The Effect of Enzyme-Inducing Antiseizure Drugs on the Pharmacokinetics and Tolerability of Procarbazine Hydrochloride

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Abstract

Purpose: Procarbazine hydrochloride (PCB) is one of the few anticancer drugs with activity against high-grade gliomas. This study was conducted to determine if the maximum tolerated dose and pharmacokinetics of PCB are affected by the concurrent use of enzyme-inducing antiseizure drugs (EIASD).

Experimental Design: Adults with recurrent high-grade glioma were divided into cohorts who were (+) and were not (−) taking EIASDs. PCB was given orally for 5 consecutive days each month. Six patients were evaluated at each dose level beginning with 200 mg/m2/d and escalated using the modified continual reassessment method. Toxicity and response were assessed. Pharmacokinetic studies were done with a new electrospray ionization mass spectrometry assay.

Results: Forty-nine patients were evaluated. The maximum tolerated dose was 393 mg/m2/d for the +EIASD group and the highest dose evaluated in −EIASD patients was 334 mg/m2/d. Myelosuppression was the primary dose-limiting toxicity. Significant hepatic dysfunction occurred in three patients in the +EIASD cohort. Four partial responses (8%) and no complete responses were observed. PCB exhibited linear pharmacokinetics with no significant differences between the two cohorts. A marked increase in peak PCB levels was noted on day 5 relative to day 1, which was not attributable to drug accumulation.

Conclusions: This study suggests that (a) EIASD use does not significantly affect the pharmacokinetics of PCB; (b) changes in the peak plasma concentration of PCB, consistent with decreased apparent oral clearance due to autoinhibition of hepatic metabolism, occur with daily dosing; and (c) severe hepatic dysfunction may accompany this administration schedule.

High-grade gliomas have proved to be quite resistant to chemotherapeutic drugs. Currently, only two chloroethylnitrosoureas (carmustine and lomustine), procarbazine (PCB), and temozolomide are considered sufficiently active to warrant “standard” use in the treatment of these tumors (1). These drugs have only modest activity, with reported response rates ranging from 15% to 40%, and only temozolomide has shown a clear survival advantage when used in the adjuvant setting (2). Nevertheless, given the temporary benefits of surgery and radiation in these malignancies and the limited chemotherapeutic options, many patients with primary brain tumors receive PCB. It is considered a vital component of the PCB, lomustine, and vincristine (PCV) regimen that was introduced into clinical practice in the 1970s (3–5). PCV is used in patients with newly diagnosed or recurrent glioblastoma multiforme, anaplastic astrocytoma, anaplastic oligodendroglioma, as well as low-grade astrocytomas and oligodendrogliomas (6–15).

Antiseizure drugs are frequently used in patients with primary brain tumors (16). Many classic antiseizure drugs, such as phenytoin, phenobarbital, and carbamazepine, are potent inducers of various cytochrome P450 (CYP) isozymes and the UDP-glucuronosyltransferases, two of the major enzyme systems involved in hepatic drug metabolism (17). The antineoplastic activity of PCB results from its conversion to highly reactive alkylating species by hepatic oxidative metabolism mediated, at least in part, by CYP enzymes (18, 19). Although this has been known for many years, the effect of enzyme-inducing antiseizure drugs (EIASD) on the pharmacokinetics, tolerability, and efficacy of PCB in patients with brain cancer has never been formally referenced in the literature.
investigated. Accordingly, the study described in this report was undertaken to determine the effect of EIASDs on the maximum tolerated dose, toxicity profile, and pharmacokinetic behavior of PCB in patients with recurrent malignant glioma.

Patient selection. Adult patients (age $\geq$18 years) with histologically proven malignant glioma that was progressive or recurrent after radiation therapy were eligible for the study. Conditions required for entry into the study included (a) measurable disease by contrast-enhanced magnetic resonance imaging or computed tomography; (b) Karnofsky performance status $\geq$60%; (c) life expectancy $>2$ months; (d) previous treatment with not more than one chemotherapy regimen. Minimum permitted time intervals from prior treatments were 3 months for radiation, 6 weeks for chloroethylnitrosoureas, and 3 weeks for all other chemotherapeutic agents. Full recovery from the effects of any earlier intervention was required. Eligibility also required showing acceptable hematologic variables (absolute neutrophil count $\geq 1,500/\mu L$; platelet count $\geq 100,000/\mu L$), renal function (serum creatinine $\leq 1.7$ mg/dL), and hepatic function (bilirubin $\leq 1.5$ mg/dL; serum levels of aspartate aminotransferase and alanine aminotransferase less than four times the upper limit of normal). Exclusion criteria included (a) a prior malignancy other than curatively-treated basal cell carcinoma or cervical carcinoma in situ; (b) previous treatment with PCB or more than two cycles of carbustine ($>460$ mg/m$^2$) or lomustine ($>220$ mg/m$^2$); (c) use of a tricyclic antidepressant within 14 days; (d) a serious concurrent infection, illness, or medical condition; (e) females who were pregnant or nursing; and (f) any other condition that would compromise treatment with reasonable safety. Agreement to practice adequate birth control methods was required for fertile patients. The protocol for this clinical study was reviewed and approved by the National Cancer Institute (Bethesda, MD) and Institutional Review Boards of each participating New Approaches to Brain Tumor Therapy institution. Each patient signed a written informed consent document, satisfying all federal and institutional policies and regulations, as a condition of registering for participation in the study.

Drug administration and dose escalation. Patients were assigned to one of two treatment groups based on preexisting use of antiseizure drugs. Patients taking phenytoin, carbamazepine, phenobarbital, primidone, and oxcarbazepine were assigned to the +EIASD group. Patients assigned to the –EIASD group were either not being treated with an antiseizure drug or taking one that does not significantly induce hepatic enzymes, such as gabapentin, lamotrigine, valproic acid, felbamate, levetiracetam, tiagabine, topiramate, and zonisamide. Inclusion in the –EIASD group also required discontinuation of any EIASDs for at least 10 days. A clinically appropriate daily dose of a corticosteroid, such as dexamethasone, was determined for each patient before beginning the first cycle of therapy. Efforts were made to maintain the same dose until the radiographic tumor measurement was done after completing the second cycle (20). PCB (Sigma-Tau Pharmaceuticals, Gaithersburg, MD) was obtained commercially as capsules containing 50 mg of drug as the hydrochloride salt. It was administered orally, once a day, for 5 consecutive days in the outpatient setting. The total number of capsules given during each cycle of therapy was calculated by multiplying the dose level, expressed as the intended daily dose normalized to body surface area, by the actual body surface area of each patient, multiplying the result by 5 days, rounding the product down to the nearest multiple of 50, and dividing this number by 50 mg. The capsules were then divided as equally as possible between each of the 5 days of treatment. Patients were counseled to avoid alcoholic beverages, foods high in tyramine, and medications with potential interactions with PCB (antihistamines, barbiturates, ephedrine, epinephrine, hypotensives, isoproterenol, narcotic analgesics, paraglyline, and phenothiazines).

Appropriate antiemics were permitted, but use of hematopoietic growth factors was prohibited, during the first cycle. Treatment with any other approved or investigational chemotherapeutic agents was not permitted.

The starting dose for both treatment groups was 200 mg/m$^2$/d. The dose was independently escalated in each group to estimate the maximum tolerated dose using the modified continual reassessment method, as previously described, with minor modifications (21). Six patients were entered into each new dose level and monitored for treatment-related toxicities. Only toxicity associated with the first cycle was used for the dose-finding determination. After all patients completed the first cycle, toxicity data were used to develop and update a two-variable logistic dose-response model. The next dose level to be evaluated was determined by using the model to calculate the dose associated with a dose-limiting toxicity rate of 33%, with the maximum increment permitted being 50% of the previous dose level. The entire process was repeated until the recommended dose remained within 10% of the preceding dose for two consecutive iterations. This dose was considered to be the maximum tolerated dose.

Treatment with the same daily dose of PCB was repeated every 4 weeks until the occurrence of dose-limiting toxicity or tumor progression. Retreatment in the event of a dose-limiting toxicity during any course was permitted after all toxicities recovered to grade $\leq$1 with a 25% dose reduction. Further decreases in the dose, in this same manner, were allowed on occurrence of a dose-limiting toxicity after treatment with a reduced dose. A maximum of three dose reductions were permitted before the patient was removed from the study. Patients were also removed from the study because of disease progression, circumstances for which continued treatment could be detrimental to the health of a patient, or noncompliance.

Patient evaluations. Evaluations done within 14 days of initiating therapy included a medical history; physical and neurologic examinations; electrocardiogram; chest X-ray; minimal status; vital signs; performance status determination; complete blood count with differential and platelet counts; blood coagulation variables; serum chemistry profile; urinalysis; and pregnancy test for women of child-bearing potential. A complete blood count with differentials and platelet count was done weekly or more often if significant myelosuppression was observed. Histories and physical examinations were repeated monthly. Toxicities were evaluated during each monthly cycle and graded according to the National Cancer Institute Common Toxicity Criteria, version 2.0. Dose-limiting toxicity was defined as any of the following treatment-related adverse events: (a) absolute neutrophil count $<500/\mu L$ for at least 3 days; (b) platelet count $<25,000/\mu L$; (c) febrile neutropenia; (d) grade 3 or 4 nonhematologic toxicities, with the exception of nausea and vomiting without adequate antiemetic prophylaxis; and (e) a delay in starting a subsequent course of treatment of more than 7 days due to incomplete recovery from toxicity.

Response to therapy was determined by magnetic resonance imaging or computed tomography imaging and neurologic examinations. The use of computed tomography was restricted to patients who were unable to undergo magnetic resonance imaging for physical or medical reasons. Imaging studies to provide tumor measurements were done within 14 days of beginning treatment (baseline) and after every second cycle of therapy until relapse. Standard New Approaches to Brain Tumor Therapy response criteria were used as previously described (22). Confidence intervals were calculated according standard methods using SAS version 9 (SAS Institute, Cary, NC).

Pharmacokinetic studies. Sampling to characterize the plasma pharmacokinetics of PCB was done during the first cycle of therapy. Blood specimens (7 mL) were drawn from a peripheral arm vein and collected in tubes containing freeze-dried sodium heparin at the following times: before dosing on days 1 and 5; at 10, 20, 40 minutes and 1.5, 2, 3, 4, and 6 hours after dosing on day 1; and at 40,
60, and 90 minutes after taking the fifth daily dose. Sample tubes were immediately placed on ice until centrifuged (2,500 × g, 5 minutes, 4°C) within 5 minutes. Plasma was promptly transferred into a cryotube and stored at −70°C until assayed. The concentration of PCB in plasma specimens was determined by reversed-phase high-performance liquid chromatography with electrospray ionization mass spectrometry, as previously described (23). During application to this study, the between-day accuracy and precision of the assay were assessed by analyzing the interpolated drug concentrations from 33 standard curves. Between-day accuracy and precision of the assay were assessed by analyzing the interpolated drug concentrations from 33 standard curves run over a period of 2 years. Means ± SDs of the between-day accuracy and precision were 100.8 ± 4.2% (range, 97.0-110.7%) and 6.3 ± 2.2% (range, 4.1-10.2%), respectively.

Actual sample times were calculated from the beginning of ingestion to the sample collection time. Individual patient plasma concentration-time curves were analyzed by standard noncompartmental methods using WinNonlin Professional version 4.0.1 (Pharsight Corp., Cary, NC). Area under the curve was estimated using the linear/log trapezoidal method to the last data point, with extrapolation to time infinity using the slope of the log-linear terminal phase. Apparent oral clearance (CL/F; where CL is the total body clearance and F is the bioavailability fraction on oral administration) was calculated as the actual dose given to each patient divided by the area under the curve. Values of the pharmacokinetic variables at each dose level are reported as the geometric mean ± SD (24, 25). Parametric statistical tests of pharmacokinetic variables were done after logarithmic transformation of the data (24, 26). All tests were two sided and P < 0.05 was the criteria for significance.

Results

Patient characteristics. Characteristics of the 49 patients enrolled in the study are summarized in Table 1. There were 37 males and 12 females with a median age of 56 years (range, 23-72 years). Sixty-nine percent of the patients had glioblastoma multiforme and 88% had a good to excellent performance status. Phenytoin was used by 81% of the 31 patients in the +EIASD arm. Forty-four percent of the 18 patients in the −EIASD arm were not taking an anticonvulsant. Dexamethasone was used by 65% of the patients in the +EIASD group and 50% of the patients in the −EIASD group.

Dose escalation and toxicities. The starting dose of PCB, 200 mg/m²/d for 5 consecutive days every 4 weeks, provided a monthly dose intensity of 1,000 mg/m² that was intermediate between the standard PCV (60 mg/m²/d × 14 days every 6 weeks = 560 mg/m²/mo) and single-agent (150 mg/m²/d × 28 days every 8 weeks = 2,100 mg/m²/mo) regimens. The dose levels evaluated, number of patients enrolled at each dose level, and clinically significant toxicities encountered during the first cycle of therapy are summarized for both treatment groups in Table 2.

In the +EIASD arm, the dose levels studied were 200, 294, 393, and 429 mg/m². No significant drug-related toxicities were observed during the first cycle of therapy at 200 mg/m². At 294 mg/m², a seventh patient was accrued in error and toxicity data from this patient were not used for dose escalation determination. No dose-limiting toxicities were seen at this dose and, thus, six patients were treated at 393 mg/m². One of these patients had grade 2 thrombocytopenia that failed to recover within 7 days after the end of the first cycle of therapy, resulting in a treatment delay and classification as a dose-limiting toxicity. At 429 mg/m², dose-limiting toxicities occurred in two of six patients: one had grade 4 fatigue and the other had grade 2 thrombocytopenia that did not normalize after a 7-day delay in scheduled retreatment. As a result, 393 mg/m² was declared the maximum tolerated dose and another six patients were enrolled at this dose level for confirmation. One of these patients experienced a dose-limiting toxicity, grade 3 thrombocytopenia that required a treatment delay of more than 7 days for complete recovery of the platelet count. The overall dose-limiting toxicity rate for the maximum tolerated dose of PCB in patients concurrently receiving EIASDs was 17% (2 of 12 patients).

Doses of 200, 294, and 334 mg/m² were studied in patients not taking EIASDs. No patients receiving 200 mg/m² experienced significant toxicity. One patient enrolled into the 294 mg/m² dose level died from unrelated cardiac failure, was invaluable for toxicity and response assessments, and was replaced with an additional patient. A dose-limiting toxicity occurred in one patient in this cohort, grade 2 thrombocytopenia that required a delay in treatment with cycle 2 of more than 7 days for recovery. Among the initial four patients evaluated at the next dose level (334 mg/m²), one patient experienced grade 3 neutropenia and thrombocytopenia.

Evidence of hepatic dysfunction in patients receiving PCB became a significant concern. This was noted in three patients in the +EIASD arm during their second treatment cycles. The first patient, who was also taking phenytoin, developed grade 3 elevations in alanine aminotransferase and total bilirubin and grade 4 aspartate aminotransferase shortly after completing the second course of treatment with PCB 393 mg/m². This was attributed to Stevens-Johnson syndrome; the patient was removed from the study and abnormalities in the liver function.

| Table 1. Patient characteristics |
|-----------------------------|------------------|
| No. patients | 49 |
| Age (y) | 56 (23-72) |
| Gender | Male 37 (76) Female 12 (24) |
| Karnofsky performance status | 70-80% 26 (53) 90-100% 17 (35) |
| Histologic diagnosis | Glioblastoma multiforme 34 (69) Anaplastic astrocytoma 12 (24) Other 3 (6) |

NOTE: Unless indicated otherwise, data are reported as number of patients with percentage of total in parentheses.
*Two patients were also receiving phenytoin or oxcarbazepine.
†Three patients were receiving two anticonvulsants.
tests completely resolved. The second patient developed grade 3 to 4 serum transaminase elevations, shortly after being removed from the study due to disease progression, ~4 weeks after completing the second course of treatment with PCB 429 mg/m². This patient was also taking zolpidem tartrate and carbamazepine and the liver function abnormalities recovered after stopping zolpidem. The third patient developed grade 2 alanine aminotransferase, grade 3 aspartate aminotransferase, and grade 4 total bilirubin elevations shortly after completing the second course of treatment with PCB 429 mg/m². This patient, who was also taking phenytoin, died of liver failure several weeks later, the cause of which was never firmly established.

When grade 2 elevations in liver function tests developed after administering the first dose of PCB to the fifth patient entered into the 343 mg/m² dose level of the -EIASD arm, the decision was made to withhold further treatment of PCB in this patient and to close the trial to further accrual.

Response. Four partial responses were documented in the 49 patients evaluated in the study, yielding an overall response rate of 8% (95% confidence interval, 2-20%). The responses occurred in 2 of 31 (6%) -EIASD patients (200 and 429 mg/m²) and 2 of 18 (11%) -EIASD patients (294 and 334 mg/m²).

Forty-seven of the 49 patients have died and the median overall survival was 6.5 months (95% confidence interval, 4.0-8.9 months). The median number of cycles of PCB administered was 2 in both treatment groups and the median time to progression was 2 months.

Pharmacokinetics. Pharmacokinetic data for the first daily dose of PCB were obtained from 41 of 49 patients. Representative plasma profiles of PCB determined in individual patients receiving the maximum tolerated dose (393 mg/m²) for the -EIASD treatment group and the highest dose evaluated (334 mg/m²) in the -EIASD arm are shown in Fig. 1. Plasma levels of the drug increased rapidly on ingestion to a peak concentration (C_max) that occurred within 25 to 60 minutes, followed by apparent biexponential decay. Mean pharmacokinetic variables for PCB determined at each dose level evaluated in both treatment groups are listed in Table 3. Figure 2 shows individual patient and mean C_l/F values for the first daily dose of PCB plotted as a function of dose for both treatment groups. ANOVA revealed no significant differences among the mean C_l/F values at each dose level studied in the +EIASD group (P = 0.38) and the -EIASD group (P = 0.44). Similarly, there was no indication of a dose-dependent trend in the apparent biological half-life (t_1/2,z) of PCB in either treatment group, further suggestive of linear pharmacokinetic behavior. The overall mean C_l/F for the entire cohort of 27 patients in the +EIASD group (870 ± 331 L/h/m²) was not significantly different from that for the 14 patients in the -EIASD group (1,024 ± 523 L/h/m²; P = 0.30). The same was true on comparison of the overall mean t_1/2,z values between the two treatment groups (P = 0.32). Therefore, the plasma pharmacokinetics of PCB does not seem to be significantly influenced by the concurrent administration of EIASDs. Similarly, the concurrent use of dexamethasone for controlling tumor-related neurologic symptoms did not affect the C_l/F of PCB, the mean value of which was 930 ± 367 L/h/m² in the 18 patients who were not taking dexamethasone as compared with 912 ± 421 L/h/m² in the 23 patients who were (P = 0.89).

Evidence of a marked alteration in the pharmacokinetics of PCB on repeated daily administration was indicated by a substantial increase in the C_max for the fifth daily dose as compared with the first dose in nearly every patient studied, as illustrated in Fig. 3. Statistical comparison of the dose normalized C_max values on days 1 and 5 yielded P values of <0.001 for both treatment groups. The increase in C_max on day 5 was clearly not a result of drug accumulation because the concentration of PCB in plasma samples collected shortly before administration of the fifth dose was either below or very close to the 0.5 ng/mL lower limit of quantitation of the analytic method. The magnitude of the effect was comparable in both treatment groups, with overall mean values of the apparent accumulation factor (calculated as the ratio of C_max values on day 5 to day 1) being 3.9 ± 3.5 for +EIASD patients and 6.0 ± 5.4 for -EIASD patients (P = 0.16).

Discussion

The primary motivation for undertaking this clinical trial was to evaluate the effect of EIASDs on the maximum tolerated dose and pharmacokinetics of PCB in patients with primary brain

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Table 2. Summary of clinical toxicities during the first cycle of therapy

<table>
<thead>
<tr>
<th>Daily dose (mg/m²)</th>
<th>+EIASD cohort</th>
<th>-EIASD cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients evaluated</td>
<td>200</td>
<td>294</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>0/0/0</td>
<td>0/1/0</td>
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<tr>
<td>Thrombocytopenia</td>
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<td>Leukopenia</td>
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<td>0/0/0</td>
</tr>
<tr>
<td>Coagulopathy</td>
<td>0/0/0</td>
<td>0/0/0</td>
</tr>
<tr>
<td>Nonhematologic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>0/0/0</td>
<td>2/0/0</td>
</tr>
<tr>
<td>Constipation</td>
<td>0/0/0</td>
<td>1/0/0</td>
</tr>
<tr>
<td>Fatigue</td>
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<td>0/0/0</td>
</tr>
<tr>
<td>Myalgia</td>
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</tr>
<tr>
<td>Transaminasltis</td>
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<td>Neurologic</td>
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<td>Dizziness</td>
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</tr>
<tr>
<td>Headache</td>
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</table>
tumors. These issues have not been studied in the past, although PCB has been used clinically in lymphomas and brain tumors since the 1960s and it remains one of the few chemotherapeutic agents with activity against malignant brain tumors (1, 27). There is considerable evidence supporting the potential for a clinically relevant pharmacokinetic interaction between PCB and EIASDs that could affect the toxicity profile of the drug and modulate its anticancer activity. Nonclinical studies have shown that the metabolism of PCB, both in vitro and in vivo, is induced by phenobarbital (28–31). Pretreating tumor-bearing mice with either phenobarbital or phenytoin enhances the efficacy of PCB, presumably as a consequence of increased conversion to cytotoxic metabolites (32). Conversely, it has also been shown that PCB is a very effective inhibitor of the metabolism and systemic clearance of a number of compounds that are CYP substrates, including barbiturates (33–35). Consistent with these findings, an unusually high incidence of hypersensitivity reactions attributed to PCB in glioma patients receiving the drug in several combination chemotherapy regimens, as compared with past experience in patients with Hodgkin’s disease, was found to be significantly associated with the concurrent use of phenytoin, phenobarbital, or carbamazepine (36, 37). Nevertheless, when used in the treatment of central nervous system malignancies, prescribing the same dose of PCB regardless of the concurrent use of EIASDs remains the accepted clinical practice.

Information on the pharmacokinetics of PCB from previous studies is extremely limited. It seems that this may be attributable to the unavailability of sufficiently sensitive and specific analytic methods for determining PCB in biological fluids when the agent was undergoing preclinical and clinical development (23). Pilot experiments done during the preclinical evaluation of PCB revealed that the drug is efficiently absorbed from the gastrointestinal tract, rapidly eliminated from systemic circulation, and that it readily crosses the blood-brain barrier (38–40). Radiotracer studies have shown that the drug and its metabolites are predominantly eliminated by urinary excretion (39), with unchanged drug in urine accounting for ~25% of the dose given i.v. or p.o. to mice, dogs, and a single cancer patient (38). Although no actual data on the pharmacokinetics of PCB in humans have been reported, the disposition of the drug and its distribution into cerebrospinal fluid in a cancer patient have been described as being similar to the dog, but without showing any supporting data (38). The only other human studies of the drug were presented as a preliminary report to define time courses for several of its metabolites in plasma from a single cancer patient during daily oral treatment with PCB (40). However, the analytic method developed for determining the metabolites was incapable of measuring the parent drug.

A new electrospray ionization mass spectrometry assay for determining PCB in human plasma was developed for use in this clinical trial (23). The assay was thoroughly validated and shown to be accurate and reproducible for measuring PCB at concentrations as low as 0.5 ng/mL. This level of sensitivity enabled the plasma concentration-time profile of the compound to be accurately defined, even in patients taking EIASDs who were treated with 200 mg/m² of PCB. The concentration of PCB in plasma increased rapidly following oral administration and maximum levels were achieved within 1 hour in most patients. In patients receiving the starting dose, which was only 33% greater than the daily dose most commonly used in the single agent regimen of PCB (150 mg/m²), peak concentrations ranged from 39 to 370 ng/mL (150–1,440 nmol/L). Subsequently, plasma levels decayed nearly 3 orders of magnitude by the end of the 6-hour sampling interval. Linearity in the plasma pharmacokinetics of PCB was indicated by the absence of a dose-dependent trend in either the CL/F or t1/2,z for the first daily dose.

There was no evidence to suggest that the pharmacokinetics of PCB was affected by the concomitant use of EIASDs. Mean values of the CL/F, t1/2,α, and tmax for the first daily dose of PCB in 27 patients who were concurrently receiving EIASDs were comparable to those for 14 patients who were either not taking an antiseizure medication or using a drug that does not induce hepatic CYP. Similarly, the concurrent administration of dexamethasone, either with or without EIASDs, had no significant effect on the CL/F of PCB. These findings are surprising in consideration of the prominent role of phenobarbital-inducible enzymes in the bioactivation of PCB. PCB is metabolized to a single initial product, azoprocarbazine, in a reaction catalyzed by CYP and monoamine oxidase in rat hepatocytes (28). It was estimated that monoamine oxidase is responsible for at least 40% of the total rate of PCB oxidation in isolated hepatocytes from untreated rats and 25% in hepatocytes from phenobarbital pretreated rats. The direct oxidation of PCB by monoamine oxidase is not affected by
phenobarbital pretreatment, raising the possibility that the initial step in the metabolism of PCB in humans is predominantly catalyzed by monoamine oxidase, rather than CYP enzymes as in rats. However, unlike other hydrazine derivatives, PCB is a competitive inhibitor of monoamine oxidase that does not irreversibly inactivate the enzyme in vitro (28), which does not provide an explanation for the significant increase in the C_max of PCB on repeated daily dosing observed in the present study. This suggestion of a decrease in the CL/F of PCB is consistent with autoinhibition of its metabolism, which is most likely a consequence of the general inhibitory effect of PCB on hepatic CYP activity that has previously been described (33–35). Pretreatment with PCB, at doses similar to those evaluated in this clinical trial, significantly prolongs pentobarbital and hexobarbital sleeping time in rodents and decreases the clearance of hexobarbital in mice (33, 34). Administering a single dose of PCB to mice resulted in a significant decrease in CYP protein in liver microsomes as well as diminished catalytic activity of several CYP enzymes (34, 35). Decreases in CYP levels and catalytic activity were both maximal 4 to 8 hours after dosing and persisted for at least 16 hours. To the best of our knowledge, whether or not treatment with PCB results in an analogous decrease in hepatic monoamine oxidase protein content and activity has not been reported.

Information presently available in the literature indicates that the phenobarbital-inducible CYP2B, CYP3A, and CYP2C enzymes are potentially involved in catalyzing the initial step of its metabolism, which is most likely a consequence of the general inhibitory effect of PCB on hepatic CYP activity that has previously been described (33–35). Pretreatment with PCB, at doses similar to those evaluated in this clinical trial, significantly prolongs pentobarbital and hexobarbital sleeping time in rodents and decreases the clearance of hexobarbital in mice (33, 34). Administering a single dose of PCB to mice resulted in a significant decrease in CYP protein in liver microsomes as well as diminished catalytic activity of several CYP enzymes (34, 35). Decreases in CYP levels and catalytic activity were both maximal 4 to 8 hours after dosing and persisted for at least 16 hours. To the best of our knowledge, whether or not treatment with PCB results in an analogous decrease in hepatic monoamine oxidase protein content and activity has not been reported.

Information presently available in the literature indicates that the phenobarbital-inducible CYP2B, CYP3A, and CYP2C enzymes are potentially involved in catalyzing the initial step in the metabolism of PCB in rat liver (29, 41, 42). Human CYP enzymes from each of these subfamilies are induced by phenytoin, dexamethasone, or both drugs (43, 44). Therefore, because the majority of patients in the +EIASD arm of the study were taking both phenytoin and dexamethasone, it would seem that the direct oxidation of PCB in humans is not catalyzed by an enzyme induced by these drugs. Unfortunately, in vitro studies on the metabolism of PCB by human liver preparations or cells expressing recombinant human CYP enzymes have not been reported. In the absence of this information, accounting for the lack of an effect of CYP inducing drugs on PCB pharmacokinetics remains an unanswered question that warrants further investigation.

The conventional administration schedule for single agent PCB in the treatment of glioblastomas is oral dosing once a day for 28 days, repeated every 8 weeks (45). An alternative schedule, once daily for 5 days every 4 weeks, was evaluated in this study to determine if tolerability to the drug could be improved by reducing the duration of repeated dosing without compromising its therapeutic benefit. This is analogous to the approved administration schedule for temozolomide. In addition, accrual was limited to minimally pretreated patients to diminish factors that could confound the determination of reliable maximum tolerated doses. In patients concurrently receiving EIASDs, the maximum tolerated dose was 393 mg/m^2/d, with myelosuppression being the primary dose-limiting toxicity. The highest dose evaluated in the –EIASD cohort was 334 mg/m^2/d. Although no dose-limiting toxicities were observed in the five patients treated with this dose, an increasing number of grade 2 toxicities related to myelosuppression suggests that the maximum tolerated dose was being approached. Accrual was stopped before formally establishing a maximum tolerated dose because of safety concerns resulting from the development of hepatic dysfunction in several patients and because the primary question addressed in this trial, the effect of EIASDs on the pharmacokinetics of PCB, had been answered.

Hepatotoxicity has not previously been identified as a clinically significant problem with the use of PCB as a single agent. However, it has been described with the nitrogen mustard/vincristine/prednisone/procarbazine (MOPP) regimen in lymphomas and rarely with the PCV regimen in high-grade gliomas (6, 36). In the three episodes of significant hepatic dysfunction seen in this trial, two of the patients were receiving phenytoin and the other was taking carbamazepine together with zolpidem. There have been

Table 3. Mean pharmacokinetic variables of procarbazine hydrochloride

<table>
<thead>
<tr>
<th>Dose (mg/m^2)</th>
<th>No. patients</th>
<th>C_max (ng/mL)</th>
<th>t_max (h)</th>
<th>AUC (ng h/mL)</th>
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<tr>
<td>185 ± 4</td>
<td>3</td>
<td>243 ± 99</td>
<td>0.57 ± 0.17</td>
<td>154 ± 64</td>
<td>0.69 ± 0.11</td>
<td>1,024 ± 525</td>
<td>&lt;0.5</td>
<td>577 ± 597</td>
<td>2.4 ± 2.7</td>
</tr>
<tr>
<td>296 ± 9</td>
<td>6</td>
<td>406 ± 204</td>
<td>0.51 ± 0.19</td>
<td>321 ± 144</td>
<td>0.83 ± 0.18</td>
<td>922 ± 381</td>
<td>&lt;0.5</td>
<td>1,075 ± 219</td>
<td>2.7 ± 1.4</td>
</tr>
<tr>
<td>388 ± 11</td>
<td>12</td>
<td>492 ± 442</td>
<td>1.26 ± 0.92</td>
<td>493 ± 218</td>
<td>0.78 ± 0.14</td>
<td>865 ± 370</td>
<td>&lt;0.5</td>
<td>2,901 ± 735</td>
<td>5.1 ± 5.1</td>
</tr>
<tr>
<td>422 ± 13</td>
<td>6</td>
<td>492 ± 442</td>
<td>1.26 ± 0.92</td>
<td>493 ± 218</td>
<td>0.78 ± 0.14</td>
<td>865 ± 370</td>
<td>&lt;0.5</td>
<td>1,916 ± 1,939</td>
<td>3.9 ± 2.9</td>
</tr>
<tr>
<td>All</td>
<td>27</td>
<td>0.84 ± 0.22</td>
<td></td>
<td>870 ± 331</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.9 ± 3.5</td>
</tr>
<tr>
<td>–EIASD cohort</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 ± 17</td>
<td>3</td>
<td>106 ± 108</td>
<td>1.56 ± 1.25</td>
<td>150 ± 29</td>
<td>0.90 ± 0.46</td>
<td>1,334 ± 254</td>
<td>&lt;0.5</td>
<td>416 ± 149</td>
<td>3.9 ± 2.3</td>
</tr>
<tr>
<td>291 ± 15</td>
<td>7</td>
<td>231 ± 117</td>
<td>0.96 ± 0.47</td>
<td>276 ± 131</td>
<td>0.86 ± 0.35</td>
<td>1,053 ± 490</td>
<td>&lt;1.7</td>
<td>1,969 ± 1,533</td>
<td>8.5 ± 7.8</td>
</tr>
<tr>
<td>332 ± 10</td>
<td>4</td>
<td>587 ± 492</td>
<td>0.83 ± 0.50</td>
<td>415 ± 285</td>
<td>1.24 ± 0.66</td>
<td>799 ± 563</td>
<td>&lt;0.5</td>
<td>2,633 ± 1,364</td>
<td>4.5 ± 4.9</td>
</tr>
<tr>
<td>All</td>
<td>14</td>
<td>0.97 ± 0.44</td>
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<td>1,024 ± 523</td>
<td></td>
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<td></td>
<td>6.0 ± 5.4</td>
</tr>
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</table>

NOTE: Data are presented as the geometric mean ± SD. Abbreviations: C_max, maximum drug concentration in plasma; t_max, observed time of the peak drug concentration; AUC, area under the plasma concentration-time curve from time 0 to infinity; t_1/2,α, half-life of the apparent terminal disposition phase; CL/F, apparent oral clearance; C(0), pretreatment drug concentration in plasma; C_max ratio [(C_max on day 5) / (C_max on day 1)].

^*Average ± SD of the actual dose delivered to the patients at each dose level.

^†Measurable drug levels ranging from 0.8 to 1.7 ng/mL were detected in four patients.
occasional reports of severe hepatotoxicity associated with carbamezapine, phenytoin, and zolpidem (46–48). Although direct involvement of PCB cannot be discounted, the hepatic dysfunction could be attributable to the anticonvulsants, secondary to their decreased clearance when administered concurrently with PCB. Regardless of the underlying mechanism, the possibility exists that the unusual incidence of hepatotoxicity resulted from administering PCB at daily doses that were considerably greater than used in standard clinical practice. Specifically, the three patients experiencing severe hepatotoxicity received daily doses of 393 or 429 mg/m². In comparison, PCB is given at daily doses of 150 mg/m² for the conventional single-agent regimen and at 60 mg/m² in the PCV combination. Pending further clinical investigations, it is recommended that administering PCB together with other drugs that have a known potential for hepatic toxicity and that are eliminated by CYP-mediated metabolism (33, 36). Diminished clearance of anticonvulsants induced by PCB may have contributed to the severe hepatic dysfunction experienced by several patients. Decreased conversion to the ultimate methylating species responsible for the antitumor activity of PCB, by sequential CYP-mediated oxidation reactions, is a presumed consequence of the increased $C_{\text{max}}$ of the parent drug resulting from its general inhibitory effect on the hepatic CYP system. This implies that repeated

113 patients with glioblastoma multiforme, the partial response rate was 5%, with a 6-month overall survival rate of 44% and an overall progression-free survival of 8.3 weeks. Much higher response rates, ranging from 13% to 52%, were reported in earlier trials where less rigorous inclusion criteria, outmoded radiographic techniques, or even clinical observations rather than radiologic response criteria were employed (5, 49–51). Our intention to assess the efficacy of the daily $\times$ 5 schedule of PCB in a phase II study was not pursued because of the unexpected incidence of hepatic dysfunction in the phase I component of the trial.

The results of this study reinforce the observations that PCB-related drug-drug interactions are more complex than the typical CYP changes noted with EIASDs and other chemo- therapeutic agents such as paclitaxel and irinotecan. Furthermore, our findings confirm the potential for clinically significant interactions between PCB and other drugs that are eliminated by CYP-mediated hepatic metabolism (33, 36). Diminished clearance of anticonvulsants induced by PCB may have contributed to the severe hepatic dysfunction experienced by several patients. Decreased conversion to the ultimate methylating species responsible for the antitumor activity of PCB, by sequential CYP-mediated oxidation reactions, is a presumed consequence of the increased $C_{\text{max}}$ of the parent drug resulting from its general inhibitory effect on the hepatic CYP system. This implies that repeated

This clinical trial also provided a preliminary assessment of the antitumor activity of PCB given by the once a day times five schedule. The overall objective response rate was 8%, consisting of four partial and no complete response, with a median time to progression of 2 months and median overall survival of 6.5 months. These findings are comparable to a recent clinical trial in which the activity of the conventional 28 day schedule of single agent PCB was evaluated (45). In

![Fig. 2. Plots depicting relationships between the apparent oral clearance (CL/F) for the first dose of procarbazine hydrochloride and the dose administered to patients in the +EIASD (A) and –EIASD (B) treatment groups. Points, observed values in each patient; horizontal bars, mean value for each dose level.](image-url)

![Fig. 3. Observed values of the maximum drug concentration in plasma ($C_{\text{max}}$) for the first and fifth daily dose of procarbazine hydrochloride in patients belonging to the +EIASD (A) and –EIASD (B) treatment groups. Observed values (O) in the same patient are connected by line segments.](image-url)
administration of PCB on a daily basis, as in every single-agent and combination regimen of the drug that has gained clinical acceptance, may actually result in suboptimal bioavailability, thereby compromising its potential therapeutic effectiveness. To address this question, electrospray ionization mass spectrometry methods to measure the concentration of the major circulating metabolites of PCB have been developed and applied to the analysis of specimens from this clinical trial. These findings will be communicated in a subsequent report.

References
The Effect of Enzyme-Inducing Antiseizure Drugs on the Pharmacokinetics and Tolerability of Procarbazine Hydrochloride
