ABSTRACT

Brequinar sodium is a quinoline carboxylic acid derivative that has shown antitumor activity in a number of in vivo murine and human tumor xenograft models. Its mechanism of action is blockade of de novo pyrimidine biosynthesis by inhibition of dihydroorotic acid dehydrogenase. In vitro and in vivo studies demonstrate the superiority of prolonged drug exposure in achieving tumor growth inhibition. This phase I study evaluated the administration of brequinar sodium by short, daily i.v. infusion for 5 days repeated every 4 weeks. Fifty-four subjects were enrolled in the study and received drug in doses ranging from 36-300 mg/m². The dose-limiting toxicities were mucositis and diffuse skin rash. Other toxicities included myelosuppression, nausea, vomiting, malaise, and burning at the infusion site. The maximum tolerated dose on the "daily times 5" schedule was 300 mg/m². The recommended phase II dose is 250 mg/m². Pharmacokinetic analysis of the day 1 drug clearance curves in 51 subjects showed slight nonlinearity in the relationship between dose and area under the clearance curve (AUC). The dose versus AUC relationship was well described using a Michaelis-Menten model of brequinar elimination kinetics with V∞ = 45 (µg/ml)/h and Kmax = 123 µg. Analysis of the day 5 drug clearance curves revealed a diminution in V∞ to 30 (µg/ml)/h. As a consequence of the reduction in V∞, brequinar plasma concentrations on day 5 were higher than predicted from day 1 drug kinetics. Pharmacodynamic analysis of the day 1 kinetic parameters and the toxicities occurring during the first cycle of drug therapy revealed significant correlations between mucositis and dose, AUC, and peak brequinar concentration; between leukopenia and AUC and peak drug concentration; and between thrombocytopenia and β elimination rate.

INTRODUCTION

Brequinar sodium (6-fluoro-2-(2'-fluoro-1,1'-(biphenyl-4-yl)-3-methyl-4-quinoline carboxylic acid sodium salt) is a novel quinoline carboxylic acid derivative that has shown antitumor activity in a number of in vivo murine tumor models (P388 leukemia, L1210 leukemia, and colon 38 carcinoma) and in vivo human tumor xenograft models (CX-1 colon carcinoma, MX-1 mammary carcinoma, LX-1 lung carcinoma, and BL/STX-1 gastric carcinoma) (1). The drug blocks pyrimidine de novo biosynthesis due to potent inhibition of the mitochondrial enzyme dihydroorotic acid dehydrogenase (2, 3). This enzyme is the fourth in the pyrimidine de novo pathway, catalyzing the oxidation of dihydroorotate to orotate (4). Cell culture studies using L1210 murine leukemia and WiDr human adenocarcinoma indicate that prolonged, continuous exposure of cells to brequinar is necessary to achieve the long-lasting depletion of intracellular UMP which is critical for the growth-inhibiting effects of the drug (5). In the in vivo murine L1210 leukemia model, schedules based upon long-term (9-16 day) daily administration of drug were more efficacious than those in which drug was given every other day (1). This in vivo observation is consistent with the in vitro findings and suggests that clinical dosing schedules resulting in prolonged drug exposure may yield superior antitumor results. The dosing schedule in this study, short, daily i.v. infusion for 5 consecutive days, is one designed to achieve a prolonged drug exposure in an outpatient clinical setting.

Preclinical toxicity studies were performed in several animal species and a consistent pattern of toxicity was observed. The principal toxicities encountered were gastrointestinal irritation and ulceration, myelosuppression, and venous irritation and phlebitis in animals treated i.v. In all animal species tested, the toxicity of brequinar was cumulative as reflected in a marked reduction in the maximum tolerated dose following multiple versus single dose administration. The gastrointestinal effects were dose limiting in all species studied. The dose producing 10% lethality in mice on a "daily times 5" schedule was 193 mg/m²/day. Preclinical studies in dogs indicated that this larger species was only able to tolerate a dose of 6 mg/m²/day on this schedule. Therefore, the initial clinical trials of the schedule in Europe were initiated at one-third of that dose, 2 mg/m²/day (6). By the time our study began, patients had been treated safely at doses as high as 36 mg/m²/day, so this dose was used as the starting dose in our study.

The purposes of this study were (a) to determine the maximum tolerated dose of brequinar given by short, daily i.v. infusion for 5 days repeated every 28 days, (b) to recommend a dose for phase II studies, (c) to characterize and quantitate the toxicities and adverse reactions seen with this schedule of administration, (d) to seek preliminary evidence for antitumor activity of brequinar, and (e) to describe the pharmacology of brequinar given on this schedule.

MATERIALS AND METHODS

Subject Population. Patients with histologically confirmed solid tumors refractory to conventional therapy or for which no effective therapy was known were candidates for entry into this study. The eligibility criteria also included age between 18 and 75 years, performance status (Eastern Cooperative Oncology Group criteria) of 3 or better (at least able to perform minimal self-care), life expectancy of at least 4 weeks, no major surgery within 14 days, no chemotherapy or radiotherapy within 28 days of entering protocol (42 days if prior therapy included mitomycin, nitrosourea, or cisplatin), adequate hematopoiesis [total WBC count > 4 x 10⁹ cells/µl (4 x 10⁹ cells/L) and platelet count > 100 x 10⁹ cells/µl (100 x 10⁹ cells/liter)], hepatic function [total bilirubin concentration < 1.5 mg/dl (26 µmol/liter)], and renal function [creatinine concentration < 1.5 mg/dl (133 µmol/liter)], and no other coexistent medical problems of sufficient severity to prevent full compliance with the study. In preclinical trials myocardial degeneration had been seen in monkeys treated at lethal doses of brequinar, so patients with active coronary artery disease, arrhythmias, or recent myocardial infarctions were excluded from study. Patients with a history of cardiac disease had multigated blood pool ventriculography performed and were excluded from study if the left ventricular ejection fraction was ≤ 45%. All subjects gave written, informed consent according to federal and institutional guidelines.

Clinical Evaluation. Prior to entry onto the study all subjects had a complete medical history and physical examination performed. At that time, tumor measurements were recorded in subjects with measurable...
disease and initial laboratory data obtained. Most subjects were seen weekly while in study and an interim history, physical examination, and laboratory data were obtained. Toxicity was graded weekly using NCI criteria (7). Tumor measurements were made every 2 cycles, or more frequently if clinically indicated, and responses graded using NCI criteria. Electrocardiography was performed on days 1 and 5 of each treatment cycle. Multigated blood pool ventriculograms were obtained every 2 cycles and subjects with ≥20% decrease in left ventricular ejection fraction were removed from study.

Drug Administration. Brequinar sodium was supplied by Dupont Pharmaceuticals (Wilmington, DE) as a lyophilized powder (100 or 500 mg/vial). The drug was dissolved in water and then diluted in saline to achieve concentrations ranging from 0.1–0.3 mg/ml, depending upon the total dose to be given and the duration of the infusion. Initially, drug was delivered over a period of 30 min. Because of a high incidence of burning at the site of infusion and other minor local toxicities, the infusion time was increased to 60 min. The drug was administered daily for 5 days every 4 weeks. The starting dose of brequinar was 36 mg/m². The dose was escalated using a modified Fibonacci scheme (48, 64, 85, 110, 135, 170, 210, 250, and 300 mg/m²). A minimum of 3 subjects were entered at each dose level. Dose escalation was permitted in individual patients if treatment at the original dose resulted in no toxicity and if at least 1 previously untreated subject had been treated at the higher dose and followed for a minimum of 4 weeks without development of significant adverse effects.

Pharmacology Studies. Plasma clearance studies were performed on the first and fifth days of drug administration during the initial treatment cycle. On the first day, blood samples (5 ml each) were collected before infusion and 2, 5, 10, 15, and 30 minutes and 1, 2, 4, 6, 8, 12, 18, and 24 h following the termination of the infusion. On the fifth day, samples were taken immediately prior to drug infusion (24 h following drug administration on the preceding day) and 15 and 30 minutes and 1, 2, 4, 6, 8, and 24 h following infusion. Plasma brequinar concentrations were measured by liquid chromatography using the method described by Arteaga et al. (8). Individual plasma clearance curves were fit using a 2-compartment linear mamillary model of drug distribution and administration. The data from the fifth day of therapy were fit to the steady-state expression of the model for drug dosing by intermittent infusion (9). The values of the following kinetic parameters were estimated for each clearance curve: α and β rate constants and associated half-lives, Vc, Va, CL, and AUC. Pharmacokinetic modeling and parameter estimation were performed by nonlinear regression analysis using PCNONLIN (Statistical Consultants, Lexington, KY). The mean kinetic parameter values that were derived from analysis of the first and fifth day clearance curves were compared using Student's t test for correlated data pairs.

Correlations among kinetic parameter values and categorical toxicity data were calculated with Spearman's rank-order correlation statistic (10).

RESULTS

The characteristics of the 54 subjects enrolled in the study are shown in Table 1. Forty-six subjects had an Eastern Cooperative Oncology Group criteria performance status of either 0 or 1. A total of 148 courses were administered. Thirty-nine subjects received at least 2 courses of brequinar and 16 patients received more than 2 courses. Thirty-three subjects received 3 courses, 9 received 4 courses, and 4 others received 7, 9, 12, and 13 courses. Table 2 indicates the number of subjects entered at each dose, the number in whom escalation was possible, and the number who required dose modification downward because of toxicity. Fourteen subjects were escalated, 7 by one dose level and 7 by more than one dose level. Only 1 subject who entered the study at a dose level higher than 135 mg/m² was escalated by more than one level. This subject, who received 13 courses of brequinar, received 3 courses at both 210 and 250 mg/m² and 7 courses at 300 mg/m².

Toxic Effects. The toxicities associated with brequinar are shown in Table 3. Only sporadic toxicity was observed up to the dose of 135 mg/m², and no subject entered at 36–135 mg/m² required dosage reduction. Of the 65 courses administered at those 6 dose levels, only 3 were associated with nausea or vomiting, 15 with grade 1 or 2 mucositis, 9 with mild skin rashes, and 6 with either grade 1 or 2 leukopenia or thrombocytopenia. A variety of other minor symptomatic complaints were reported by subjects (malaise, diarrhea, and abdominal pain), but none could be conclusively associated with drug administration. Similarly, minor abnormalities in several laboratory studies were observed. These included elevated serum concentrations of aminotransferases, alkaline phosphatase, creatinine, urea, and amylase. Proteinuria was also found occasionally. It was concluded that these findings were not related to receiving brequinar because the incidence and severity of the abnormalities did not increase with dose escalation and because both the clinical and laboratory findings were consistent with the status of the underlying malignancy. Burning and discom-
The dose-limiting toxicities of brequinar given on this schedule were mucositis and skin rash. The mucositis was noted between days 4 and 8 with the most severe episodes generally having an earlier onset. Resolution of symptoms was also dependent on the severity of the mucositis with 5–7 days generally required for recovery from grade 2 or 3 toxicity. Discomfort from the mucositis occasionally seemed disproportionate to the findings on physical examination. Many subjects were very symptomatic with only modest degrees of mucosal ulceration. In these subjects the change in the oral mucosa was often that of erythema and swelling of the tissue rather than frank ulceration. The skin rash also had some distinctive features. The onset of the rash was generally between days 6 and 9 and in subjects developing both rash and mucositis usually occurred 1 or 2 days later than the mucositis. The rash was diffuse with a predilection for the face, neck, and upper trunk. The most severely affected areas were often the intertriginous areas including nasal creases, axillae, breasts, groin, and perineum. In its milder form the rash was a macular scaling eruption often that of erythema and swelling of the tissue rather than frank ulceration. In these subjects the change in the oral mucosa was generally indistinguishable from other drug eruptions. No vasculitic changes were noted. In two patients treated at 170 and 250 mg/m², skin toxicity took a different form. They developed exquisite tenderness, redness, and swelling of hands and feet which was clinically indistinguishable from that seen with continuous infusion schedules of 5-fluorouracil.

At the highest doses, several other minor adverse reactions were observed. Despite the prolongation of the daily infusions to 60 min, 20% of courses were still associated with burning over the infused vein. Diarrhea or an increased frequency of bowel movements was reported by subjects during 20% of courses, and malaise which could temporally be associated with drug administration was seen in 15%.

No convincing evidence of cardiotoxicity was revealed in this study.

Therapeutic Responses. No complete or partial responses were seen. Minor responses were recorded in 2 subjects, one with colon cancer and one with renal cell cancer. They were very brief in duration and not clinically significant.

Pharmacokinetics. All of the subjects had complete plasma brequinar clearance curves obtained on day 1. Three of the curves were not well fit by a 2-compartment model due to underestimation of drug concentrations at early time points. The remainder were well described by the model. Twenty-seven subjects had complete data for day 5. All of these clearance curves were fit well by a 2-compartment model.

The mean day 1 pharmacokinetic parameter values are listed in Table 4. Slight, but significant, negative correlations exist between dose and $V_c$ ($r = -0.400; P < 0.005$) and $V_m$ ($r = -0.354; P < 0.02$). Inspection of the scatterplot of brequinar dose versus AUC reveals a curvilinear relationship between the two. This behavior is suggestive of saturable elimination kinetics. To distinguish from other drug eruptions. No vasculitic changes were noted. In two patients treated at 170 and 250 mg/m², skin toxicity took a different form. They developed exquisite tenderness, redness, and swelling of hands and feet which was clinically indistinguishable from that seen with continuous infusion schedules of 5-fluorouracil.

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explore that possibility, the dose versus AUC data were analyzed by performing a computer simulation study using a 2-compartment kinetic model with elimination of drug treated as a Michaelis-Menten process. The intercompartmental exchange rates for the model were set equal to the average values from the individual kinetic analyses. Simulations were run for paired values of the Michaelis parameters, and the AUCs from the simulated clearance curves were compared to the observed data. Using the iteratively reweighted sum of squared residuals as the criterion of goodness of fit of the simulated to the observed values, a grid search was undertaken to identify the \( V_{\text{max}} K_m \) pair yielding the best AUC fit. An excellent fit of the data is achieved with the following parameter values: \( V_{\text{max}} = 45 \) (\( \mu g/ml \))/h and \( K_m = 123 \) \( \mu g/ml \) (Fig. 1, top).

Fig. 2 shows the simulated plasma clearance curves using the 2-compartment model with the best-fit Michaelis parameter values and the same model with linear elimination of drug at a rate equal to \( V_{\text{max}} K_m \). The curves represent the drug levels after a 300 mg/m\(^2\) dose administered as a 1-h infusion. Notice that even at this dose, which results in the greatest disparity in the elimination kinetics of brequinar, the clearance curves are only slightly different. This illustrates how it is that individual clearance curves do not suggest the presence of nonlinear kinetics. Only the AUC data at multiple dose levels demonstrate the nonlinearity.

Predictions of the individual plasma drug concentrations for day 5, based upon the same individual’s pharmacokinetic parameter estimates derived from the day 1 data, underestimate the measured values in most subjects (Fig. 3). Separate analyses of the day 5 clearance curves yielded estimates of \( V_{\text{ci}} \) and \( V_{\text{c}} \) which are similar to the day 1 values [day 1: 3.05 ± 0.72 (SD) liter/m\(^2\), day 5: 3.04 ± 0.87 liter/m\(^2\) for \( V_{\text{c}} \); day 1: 7.91 ± 2.35 liter/m\(^2\), day 5: 8.51 ± 2.44 liter/m\(^2\) for \( V_{\text{ci}} \)]. The day 5 AUC estimates are, on average, 53% larger than the day 1 values (P < 0.001). Examination of the dose versus AUC scatterplot for the day 5 clearance curves shows a curvilinearity similar to that of the day 1 data. An identical modeling approach to that used for the day 1 data again resulted in an excellent fit of the data (Fig. 1, bottom). The parameter values for this fit are \( V_{\text{max}} = 30 \) (\( \mu g/ml \))/h and \( K_m = 127 \) \( \mu g/ml \). The \( K_m \) estimates for days 1 and 5 are nearly identical. This indicates that the differences between the AUCs on days 1 and 5 are completely explained by a 33% decrease in the \( V_{\text{max}} \) of the elimination process.

Pharmacodynamics. Correlation analysis of drug toxicity (measured as NCI common toxicity grades) and day 1 pharmacokinetic parameters reveals significant relationships (at the P ≤ 0.05 level) between dose, AUC, and peak brequinar concentration and the development of mucositis, rash, and leukopenia (Table 5). Even when the extremely conservative Bonferroni adjustment for multiple tests of significance is made, in this case indicating a significant P level as one <0.002, significant correlations exist between mucositis and dose, AUC, and peak brequinar concentration and between leukopenia and AUC and peak drug concentration. A significant correlation (at the P ≤ 0.05 level) also exists between thrombocytopenia and \( \beta \) elimination rate. As \( \beta \) half-life is related to a subject’s duration of exposure to lower drug concentrations, the finding of a correlation with thrombocytopenia is consistent with, but does not prove, a duration-threshold concentration basis for this toxicity. The distinctly different dynamic relationships for leukopenia and thrombocytopenia suggest that the mechanism of cytotoxicity of brequinar differs in the two hematopoietic cell types. None of the correlations are of a magnitude that the measurement of a pharmacokinetic parameter value could be used as a reliable predictor of toxicity.

DISCUSSION

Since the efficacy of brequinar in vitro depends upon maintaining an inhibitory concentration of drug for 48 h or more (5), it seems useful to determine whether the plasma concentrations of brequinar in our study subjects were maintained at levels comparable to those in the in vivo models. Brequinar concentrations have been measured in mice given i.v. drug (11) and the minimum drug concentrations in mice receiving the optimal i.v. dose on a daily times 9 schedule in the in vivo tumor studies (1) can be calculated to be 3 \( \mu g/ml \). Nine subjects in our study (1 at a dose of 170 mg/m\(^2\), 4 at a dose of 250 mg/m\(^2\), and 4 at a dose of 300 mg/m\(^2\)) maintained plasma concentrations above this level throughout the 5 days of therapy. Therefore, on a daily dosing schedule humans can maintain plasma brequinar concentrations at or above levels that are efficacious in in vivo models although, even at the highest dose
administered in this study, only two-thirds of the subjects achieved such levels.

The dose-dependent pharmacokinetic nonlinearity demonstrated in this study is a somewhat subtle finding, the recognition of which was greatly helped by the large number of subjects enrolled in the study. Examination of the AUC versus dose data reported by Arteaga et al. (8) does not suggest nonlinear kinetic behavior except at a dose of 300 mg/m². Their data which is based upon a study of 28 subjects are not inconsistent with ours. Their data, when graphed, are simply less suggestive of curvilinearity than are ours. Arteaga et al. (8) report the same time-dependent nonlinear kinetic behavior described here. In their study, there was a mean 23% increase in the day 5 AUCs compared to day 1. Our study revealed a mean 49% increase in the day 5 AUCs. Our modeling of the AUC data using Michaelis-Menten elimination kinetics produced good fits of the days 1 and 5 data and reveals that the increased day 5 AUCs can be attributed to a decline in \( V_{\text{max}} \) from 45 to 30 (\( \mu g/ml \))/h.

This study indicates that the dose-limiting toxicities of brequinar sodium given on this schedule are skin rash and mucositis. The seven subjects in this study who were treated initially at 300 mg/m² were of good performance status and had minimal prior therapy, yet six developed grade 2 or 3 mucositis and/or rash. This incidence of mucocutaneous toxicity, seen also in other studies (8, 12), indicates that this is too high a starting dose. We, therefore, suggest 250 mg/m² as an appropriate phase II starting dose. Our experience indicates that patients who tolerate that dose well can have their dose safely escalated to 300 mg/m². Our sample size is small and this will need to be verified in subsequent phase II studies which utilize the same approach. Our protocol did not allow escalation beyond 300 mg/m², but subsequent studies should evaluate the feasibility of escalating the dose in patients who are nontoxic at 300 mg/m².

Experience with other antimetabolites given on a repeated dose schedule would suggest that this is an important step to take and one which will increase the chance of yielding antitumor activity. Dose escalation should proceed cautiously, however, because of the uncertainty of the drug exposures due to the nonlinear kinetics of brequinar at such doses. A conservative recommendation for the phase II starting dose in patients considered to be at increased risk for toxic effects would be 170 mg/m².

**REFERENCES**


Phase I and Pharmacokinetic Study of Brequinar Sodium (NSC 368390)
