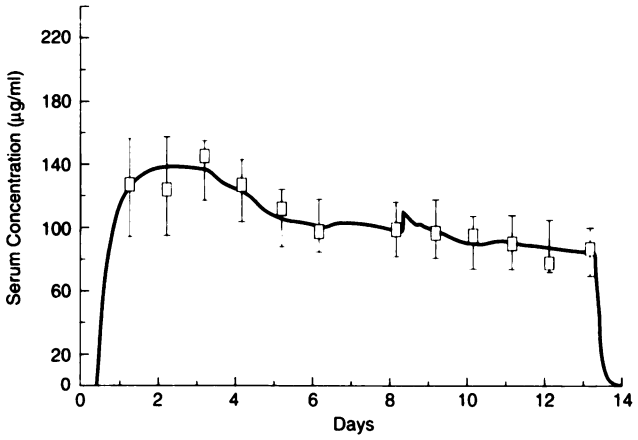

Fig. 3. Declining phenylacetate concentrations over time during CIVI (250 mg/kg/day) in one patient, suggestive of clearance induction. □, measured serum phenylacetate concentrations; *bars*, 95% confidence limits of the model's fit to the data.

Refinement of these initial parameter estimates by nonlinear least squares fitting of the entire concentration *versus* time profile for each bolus dose yielded the following estimates: K_m = 105.1 ± 44.5 μg/ml; V_{max} = 24.1 ± 5.2 mg/kg/h; and V_d = 19.2 ± 3.3 liters. The differences between the two methods of estimation were not statistically different, as measured by Student's *t* test (P = 0.89).

Induction of Phenylacetate Clearance. In some patients treated at dose levels 1 and 2, we observed a tendency for the serum phenylacetate concentration to decrease with time. An example of this phenomenon is shown in Fig. 3. Considering the 12 cycles of therapy delivered at these levels, a comparison of the serum drug concentration measured on day 2 of CIVI to that observed on day 11 demonstrated a 23% mean decline in concentration over this time period (Wilcoxon signed rank test, P = 0.016).

At dose levels 3 and 4, attempts at maintaining targeted serum phenylacetate concentrations using adaptive control with feedback led to variable rates of drug infusion over time, which precluded a simple comparison of drug concentrations at the beginning and end of therapy. We therefore analyzed all cycles of therapy at all four dose levels and compared the performance of the single compartment nonlinear model described above with the same model modified to allow V_{max} to increase with time. The formula used to describe this increase was

$$V_{max}\ (t) = V_{max}\ (t = 0) \times \{1.0 + [(IF - 1.0) \times (1.0 - e^{IR \times t})]\}$$

where t is the time elapsed (in h) since the initiation of therapy, IF is an induction factor representing the maximum-fold increase in V_{max} at infinite time, and IR is a first order rate constant (h^{-1}) describing the rate at which V_{max} increases over time. Each cycle of therapy (n = 21) was evaluated by comparing the difference in the weighted sum of errors squared generated by nonlinear least squares fitting with each model. The significance of the difference was evaluated using the F distribution. In 9 of the 21 cycles, allowing V_{max} to increase with time yielded an improved fit (induction parameters, IF = 1.87 ± 0.37, IR = 0.0028 ± 0.003 h − 1). The Spearman rank correlation method did not demonstrate a correlation between rate of drug administration and the need to incorporate the two induction parameters into the model (rank correlation coefficient, −0.39; P = 0.084). The dose rates administered ranged from 450 to 1850 mg/h.

Review of concomitantly administered medications revealed no association between specific drugs and the occurrence of a time-dependent increase in phenylacetate clearance. In the seven patients with primary CNS tumors, treatment with anticonvulsants always

Mechanisms of Phenylacetate Clearance. As shown in Fig. 2, phenylacetate underwent rapid conversion to phenylacetylglutamine. In the three patients who received 150 mg/kg of phenylacetate over 2 h, the peak serum concentration of phenylacetylglutamine was 224 ± 81 μg/ml, 325 ± 72 min postinfusion. After the 60-mg/kg boluses, the peak serum phenylacetylglutamine concentration was 104 ± 33 μg/ml at 86 ± 33 min.

The plasma glutamine concentration prior to bolus treatment with phenylacetate was 109 ± 29 μg/ml (n = 16), similar to values reported in the literature for normal volunteers (2, 3). The largest reduction in circulating plasma glutamine levels (46%) was observed in a patient receiving a 150-mg/kg bolus. Since phenylacetate is conjugated with glutamine to yield phenylacetate, the molar excretion of glutamine was found to increase in direct proportion to the dose of drug administered.

The molar excretion of phenylacetylglutamine was determined from 24-h urine collections. It accounted for 99 ± 23% (n = 18) of the dose of phenylacetate administered over the same period of time. The recovery of free, nonmetabolized drug was only 1.5 ± 2.4% of the total administered dose. A strong phenylacetate odor was detectable on patients' clothes and on examiners' hands after physical examination. This suggests that phenylacetate may also be excreted to some extent transdermally.

Distribution of Phenylacetate and Phenylacetylglutamine into the CSF. Clinical circumstances required evaluation of the cerebrospinal fluid in two patients who had metastatic prostate cancer and were free of CNS metastases. The first had reached steady-state phenylacetate and phenylacetylglutamine concentrations of 141 and 199 μg/ml, respectively. The corresponding simultaneous CSF concentrations were 74 and 5 μg/ml, respectively. At the time of simultaneous serum and CSF sampling, the second patient had not received further therapy for 6 h after having reached a serum phenylacetate concentration of 1044 μg/ml. Measurements in serum and CSF were 781 and 863 μg/ml for phenylacetate and 374 and 46 μg/ml for phenylacetylglutamine, respectively.

Clinical Toxicities. No toxicity was associated with bolus administration of the drug. The highest peak serum concentrations were measured after the 150-mg/kg bolus over 2 h (533 ± 94 μg/ml). Table 2 lists the average serum phenylacetate concentrations per dose level. Although those achieved at dose levels 3 and 4 are close to their target, the large associated standard deviations reflect our inability to maintain serum phenylacetate concentrations within the desired range, even when using adaptive control with feedback.

Drug-related toxicity was clearly related to the serum phenylacetate concentration. Three episodes of CNS toxicity, limited to confusion and lethargy and often preceded by emesis, occurred in patients treated at dose levels 3 and 4. They were associated with drug concentrations of 906, 1044, and 1285 μg/ml (1078 ± 192 μg/ml), respectively. Symptoms resolved within 18 h of terminating the drug infusion in all instances.

Antitumor Activity. Prostatic specific antigen (measured weekly) remained stable for more than 2 months in 3 of the 9 patients with prostate cancer treated at dose levels 2, 3, and 4. A fourth patient taking 180 mg of morphine daily experienced marked improvement in bone pain control and was able to substitute a nonsteroidal antiinflammatory drug to his narcotic regimen. The mean phenylacetate concentration of the four responders was 244 ± 33 μg/ml (186, 197, 269, and 325 μg/ml). These values were not statistically different from those achieved in the other six patients with prostate cancer. One of six patients with glioblastoma multiforme that recurred after surgery, standard radiation therapy and chemotherapy with bischloroethylnitrosourea has maintained improvements in performance status aphasia for more than 9 months. Although no change in the size of the tumor mass was noted, reduction in peritumoral edema was documented by magnetic resonance imaging (see Fig. 4). His steroid regimen had not been changed for 2 weeks prior to starting therapy with phenylacetate and has been kept unchanged since.

DISCUSSION

Previous descriptions of the pharmacokinetics of phenylacetate have been fragmentary. Simell *et al.* (3) reported the drug to have first order elimination kinetics with a half-life of 4.2 h following bolus dose administration (270 mg/kg) in children. The failure to recognize the nonlinear nature of phenylacetate pharmacokinetics probably resulted from the smaller total doses given to these patients compared to those given in our study. The saturable pharmacokinetics of phenyl-

Table 2 *PA[a] and PAG concentrations per dose level during CIVI*

Dose level	PA[a] dose (mg/kg/day)	PA (μg/ml)	PAG (μg/ml)
1	150	49 ± 19[b]	90 ± 34
2	250	104 ± 40	150 ± 63
3	266 ± 40	178 ± 85	188 ± 55
4	374 ± 95	397 ± 244	306 ± 51

[a] PA, phenylacetate; PAG, phenylacetylglutamine.
[b] Mean ± SD.

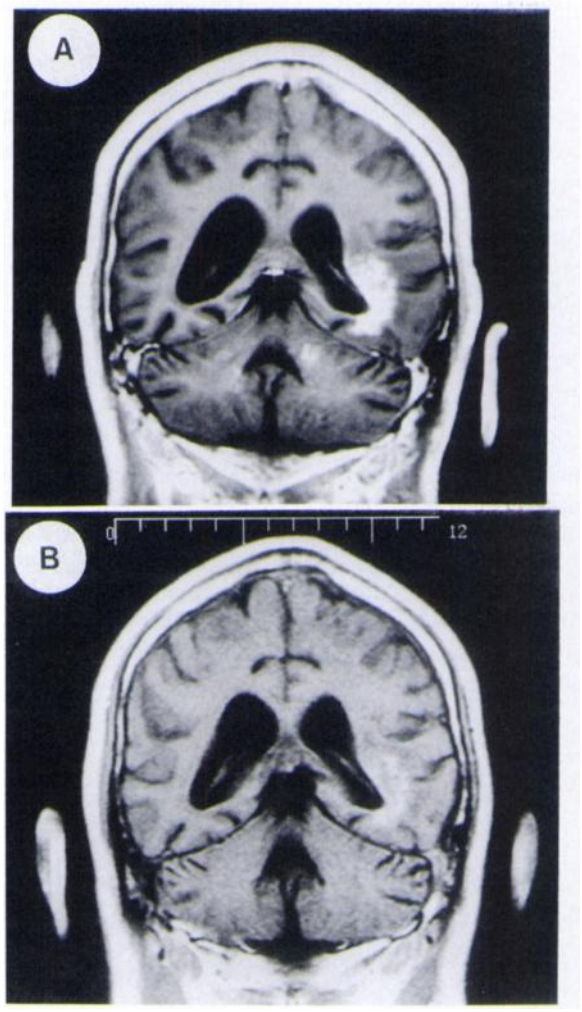

Fig. 4. (*A*) Pretreatment gadolinium-enhanced brain magnetic resonance imaging in a patient with glioblastoma multiforme. (*B*) Posttreatment gadolinium-enhanced magnetic resonance imaging after 1 cycle of phenylacetate (150 mg/kg/day) illustrating resolution

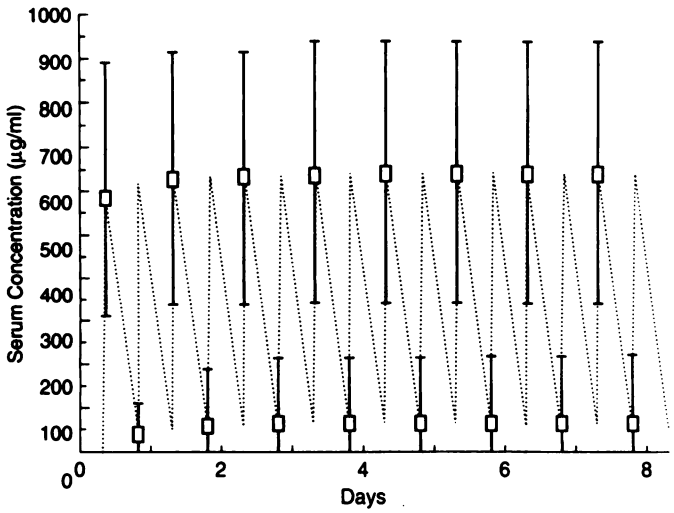

Fig. 5. Simulation of an every-12-h phenylacetate regimen (200 mg/kg/dose, 1-h infusion) in a pharmacokinetically average patient. For simplicity, induction of clearance was not factored in. *Bars*, 95% confidence limits expressing the anticipated range of concentrations in a population of patients.

acetate is consistent with an enzymatic process and our calculations from the 24-h urinary excretion of phenylacetylglutamine confirm that this is the major route of elimination. Evidence that drug clearance may increase with time was derived from the comparison of drug levels on days 2 and 11 of the CIVI, adding another layer of complexity to the pharmacokinetics of phenylacetate. To explain this phenomenon, we first considered the potential role of concomitantly administered medications but failed to demonstrate any association. Our analysis of a possible relationship between an increase in drug clearance with time and the rate of drug administration did not reach statistical significance but suffered from the small number of cycles of therapy available for analysis. It should also be noted that, relative to the 14-day period over which it was assessed, V_{max} tended to increase slowly, with an average half-time calculated from the induction rate of 9.6 days. Longer periods of CIVI would allow this process to be more thoroughly characterized.

Phenylacetate was delivered by CIVI in order to mimic the preclinical conditions that had demonstrated antitumor activity, namely, continuous exposure to concentrations equal to or higher than 275 μg/ml for at least 2 weeks (8–11). The results of Table 2 indicate that attempting to maintain serum phenylacetate concentrations at 400 μg/ml using adaptive control with feedback was problematic, with drug concentrations that often greatly exceeded the level-specific targets. Lower concentrations (200–300 μg/ml) were safely maintained. Phenylacetate serum concentrations in excess of 900 μg/ml were typically associated with CNS toxicity. As expected for such a small and lipophilic molecule, phenylacetate readily penetrates into the CSF (this study and Ref. 1). While the ability to cross the blood-brain barrier may underlie the clinical improvement seen in the patient with glioblastoma, it could also explain the dose-limiting side-effects of the drug, *i.e.*, nausea, vomiting, sedation, and confusion.

In the average patient, the drug must be infused at a rate equal to 75% of V_{max}, in order to maintain a constant serum phenylacetate concentration of 400 μg/ml, which is 4 times greater than K_m. Thus, the slightest error in the estimation of individual pharmacokinetics or in the rate of drug infusion results in large changes in drug concentration. An alternative strategy is to deliver the drug by repeated short infusions. Our limited experience with the 150-mg/kg i.v. boluses suggests that serum phenylacetate concentrations occurring transiently above 500 μg/ml are well tolerated. In addition, intermittent drug infusion should permit some drug washout to occur, thereby minimizing drug accumulation. A regimen of 200 mg/kg every 12 h (1-h infusion) is simulated in Fig. 5. It assumes that the pharmacokinetic parameters determined from our 17 patients are representative of the cancer population at large and that V_{max} does not change with time. It predicts that a wide range of peak drug concentrations will be observed. However, it is possible that these would be sufficiently transient so as not to produce CNS toxicity and the troughs not so prolonged as to abrogate the antitumor activity of the drug.

Although dosing alternatives should be explored, our study indicates that phenylacetate can be safely administered by CIVI and result in clinical improvement in some patients with hormone-refractory prostatic carcinoma and glioblastoma multiforme who failed conventional therapies.

ACKNOWLEDGMENTS

We are grateful to Dr. Nicholas Patronas for his expert radiological assistance during the conduct of this study and to Frank N. Konstantinides for assaying glutamine levels in plasma.

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