Disposition of Phenylbutyrate and its Metabolites, Phenylacetate and Phenylacetylglutamine

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Phenylacetate, an inducer of tumor cytostasis and differentiation, shows promise as a relatively nontoxic antineoplastic agent. Phenylacetate, however, has an unpleasant odor that might limit patient acceptability. Phenylbutyrate, an odorless compound that also has activity in tumor models, is known to undergo rapid conversion to phenylacetate by beta-oxidation in vivo. This phase I study examined the pharmacokinetics of phenylbutyrate and characterized the disposition of the two metabolites, phenylacetate and phenylacetylglutamine. Fourteen patients with cancer (aged 51.8 ± 13.8 years) received a 30-minute infusion of phenylbutyrate at 3 dose levels (600, 1200, and 2000 mg/m²). Serial blood samples and 24-hour urine collections were obtained. Samples were assayed by high-performance liquid chromatography. A model to simultaneously describe the pharmacokinetics of all three compounds was developed using ADAPT II. Data were modeled as molar equivalents. The model fit the data well as shown by mean (±SD) coefficients of determination (r^2) for phenylbutyrate, phenylacetate, and phenylacetylglutamine, which were 0.96 ± 0.07, 0.88 ± 0.10, and 0.92 ± 0.06, respectively. The intrapatient coefficient of variation percentage (CV%) around the parameter estimates were small (range 7.2–33.5%). Phenylbutyrate achieved peak concentrations in the range of in vitro tumor activity (500–2000 μmol/L) and exhibited saturable elimination (K_m = 34.1 ± 18.1 μg/mL and V_{max} = 18.1 ± 18 mg/h/kg). Metabolism was rapid; the times to maximum concentration for phenylacetate and phenylacetylglutamine were 1 and 2 hours, respectively. The conversion of phenylbutyrate to phenylacetate was extensive (80 ± 12.6%), but serum concentrations of phenylacetate were low owing to rapid, subsequent conversion to phenylacetylglutamine. The ratio of phenylbutyrate AUC to phenylacetate AUC was 2.66. Thus, phenylbutyrate may not be a prodrug for phenylacetate and should be pursued as an independent antitumor agent.

The amino acid phenylalanine is degraded by a combination of hydroxylation and deamination, leading to a range of metabolic products including phenylacetate, a compound used to treat children with hyperammonemic urea cycle disorders.[1] Man and higher primates conjugate phenylacetate with glutamine to form phenylacetylglutamine, whereas in rodents this compound is conjugated with glycine.[2] The fact that phenylacetate is conjugated with and depletes circulating glutamine is of special interest, because tumor cells are highly dependent on this amino acid, rendering glutamine a target for therapeutic intervention. In addition to potential glutamine starvation, phenylacetate can arrest tumor growth by modulating the expression of genes critical to growth control and differentiation.[3–6]

Recently, phenylacetate has been shown to possess cytostatic and differentiating properties against a va-

riety of hematologic and solid tumors in laboratory models.[3-6] When given to healthy subjects, phenylacetate undergoes hepatic conjugation with glutamine by phenylacetyl coenzyme A: glutamine acyltransferase, which yields phenylacetylglutamine, the major urinary metabolite.[2] Although previously shown to follow first-order pharmacokinetics,[7] the drug exhibits nonlinear, saturable pharmacokinetics at doses currently being evaluated in patients with cancer.[8] Phenylacetate, however, has an unpleasant odor that might limit its acceptance and development as an oral drug.

In contrast, phenylbutyrate is an odorless compound and has also been safely given to children for hyperammonemic urea cycle disorders.[9,10] Recent laboratory studies have documented that phenylbutyrate, like phenylacetate, can (1) induce selective cytostasis and maturation of cultured tumor cells derived from various erythropoietic and solid neoplasms (including adenocarcinomas of the prostate, breast, ovary, colon, and lung, as well as central nervous system tumors and malignant melanoma); (2) modulate the expression of genes implicated in tumor growth, metastasis, and immunogenicity; and (3) enhance the efficacy of other agents of clinical interest including retinoids, interferon alfa, suramin, 5-aza-2'-deoxycytidine, and hydroxyurea (Samid et al.[6]; Liu et al., Hudgins et al., Figg et al., submitted; Sand et al., unpublished data). Phenylbutyrate is converted in vivo to phenylacetate by mitochondrial beta-oxidation.[11] Therefore, phenylbutyrate is currently being investigated as a new antineoplastic agent, and as a prodrug for phenylacetate in the treatment of cancer.

To better understand the disposition of these compounds after intravenous administration of phenylbutyrate, a pharmacokinetic model that simultaneously characterizes the disposition of phenylbutyrate, phenylacetate, and phenylacetylglutamine was developed from plasma and urine data collected during a phase I clinical trial.

METHODS AND DEVELOPMENT OF MODEL

Adults with advanced solid tumors refractory to conventional therapy, a performance status greater than 60% on Karnofsky's scale,[12] normal hepatic transaminases and bilirubin, a serum creatinine less than 1.5 mg/dL, and normal leukocyte and platelet counts were eligible for this study. The clinical protocol was approved by the National Cancer Institute's Institutional Review Board, and all patients gave written informed consent before participating in the study.

Patients were enrolled into the study in cohorts of at least 3 per dosage level (600, 1200, and 2000 mg/m²). Each patient received a single 30-minute infusion of phenylbutyrate, and serial blood samples were collected before, immediately post-dose, and at 0.16, 0.3, 0.5, 0.75, 1, 1.5, 2.5, 3.5, and 5 hours after the infusion. Blood samples (5 mL) were collected in 5-mL glass tubes (Vacutainer®; Becton Dickinson, Rutherford, NJ) either via an intravenous catheter (separate from the drug administration catheter) or venipuncture. Blood was centrifuged, and the serum was transferred to 5-mL polypropylene tubes and frozen at −85°C until the time of analysis. A 24-hour urine collection for cumulative phenylacetylglutamine excretion was done in a subset of patients.

The reversed phase high-performance liquid chromatography method for measuring serum concentrations of phenylacetate, phenylbutyrate, and phenylacetylglutamine has been previously described.[13] Briefly, 100 μL of 10% perchloric acid was used to precipitate the proteins of a 200-μL aliquot of serum, which was then centrifuged. The supernatant was neutralized with 25 μL of a 20% solution of potassium bicarbonate. After centrifugation, 20 μL of supernatant was injected onto a C-18 column heated at 60°C. Urine samples were processed similarly, after a 1:20 dilution with water. Elution was done with an increasing gradient of acetonitrile in water from 5 to 30% over 45 minutes. Its progress was followed by monitoring ultraviolet absorbance at 208 nm. Characteristic elution times for phenylacetylglutamine, phenylacetate, and phenylbutyrate were 10.1, 17.4, and 27.8 minutes, respectively. The assay yielded a lower limit of detection of 2 μg/mL and was linear for concentrations as high as 2,000 μg/mL. Between 20 and 1,000 μg/mL, the interassay CV% was less than 10%.

A model to simultaneously describe the pharmacokinetics of all three compounds was developed using ADAPT II.[14] Several models were constructed to compare one and two compartments for each drug, as well as the possibility of nonlinear pharmacokinetics. Model selection was determined by Akaike's Information Criterion (AIC),[15] and by visual inspection of the difference between measured and computer-fitted concentrations (residuals). Data were modeled as molar equivalents. The pharmacokinetic parameters were estimated using weighted nonlinear least squares by an adaptive process that used sequential updating of priors for parameter values. Weighting was by the inverse of the observation variance for all compounds.

Drug input was by intermittent intravenous infusion. To make the model identifiable, the volume of distribution of phenylacetate was fixed at 0.3 L/kg based on previous phase I data in which phenylacetate was given intravenously.[8] Complete conversion of phenylacetate to phenylacetylglutamine and elimination of all phenylacetylglutamine in the
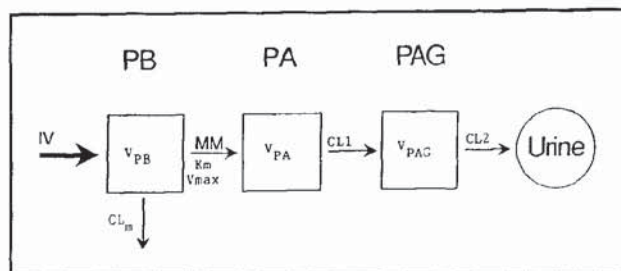
Figure 1. Model to describe the disposition of phenylbutyrate (PB), phenylacetate (PA), and phenylacetylglutamine (PAG) illustrating the pharmacokinetic parameters.

Abbreviations: V_{PB}, volume of distribution for PB; V_{PA}, volume of distribution for PA; V_{PAG}, volume of distribution for PAG; MM, Michaelis-Menten elimination; K_m, Michaelis-Menten constant; V_{max}, maximum elimination rate; CL_1, formational clearance of PA to PAG; CL_2, clearance of PAG into the urine; CL_m, clearance of PB out of the central compartment.

urine was also assumed based on our previous phase I experience.[8] Thus, the fraction of phenylbutyrate converted to phenylacetate was determined using the following equation:

$$\frac{\text{urinary phenylacetylglutamine } (\mu mol)}{\text{dose of phenylbutyrate } (\mu mol)}.$$

The pharmacokinetic parameters for phenylacetylglutamine are dependent on this fraction, which is analogous to oral drugs where clearance and volume are dependent on the value of bioavailability (i.e., CL/F or V_{ss}/F).

The model eventually used was a one-compartment nonlinear model for phenylbutyrate with conversion to a one-compartment linear model for phenylacetate, and further conversion of phenylacetate to phenylacetylglutamine (one-compartment). Phenylbutyrate was parameterized by a central volume (V_{PB}), a minor elimination pathway (CL_m), and a nonlinear function consisting of intrinsic clearance (CL_{int}) and the Michaelis-Menten constant (K_m). The V_{max} is equal to $CL_{int} \cdot K_m$. The CL_1 and CL_2 describe the clearances of phenylacetate to phenylacetylglutamine and phenylacetylglutamine into the urine, respectively. The V_{PAG} describes the volume of distribution (V_z) for phenylacetylglutamine. The V_{PA} represents the volume of distribution of phenylacetate. The model displaying the pharmacokinetic parameters is shown in Figure 1.

The area under the serum concentration versus time curve (AUC) was calculated by the trapezoidal rule according to Gibaldi and Perrier.[16] The AUC was determined from time zero until the last time point (5 hours), because concentrations of each compound were usually below detectable limits at this point and because of the difficulty in determining an elim-

ination rate constant for the metabolites owing to sparse data describing the terminal slope.

RESULTS

Patients

Fourteen male patients were included in the study. Three patients received 600 mg/m² of phenylbutyrate, 8 received 1200 mg/m², and 3 received 2000 mg/m². Patient demographics are shown in Table I.

Pharmacokinetics

The model fit the data well as shown by mean (±SD) coefficients of determination (r^2) for phenylbutyrate, phenylacetate, and phenylacetylglutamine, which were 0.96 ± 0.07, 0.88 ± 0.10, and 0.92 ± 0.06, respectively. Pharmacokinetic parameters are shown in Table II. The intrapatient CV% around the parameter estimates were small, ranging from 7.2 to 33.5% of the fitted values. The mean interpatient CV% for parameter values ranged from 11.85 to 34.6%.

Serum concentration-time plots for a representative patient in each dosage group are shown in Figure 2. Similar fits were seen for the other patients. Peak concentrations of phenylbutyrate after 600 mg/m² ranged from 31 to 57 µg/mL. After 1200 mg/m² and 2000 mg/m², peak concentrations in serum ranged from 57 to 115 µg/mL and 114 to 184 µg/mL, respectively. Concentrations at 5 hours after dosing were 2

TABLE I					
Individual and Mean Patient Demographics					
Patient No.	Age (yr)	Height (cm)	Weight (kg)	Dose/m²	Dose (mg)
1	75	174	63.9	600	1080
2	60	188	87.0	600	1278
3	55	177	79.9	600	1188
4	66	164	70.6	1200	2112
5	55	180	101.4	1200	2640
6	61	180	101.4	1200	2352
7	39	167	80.2	1200	2292
8	29	179	70.6	1200	2340
9	48	170	71.4	1200	2148
10	35	169	72.5	1200	2196
11	42	184	103.0	1200	2700
12	46	152	54.0	2000	3000
13	71	180	82.0	2000	4020
14	43	158	48.7	2000	2940
Mean	51.8	173.0	77.6		
SD	13.8	10.2	16.7		

TABLE II

Pharmacokinetic Parameters Derived From the Model

	Mean	SD
V_{PB} (L/kg)	0.21	0.08
CL_m (L/hr/kg)	0.10	0.04
K_m (µg/mL)	34.1	18.1
CL_{int} (L/hr/kg)	0.50	0.30
V_{max} (mg/hr/kg)	18.1	18.0
V_{PA} (L/kg)	0.30	Fixed
CLt1 (L/hr/kg)	0.37	0.13
V_{PAG} (L/kg)	0.19	0.11
CLt2 (L/hr/kg)	0.17	0.11
AUC PB 600 mg/m²	265.4	139.6
AUC PB 1200 mg/m²	557.8	167.9
AUC PB 2000 mg/m²	1214.5	689.2
AUC PA 600 mg/m²	120.0	16.6
AUC PA 1200 mg/m²	220.2	90.6
AUC PA 2000 mg/m²	608.3	160.0
AUC PAG 600 mg/m²	401.3	119.2
AUC PAG 1200 mg/m²	438.0	269.7
AUC PAG 2000 mg/m²	1055.4	389.6

V_{PB} = volume of distribution for PB; V_{PA} = volume of distribution for PA; V_{PAG} = volume of distribution for PAG; K_m = Michaelis–Menten constant; CL_{int} = intrinsic clearance; V_{max} = maximum elimination rate; CLt1 = formational clearance of PA to PAG; CLt2 = clearance of PAG into the urine; CLm = clearance of PB out of central compartment; AUC = area under the curve from time 0 to 5 hours post-dose; PB = phenylbutyrate; PA = phenylacetate; PAG = phenylacetylglutamine.
AUC values are represented as µmol·hr/L.

µg/mL or lower in all patients. Phenylbutyrate exhibited saturable elimination pharmacokinetics as evidenced by concave log-linear plots on visual inspection, an AUC_{0-5} disproportionate to dose (Figure 3), and improved fits at high doses using a nonlinear function as assessed by AIC. Final estimates for Michaelis-Menten parameters were a K_m of 34.1 ± 18.1 µg/mL and a V_{max} of 18.1 ± 18.0 mg/h/kg.

Six patients had complete 24-hour urine collections for determination of phenylbutyrate conversion to phenylacetate, 1 at 600 mg/m², and 5 at 1200 mg/m². The percentage of conversion was high with a mean (±SD) of 80.0 ± 12.6%. The conversion ranged from 68 to 100% of phenylbutyrate accounted for in the urine by phenylacetylglutamine.

Phenylacetate was detectable in plasma immediately after phenylbutyrate infusion with mean (±SD) peak concentrations of 20.7 ± 13.6 µg/mL. The time to maximum concentration most commonly occurred 30 to 60 minutes after the infusion. The serum concentrations of phenylacetate that were seen in this study were much lower than those after intravenous administration of phenylacetate.[8] After phenylbutyrate administration, phenylacetate followed

first-order elimination. The Michaelis-Menten constant of phenylacetate from our previous trial[8] was 105.1 ± 44.5 µg/mL. The highest concentration of phenylacetate achieved in this study was 57 µg/mL with 11 of the 14 patients exhibiting peak concentrations less than 30 µg/mL. Because the peak phenylacetate concentrations were less than or equal to one-half the K_m, the nonlinear function of phenylacetate collapses to a first-order rate constant.[16] As a comparison of the total exposure between the 2 compounds, the mean (±SD) ratio of phenylbutyrate AUC to phenylacetate AUC was 2.66 ± 1.57.

Phenylacetylglutamine serum concentrations were also observed immediately after phenylbutyrate dosing. However, peak concentrations appeared 1 to 3.5 hours after the infusion, which was later than those of phenylacetate. Phenylacetylglutamine achieved maximum serum concentrations of 59.5 ± 34.2 µg/mL, which ranged from 27 to 129% of those of phenylbutyrate (mean ± SD, 61.2 ± 29.9%). Comparatively, phenylacetate achieved peak concentrations that were only 38.8 ± 19.2% of those of phenylacetylglutamine.

DISCUSSION

Pharmacokinetic models of anticancer agents can be used for a variety of purposes. In addition to describing a drug's disposition, these models can be used to (1) determine sampling schemes based on a small number of blood samples (using optimal sampling theory[17]); (2) predict plasma concentrations of new regimens; or (3) optimize dosing for maximal efficacy and minimal toxicity in patients receiving multiple courses of therapy.

The simultaneous modeling approach used in this analysis accurately characterized the conversion and disposition of phenylbutyrate and its two metabolites, phenylacetate and phenylacetylglutamine. There is increasing interest in phenylacetate as a relatively nontoxic antitumor agent.[3-6,8] The unpleasant odor of phenylacetate, however, may limit its acceptance by patients. Phenylbutyrate is the odorless precursor of phenylacetate, with demonstrable antitumor activity in laboratory models. Phenylbutyrate was converted to phenylacetate with subsequent conversion to phenylacetylglutamine. These conversions were rapid with detectable amounts of both metabolites occurring less than 10 minutes after initiation of the phenylbutyrate infusion. Phenylbutyrate was characterized by nonlinear elimination pharmacokinetics with a K_m of 34.1 µg/mL and a V_{max} of 18.1 mg/h/kg.

Our group has previously reported the results of a phase I study of intravenous phenylacetate that showed nonlinear pharmacokinetics for phenylacet-
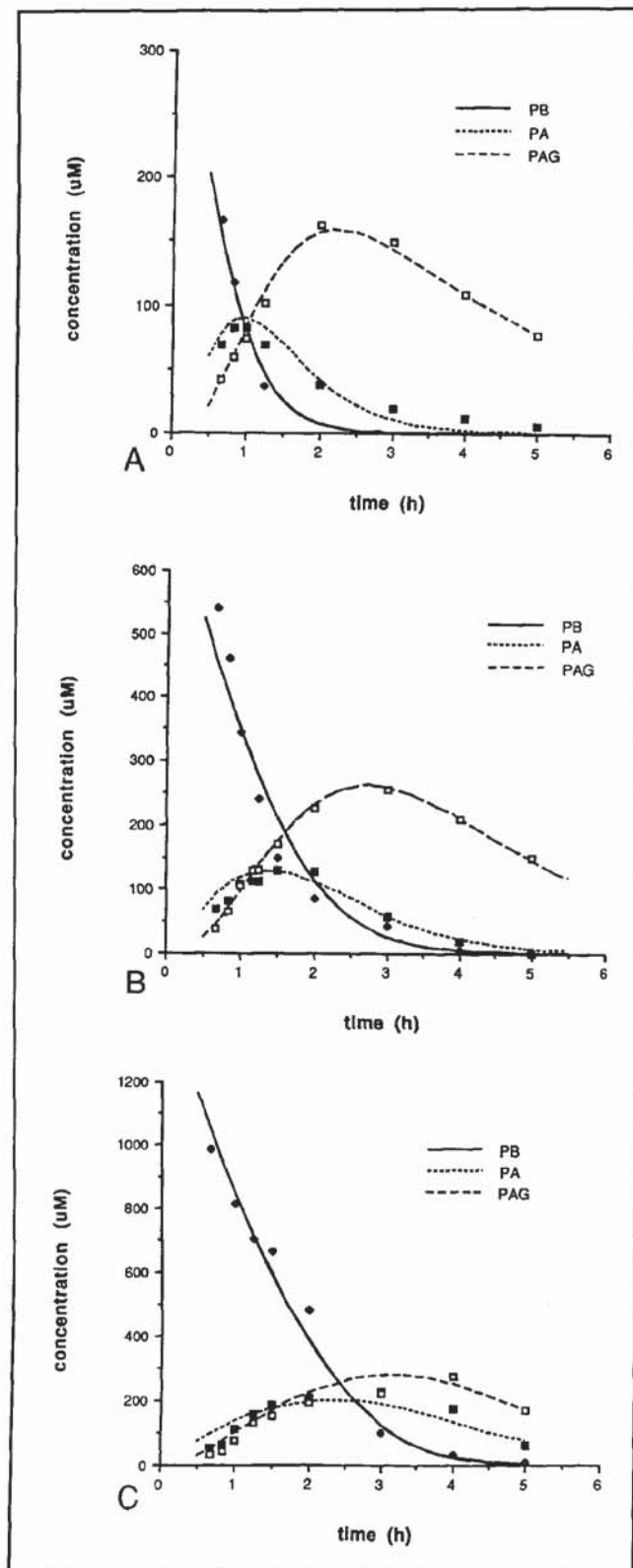
Figure 2. Actual (squares) and computer-fitted (line) concentration-time profiles of phenylbutyrate, phenylacetate, and phenylacetylglutamine in (A) patient 1 receiving 600 mg/m² of phenylbutyrate; (B) patient 5 receiving 1200 mg/m² of phenylbutyrate; (C) patient 12 receiving 2000 mg/m² of phenylbutyrate.

←

ate characterized by saturable metabolism to phenylacetylglutamine.[8] In this study, where concentrations of phenylacetate were smaller than the reported K_m, the Michaelis-Menten function reduces to a first-order rate constant. Thus, no saturability of phenylacetate was observed. The low concentrations of phenylacetate seen in this study may also be related to the small doses of phenylbutyrate used here compared with the initial phase I trial, which used a 150-mg/kg (approximately 6000 mg/m²) bolus of phenylacetate.[8]

Preclinical antitumor activity has been observed for phenylbutyrate at concentrations of 500 to 2000 μmol/L (94–376 μg/mL). This concentration range was shown here to be clinically achievable after a 30-minute infusion. It will be important to further evaluate the pharmacokinetics of phenylbutyrate using alternative dosing strategies (e.g., continuous infusion) or higher doses to determine whether these concentrations can be maintained for longer periods of time. In addition, continuous infusion may yield higher phenylacetate concentrations, especially if saturation of phenylacetate is achieved.

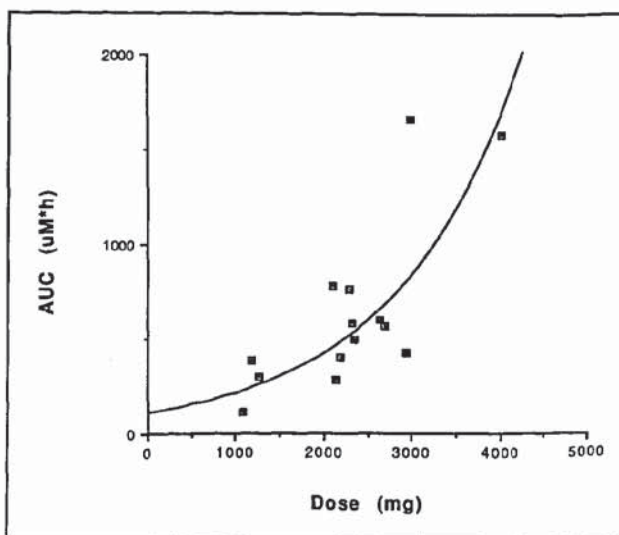Phenylbutyrate is known to undergo rapid conversion to phenylacetate in vivo by beta-oxidation.

Figure 3. Plot of phenylbutyrate dose (mg) and area under the curve. Line of best fit is shown; $y = 105.95 \cdot 10^{(0.000297x)}$, (R = .78).

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