A Phase I Dose Escalation and Bioavailability Study of Oral Sodium Phenylbutyrate in Patients with Refractory Solid Tumor Malignancies[1]

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ABSTRACT

Purpose: **Phenylbutyrate (PB) is an aromatic fatty acid with multiple mechanisms of action including histone deacetylase inhibition. Preclinically, PB demonstrates both cytotoxic and differentiating effects at a concentration of 0.5 mM. We conducted a Phase I trial of p.o. PB patients with refractory solid tumor malignancies to evaluate toxicity, pharmacokinetic parameters, and feasibility of p.o. administration.**

Experimental Design: **Twenty-eight patients with refractory solid tumor malignancies were enrolled on this dose-escalation to maximally tolerated dose trial. Five dose levels of PB were studied: 9 g/day ($n = 4$), 18 g/day ($n = 4$), 27 g/day ($n = 4$), 36 g/day ($n = 12$), and 45 g/day ($n = 4$). Pharmacokinetic studies were performed and included an p.o. bioavailability determination. Compliance data were also collected.**

Results: **The recommended Phase II dose is 27 g/day. Overall the drug was well tolerated with the most common toxicities being grade 1–2 dyspepsia and fatigue. Nonoverlapping dose-limiting toxicities of nausea/vomiting and hypocalcemia were seen at 36 g/day. The p.o. bioavailability of PB was 78% for all dose levels, and the biologically active concentration of 0.5 mM was achieved at all dose levels. Compliance was excellent with 93.5% of all possible doses taken. No partial remission or complete remission was seen, but 7 patients had stable disease for more than 6 months while on the drug.**

Conclusions: **PB (p.o.) is well tolerated and achieves the concentration *in vivo* that has been shown to have biological activity *in vitro*. PB may have a role as a cytostatic agent and should be additionally explored in combination with cytotoxics and other novel drugs.**

INTRODUCTION

Differentiating agents in cancer therapy may potentially alter tumor growth and progression, slow or inhibit metastases and/or effect response to other forms of therapy. Sodium PB[3] is an aromatic fatty acid that is converted *in vivo* to PA by β-oxidation in liver and kidney mitochondria. Both substances have been shown to act as differentiating agents (1). In various tumor model systems, PA/PB exert broad effects on tumor cytostasis and tumor differentiation, altering gene expression for tumor growth, invasion, angiogenesis, and immunogenicity, among others. Putative mechanisms of action include inhibition of histone deacetylase, hypomethylation, modification of lipid metabolism, and activation of peroxisome proliferation activator receptor (2–9). PB (buphenyl) has been FDA approved for use clinically in patients with hyperammonemia secondary to urea cycle disorders, and PB has been used for hyperammonemia after high-dose chemotherapy or transplant. It also increases fetal hemoglobin production in patients with sickle cell anemia or B-thalassemia (10–14).

The induction of apoptosis has also been shown for other tumor types including primary acute myelogenous leukemia as samples grown in suspension culture demonstrated apoptosis at concentrations beginning at 0.5 mM. The differentiating effects of PB have also been demonstrated *in vitro*. In solid tumor cell lines, PB was shown to induce G_1/G_0 arrest and to induce $p21^{waf1/cip1}$, a cell cycle checkpoint protein associated with differentiation, within 24 h of treatment. Growth arrest was also accompanied by induction of $p57^{kip1}$, another protein associated with differentiation (16).

A Phase I clinical trial of i.v. continuous infusion of PB has shown that the drug is safe and tolerated at a continuous infusion i.v. dose of 410 mg/kg/day. The study evaluated continuous i.v.

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[3] The abbreviations used are: PB, phenylbutyrate; PT, prothrombin time; PTT, partial thromboplastin time; ECOG, Eastern Cooperative Oncology Group; T_{max}, time to maximum plasma concentration; C_{max}, maximum plasma concentration; PG, phenylacetylglutamine; PA, phenylacetate; AUC, area under the curve; QOL, quality of life assessment; $t_{1/2}$, half-time; EORTC, European Organization for Research and Treatment of Cancer; CNS, central nervous system; MTD, maximum tolerated dose; PSA, prostate-specific antigen; HDAC, histone deacetylase inhibitor.

infusion of PB for 5 days every 21 days in patients with refractory solid tumor malignancies. No complete responses were noted; however, one patient did have a partial response. Dose-limiting toxicity was predominantly neurocortical.[4]

Histone deacetylase inhibition is an interesting target. Several agents have recently entered clinical trials that target this mechanism. PB is one of such agents to be tested in the clinic. The objectives of this Phase I trial of p.o. PB in refractory solid tumors were to determine the toxicities, p.o. bioavailability, pharmacokinetic behavior, and feasibility of the p.o. formulation. Also, the study sought to determine the recommended Phase II dose of chronic p.o. administration of this differentiating agent for potential combination with other chemotherapeutic agents.

PATIENTS AND METHODS

Patient Eligibility. Patients eligible for this trial had to meet the following criteria: pathological confirmation of advanced cancer; no available therapy known to improve survival; last dose of radiation therapy or chemotherapy (not including steroids) $\geq$4 weeks before study entry (including a 4–6 week period of antiandrogen withdrawal); ECOG performance status of $\geq$2 and a life expectancy of $\geq$3 months; adequate bone marrow function (WBC $>$2000/mm^3 or ANC $>$1500/mm^3, platelets $>$100,000/mm^3, and hemoglobin $>$9 g/dl), adequate renal function (serum creatinine $<$2.0 mg/dl), and adequate hepatic function (total bilirubin $<$1.5 mg/dl and aspartate aminotransferase/alanine aminotransferase $<1.5 \times$ the upper limit of normal); adequate cardiac function with LVEF $>$40% by echocardiography or multiple-gated acquisition scan; and adequate pulmonary function with FEV_1 $>$1.5 liter/min. These studies were required because of the fact that PB was shown to cause sodium retention, edema, and pleural effusions in animal studies.

Ineligible patients included: patients with persistent nausea or moderate to severe anorexia; patients with active infectious processes including HIV; patients with medical or psychiatric problems unrelated to the malignancy that might jeopardize compliance or put them at undue risk; pregnant or breastfeeding women; patients with connective tissue auto-immune diseases; patients with known CNS metastases or any history of CNS involvement or patients with active seizure disorders; and patients who have been treated with prior sodium PB, antineoplaston, or sodium PB. Before enrollment of any patients, the study received approval from the Johns Hopkins Joint Committee on Clinical Investigation and from the Cancer Therapeutics and Evaluation Program of the National Cancer Institute, NIH.

Patient Evaluations. Patient evaluations before receiving the first dose of PB included: complete history and physical exam; performance status assessment; mini-mental cognitive exam; tumor measurements; complete blood count with leukocyte differential; serum sodium; potassium; chloride; CO_2; blood urea nitrogen; creatinine; calcium; magnesium; phosphorous; total bilirubin; liver transaminases; alkaline phosphatase; total protein; albumin; uric acid; PT; PTT; urinalysis; electrocardiogram, multiple-gated acquisition scan, or echocardiogram; pulmonary function tests (spirometry with lung volumes); chest X-ray (or chest computed tomography if part of tumor evaluation); pathological confirmation of cancer diagnosis; and appropriate tumor markers.

Study evaluations during the first course of therapy included: weekly complete blood counts with leukocyte differential; serum sodium; potassium; chloride; CO_2; blood urea nitrogen; creatinine; magnesium; phosphorous; calcium; PT; PTT; and tumor markers. These were obtained weekly for the first 3 weeks.

For subsequent courses, after 3 weeks at a stable dose level, blood counts with leukocyte differential, serum sodium, potassium, chloride, CO_2, blood urea nitrogen, creatinine, PT, PTT, and tumor markers were performed every other week.

Treatment Plan and Drug Administration. PB was given in three equally divided daily doses until disease progression was documented, decline in performance status to ECOG 3 or 4 occurred, or patients requested to be withdrawn from study. Two tablet sizes were used during this study. Initially, 375-mg tablets of PB were used, and the study was completed with 500-mg PB tablets. Dose level one was 9 g/day PB (24 375-mg tablets/day or 18 500-mg tablets/day divided into three equal daily doses). Dose level two was 18 g/day PB (48 375-mg tablets/day or 36 500-mg tablets/day divided into three equal daily doses). The last three dose levels used only 500-mg tablets. They were 27 g/day, 36 g/day, and 45 g/day.

During cycle 1, each patient took part in the bioavailability assessment. Patients received a dose of PB either p.o. or i.v. on day 1 and then received the alternative formulation on day 2 in an equivalent dose. When administered i.v., PB was given over 1 h. From previous pharmacokinetic studies of PB, the half-life was reported to be 1 h, and therefore, a 24-h interval between p.o. and i.v. administration was considered to be an adequate washout period (1). The formal study of multiple p.o. daily dosing commenced on day 3. One course of therapy consisted of 28 days of chronic p.o. treatment with PB.

Four patients were treated at each dose level. All four patients in a given cohort must have started treatment, and at least three patients must have completed $\geq$3 weeks of therapy with the fourth patient being $\geq$1 week into therapy without meeting the criteria for the MTD before patients were enrolled at the next dose level. If two or more patients in a dose level experienced a dose-limiting toxicity, then the MTD was considered to be exceeded, and three more patients would be treated at the next lower dose. If one of four patients experienced a dose-limiting toxicity at a given dose, then three more patients were accrued at the same dose. The MTD was defined as the highest dose in which 1 or fewer of seven patients experienced a dose-limiting toxicity, (*e.g.*, grades 3 or 4). Three additional patients were entered on the 36 g/day dose level because this was initially thought to be the MTD.

Intrapatient dose escalation was permitted; however, no patient received dose escalation. If a patient had received at least two courses of treatment without toxicity greater than grade 2,

[4] Carducci, M. A., Gilbert, J., Bowling, M. K., Noe, D., Eisenberger, M., Sinibaldi, V., Zabelina, Y., Chen, T., Grochow, L. B., and Donehower, R. C. Phenylbutyrate for refractory solid tumors: Phase I clinical and pharmacological evaluation of i.v. and oral phenylbutyrate, manuscript in preparation.

then the patient was eligible to receive the next higher dose level for subsequent treatments. However, before such an escalation could occur, two PB-naïve patients should have completed at least one course at the next higher dose level without any dose-limiting toxicity.

Drug was administered as either a 375-mg or 500-mg tablet. Once the tablets containing 375 mg of PB were exhausted, the 500-mg tablets were used. Drug was supplied by Targon to the National Cancer Institute, NIH, Bethesda, Maryland. The drug was administered with meals when possible. Qualified nursing personnel monitored the p.o. administration during the first 3 days of therapy while the patient was hospitalized on the Johns Hopkins General Clinical Research Center and followed in the clinic for pharmacokinetic studies. Subsequently, the patients self-administered the doses of medication per schedule. Vial counts were used to monitor compliance as well as patient calendars. Patients also recorded any partial doses. If a patient regurgitated a dose, that dose was not repeated, but the patient resumed his/her schedule for subsequent doses. A patient "designee" (*i.e.,* acquaintance, friend, or family member) was also asked to follow and record any toxicities, including changes in cognitive ability. Additionally, the designee assisted the patient in the recording of doses.

Toxicity. Toxicities were scored according to the National Cancer Institute common toxicity criteria, version 1.0. Nonhematological toxicity of grade 3 or 4 (or in some instances of grade 2, especially chronic renal, pulmonary, and some neurological, cardiac, and local toxicities) was considered dose limiting. Hematological toxicity criteria for dose-limiting toxicity were absolute neutrophil count $<500/mm^3$, either with fever or persisting >5 days or thrombocytopenia $<50{,}000/mm^3$.

Patient compliance in light of the large numbers of tablets taken 3 times/day was documented because of the realization that decreased compliance might also be a dose-limiting factor and make toxicity assessment difficult. Patients on a particular dose level had to take $\geq$4 weeks of treatment, ingest $\geq$50% of the intended dose, and not miss $>$6 doses/week to be considered fully evaluable as compliant. If two or more patients on a dose level did not meet the above criteria, then two more patients could be added to that dose level to assess compliance. If 3/6 patients were not able to meet the compliance standards, then that was considered a dose-limiting event.

Antitumor Response. Evaluation of tumor measurement/sites of disease response was completed after 8 weeks of p.o. PB. Patients could be evaluated earlier than 8 weeks if there was suggestion of tumor progression. Patients removed from study earlier than 8 weeks had tumor evaluation at the time of removal of the study if $\geq$3 weeks of therapy had been administered. A complete response was defined as disappearance of all clinically detectable malignant disease for $\geq$4 weeks. Patients with bony metastases required normalization of all bone X-rays. A partial response was defined as $\geq$50% decrease in tumor size (sum of the product of the largest perpendicular diameters) for $\geq$4 weeks without increase in size of any area of known malignant disease or appearance of new areas of malignant disease. Stable disease was defined as no significant change in measurable or evaluable disease for $\geq$4 weeks (8 weeks for bone metastases), no increase in size of any known malignant disease, or appearance of any new areas of disease. This designation included decrease in malignant disease of $<$50% or increase in malignant disease of $<$25%. Progression was defined as significant increase in size and number of malignant lesions, including $\geq$25% increase over original measured area or the appearance of any new malignant lesions. If bone scans were the only measure of disease, then progression by bone scan had to be seen on two serial bone scans if the patient remained asymptomatic. Rise in any tumor marker did not constitute progression unless evidenced by new sites of disease or increase in the size of lesions identified previously.

Pharmacokinetic Sampling and Analytical Assay. As noted earlier, bioavailability assessments were obtained for each patient on days 1 and 2 during cycle 1. On an alternating schedule, patients received a single dose of p.o. or parenteral PB on day 1 and the alternative route as a single, equivalent dose on day 2. After p.o. administration on days 1 or 2, venous blood samples were obtained pretreatment at 0.25, 0.5, 0.75, 1, 1.25, 1.50, 1.75, 2, 2.5, 3, 6, 9, 12, and 24 h. Plasma samples were also obtained on day 3 (p.o. PB) and on the final day of p.o. PB administration when possible. After administration of i.v. PB on days 1 or 2, venous blood samples were obtained on a schedule as follows: preinfusion; 0.25 h and 0.50 h into the infusion; immediately before termination of the infusion; and 10 min, 20 min, 40 min, 1.5 h, 3 h, 6 h, 9 h, 12 h, and 24 h after termination of the infusion. During the chronic p.o. dosing schedule, samples were obtained after treatment with the first p.o. dose on day 3 and on the final day of PB administration (optional) at the following times: pretreatment, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, and 8 h. Samples were centrifuged at $1500 \times g$ for 10 min and plasma was isolated and frozen at -20°C until the time of assay.

Plasma PB, PA, and PG concentrations were determined in all plasma specimens by reverse-phase high-performance liquid chromatography assay. Plasma (200 units/liter) containing compound were transferred to a microcentrifuge tube, followed by the addition of 50 ml of 10% perchloric acid (Sigma Chemical Co., St. Louis, MO) for protein extraction. The samples were vortexed and centrifuged (4°C, 8500 rpm, 10 min). Supernatant (150 μl) was added to 5 μl of super-saturated potassium bicarbonate solution for neutralization and centrifuged. All of the supernatant was transferred to an autosampler vial for analysis. The chromatographic apparatus consisted of a Thermoquest Liquid Chromatograph (Thermo Separation Products, Piscataway, NJ) with autosampler compartment AS3000, solvent delivery system, and a diode-array UV absorbance detector with a resolution of 2 nm. The absorbance wavelength was 208 nm (bandwidth, 10 nm). Mobile phase A consisted of 100% deionized water (Milli-QUV Plus; Millipore Corp., Bedford, MA) with 0.005 M phosphoric acid (Sigma Chemical Co.) buffer. Mobile phase B consisted of 100% high-performance liquid chromatography grade acetonitrile (J.T. Baker, Phillipsburg, NJ) with 0.005 M phosphoric acid buffer. All mobile phases were run at a combined flow rate of 1 ml/min for a run time of 45 min using gradient profile. A Waters Nova-Pak C18 guard column was placed in line before the analytical column. The samples were injected onto a reverse-phase (Waters Bond-Pak C18, 3.9×300 mm; Millipore Corp., Jilford, MA) column, which was maintained at 60°C. PA, PB, and PG had retention times of ~18.2, 31.4, and 10.2 min, respectively. Chromatographic peak area was used for quantification by linear regression analysis.

The lower limit of the assay for detection of PB and metabolites was 10 μM. Quality control samples were assayed at concentrations of 25, 500, and 2000 μM, and the inter- and intra-day coefficients of variation were <10%.

Pharmacokinetic Analysis. Individual plasma concentrations for PB, PA, and PG in plasma were analyzed using model-independent methods (17). The C_{max}, T_{max}, and the time that PB plasma concentrations remained >0.5 mM were the observed values from inspection of the concentration-*versus*-time curves. The terminal rate constant, k, was calculated as the negative slope of the log-linear regression. During the phase of the study to assess bioavailability (days 1 and 2) the AUC from time 0 to the time of the last quantifiable sample, AUC_{last}, was calculated using the linear trapezoidal method as implemented in WinNonlin version 2.0 (Pharsight, Mountain View, CA). The AUC was extrapolated to infinity (AUC_{inf}) by dividing the last measured concentration by λ_z, which was determined from the slope of the terminal phase of the drug concentration-*versus*-time curve. Absolute p.o. bioavailability (F) expressed as a percentage was calculated by dividing the p.o. AUC by the i.v. AUC normalized to dose ($F\% = [AUC_{p.o.}/AUC_{i.v.}] \times [Dose_{i.v.}/Dose_{p.o.}] \times 100$). On day 3, when p.o. PB was administered 3 times/day, AUC_{last} was calculated. Systemic exposure of PA and PG relative to that of PB was calculated as the AUC_{last} ratio of PA:PB and PG:PB, respectively.

Pharmacokinetic data were described using descriptive statistics. Univariate correlation analysis was used to assess the relationship between PB dose and PB, PA, and PG exposure. Nonparametric methods (Wilcoxon's rank sum and Kruskal-Wallis tests) were used to determine the relationship between drug and metabolite exposure and National Cancer Institute toxicity grade. The *a priori* level of significance was set at 0.05. Statistical analysis was performed using the JMP version 3.2.6.

QOL. QOLs were made using the EORTC Quality Questionnaire QLQ-C30. Twenty-eight patients were given the QOL questionnaire. The ECOG performance status scale was also used. Patients were asked to complete the EORTC QLQ-C30 before starting study medication and monthly for the duration of their time on study. The Statistical Product and Service Solutions software package was used for analysis of the QOL questionnaire response data. Individual question responses and demographic data were hand-entered by the author. One-way ANOVA and two-tailed *t* test were used according to their applicability. *P* values of ≤0.05 were interpreted as indicating significant differences.

Compliance Data. Patient compliance data were obtained for 22 patients. Patients filled out prewritten medication administration records. Each patient had a designated assistant to help fill out and verify the information on the patient diaries. Patients were to record each dose taken and the times. Also, they were to record problems/side effects associated with a particular dose. Patients were to fill out the diaries for the length of their time on trial.

RESULTS

Twenty-eight patients were enrolled in this study for toxicity and compliance, 23 of whom had detailed pharmacokinetic studies performed. Patient characteristics are listed in Table 1.

Table 1 Patient characteristics

Characteristic	No. of patients
Total patients	28
No. of courses	96
Sex	
Male	22
Female	6
Age, ys	
Median	57
Range	26–75
ECOG performance status	
0	13
1	15
Prior therapy	
No prior therapy	2
Prior chemotherapy	
1–2 prior regimens	12
≥3 prior regimens	7
Prior hormone/immune therapy	
1 prior regimen	9
≥2 prior regimens	10
Prior radiation therapy	11
Diagnosis	
Prostate	12
Renal	6
Breast	5
Colorectal	2
Thyroid	2
Bladder	1

Patient Compliance. Of the 28 patients studied, complete patient diaries were obtained on 22 patients. Patients had to complete at least 1 full cycle to be evaluated for overall compliance with this treatment approach. Six patients were excluded from this analysis as a result of lost or incomplete diaries.

Of the 22 patients, a total of 81 cycles were recorded in the diaries (84 total cycles were given) for this group of patients. The total number of possible doses given was 6768. The total number of missed doses was 438 with 6.5% of the possible doses missed or 93.5% of possible doses taken by the patients. At least half of the pills had to be taken for a given dose to be counted as completed. Only one patient recorded that he had taken half the required number of pills, and this occurred for 9 doses. Compliance did decrease as the dose of PB increased. The percentage of missed doses increased from 0.2% at the 9-g dose to 1.6, 3.5, 7.1, and 15% at the 18, 27, 36, and 45-g dose levels, respectively.

Dose Adjustments, Toxicities, and Determination of MTD. The principal toxicities are illustrated in Table 2. On analysis of the toxicity data, the recommended Phase II dose of p.o. PB is 27 g/day. The majority of dose-limiting toxicities occurred at the 36 and 45-g dose levels. However, these toxicities were not necessarily the same for each patient and will be additionally discussed.

At the lowest dose level, 9 g/day, no dose-limiting toxicities were seen. One patient at the lowest dose level did experience grade 3 fatigue. The patient had thyroid cancer, and after additional evaluation his fatigue was attributed to T3 thyrotoxicosis. The patient had experienced this condition in the past with a similar level of fatigue. With appropriate treatment of his

Table 2 Nonhematologic toxicity as a function of dose level[a]

Dose level (g)	9 (n = 4)		18 (n = 4)		27 (n = 4)		36 (n = 7)		45 (n = 4)	
Grade of toxicity	1–2	3–4	1–2	3–4	1–2	3–4	1–2	3–4	1–2	3–4
Toxicity										
Nausea and/or vomiting	5 (3)		4 (3)		4 (2)		15 (4)	1 (1)	4 (3)	1 (1)
Dyspepsia	5 (3)		6 (3)		10 (3)		12 (4)			
Fatigue	13 (4)		4 (3)		17 (3)		18 (4)		6 (3)	1 (1)
Neurocortical			1 (1)		11 (3)		15 (3)			1 (1)
Odor					2 (2)		13 (3)			
Edema							5 (1)			1 (1)
Hypocalcemia								1 (1)		

[a] Data represent no. of cycles (no. of patients).

thyroid disorder, his fatigue subsided while he continued the same dose of PB.

At the 36 g/day dose-level, several potential dose-limiting toxicities were seen in two of seven patients. Grade 4 hypocalcemia (calcium 5.6 and albumin 4.0) occurred in one patient receiving PB 36 g/day during cycle 1 of therapy. The patient experienced fatigue but not tetany. He received i.v. calcium supplementation followed by chronic p.o. calcium supplementation. His calcium normalized after a dose reduction to 27 g/day for his second cycle and after calcium supplementation. One patient experienced grade 3 nausea and vomiting at the 36 g/day dose level and was subsequently dose-reduced to 27 g/day with resolution of the nausea and vomiting. The two dose-limiting toxicities (grade 4 hypocalcemia and grade 3 nausea and vomiting) occurred in two separate patients.

At the 45 g/day dose-level, two of four patients experienced dose-limiting toxicities. One patient experienced grade 3 fatigue. The patient had prostate cancer and had been pretreated with two prior chemotherapy regimens. Additionally, he was on furosemide for significant lower extremity, penile, and scrotal edema and developed hypokalemia with a potassium of 2.6 meq/liter. The patient did have baseline edema before study initiation; however, it significantly worsened while on the PB. Of note, a prestudy echocardiogram demonstrated a left ventricular ejection fraction of >55%, and computed tomography of the abdomen and pelvis demonstrated adenopathy but no evidence of thrombosis. The patient was taken off the study after 1 cycle secondary to toxicity. The edema resolved within 3 weeks of stopping the drug. Serious dose-limiting neurocortical toxicity was seen in one patient at a dose of 45 g/day. The toxicity consisted of slurred speech, decreased concentration, decreased coordination, and confusion. It occurred within 2 days of starting the drug. The patient self-discontinued the drug on days 8 and 9 of therapy with some resolution of neurotoxicity. He subsequently received a dose-reduction to 36 g/day 10 days after starting his first cycle, but the neurotoxicity again became dose-limiting, and the patient discontinued the drug on days 12 and 13. On day 14, he started 27 g/day with no additional neurotoxicity on the reduced dose.

Various nonhematological toxicities, which were not dose-limiting, proved common. Nineteen of 23 patients experienced some degree of fatigue, but 17 of the 19 had only grade 1 or 2 fatigue. Grade 1 or 2 nausea and vomiting was seen in 13 of the 23 patients studied for toxicity. These symptoms were treated with supportive measures including metoclopramide and prochlorperazine. The nausea and vomiting most often occurred within 30 min after taking the drug, and the symptoms rapidly subsided after drug discontinuation. Symptoms of dyspepsia including heartburn, indigestion, and "gassy feeling" were noted in 13 of 23 patients. Patients were treated symptomatically with metoclopramide and H2-blockers with only moderate symptom resolution. The symptoms occurred mainly around the time of pill ingestion and were usually self-limited. Dyspepsia did not appear to be related to dose as it was evenly distributed among patients.

Mild neurotoxicity was seen in 5 patients and consisted of grade 1 drowsiness and/or grade 1 confusion at the 18, 27, and 36 g dose levels. Four of 23 patients developed an odor, which was described as smelling like "sweat" or "rancid butter." The odor was not always apparent to the patient but was noted by the caregiver in most cases. Three of the 4 cases occurred in the cohort receiving 36 g/day. One case occurred in a patient receiving 27 g/day and only noted on the ninth of 9 cycles of PB.

Pharmacokinetic Studies. Of 28 patients treated, pharmacokinetic studies were completed in 23 patients during the bioavailability study period (days 1 and 2) and after administration of the first dose on day 3. For one patient treated at the 9 g/day dose level, the extrapolated PB AUC after p.o. administration during the bioavailability study period represented 67% of the AUC_{inf} (average PB % AUC extrapolated was <5%). Therefore, PB bioavailability was not determined for this patient. Five patients were added at the 36 g/day dose level to additionally assess compliance. Pharmacokinetics were not obtained on these five patients.

Pharmacokinetics of PB. Bioavailability (p.o.) and pharmacokinetic parameters for PB after i.v. and p.o. administration on days 1 or 2 are listed in Table 3. Representative plasma concentration-time plots at the 27 and 36 g/day dose levels, the recommended Phase II dose, and the dose at which dose-limiting toxicities were seen in two of seven patients, respectively, are illustrated in Fig. 1. After administration of a single p.o. dose of PB, absorption was rapid with a mean T_{max} value of 1.8 h. The absolute bioavailability of p.o. PB was near complete, with a mean (SD) value of 78% (24%). PB pharmacokinetics (p.o.) were linear within the dose range studied, as evidenced by a 4.4-fold increase in AUC values as the PB dose was increased 4-fold from 9 to 36 g/day (Table 3). After p.o.

Table 3 Oral bioavailability and pharmacokinetic parameters for PB and metabolites determined using noncompartmental methods

PK Parameter[a]	PB dose (g/day) 9 (n = 4)	18 (n = 4)	27 (n = 4)	36 (n = 7)	45 (n = 4)	All dose levels
i.v. period						
PB						
C_{max} (μmol/liter)[b]	826 (35)	1671 (305)	2327 (488)	3508 (1188)	2603 (903)	
T_{max} (hr)	1.0 (0.06)	1.0 (0.06)	0.95 (0.03)	1.2 (0.36)	1.5 (1.1)	1.1 (0.47)
AUC (μmol/liter × hr)	1330 (285)	2978 (1246)	4517 (1388)	7954 (1651)	6008 (1313)	
T > 0.5 mM (hr)	1.7 (0.1)	2.2 (0.8)	2.4 (0.8)	3.9 (0.3)	3.7 (1.0)	
PA						
C_{max} (μmol/liter)	107 (37)	218 (72)	510 (252)	419 (51)	683 (173)	
T_{max} (hr)	1.8 (0.55)	2.7 (0.98)	3.3 (0.92)	4.1 (0.10)	5.3 (1.5)	3.5 (1.4)
AUC (μmol/liter × hr)	374 (74)	883 (502)	2201 (1545)	2614 (278)	4696 (962)	
PA:PB AUC ratio	0.27 (0.15)	0.29 (0.05)	0.45 (0.24)	0.33 (0.06)	0.77 (0.22)	0.46 (0.25)
PG						
C_{max} (μmol/liter)	189 (33)	324 (86)	503 (138)	507 (202)	508 (243)	
T_{max} (hr)	2.3 (0.41)	3.7 (0.77)	3.7 (0.50)	5.5 (1.7)	6.8 (2.6)	4.6 (2.1)
AUC (μmol/liter × hr)	955 (125)	1854 (641)	3384 (1771)	4524 (2819)	4736 (2968)	
PG:PA AUC ratio	0.74 (0.25)	0.68 (0.29)	0.72 (0.20)	0.59 (0.41)	0.84 (0.58)	0.70 (0.32)
p.o. period						
PB						
C_{max} (μmol/liter)	517 (243)	771 (254)	1574 (639)	1906 (996)	1495 (584)	
T_{max} (hr)	1.5 (0.90)	1.5 (0.19)	1.4 (0.79)	1.5 (0.46)	1.9 (0.37)	1.8 (0.72)
AUC (μmol/liter × hr)	1127 (491)	2176 (1096)	4098 (1870)	5613 (2380)	5321 (1210)	
T > 0.5 mM (hr)	1.1 (0.7)	2.4 (0.8)	3.9 (0.8)	4.1 (0.6)		
F	0.88 (0.24)	0.71 (0.15)	0.89 (0.22)	0.69 (0.20)	0.94 (0.36)	0.78 (0.24)
PA						
C_{max} (μmol/liter)	98 (39)	181 (54)	503 (202)	449 (209)	715 (174)	
T_{max} (hr)	2.7 (0.64)	3.8 (1.5)	3.8 (1.4)	5.3 (1.8)	5.6 (1.1)	4.4 (1.6)
AUC (μmol/liter × hr)	350 (106)	879 (434)	2704 (1713)	2906 (1441)	5420 (1502)	
PA:PB AUC ratio	0.38 (0.22)	0.43 (0.14)	0.63 (0.20)	0.54 (0.18)	0.95 (0.25)	0.66 (0.31)
PG						
C_{max} (μmol/liter)	188 (47)	303 (62)	511 (195)	475 (193)	621 (329)	
T_{max} (hr)	2.9 (0.28)	3.7 (1.5)	3.8 (1.5)	6.2 (0.24)	6.3 (3.1)	4.8 (2.0)
AUC (μmol/liter × hr)	1067 (211)	2056 (629)	3840 (1979)	4253 (2340)	6547 (5456)	
PG:PB AUC ratio	1.1 (0.38)	1.1 (0.48)	0.92 (0.28)	0.85 (0.47)	1.1 (0.71)	1.0 (0.42)

[a] Values are mean (SD).
[b] T > 0.5 mM, time plasma concentration remains above 0.5 mM; F, bioavailability.

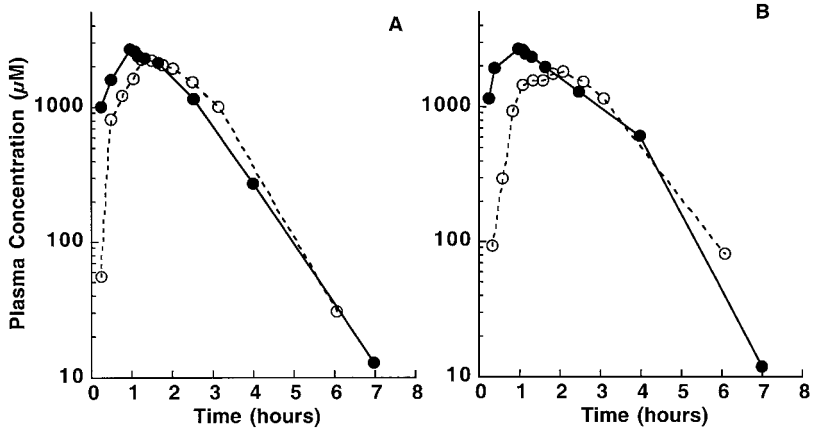

Fig. 1 Representative PB plasma concentration-time profiles at the 27 g/day (*A*) and 36 g/day (*B*) dose levels. ———, i.v. PB; ----, p.o. PB.

administration on day 1 or 2, the average $t_{1/2}$ was 1 h and "apparent" p.o. clearance was 15 liters/hr. These parameters were independent of PB dose. Plasma concentrations of PB remained above 0.5 mM for ~1.1, 2.4, 3.2, 3.9, and 4.1 h at the 9, 18, 27, 36, and 45 g/day dose levels, respectively. PB pharmacokinetic parameters were similar after administration of a single i.v. or p.o. dose (Table 3) and after administration of the first p.o. dose on day 3 (Table 4). Five patients had pharmacokinetic studies performed on the last day of treatment, after receiving an average (range) of 4 (2–8) courses. Plasma sampled before treatment of the last day of PB administration had measurable concentrations of PB in 2 of 5 patients; comparison

Table 4 Pharmacokinetic parameters for PB and metabolites on day 3

PK parameter[a]	PB dose (g/day)					
	9 (n = 4)	18 (n = 4)	27 (n = 4)	36 (n = 7)	45 (n = 4)	All dose levels
PB						
C_{max} (μmol/liter)	457.9	949.9	1679.8	1878.3	1669.4	
T_{max} (hr)	1.93	1.40	1.26	2.17	1.84	1.76
AUC (μmol/liter × hr)	925.5	2170.6	3778.6	4687.4	4941.4	
T > 0.5 mM (hr)[b]	1.3	2.4	3.1	3.6	4.2	3.16
$t_{½}$ (hr)	0.76	0.61	0.58	0.94	1.16	0.78
Clearance (liter/hr)	18.7	16.0	14.0	14.8	19.8	15.97
PA						
C_{max} (μmol/liter)	104.2	224.0	589.1	447.6	739.9	
T_{max} (hr)	3.30	3.45	3.51	4.49	5.15	3.98
AUC (μmol/liter × hr)	404.5	633.3	2394.8	1349.4	2648.2	
PA:PB AUC ratio	0.38	0.43	0.63	0.54	0.95	0.66
PG						
C_{max} (μmol/liter)	187.5	303.1	511.1	474.8	620.5	
T_{max} (hr)	2.92	3.74	3.76	6.15	6.33	4.76
AUC (μmol/liter × hr)	1014.9	1967.8	3488.6	4051.5	6046.4	
PG:PB AUC ratio	1.11	1.07	0.92	0.85	1.09	1.02

[a] Values are mean (SD).
[b] T > 0.5 mM, time plasma concentration remains above 0.5 mM.

of PB C_{max} and AUC values to those on days 1 or 2 and 3 showed similar values.

Pharmacokinetics of PA and PG. PA and PG pharmacokinetic parameters on days 1 and 2 are summarized in Table 3. After p.o. administration, T_{max} values for PA and PG were achieved later than those for PB (4.4 and 4.8 h, respectively); mean (range) apparent $t_{1/2}$ values for PA and PG were 1.8 (1–5.3) and 2.8 (1.6–7.1) h, respectively. PA AUC increased disproportionately with PB dose; when the PB dose was increased 4-fold from 9 to 36 g/day, the average PA AUC value increased 8-fold. PG exposure appeared to increase in proportion with dose. After i.v. and p.o. administration, mean systemic exposure (AUC) to PA represented approximately 46–66% of that for PB; at the highest dose level (45 g/day), PA represent an average of 77–95% of PB exposure, which is consistent with a disproportionate increase in PA exposure at the higher PB dose-levels. Exposure to PG represented approximately 70–100% of that for PB and appeared to be independent of PB dose. PA and PG pharmacokinetic parameters were similar after administration of the first p.o. PB dose on day 3 (Table 4).

Relationship between Drug Exposure and Effect. The time that PB concentrations remained above 0.5 mM were greater (4.2 and 5.3 h) in the two patients who experienced dose-limiting neurocortical toxicity during course 1 relative to PB concentrations in patients who experienced no or less severe toxicity (mean, 3.0 h). This did not reach a level of significance, most likely attributable to the small number of patients who experienced severe toxicity. PA exposure was not related to the incidence of neurocortical toxicity.

Antitumor Responses. Of the 28 patients studied, 23 were evaluable for tumor response. Five patients received less than a full cycle because of toxicity (1 patient) and tumor progression (4 patients). No patient had a partial or complete response. Twelve of the 23 evaluated patients demonstrated stable disease (for ≥2 cycles) as the best response. Seven of the patients demonstrated stable disease for >6 months. Eleven patients demonstrated progressive disease without a period of stable disease. Of the patients with stable disease, the median number of cycles during which disease remained stable was 6.1. Of the 12 patients with stable disease, 1 patient was treated at dose level 1 (4 patients entered at 9 g/day), 2 patients were treated at dose level 2 (4 patients entered at 18 g/day), 2 patients were treated at dose level 3 (4 patients entered at 27 g/day), 6 patients were treated at dose level 4 (7 patients entered at 36 g/day), making this dose level the one with the greatest number of patients with stable disease. One patient was treated at dose level 5 (4 patients entered at 45 g/day).

After 2 weeks of PB therapy, 9 of 12 prostate cancer patients had PSA values that were measured. Seventy-eight percent had a rise in PSA after 2 weeks of continuous PB exposure (range of increase PSA 40–213%), pointing to a possible differentiating effect of the PB. Twenty-two percent had no change or a minimal decline in PSA after 2 weeks. There was no discernable trend in CEA and calcitonin values in the patients with colorectal cancer and medullary carcinoma of the thyroid, respectively.

QOL. Twenty-three of 28 patients entered into this study completed 2 or more EORTC QLQ-C30 questionnaires. To study QOL changes over time, the medians of each question were compared for 3 paired groups. The pretreatment response was compared with the 1-month response, the 2-month response, and the end-of-treatment response. The comparisons were made across all of the patients and then by individual dose level. For questions in the symptom scale portion of the questionnaire, only one question ("Were you tired?") of three demonstrated a statistically significant difference between time intervals. On the functioning scale portion of the questionnaire, one of the two cognitive questions ("Have you had difficulty concentrating on things like reading a newspaper or watching television?") revealed a significant difference over the time intervals examined.

The statistically significant question on the symptom scale

asked "Were you tired?" On comparison of the pretreatment, 1-month, and 2-month responses, the patients were found to be more tired ($P = 0.009$ at 1 month and $P = 0.001$ at 2 month) as treatment progressed. However, by the end of study treatment, the significance had disappeared ($P = 0.512$).

The functioning scale addressed level of patient concentration. For all patients, pretreatment values compared with 1-month ($P = 0.009$), 2-month ($P = 0.010$), and end-of-treatment ($P = 0.05$) were significant.

Two questions addressed global quality of life. At the 1-month time point there was no significant difference in patient ratings. However, by the 2-month and end-of–treatment points, patient ratings of their overall physical condition and overall quality of life had significantly decreased. For "overall physical condition," the *P* at 2 months was 0.015 and at end-of-treatment was 0.051, when compared with pretreatment. For "overall quality of life," the results were $P = 0.008$ and $P = 0.022$ at 2 months and end-of-treatment. When each of the five dose levels were analyzed individually, there was no statistical difference within groups to the "overall quality of life" questions.

DISCUSSION

This Phase I clinical trial of p.o. PB has demonstrated the safety of p.o. PB in patients with solid tumor malignancies at the recommended Phase II dose of 27 g/day. No patients demonstrated significant hematological toxicity. Grade 1 and 2 nonhematological toxicities were evenly distributed at all dose levels. Dose-limiting toxicities were seen at the higher dose levels of 36 and 45 g with two of seven patients and two of four patients experiencing dose-limiting toxicities, respectively. These were nonhematological in nature and consisted of individual episodes of grade 4 hypocalcemia and grade 3 nausea and vomiting at 36 g. At the 45 g dose level, there was 1 episode of grade 3 fatigue and grade 3 edema in the same patient and neurocortical toxicity was dose-limiting in one of four patients at the highest dose level. That patient experienced grade 3 decreased concentration, grade 3 decreased coordination, and grade 3 slurred speech. Although neurocortical symptoms may be progressive in patients with prolonged administration at a particular dose, the patient received 2 dose reductions to 27 g/day with resolution of his neurocortical toxicity while remaining on the drug. In fact, on analysis of the dose-limiting toxicities of the p.o. PB in this non-CNS study, the two toxicities, hypocalcemia and nausea and vomiting only appeared in one patient each and did not appear to be related to any pharmacokinetic parameter. The severity of the nausea and vomiting may be dose related, but only 1 of 7 patients at this dose level experienced this DLT, and 36 g/day may still be a safe dose.

The potential importance of the higher dose of 36 g/day is underscored by the preclinical data. Gore *et al.* (18) assessed the dose-response characteristics of PB-induced histone acetylation. *In vitro* ML-1 cells were incubated with various doses of PB for 4–24 h. Induction of histone acetylation occurred at concentrations as low as 0.25 mM. Additionally, both Gore and Warrell *et al.* (19) have noted histone acetylation in as little as 4–6 h after exposure to PB. Additionally, the dose-response curve for induction of histone acetylation by PB in ML-1 cells closely paralleled its dose-response characteristics for cell cycle arrest. Carducci *et al.* (4) have also verified 0.5 mM as the plasma concentration of drug at which biological effect is noted *in vitro*. At 36 g/day the T $>$0.5 mM (hr) was 3.9 h and, thus, achieves a potentially active drug concentration for an extended period of time. At 27 g/day, the T $>$0.5 mM was 3.2 h. It remains unclear as to what length of time is required for therapeutic threshold for biological effect, but this will have to be additionally explored in a Phase II investigation. Of note, the greatest percentage of patients with stable disease was at the 36 g/day dose level.

The AUCs of PB and PG increased in a linear manner. Absorption (p.o.) of PB is nearly complete and T_{max} is achieved quickly at a mean of 1.9 h. Importantly, as noted earlier, the time at which PB plasma levels remain above the level at which biological activity is seen in preclinical studies, 0.5 mM/h, is ~4 h at the 36 and 45 g dose levels. This shows that PB is rapidly and nearly completely absorbed and sustains clinically significant plasma drug levels for an extended period of time. The AUCs of PA did not increase linearly in proportion with dose, which has been noted in previous trials of i.v. PB.[4] In our study, at the highest dose level, there was a disproportionate increase in PA exposure. It should be noted that the dose-limiting neurotoxicity did occur at the highest dose level. Perhaps PA exposure after p.o. administration does correlate with neurotoxicity and fatigue at this level. However, the sample size is too small to determine whether this observation is statistically significant.

It should be realized that PB has differentiating properties and, therefore, may not demonstrate evidence tumor response but may actually demonstrate a subset of patients with stable disease and delay progression. Whereas no patients exhibited a partial or complete response, 25% of patients (4/12 men with prostate cancer, 2/4 with renal cell carcinoma, and 1 patient with thyroid cancer) had stable disease for $>$6 months and, therefore, received $>$6 cycles of the drug. The longest patient course was 11 months. Two are still alive at 1048 and 418 days from study initiation. It should be noted that 3 of 6 patients with renal cell carcinoma had advancing disease progression after first line therapy before initiation of therapy and had stable disease for $\geq$6 months while on the study drug.

Despite few DLTs and overall good tolerability, the EORTC QLQ C-30 quality of life measurement demonstrated that there was a statistically significant decrease in overall quality of life of patients from study initiation to end. The ratings of physical condition decreased over time. Yet it is not certain whether this was a result of the drug or disease progression or both, and, therefore, the clinical significance is uncertain.

This study required patients to take a large amount of pills daily. Therefore, patient compliance was important to assess. Overall, compliance was good with 93.5% of all possible doses taken by the patients. The difficult nature of taking a large amount of tablets may have been balanced by the convenience of drug administration at home and, therefore, p.o. PB remains a plausible option for studies with this agent in the future.

Given the relatively mild toxicity profile of PB and its potential cytostatic abilities, several Phase I trials have been proposed using this drug in combination with other agents in refractory solid tumor malignancies. One combination involves p.o. PB and 13-*cis*-retinoic acid. The rationale for combining

these two agents stems from their distinct activity at steroid nuclear receptors, such as the human peroxisome proliferator-activated receptors α and γ, which act as transcription factors controlling the expression of several genes involved in peroxisomal and mitochondrial pathways of lipid metabolism (20). Another strategy for the clinical development of PB with other agents with potentially synergistic effects involves the combination of 5-azacytidine, an agent that inhibits DNA methyltransferase with PB. *In vitro* studies on multiple human cancer cell lines have shown that the combination of the methyltransferase inhibitor and a histone deacetylase inhibitor can reactivate the expression of silenced genes, including tumor suppressor genes (21).

PB proves to be an interesting HDAC inhibitor with a wide variety of possible mechanisms of action and effects. Importantly, it is relatively well tolerated, and biologically active plasma concentrations can be achieved and maintained for several hours using the p.o. formulation. Its cytostatic potential has been encouraging given the duration of stable disease seen in several patients. Even more encouraging is the observation that PB treatment in three renal cell carcinoma patients with prior rapidly progressive disease resulted in prolonged stabilization of disease. The inhibition of HDAC is a promising strategy for cancer therapy both alone and in combination with other agents. Second-generation HDAC inhibitors are presently in clinical development. PB provides a template on which to build this rapidly expanding arena of HDAC inhibitors in drug development.

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