Phase I and Clinical Pharmacology Trial of Crisnatol (BWA770U Mesylate) Using a Monthly Single-Dose Schedule


ABSTRACT

Crisnatol is a novel lipophilic arylmethylaminopropanediol with significant antineoplastic activity in a variety of murine and human tumor models which functions as a DNA intercalator. In this Phase I trial, a 6-hour infusion of the drug was administered i.v. in 700 to 1500 ml of 5% dextrose in water every 28 days. Eighty-five courses at doses of 7.5 to 516 mg/m² were administered to 43 patients with refractory solid tumors. Reversible neurological toxicity was dose limiting at 516 mg/m² and was manifested as somnolence, dizziness, blurred vision, unsteady gait, and α-slowing on electroencephalogram at the end of infusion. All neurological signs and symptoms were reversible. No hematological toxicity was observed. Other toxicities included phlebitis, mild to moderate nausea and vomiting, reversible sinus node arrest in one patient, and hypertension. Crisnatol plasma concentrations were determined by high-pressure liquid chromatography. After infusion, plasma concentrations declined biexponentially with a terminal τ₁ of 2.9 h. Using a two-compartment model, the mean apparent volume of distribution at steady state and total-body clearance were 58.8 liters/m² and 18.3 liters/h/m², respectively, indicative of extensive tissue distribution and rapid hepatic clearance. Peak plasma levels occurred at the end of infusion and correlated with the onset of neurological toxicity. The recommended Phase II dose for this schedule is 388 mg/m².

INTRODUCTION

Crisnatol, 2-[(6-chrysenylmethyl)amino]-2-methyl-1,3-propanediol (BWA770U mesylate), is a member of the novel arylmethylaminopropanediol series of antineoplastic agents (Fig. 1). This specific compound was chosen for clinical development on the basis of its lipophilicity and preclinical antitumor activity. In murine and human tumor models, the drug was found to have substantial antineoplastic activity. After preclinical toxicological and pharmacological evaluation, the drug was advanced to Phase I clinical trial.

Studies of the DNA binding properties of polycyclic aromatic derivatives containing amine side chains led to the discovery of antitumor activity in the series of agents with the general structure (aryl-) CH₂NHR. All active compounds were potent DNA intercalators. Variation of the side chain yielded crisnatol, which produced a 180% increase in life span in P388 murine leukemia at the optimal dose of 100 mg/kg. This was the best result among the 6-(chrysenyl)-CH₂NH[R(CH₂OH)₂] derivatives (1). Screening against experimental tumors also showed significant activity against L1210 murine leukemia, B16 melanoma, M5076 sarcoma, Lewis lung carcinoma, and reticulum cell sarcoma 1. The drug had borderline activity against colon 38 and was inactive against a mammary xenograft. It had variable activity against DNA intercalator-resistant P388 sublines. Delayed outgrowth and regression of P388 footpad implants established the systemic effectiveness of the i.p. route (2). Preclinical evaluation also included testing in a human tumor cloning system, results of which are published elsewhere (2).

Toxicology studies were conducted using nonfasted male CD-1 (Institute for Cancer Research) mice, Sprague-Dawley male CD rats, and beagle dogs (3). The major toxicities noted involved the central nervous system in all species tested. In rats receiving 30 to 40 mg/kg as a single dose, seizures and acute death were noted. At 20 mg/kg, ataxia, prostration, salivation, irregular breathing, and tail necrosis were noted. No drug-related hematological toxicity occurred. The acute LD10 was found to be 149 mg/m² in rats and 195 mg/m² in mice. Beagle dogs treated on the single-dose schedule had convulsions at doses of 360 and 720 mg/m². Emesis (dose-related in incidence and frequency), ataxia, salivation, tremors, scleral injection, gingival injection, and phlebitis were also noted at these dose levels. No significant toxicity was seen in dogs at 180 mg/m². No hematological abnormalities were noted at any doses. Chemistry tests showed minimal increases in alkaline phosphatase, creatinine, and glucose. Serial ophthalmological examinations and electrocardiograms were normal. Further study with all species indicated that CNS toxicities were dose rate (mg/min) related.

The initial dose for human Phase I study was derived from 1/10 of the LD10 in rodents (14.9 mg/m² in rats and 19.5 mg/m² in mice). Given the novelty of the compound and the potential CNS toxicities, a conservative starting dose of 1/20 of the LD10 in rats, or 7.5 mg/m², was selected.

MATERIALS AND METHODS

Patient Selection. The clinical characteristics and diagnoses for the patients in this trial are displayed in Table 1. Forty-three patients were entered in the study. All patients had histologically documented, advanced solid tumors, refractory to all known forms of effective therapy. Measurable or evaluable disease was required for admission to the study. Written informed consent as required by institutional and federal regulations was obtained from all patients. Patients were required to have adequate organ function including: bone marrow - WBC, >3000/mm³; platelets, >10,000/mm³; hemoglobin, >10 g/100 ml; hepatic-bilirubin, <2.5 mg/100 ml; serum glutamic oxaloacetic transaminase, <2.0 times normal (higher values of serum glutamic oxaloacetic transaminase were allowed if due to patient's gingival injection, and phlebitis were also noted at these dose levels. No significant toxicity was seen in dogs at 180 mg/m². No hematological abnormalities were noted at any doses. Chemistry tests showed minimal increases in alkaline phosphatase, creatinine, and glucose. Serial ophthalmological examinations and electrocardiograms were normal. Further study with all species indicated that CNS toxicities were dose rate (mg/min) related.

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continuous EEC monitoring was performed. Vital signs and eligibility criteria were met, treatment was begun. During treatment, base-line EEC was performed. If the EEC was normal and other metabolic — normal urinalysis; creatinine, <2.0 mg/100 ml; neurological — normal pretreatment neurological examination and EEC; renal — normal urination; creatinine, ≤2.0 mg/100 ml; no evidence or history of CNS disease (seizure disorder, CNS metastases) with normal pretreatment neurological examination and ECG; and blood glucose, <200 mg/100 ml. Patients were required to have a Karnofsky performance status of 60 or better and must have been off previous anticancer therapy for at least 3 wk (6 wk if the previous therapy was a nitrosourea or mitomycin C).

Treatment Plan. Patients were hospitalized for each dose of crisnatol. The formulation of the drug for clinical use was supplied by Burroughs Wellcome as a sterile powder in 50-mg vials. The vials were reconstituted with 10 ml of bacteriostatic water for injection. The solution was then passed through a 0.22-μm filter and further diluted with 5% dextrose in water to the final volume of administration. The total volume was 700 ml for dose levels ≤292 mg/m² and 1500 ml for dose levels ≥292 mg/m². The drug was administered through a free-flowing i.v. line, using an infusion pump, over 6 h as a single dose repeated every 28 days.

Prior to treatment, each patient was seen by a neurologist, and a base-line EEG was performed. If the EEG was normal and other eligibility criteria were met, treatment was begun. During treatment, continuous EEG monitoring was performed. Vital signs and electrocardiographic monitoring were performed during and for 24 h after the infusion. Patients were seen in follow-up by the neurologist if neurological toxicity was suspected. At the highest dose level (516 mg/m²), patients were also seen by an ophthalmologist before and after treatment. After 28 days, the patients were fully evaluated for tumor response. If the disease was stable or if there was evidence of tumor regression, the patient received further courses of treatment as above. EEG monitoring was not done on subsequent courses.

Dose escalation followed a modified Fibonacci scheme, beginning at 7.5 mg/m² and increasing to 516 mg/m² (Table 2). At least three evaluable patients were entered at each dose level. The first patient at each level was observed for 4 wk. If toxicity was acceptable, two additional patients were entered. Six patients were entered at 388 and 516 mg/m² to further define toxicity at these levels. For patients having stable disease and no toxicity at the initial dose level, additional doses were allowed at the highest dose level which had been safely completed in at least three patients.

Study Parameters. Base-line studies on all patients included history, physical examination, complete blood counts, serum chemistries, prothrombin time, partial thromboplastin time, urinalysis, erythrocyte sedimentation rate, C₃, chest radiographs, electrocardiogram, and appropriate base-line scans or radiographs needed to measure or evaluate tumor response. After treatment, patients were seen in the clinic weekly for 4 wk and on Days 36 and 49 after treatment if subsequent courses were not administered. At each visit, evaluations included history, physical examination, toxicity notation, complete blood counts, serum chemistries, prothrombin time, partial thromboplastin time, urinalysis, erythrocyte sedimentation rate, and C₃. In addition, studies for tumor measurement or evaluation were performed prior to each subsequent course. Standard response criteria and WHO toxicity criteria were used for evaluation.

Termination of Study. Patients were taken off study after completion of follow-up evaluation if their disease was found to be progressing by Day 29 of any course. They were also removed from study if toxicity was deemed unacceptable or if intercurrent illnesses made further treatment inadvisable. Patients were also free to terminate their participation in the study at any time. The Phase 1 study was terminated when the maximum tolerated dose was established.

Blood Sampling and Urine Collections. Blood samples for pharmacokinetic studies were obtained from an indwelling i.v. heparin lock in the arm contralateral to the infusion line. Serial 8-ml specimens were obtained in EDTA tubes before infusion; 1, 2, and 4 h into infusion; and at the end of infusion. Postinfusion samples were collected at 10, 20, 40, and 60 min, and 1.5, 2, 4, 6, 8, 12, 24, and 48 h postinfusion. One ml of whole blood was removed from each tube, labeled, flash frozen, and stored. The remaining sample was centrifuged, and plasma was harvested, flash frozen, and stored at −20°C in polyethylene tubes. A base-line urine specimen was obtained, and serial urine samples were collected during and after infusion with the last collection period ending at 48 h. The total volume of each sample was recorded; the urine sample was shaken; and a 20-ml aliquot was removed, labeled, and stored at −20°C. The concentration of drug in each specimen was measured by HPLC.

HPLC Analysis. A normal phase HPLC method was developed for the analysis of crisnatol in plasma and urine samples. A structurally related analogue, BWA1195U [2-methyl-2-{(pyrenylmethyl)amino}]-
1,3-propanediol hydrochloride), was added to each plasma/urine sample as an internal standard prior to extraction. Stock solutions and urine/plasma standard curves were prepared on the day of each procedure. A 0.5-ml volume of plasma or urine was alkalinized with 50 μl of 8 N KOH and extracted with 2.5 ml of chloroform:methanol (9:1). The mixture was gently rotated on a multipurpose rotator 10 min and centrifuged for 10 min at 800 x g. The organic layer was transferred to a 12 x 75-mm disposable glass culture tube and evaporated to dryness in a 45°C water bath under a gentle stream of nitrogen. The extraction yield was 80 to 90% for crisnatol and 87% for the internal standard. The extracted residue was then reconstituted with 250 μl of chloroform and 75 μl injected [Perkin-Elmer (ISS-100) autosampler; Norwalk, CT] onto a 5-μm silica column (4.6 mm x 25 cm; ES Industries, Marlton, NJ). The elution system consisted of 5% methanol in dichloromethane (HPLC grade) with 0.02% perchloric acid pumped (Model 510; Waters, Milford, MA) at a flow rate of 1 ml/min with constant helium purge. UV absorbance (Spectro-Monitor D; Milton/Roy, Rivera Beach, FL) was monitored at 269 nm. Chromatograms and peak height areas were stored and analyzed on a Model DS-80Z microcomputer (Digital Specialties, Chapel Hill, NC). Retention times for crisnatol and BWA1195U were 8 and 9 min, respectively. A typical chromatogram is illustrated in Fig. 2. The coefficient of variation was less than 5% over the linear range of the assay (5 to 500 ng/ml), with the lower limit of sensitivity of the assay set at 5 ng/ml.

Pharmacokinetic Analysis. The plasma concentration-time data were computer fitted to a 2-compartment open model with zero-order infusion using NONLIN (4) with a weighting of 1/y. The values for A, B, α, and β were used to obtain values for the pharmacokinetic parameters t1/2b, Vc, Vm, and CL. The area under the plasma concentration-time curve was estimated by the linear trapezoidal method up to the last measurable data point and extrapolated to infinity.

RESULTS

Forty-three patients received a total of 85 courses of crisnatol. Only one patient failed to receive the full intended dose of drug. This patient developed cardiac toxicity early in the infusion of a planned dose of 516 mg/m². All other patients were evaluable for acute toxicity. Five patients died within 28 days of a dose of crisnatol; each was attributable to disease, and each had completely recovered from any drug toxicity by the time of death. Two of these patients died before evaluation of the first course of therapy, one on Day 12 and one on Day 26. Two others died during follow-up after the second dose, and one after a third dose. All other patients were followed for at least 28 days after each dose of drug.

Neurological Toxicity. Table 3 summarizes the neurological toxicities at the two highest doses. Patients experienced no neurological toxicity at doses less than 388 mg/m². At 388 mg/m², 4 of 6 patients had mild to moderate dizziness, with 2 of these patients having positional vertigo. One patient also had subjective complaints of blurred vision and generalized mild numbness. Slight ataxia and mild nystagmus accompanying the vertigo were the only objective neurological findings. The patient with blurred vision also had mild background slowing of the α-activity on his EEG. At 516 mg/m², all 6 evaluable patients experienced neurological toxicity. In 4 of the 6, blurred vision was a prominent complaint. None of these had objective ophthalmological changes. Dizziness was noted by 5 of 6 patients, 2 having true vertigo with mild nystagmus on exam. One of these patients had moderately severe ataxia. In the other 4 patients with dizziness, mild ataxia was noted. The 2 patients with vertigo were also noted to have mild diffuse slowing of background α-activity on EEG. In all patients, the neurological symptoms began 2 to 3 h before the end of infusion and resolved 2 to 6 h after completion of drug therapy. Two patients developed seizure activity while on study. Each had been previously treated at 516 mg/m². A 54-yr-old Hispanic female with metastatic colorectal carcinoma experienced a generalized seizure on Day 42 after her second course of crisnatol. Computerized axial tomographic scans revealed metastatic disease to the brain. The second patient was a 70-yr-old male with metastatic melanoma who experienced a generalized seizure which began as a focal right upper extremity seizure on Day 50 after his only dose of crisnatol. Evaluation with computerized tomographic scanning, EEG, and lumbar puncture failed to reveal the etiology of the seizure. Due to the lack of any chronological relationship between the onset of seizure activity and crisnatol therapy, the occurrence was considered unrelated.

Other Toxicity. Nonneurological toxicity is summarized in Table 4. No significant hematological toxicity was noted in any patient. Phlebitis first occurred at the 105-mg/m² dose level. At 292 mg/m², phlebitis above the i.v. site was moderate to severe in 3 of 4 patients. Due to this, the volume of infusion was increased from 700 ml to 1500 ml at higher dose levels. With the more dilute solution, phlebitis was mild to moderate in all patients treated at 388 and 516 mg/m². The phlebitis responded to symptomatic treatment and resolved within 3 to 4 days of treatment.

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Gastrointestinal toxicity was mild and sporadic at doses less than 388 mg/m². At 388 and 516 mg/m², 3 of 6 and 6 of 6 patients, respectively, experienced mild to moderate nausea and vomiting. The nausea and vomiting were more severe in patients who received 516 mg/m². This typically began 2 to 3 h before the end of the 6-h infusion and lasted 2 to 6 h after the end of infusion. In all cases where treatment was given, phenothiazine antiemetics resulted in adequate control of nausea and vomiting. Phenothiazines were only administered after the posttreatment EEG had been obtained, and therefore, prophylactic effects of antiemetics could not be evaluated.

A probable drug-related cardiac arrhythmia was observed in one patient, a 76-yr-old Latin American female with metastatic fibrosarcoma and no known history of heart disease who was enrolled at the 516-mg/m² dose level. At 50 min into the infusion, the patient developed an asymptomatic sinus arrest with a junctional escape rhythm which resulted in no significant change in her cardiovascular status. The infusion was stopped at 65 min, after she had received a total dose of 142.3 mg, or 88.3 mg/m². She remained asymptomatic and returned to normal sinus rhythm 140 min later. She was monitored for 24 h further with no recurrence of the electrocardiographic change.

Hypertension was noted as a possible toxicity. A significant elevation (increase in systolic or diastolic pressure by >25 mm Hg from base line) over base-line blood pressure occurred in 3 of 6 patients at 388 mg/m² and in 3 of 6 evaluable patients at 516 mg/m². This was noted in five of the six courses given to the 3 patients at 388 mg/m² and in all six courses in the 3 patients at 516 mg/m². When it occurred, the blood pressure began a gradual increase 2 to 4 h after the beginning of infusion. The highest blood pressure occurred near the end of infusion, and all blood pressures returned to base line by 2 to 4 h after the end of infusion.

Antitumor effects. No partial or complete tumor responses were seen during this Phase I study. Twenty patients had stable disease for one to nine monthly courses of therapy with all others having progressive disease after one course. The longest duration of stable disease (9 mo) occurred in a patient with metastatic squamous cell carcinoma of the pharynx, who received nine courses at doses of 105 to 292 mg/m². Another patient with metastatic renal cell carcinoma remained stable through six courses ranging from 292 to 388 mg/m².

Pharmacokinetics. The pharmacokinetic results are summarized in Table 5. Plasma concentration versus time profiles for representative individual patients at the 105-, 388-, and 516-mg/m² dose levels are shown in Fig. 3. The plasma concentrations declined biexponentially with a harmonic mean terminal t½ of 2.85 h. The mean apparent volume of distribution at steady state was 58.8 liters/m², and the mean total body clearance was 18.3 liters/h/m². Less than 1% of the total dose of crisnatol was excreted unchanged in urine over 48 h. Metabolites were not detected in either plasma or urine. The mean peak plasma levels were 5.48 and 5.44 μg/ml at 388 and 516 mg/m² occurring at the end of infusion. Examining the relationship between AUC and dose over the entire dosage range (7.5 to 516 mg/m²), nonlinearity could not be demonstrated. Deviation from strict linear increases in AUC with increased dose is presumed to be due to patient variability. With respect to clearance, there appeared to be a trend toward a decrease in clearance with increase in dose ≥219 mg/m². However, the small number of patients at the earlier dose levels may have accounted for this apparent dose dependency. As shown in Fig. 4, peak plasma levels appear to correlate with the severity of clinical neurological toxicity (r = 0.85, P < 0.002).

DISCUSSION

Murine models and in vitro human tumor sensitivity suggest that crisnatol has significant antitumor activity. The goal of this Phase I study was to define the toxicity in humans treated
with a 6-h infusion of the drug and to determine a maximally tolerated dose with this schedule.

This schedule was chosen in an attempt to maximize the single dose that could be administered without encountering acute, delivery rate-related central nervous system toxicities that were seen in dogs. The maximally tolerated dose was determined to be 516 mg/m²; the dose-limiting toxicity was neurological toxicity which was reversible within 6 h of the end of therapy in all patients. Severe acute central nervous system toxic effects, e.g., seizures, were not encountered. Linear regression analysis of peak plasma concentrations versus neuropo
ticity \( (r = 0.85) \) suggests that plasma levels between 4.34 and 7.33 \( \mu g/ml \) correlated with the central nervous system toxicity encountered. Gastrointestinal toxicity and phlebitis were noted in the majority of patients at the highest dose levels but were not of dose-limiting severity at either the 388- or 515-mg/m² dose levels. The incidence and severity of phlebitis were reduced when the concentration of infused drug was maintained at \( \leq 1 \) mg/ml. Nausea and vomiting were easily controlled with phe
othiazine antiemetics. A single episode of cardiac toxicity occurred; the significance of a single incidence of asymptomatic sinus arrest in this elderly female is uncertain. Hypertension was noted at both the 388- and 516-mg/m² dose levels, but was not associated with any symptomatology. Since the blood pressure elevations usually occurred during episodes of neurological and gastrointestinal toxicity, it is difficult to determine if this is a primary or secondary drug-related effect. The absence of any hematological toxicity is remarkable.

Pharmacokinetic studies demonstrated a relatively short half-life. The large volume of distribution, high total-body clearance, and lack of urinary excretion are indicative of extensive tissue distribution and rapid hepatic clearance. These data in conjunction with the apparent correlation of peak plasma levels with dose-limiting neurological toxicity would suggest that exploration of more prolonged infusion schedules may result in an increase in the therapeutic index of crisnatol.

If this schedule of crisnatol administration were to be utilized for Phase II studies, the recommended dose of such trials would be 388 mg/m². However, since significant neurological toxicities appear to be related to attainment of a threshold plasma concentration (>4.5 \( \mu g/ml \)), it would be desirable to define an administration schedule that would maintain the peak plasma level below this threshold for as long a duration as possible. This administration objective is consistent with the in vitro data in which antitumor activity was greater with continuous drug exposure when compared to 1-h pulses (2). This exploration of higher doses of crisnatol administered over a longer period of time may maximize the chance of identifying a regimen of clinical utility. This concept is currently being explored.

REFERENCES

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Glenn S. Harman, John B. Craig, John G. Kuhn, et al.