A Phase I and Pharmacological Study of Protracted Infusions of Crisnatol Mesylate in Patients with Solid Malignancies

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ABSTRACT

This Phase I and pharmacological study was performed to assess the feasibility of administering the polycyclic aromatic hydrocarbon crisnatol in increasingly prolonged continuous i.v. infusions to patients with advanced solid malignancies. The study also sought to characterize the principal toxicities of crisnatol on this schedule, to recommend doses for subsequent disease-directed studies, and to characterize possible associations between pharmacological parameters and toxicity. Sixteen patients were treated with 40 courses of crisnatol administered as a continuous i.v. infusion. The initial dose-schedule was 750 mg/m²/day for 6 days, and the duration of the infusion was to be progressively increased by 3-day increments to 9, 12, 15, 18, and 21. Courses were to be repeated every 4 weeks. Moderate to severe central nervous system (CNS) toxicity precluded the administration of crisnatol administered as a continuous i.v. infusion. The initial dose-schedule was 750 mg/m²/day for 6 days, and the duration of the infusion was to be progressively increased by 3-day increments to 9, 12, 15, 18, and 21. Courses were to be repeated every 4 weeks. Moderate to severe central nervous system (CNS) toxicity precluded the administration of crisnatol 750 mg/m²/day for longer than 6 days, and, therefore, the dose of crisnatol was reduced to 600 mg/m²/day. At this dose, three of five patients receiving a 12-day infusion experienced dose-limiting toxicity, which consisted of pulmonary thromboembolism (two patients) and grade 4 thrombocytopenia (one patient). None of the six patients completing a 9-day infusion at 600 mg/m²/day developed dose-limiting toxicity during the first or second course of crisnatol. At this dose level, the plasma concentrations at steady state (Cₚ₀) averaged 1607.8 ± 261.1 ng/ml, which exceeds minimal inhibitory concentrations for most tumors in vitro (1000 ng/ml). In fact, the administration of crisnatol at a dose of 600 mg/m²/day for 9 days resulted in the longest duration that biologically relevant plasma crisnatol concentrations have been sustained. Plasma Cₚ₀ values were significantly higher in patients who experienced severe CNS toxicity compared with those who did not (2465.3 ± 1213.5 versus 1342 ± 447.3 ng/ml; P = 0.04). There were no relationships evident between the clearance of crisnatol and indices reflecting renal and hepatic functions. One patient with a glioblastoma multiforme experienced a partial response lasting 14 months. The relative lack of intolerable CNS toxicity at the recommended dose for Phase II studies of crisnatol, 600 mg/m²/day for 9 days, as well as the magnitude of the Cₚ₀ values achieved and the antitumor activity observed at this dose, are encouraging. However, the mechanisms for the apparently increased thrombogenicity observed in this trial are unclear and require further elucidation.

INTRODUCTION

Crisnatol (2-[(6-chrysenylmethyl) amino]-2-methyl-1, 3-propanediol; Fig. 1) is the prototypical compound of a series of polycyclic aromatic hydrocarbon anticancer agents, known as the AMAPs, which have a common methyaminopropanediol side chain linked to variable carbocyclic rings (1–3). The precise mechanism of action of crisnatol, a chrysen-based molecule, is not known; however, DNA binding studies and patterns of cross-resistance in preclinical studies strongly suggest that it is a DNA intercalating agent (1, 3, 4). Crisnatol also inhibits topoisomerase II, which results in protein-associated DNA double-strand breaks (4). In human tumor cells growing in tissue culture, the agent blocks macromolecular synthesis, with IC₅₀ values of 5 and 18 μM for inhibition of DNA and RNA synthesis, respectively (5). Much higher concentrations are required to inhibit protein synthesis, and crisnatol did not alter nucleoside precursor uptake, phosphorylation, or retention.

Activity against a broad range of tumor types was noted in preclinical studies, with prominent activity against P388 murine leukemia sublines with acquired resistance to several antimetabolites, alkylating agents, and antimicrotubule agents, although sublines with acquired resistance to DNA-intercalating agents were cross-resistant (6). Crisnatol demonstrated impres-

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3 Deceased.

4 The abbreviations used are: AMAP, arylmethylaminopropanediol; ALT, alanine amino transferase; AST, aspartate aminotransferase; AUC, area under the concentration versus time curve: AUCₚ₀, AUC from 0 to last sampling time; CL, clearance; CNS, central nervous system; Cₚ₀, concentrations at steady state; DLT, dose-limiting toxicity; PK, pharmacokinetics.
Phase I and Pharmacological Study of Crisnatol Mesylate

Crisnatol was initially evaluated as a 6-h i.v. infusion in the L1210 leukemia and i.v. Lewis lung metastasis models, a significant prolongation in survival and in cures were noted (1). Prominent activity was also noted in the B16 melanoma, M5076 sarcoma, and reticulum cell sarcoma models. Less impressive activity, however, was noted against the human colon 38 xenograft, and crisnatol was inactive against the human MX-1 mammary tumor xenograft (1). In the human tumor cloning assay, crisnatol was demonstrated to induce prominent dose and schedule-dependent anticancer activity against malignant glioma, melanoma, and ovarian adenocarcinoma specimens taken directly from patients (3). After short-term (1-h) treatment with 10 $\mu$g/ml crisnatol, responses (≥50% reduction in new colony formation) were noted in 33% of specimens, whereas 39 and 60% of tumors responded after continuous treatment with 1 and 10 $\mu$g/ml, respectively, for 14 days. In vitro studies using MCF-7 breast cancer cells have also yielded important information regarding the relative contributions of drug concentration and duration of exposure to crisnatol’s antitumor activity (7). Exposure times of less than 6 h required high drug concentrations to achieve cytotoxicity. When drug concentrations and duration of exposure were varied to yield identical AUCs, the level of cytotoxicity was similar for a 3-h treatment period and a 48-h treatment with one-fifth of the drug concentration.

In toxicological and pharmacological studies performed in rodents and dogs, acute CNS toxicity characterized by ataxia, prostration, and irregular breathing, were the principal DLTs of crisnatol (2). The propensity to develop CNS toxicity seemed to be related to the drug concentration and administration rate. Dogs treated with high doses (370 and 720 mg/m$^2$ i.v.) also developed phlebitis, ataxia, tremors, emesis, salivation, and scleral and gingival injection. Myelosuppression was not noted. After i.v. administration of $^{14}$C-radiolabeled crisnatol, the drug was distributed primarily to organs with the highest blood perfusion; drug concentrations in brain, lung, kidney, spleen, pancreas, and liver were nine times that in plasma 1 h after treatment (8). The principal mechanism of drug disposition in dogs is hepatic microsomal metabolism, with 81–92% and 6–12% of the administered dose of crisnatol recovered as three metabolites in the feces and urine, respectively (9, 10).

Crisnatol was initially evaluated as a 6-h i.v. infusion repeated every 28 days in a Phase I clinical trial in patients with solid malignancies (11). Reversible CNS manifestations consisting of somnolence, confusion, ataxia, unsteady gate, blurred vision, and diffuse slowing of the background rhythm on electroencephalograms were noted consistently at the 516 mg/m$^2$ dose level, and a dose of 388 mg/m$^2$ was recommended for Phase II studies. The PK of crisnatol were well described by a two-compartment model; total body CL was rapid (mean, 18.3 liter/h/m$^2$), with the elimination $t_{1/2}$ averaging 2.85 h, and the volumes of distribution at steady-state averaging 58.8 liter/m$^2$. Peak plasma concentrations correlated with CNS toxicity, and concentrations greater than 6 $\mu$g/ml were associated with more severe CNS manifestations. CNS toxicity also limited the dose escalation of crisnatol on more protracted administration schedules; severe CNS effects were noted after treatment with crisnatol doses of 900 and 2700 mg/m$^2$ or greater, infused over 18 and 72 h, respectively (12, 13). Phlebitis near the infusion site also was noted when crisnatol concentrations in the infusion approached 0.25 mg/ml, and central venous access devices were recommended for drug concentrations of at least 0.5 mg/ml. Mild to moderate hypertension, nausea, and vomiting were also noted. The most notable antitumor activity was noted in a Phase II trial involving 22 patients with advanced malignant glioma in whom two complete responses, two minor responses, and durable disease stability in several other patients were recorded (14). In this study, patients were treated with crisnatol 2250 mg/m$^2$ infused over 72 h. Initially, dose adjustments were based on PK parameters, but subsequent dose adjustments were based solely on the occurrence of CNS toxicity. Toxicities included mild to moderate vertigo, blurred vision, and ataxia, and one subject experienced grade 2 leukopenia and transaminase elevations. Major seizures were encountered in approximately 5% of the courses that were dose-modified based on PK parameters, and this incidence was further reduced to 2–3% when dose reductions were based on CNS toxicity. Using this dose adjustment scheme and prophylactic antiemetics, the therapy seemed to be well tolerated.

The results of preclinical studies indicating that the duration of infusion of crisnatol is perhaps one of the most important determinants of cytotoxicity, and the relationship of CNS toxicity to peak drug concentration support a rationale for the evaluation of protracted administration schedules. The principal objectives of this Phase I and PK study were to: (a) characterize the principal toxicities of crisnatol when administered as an increasingly protracted infusion; (b) determine the maximum tolerated dose and maximum tolerated infusion duration of crisnatol, and recommend safe starting doses for Phase II studies; (c) characterize the pharmacological behavior of crisnatol; and (d) seek preliminary evidence of antitumor activity in patients with advanced solid malignancies.

PATIENTS AND METHODS

Eligibility. Patients with histologically confirmed solid malignancies refractory to conventional therapy or for whom no effective therapy existed were candidates for this study. Eligibility criteria included: (a) age ≥18 years; (b) Eastern Cooperative Oncology Group performance status ≤2 (ambulatory and capable of self-care); (c) life expectancy >12 weeks; (d) adequate hematopoietic (WBC ≥3000/µl, absolute neutrophil

![Fig. 1 Structure of crisnatol mesylate.](image-url)
count \( \geq 1500/\mu l \) and platelet count \( \geq 100,000/\mu l \); hepatic (total bilirubin \( \leq 1.5 \) mg/day; AST, ALT, and alkaline phosphatase \( \leq 2 \) times the upper limit of normal or \( \leq 5 \) times the upper normal limit if the elevations were due to hepatic metastases), and renal (serum creatinine \( \leq 2.0 \) mg/dl) functions; (e) no other coexisting medical problems of sufficient severity to prevent full compliance with the study, including congestive heart failure, angina pectoris, myocardial infarction, or uncontrolled hypertension within 6 months prior to study entry; and (f) no major surgical procedures within 30 days nor wide-field radiotherapy and/or chemotherapy or hormonal therapy within 3 weeks of entering onto protocol (or 6 weeks in those treated with a nitrosourea or mitomycin C). All of the patients gave written informed consent according to federal and institutional guidelines before treatment.

**Drug Administration.** Crisnatol was supplied by ILEX Oncology, Inc. (San Antonio, TX) in sterile amber 30-ml vials containing the equivalent of 150 mg of the free base. All of the doses and labeling were expressed as the free base and not as the mesylate salt; 500 mg/m\(^2\) free base is equivalent to 639 mg/m\(^2\) crisnatol mesylate. The 150-mg vials were reconstituted with 20 ml of sterile water for injection, yielding a solution containing crisnatol 7.5 mg/ml. The dose of crisnatol for a 72-h period was further diluted in 1000 ml of 5% dextrose solution, in polyvinyl chloride infusion bags, and then administered as a continuous i.v. infusion through a central venous catheter using a Harvard Apparatus ambulatory infusion pump (Harvard Apparatus Inc., South Natick, MA). Because the infusate was demonstrated to be chemically stable at room temperature for up to 96 h if protection from light was sufficient, the infusate solution was replaced after each 72-h period. Coumadin 1 mg/day was administered prophylactically because of a higher than expected incidence of central venous catheter-related thrombotic events in prior clinical studies of crisnatol.\(^5\)

**Dosage and Dose Escalation.** The starting daily dose of crisnatol was 750 mg/m\(^2\)/day as a continuous i.v. infusion for 6 days. This dose-rate was selected because it is considered the overwhelming body of preclinical and previous clinical data indicated that CNS toxicity was related to both the rate of crisnatol administration and the peak plasma concentration, instead of the total dose administered. Therefore, the dose rate of 750 mg/m\(^2\)/day, which was previously demonstrated to be safe as a 72-h continuous i.v. infusion, was administered over 6 days at the starting dose level (12, 13). The duration of the infusion at this fixed dose was to be increased by 3-day increments (6, 9, 12, 15, 18, and then 21 days) with the treatment repeated every 28 days. A minimum of one patient was to be treated at each infusion-duration level, and all of the patients at any dose level were to be assessed for toxicity for at least 28 days before a subsequent patient could be enrolled at the next higher dose level. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria. (15). The duration of infusion was to be increased in successive new patients as long as patients treated at the previous infusion-duration level did not experience nonhematological toxicity that was greater or equal to grade 2 in severity, and hematological toxicity was not equal or greater than grade 3. An additional patient (two total patients) was treated at a particular infusion-duration level if any grade \( \geq 2 \) nonhematological (excluding alopecia) or any \( \geq 3 \) hematological toxicity was observed in the first patient treated at that level. If no significant toxicity was observed in this second patient, the duration of infusion was increased for subsequent patients, whereas a total of three patients were treated if significant toxicity was observed in the second subject treated. If an episode of DLT was observed at a particular infusion-duration level, at least six patients were treated. DLT was defined as at least one of the following: (a) grade 3 irreversible (>2-days duration) nonhematological toxicity; (b) grade 3 nausea/vomiting despite treatment with an optimal antiemetic regimen; (c) grade 3 hypertension; (d) grade 3 thrombocytopenia; and grade 4 neutropenia of any duration during the first or second course of treatment. Reversible (<2-days duration) nonhematological toxicity including reversible CNS toxicity was not considered DLT. The intolerable duration level was defined as the infusion-duration at which at least 50% of the patients experienced DLT. The infusion-duration level immediately below the intolerable dose was defined as the maximum tolerated dose and the infusion-duration level recommended for Phase II clinical trials.

Because the predominant toxicity in prior Phase I and II trials of crisnatol was reversible CNS toxicity (11–13), a dose-modification schedule for CNS toxicity was devised prospectively based on the results of this scheme in these previous studies (11–13). The infusion was discontinued when patients developed severe (grade 3–4) CNS toxicity. When the toxicity resolved, the infusion rate was decreased by 10% or 20% for grade 3 or 4 neurotoxicity, respectively.

**Pretreatment Assessment and Follow-Up Studies.** Histories, physicals, and routine laboratory studies were performed pretreatment, weekly during treatment, and before each course. Routine laboratory studies included serum electrolytes, chemistries, renal and hepatic function tests, complete blood cell counts with differential WBC, prothrombin times, and urinalysis. Electrocardiograms were performed pretreatment and before each course. Formal tumor measurements were obtained after every two courses, and patients were able to continue treatment if they did not develop progressive disease. A complete response was defined as the disappearance of all disease on two measurements separated by a minimum period of 4 weeks. A partial response required a 50% or greater reduction in the sum of the products of the bidimensional measurements of all of the measurable lesions documented by two assessments separated by at least 4 weeks.

**Plasma Sampling and Assay.** Blood was sampled before treatment, immediately before the end of each 72-h period, and immediately before the end of the crisnatol infusion during the first course to measure the \( C_{ss} \) of crisnatol in plasma. Blood samples were also obtained immediately before the second course of treatment and immediately before the discontinuation of crisnatol due to toxicity, whenever possible. Six-ml samples of whole blood were collected in EDTA-containing glass vacutainer tubes. The samples were centrifuged at 2500 rpm for 10 min, and the plasma was separated, frozen immediately, and stored at \(-20^\circ C\) until analysis. Samples were assayed using a high-performance
light chromatography method as described previously (11). Briefly, a structurally related AMAP, BWA1195U (2-methyl-2-[(pyrenylmethyl) amino]-1,3-propanediol hydrochloride), was added as the internal standard to the standard curve samples, as well as to the quality control and patient samples before extraction. A 250-μl aliquot of plasma was then alkalinized with 25 μl of 0.8 N KOH and extracted with a 2.5-ml mixture of methanol-chloroform (1:9 v/v). The tube rotated for 10 min and was centrifuged for 10 min at 3000 rpm, and the organic layer was next transferred to a clean glass tube and evaporated to dryness at 35°C under a gentle stream of nitrogen. The percent recovery for both the internal standard and the crisnatol exceeded 80%. The extracted residue was then reconstituted with 200 μl of methylene chloride, and a 75-μl aliquot was injected using a Waters Model 710B WISP (Waters, Milford, MA) autosampler onto a 5-μm silica column (4.6 mm × 25 cm, Chromogasphere Si60, ES Industries, Marlton, NJ) preceded by a LiChrosorb Si60 guard column (ES Industries). The elution system consisted of 5.5% methanol in dichloromethane with 0.02% perchloric acid, at a flow rate of 1 ml/min with constant helium purge. UV absorbance (Waters 486) was monitored at 269 nm. Chromatograms and peak height areas were stored and analyzed using a Waters Maxima data acquisition software system. The lower limit of quantitation was set at 50 ng/ml. At this level the coefficient of variation of the measured concentration was 7.7%, and the deviation of the mean of the measured concentration was 1.83% of the theoretical value. The calibration curves for crisnatol were linear over the range of 50–4000 ng/ml using a weighting factor of 1/concentration. Coefficients of determination were greater than 0.999 for all of the standard curves. Interday precision, as measured by the relative SD of the calculated concentration of the quality control samples (n = 6), did not exceed 4.9% for the three quality control sample concentrations (100, 500, and 2000 ng/ml). The mean interday accuracy, which was assessed by comparing the measured concentrations with the theoretical concentration and was expressed as the percent deviation from the theoretical concentration, ranged from 0.9% for the low control (100 ng/ml) to 1% for the high (2000 ng/ml) control.

Pharmacokinetic and Pharmacodynamic Analysis. Because plasma crisnatol concentrations were measured beginning 72 h after treatment was begun, which represents approximately 25 half-lives (t 1/2 = 2.9 h; Ref. 11), C ss values were the mean plasma concentration during the infusion. CL was estimated by dividing the rate of infusion by C ss , which was normalized to body surface area. The AUC ss was calculated by dividing the total cumulative dose by CL. Descriptive statistics were used to summarize all of the PK parameters. Univariate correlation analyses were performed to examine the relationships between CL and pretreatment indices of both hepatic (ALT, AST, total bilirubin, alkaline phosphatase) and renal (serum creatinine and creatinine CL) functions, as well as with pretreatment serum albumin concentrations. The relationships between PK parameters indicative of drug exposure and the development of ≥ grade 2 CNS toxicity or venous thromboembolic events were also explored using the Wilcoxon rank-sum test. Statistical analyses were performed using JMP Version 3.1 Statistical software program (SAS Institute, Cary, NC).

RESULTS

General. Sixteen patients, whose pertinent characteristics are shown in Table 1, received 40 total courses of crisnatol at dose schedules ranging from 750 mg/m²/day for 6 days to 600 mg/m²/day for 12 days. Eight patients had primary CNS malignancies and five patients had metastatic disease to the brain that had been documented before treatment. The numbers of patients who were treated and the courses that were administered as a function of dose schedule are shown in Table 2. All four of the patients treated with crisnatol at a daily dose of 750 mg/m²/day, including two patients each treated for 6 days (level 1) and 9 days (level 2), developed CNS toxicity and/or grade 3 nausea and/or vomiting during either course 1 or 2. The median onset of CNS toxicity was 3 (range, 1–6) days, which suggested that 750 mg/m²/day is at or exceeds the threshold of dosing tolerance for the 6- and 9-day administration schedules. Therefore, the crisnatol dose-schedule was subsequently reduced to 600 mg/m²/day for 9 days (level 3), and it was elected to treat at least three patients at each level before the duration of the infusion was progressively increased in each cohort of new patients. After demonstrating a sufficient level of safety in patients treated with crisnatol 600 mg/m²/day for 9 days, the infusion duration was subsequently increased to 12 days (level 4). At 600 mg/m²/day for 12 days, three of five patients experienced DLT during either course 1 (two patients) or course 2 (one patient). Thus, further experience was ascertained at the 600 mg/m²/day for 9-day dose-schedule level, which resulted in no dose-limiting events during either course 1 and 2.

CNS Toxicity. CNS toxicity was the principal toxicity of crisnatol in this study. Although both neurocerebellar and neu-
Gob cortical toxicities were observed, cerebellar manifestations predominated, and neurocortical toxicities were typically encountered in association with neurocerebellar effects. Similarly, nausea and/or vomiting were usually noted concomitant with cerebellar effects. The neurocerebellar manifestations included ataxia, dysdiadokinesis, dysmetria, nystagmus, and diplopia, whereas somnolence was the most common neurocortical effect. The incidences of neurocerebellar and neurocortical effects, as well as nausea and/or vomiting, as a function of crisnatol dose-schedule level are depicted in Table 3.

At the 750-mg/m²/day for 6-day dose level, (level 1) two of two subjects experienced grade 1 cerebellar toxicity, consisting of mild ataxia and dysdiadokinesis that started on days 1 and 4. Neurocerebellar toxicity was also evident in two of two subjects treated with crisnatol at the next higher dose level (750 mg/m²/day for 9 days). At this level, one patient experienced grade 3 neurocerebellar toxicity, consisting of transient ataxia and diplopia, on the first day of course 1, and the second patient developed grade 2 neurocerebellar toxicity, characterized by ataxia, dysmetria, and nystagmus on the 6th day of course 2. These toxicities resolved completely within several hours after discontinuation of the treatment. However, the neurocerebellar

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**Table 2** Dose escalation scheme

<table>
<thead>
<tr>
<th>Level</th>
<th>Starting dose (mg/m²/day)</th>
<th>Infusion duration (days)</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>6</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>750</td>
<td>9</td>
<td>2</td>
</tr>
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<td>600</td>
<td>9</td>
<td>2</td>
</tr>
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<td>0</td>
</tr>
<tr>
<td>5</td>
<td>540b</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>480b</td>
<td>12</td>
<td>0</td>
</tr>
</tbody>
</table>

Total 16 40

*During course 1 or 2.
*The dose was reduced from 600 mg/m²/day.

**Table 3** Neurotoxicity

<table>
<thead>
<tr>
<th>Level</th>
<th>Starting dose (mg/m²/day)</th>
<th>Infusion duration (days)</th>
<th>Crisnatol dose schedule</th>
<th>No. of courses with neurotoxicitya</th>
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</thead>
<tbody>
<tr>
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<td>6</td>
<td>1</td>
<td>2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>2</td>
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<td>9</td>
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<td>4</td>
<td>1 0 1 0 1 1 1 0 1 2 0 1 0</td>
</tr>
<tr>
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<td>540b</td>
<td>9</td>
<td>8</td>
<td>0 0 0 0 2 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>6</td>
<td>480b</td>
<td>12</td>
<td>1</td>
<td>0 0 0 0 1 0 0 0 0 0 0 0</td>
</tr>
</tbody>
</table>

*a* Toxicity Grading: Neurocerebellar: 1, slight incoordination, dysdiadokinesis; 2, intention tremor, dysmetria, slurred speech, nystagmus; 3, locomotor ataxia; 4, cerebellar necrosis. Neurocortical: 1, mild somnolence or agitation; 2, moderate somnolence or agitation; 3, severe somnolence, agitation, confusion, disorientation, or hallucinations; 4, coma, seizures, toxic psychosis. Nausea/Vomiting: 1, one episode of vomiting, reasonable intake; 2, 2–5 episodes of vomiting in 24 h, intake significantly decreased but can eat; 3, 6–10 vomiting episodes in 24 h, no significant intake; 4, >10 episodes in 24 h or requiring parenteral support.

*The dose was reduced from 600 mg/m²/day because of toxicity during the previous course.

**Table 4** Nonneurological toxicities

<table>
<thead>
<tr>
<th>Level</th>
<th>Starting dose (mg/m²/day)</th>
<th>Infusion duration (days)</th>
<th>Total no. of patients (courses)</th>
</tr>
</thead>
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<td>2 (2)</td>
</tr>
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<td>5 (7)</td>
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<tr>
<td>5</td>
<td>540b</td>
<td>9</td>
<td>1 (8)</td>
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<tr>
<td>6</td>
<td>480b</td>
<td>12</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

*a* The dose was reduced from 600 mg/m²/day because of toxicity during the previous course.

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effects recurred in both individuals soon after the resumption of treatment with crisnatol at doses that had been reduced by either 10% (675 mg/m²/day) or 20% (600 mg/m²/day). One of the individuals completed the 9-day course after a second dose reduction to 565 mg/m²/day, whereas the other subject requested to be taken off-study on day 8 of course 2.

After the dose of crisnatol was reduced to 600 mg/m²/day, patients were better able to tolerate protracted infusions. At level 3 (600 mg/m²/day for 9 days), neurotoxicity precluded completion of treatment during courses 1 and 2 in only 1 of 7 new patients during both courses 1 and 2. This patient, a 48-year-old male with an anaplastic astrocytoma, developed locomotor ataxia, urinary incontinence, and mild somnolence on day 2 of his first course. These toxicities resolved completely within 12 h after treatment was discontinued but recurred 24 h after the resumption of treatment with the same dose of crisnatol. The crisnatol dose was reduced by 10% (540 mg/m²/day) until day 8, when a second 10% dose reduction to 480 mg/m²/day was required due to the reemergence of CNS toxicity. An additional patient at this dose level developed severe (grade 3) locomotor ataxia on day 3 of his fourth course, but a 10% dose reduction enabled the completion of the treatment course.

At the 600-mg/m²/day for 12 days dose level (level 4), a 48-year-old female with brain metastasis experienced a grand mal seizure 4 days after completing her first course of treatment (day 17). She had also developed severe (grade 3) cerebellar toxicity, grade 3 nausea and/or vomiting, and grade 2 somnolence during the 12-day treatment period, resulting in two successive 10% dose reductions to 540 mg/m²/day on day 4 and 480 mg/m²/day on day 5. Her long-term maintenance dose of corticosteroids had been decreased abruptly immediately before the development of the seizure. No further seizure activity was noted during a second course of crisnatol (480 mg/m² for 12 days) after treatment with anticonvulsants and appropriate corticosteroid doses.

All of the episodes of nausea and vomiting that were either moderate or severe in this trial occurred in patients who developed neurocerebellar toxicity. Nausea and vomiting were well managed with antiemetics, particularly serotonin antagonists. Similar to neurocerebellar toxicities, nausea and vomiting resolved soon after dosage reduction or drug discontinuation.

Patients with malignant involvement of the CNS were not more susceptible to CNS toxicity than those without CNS disease; however, the small number of patients in each relevant subgroup precluded formal analysis of the data. In the eight patients with primary CNS malignancies, there were three CNS toxicities (one grade 3 and two grade 1), and three toxic events [grade 4 (one), grade 2 (one), and grade 1 (one)] developed in the five patients with metastatic disease to the CNS, whereas three CNS toxicities [grade 2 (one) and grade 1 (two)] were documented in the three individuals who had no CNS involvement.

The incidence and severity of CNS toxicity did not seem to be significantly greater in patients who were concurrently receiving anticonvulsants and/or corticosteroids, although such comparisons involve small numbers of patients and the distribution of patients treated at each dose level also differed. Eight patients were receiving anticonvulsants before and during treatment with crisnatol [phenytoin alone (6), phenytoin and gabapentin (1), and carbamazepine (1)]. Of the seven patients who were concurrently receiving phenytoin, two individuals developed CNS toxicity (grade 1), and the patient receiving carbamazepine developed grade 3 CNS toxicity, whereas six of the eight patients who were not receiving anticonvulsants developed CNS toxicity [grade 4 (one), grade 3 (one), grade 2 (one), and grade 1 (three)]. It must also be noted that the distributions of patients who were and were not receiving anticonvulsant medications as a function of dose level also differed. For example, none of the patients treated at the 750-mg/m²/day dose level, which was associated with a relative high rate of CNS toxicity, were receiving anticonvulsant medications. With regards to corticosteroids, 6 of the 16 study patients were receiving dexamethasone at study entry, and all 6 of the subjects were initially treated with crisnatol at the 600-mg/m²/day dose level. Three of these subjects who were concurrently treated with dexamethasone developed CNS toxicities, whereas two of six subjects who were not receiving concurrent treatment with dexamethasone developed CNS toxicity after treatment with crisnatol at the 600-mg/m²/day dose level.

**Venous Thromboembolic Events.** Venous thromboembolic events, consisting of pulmonary thromboembolism and major catheter-related thrombosis with venous extension occurred in five patients during six courses (Table 4). Although a direct causal relationship between thromboembolic events and crisnatol could not be definitively established, the relatively high incidence of these events as well as the temporal relationship of the thromboembolic events to drug administration mandated that severe events be considered dose-limiting. Three patients experienced pulmonary thromboembolic events including two of five patients during either course 1 or 2 of crisnatol 600-mg/m²/day for 12-day dose level (level 4). Another subject developed a pulmonary thromboembolic event during courses 7 and 12. This patient was initially treated at the 600-mg/m²/day × 9-day dose level; the dose was reduced to 540 mg/m²/day for nine days because of severe CNS toxicity during course 4. The median onset of pulmonary embolism was on day 11 (range, days 4–28), and nuclear lung ventilation-perfusion scans were highly suggestive of pulmonary embolism in all of the

### Table 5 Crisnatol pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Dose (mg/m²/day)</th>
<th>Cₘ₈ (ng/ml)</th>
<th>AUC₉₆₄ (mg-h/liter)</th>
<th>CL (liter/h/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 2)</td>
<td>750 × 6 days</td>
<td>2394 ± 35</td>
<td>345 ± 5</td>
<td>13.1 ± 0.2</td>
</tr>
<tr>
<td>2 (n = 1)</td>
<td>750 × 9 days</td>
<td>2500</td>
<td>542</td>
<td>12.5</td>
</tr>
<tr>
<td>3 (n = 5)</td>
<td>600 × 9 days</td>
<td>1607.8 ± 261.1</td>
<td>347.8 ± 56.7</td>
<td>17.3 ± 4.9</td>
</tr>
<tr>
<td>4 (n = 4)</td>
<td>600 × 12 days</td>
<td>1510.3 ± 360.1</td>
<td>435.3 ± 104.1</td>
<td>16.9 ± 3.5</td>
</tr>
</tbody>
</table>

*Values are means ± SD.*
cases. In addition, all three of the subjects had been receiving low doses of Coumadin (1 mg/day) to prevent the development of catheter-related venous thrombotic complications, and all of these individuals had normal venous doppler studies of their lower extremity veins. Management consisted of anticoagulation with i.v. heparin followed by therapeutic doses of Coumadin. Another patient, a 34-year-old female with malignant melanoma and brain metastasis who had not been receiving prophylactic low-dose anticoagulation due to a prior CNS hemorrhagic event, developed acute dyspnea, unilateral pleuritic chest discomfort, and hypoxia on day 14 of her first course of crisnatol at the 600-mg/m²/day × 12-day dose level; however, pulmonary angiography was unremarkable.

Two patients developed extensive venous thromboses extending from their central venous catheters. One patient, who was to be treated with crisnatol at the 600-mg/m²/day for nine-day level, developed a large thrombus extending from the central venous catheter on day 5 while receiving prophylactic treatment with Coumadin 1 mg/day. Further evaluation revealed a tumor mass compressing the superior vena cava, resulting in the discontinuation of crisnatol and irradiation of the tumor mass. The second individual, who was initially treated with 600 mg/m²/day for 12 days of crisnatol that was subsequently reduced to 480 mg/m²/day because of CNS toxicity, developed an extensive venous thrombus extending from the central venous catheter to the right atrium during course 2. The patient had not been receiving a prophylactic low-dose anticoagulant because of the development of moderate thromboembolism during course 1.

Miscellaneous Toxicities. Clinically significant myelosuppression was uncommon, with only two patients experiencing moderate or severe toxicity during two courses. The most profound event was experienced by a heavily pretreated patient with concurrent pancreatic and non-small cell carcinomas and brain metastasis whose prior therapy included mitomycin C and carboplatin. The patient, who was initially treated at the 600-mg/m²/day for 12-day dose level and later dose-reduced to 480 mg/m²/day because of CNS toxicity, developed an extensive venous thrombus extending from the central venous catheter to the right atrium during course 2. The patient had not been receiving a prophylactic low-dose anticoagulant because of the development of moderate thromboembolism during course 1.

Antitumor Activity. A 48-year-old male with a glioblastoma multiforme, which had progressed soon after a partial surgical resection and radiation therapy, had a partial response lasting 14 months. The patient was concurrently receiving treatment with a stable dose of dexamethasone. The response was documented after six courses of crisnatol. He was treated initially with four courses of crisnatol at the 600-mg/m²/day for 9-day dose level followed by eight courses at 540 mg/m²/day for 9 days because of grade 3 CNS toxicity during his fourth course. Progressive disease was documented 3 months after treatment was discontinued.

Therapy regimens (consisting of carboplatin, vincristine, carmustine, procarbazine and paclitaxel), and who was not receiving corticosteroids, had stable disease documented on a magnetic imaging resonance scan after treatment with six courses of crisnatol at the 600-mg/m²/day for nine-days dose level. A positive emission tomography scan, performed 7 months after drug discontinuation, revealed no accumulation of 18-fluoro-2-deoxyglucose, and there has been no symptomatic or radiographic evidence of disease progression 20 months after beginning treatment with crisnatol.

Pharmacological Studies. Thirteen of the 16 patients were treated with crisnatol infusion rates that were not modified due to toxicity, and mean Csa values in course 1, as well as respective CL and AUCsa values, were calculated in 12 of these subjects (Table 5). The Csa values averaged 1510 ± 585 ng/ml (range, 776-2740 ng/ml) and 2430 ± 66 ng/ml (range, 2370–2500 ng/ml) for patients treated with unmodified crisnatol infusions at crisnatol doses of 600–(n = 9) and 750–(n = 3) mg/m²/day dose levels, respectively. Another subject, who was initially treated at the 750-mg/m²/day for 6-days dose level and required several dose reductions during course 1 because of the development of severe CNS toxicity, had a mean Csa of 4710 ng/ml. Respective CL values averaged 17.8 ± 5.3 liter/h/m² and 12.9 ± 0.4 liter/h/m² (P = 0.21, paired t test). At 600 mg/m²/day, AUCsa values averaged 347.8 ± 56.7 mg-h/liter and 435.3 ± 104.1 mg-h/liter in patients treated with unmodified crisnatol infusions lasting 9 and 12 days, respectively, whereas mean AUCsa values for 750-mg/m²/day infusions were 345 ± 4.95 mg-h/liter (n = 2) and 542 mg-h/liter (n = 1) in patients receiving unmodified crisnatol infusions lasting 6 and 9 days, respectively.

Relationships between Csa and laboratory indices reflecting renal function (pretreatment serum creatinine and estimated creatinine CL) and hepatic function (pretreatment serum albumin, AST, ALT, bilirubin, and alkaline phosphatase) were sought and no pharmacodynamic relationships, except for a possible relationship between Csa and serum alkaline phosphatase, were evident. Although the magnitude of serum alkaline phosphatase and mean Csa correlated weakly (r² = 0.38), Csa values in patients with and without elevated concentrations of alkaline phosphatase were significantly different (2999.5 ± 1140 versus 1509.6 ± 585.5 ng/ml; P = 0.02, Wilcoxon rank-sum test). Relationships between PK parameters reflecting dose exposure and relevant toxicities were also explored; however, PK values that could not be accurately calculated in two individuals who developed severe CNS toxicity requiring drug interruptions and dosage modifications precluded solid conclusions regarding this analysis. Nevertheless, patients who experienced CNS toxicity had significantly higher Csa values than patients who developed no CNS toxicity (2465.3 ± 1213.5 versus 1342 ± 447.3 ng/ml; P = 0.04, Wilcoxon rank-sum test) as displayed in Fig. 2. There were no such differences between patients who did and did not develop serious thromboembolic events.

Discussion

Crisnatol, the prototypical AMAP, was selected for clinical development because of its unique structural character-
Phase I and Pharmacological Study of Crisnatol Mesylate

CNS penetration. In this study, however, there were no relationships evident between the CL of crisnatol and plasma albumin and the indices of hepatic function. Despite unvaluable PK parameters in two subjects with severe CNS toxicities, patients experiencing CNS toxicity had significantly higher plasma $C_{ss}$ values compared with patients who did not develop CNS toxicity ($2465.3 \pm 1213.5$ ng/ml versus $1342 \pm 447.3$ ng/ml; $P = 0.04$). With regard to other determinants that may increase susceptibility to CNS toxicity, malignant involvement of the CNS did not seem to be an important risk factor, although an analysis of these determinants was precluded by the small number of patients in the study. Among the comediations that could potentially modulate the toxicity and pharmacological behavior of crisnatol, concurrent treatment with anticonvulsants must be considered, particularly in light of the high usage in the target population for which crisnatol is being developed. For example, phenytoin has been shown to induce cytochrome P450 3A, thereby increasing the CL of chemotherapy agents, which are largely metabolized by this microsomal system (18-20). Although the precise pathways responsible for the metabolism of crisnatol have not been elucidated, potential relationships between phenytoin coadministration and the CL of crisnatol were explored. When controlled by the dose received, patients receiving phenytoin were not more likely to have lower $C_{ss}$ ($P = 0.21$) nor higher CL rates ($P = 0.47$) compared with patients who were not receiving phenytoin.

The causal relationships between crisnatol and the other severe effects observed, particularly venous thromboembolic events, are less clear. Three patients developed pulmonary embolism during four courses, and another subject developed a clinical picture highly suggestive of pulmonary embolism, albeit unconfirmed by pulmonary angiography. Two other patients experienced other types of venous thromboembolic events. Because all five of the patients who experienced thromboembolic events either had primary or metastatic brain neoplasms, it is possible that the neoplasms themselves were the principal responsible factors. Patients with both metastatic and primary malignancies involving the brain have been demonstrated to have higher than expected incidences of thromboembolic events, most likely due to the release of procoagulants factors from brain tissues (21, 22). In a retrospective review of a series of 381 patients with malignant glioma, Ruff et al. (21) reported 109 cases of venous thromboses and three episodes of pulmonary emboli. The majority of cases occurred within 6 weeks of craniotomy. Similarly, in a prospective study of 77 patients on adjuvant radiochemotherapy after surgery for high-grade gliomas, Brandes et al. (22) observed a 20.8% risk of developing deep vein thrombosis at 12 months and a 31.7% risk at 24 months; four patients (5%) experienced pulmonary embolism. Risk factors identified were: (a) the presence of paresis predisposing to venous stasis; and (b) having a tumor with a high histological grade.

In the present study, venous flow studies were performed in all of the patients experiencing pulmonary embolism. However, there was no evidence of thrombosis or venous stasis in the lower extremities of these subjects, and only one individual experiencing pulmonary embolism had exhibited some degree of CNS toxicity resulting in some immobility during treatment.
In addition the presence of a fibrin clot, extending from the distal end of the central venous catheter to the tricuspid valve in one subject, and the presence of a thrombus in the lumen of the central catheter in another individual indicate that the thromboses in these individuals may have been initiated at the infusion site. Although venous thromboembolic events did not correlate with PK parameters indicative of crisnatol exposure, such as CL, $C_{\text{av}}$, or $AUC_{0-\infty}$, crisnatol was unexpectedly measured in pretreatment blood sampled from the central venous catheter on day 28 in four of the five patients who developed thromboses compared with only one of seven patients who did not experience thromboembolic complications during treatment with crisnatol.

In view of the above findings, an interim safety data analysis was performed in a Phase III randomized study that was being conducted in parallel with the present Phase I study. The Phase III study, which evaluated crisnatol (750 mg/m²/day for 3 days) versus BCNU after surgery and radiation treatment of glioblastoma multiforme, showed a much higher incidence of thromboembolic events in patients receiving crisnatol. At the time of the interim analysis, thromboembolic events of grade-3–4 severity had occurred in 6 (22%) of 27 patients in the crisnatol arm and in none of 19 patients receiving BCNU. Six additional patients treated with crisnatol experienced thromboembolic events of lesser severity.

The occurrence of a higher than expected rate of venous thromboembolic events with crisnatol treatment may represent a significant hurdle in the development of crisnatol as an anticancer agent, and understanding the potential mechanisms for this toxicity is highly important for the successful development of the agent. Various other cytotoxic chemotherapeutic agents have been demonstrated to possess procoagulant or anticoagulant properties (23). For example, doxorubicin, daunorubicin, and asparaginase reduce the activity of fibrin-stabilizing factors, whereas methotrexate, cyclophosphamide, and the nitrogen mustard family have been shown to increase fibrinolysis (24–26). Importantly, toxic vasculitis has been demonstrated in dogs undergoing histopathological evaluation after treatment with crisnatol as an i.v. infusion (6), and it could be speculated that this entity may predispose to the development of pulmonary embolism and other thromboembolic events. The presence of detectable crisnatol levels at the port site, 16 days after discontinuation of the crisnatol infusion in five individuals, including four in which thrombotic events were observed, also suggests at least two other potential mechanisms of thrombus formation. Due to the low solubility (0.28 mg/ml) of crisnatol when mixed in normal saline, crisnatol precipitates have been observed when normal saline is used to prepare solutions containing this agent (6). In the present study, crisnatol infusions were prepared in 5% dextrose solutions at a concentration of 1.5 mg/ml, which is not likely to result in drug precipitation. However, it could not be absolutely ascertained whether the indwelling venous catheters of some patients may have been flushed with small quantities of normal saline when the infusion systems were changed, thereby predisposing to the crystallization of crisnatol in the central veins. Alternatively, if the ports of the indwelling venous devices were not flushed at the end of the infusion, residual crisnatol in the reservoir might have lead to thrombus formation.

The results of the present study indicate that at doses of 600 mg/m²/day plasma crisnatol concentrations of approximately 1500 ng/ml, which are biologically relevant, can be maintained for as long as 9 days without intolerable CNS toxicity. The durable antitumor activity in patients with high-grade glial malignancies in this study is encouraging, particularly in view of the poor prognosis and lack of effective therapies for these neoplasms. On the basis of these results, additional studies to further evaluate the antineoplastic activity of crisnatol, as well as to elucidate the mechanisms for its potential thrombogenicity and reduce the incidence of related thromboembolic events, are warranted.

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Clinical Cancer Research

A Phase I and Pharmacological Study of Protracted Infusions of Crisnatol Mesylate in Patients with Solid Malignancies

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