Phase I and Pharmacokinetic Study of Brequinar Sodium (NSC 368390)\(^1\)


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/m\(^2\). 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/m\(^2\). The recommended phase II dose is 250 mg/m\(^2\). 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 \(V_{\text{max}} = 45\) (\(\mu\)g/ml)/h and \(K_{\text{m}} = 123\) mg. Analysis of the day 5 drug clearance curves revealed a diminution in \(V_{\text{max}}\) to 30 (\(\mu\)g/ml)/h. As a consequence of the reduction in \(V_{\text{max}}\), 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 \(\beta\) 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 toxicology 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/m\(^2\)/day. Preclinical studies in dogs indicated that this larger species was only able to tolerate a dose of 6 mg/m\(^2\)/day on this schedule. Therefore, the initial clinical trials of the schedule in Europe were initiated at one-third of that dose, 2 mg/m\(^2\)/day (6). By the time our study began, patients had been treated safely at doses as high as 36 mg/m\(^2\)/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 large-field radiotherapy within 28 days of entering protocol (42 days if prior therapy included mitomycin, nitrosourea, or cisplatin), adequate renal function [creatinine concentration < 1.5 mg/dl (133 \(\mu\)mol/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...
BREQUINAR PHARMACOKINETICS

**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-one subjects received at least 2 courses of brequinar and 16 patients received more than 2 courses. 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 rash, 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-
fort at the site of drug administration were reported in 7 of 11 courses at 100 mg/m².

The characteristic toxicities for brequinar became evident at 170 mg/m² (Table 3). Nausea and vomiting associated with brequinar was infrequent and generally mild. Only 1 episode of protracted vomiting occurred in 83 courses at the 4 highest dosages. Myelosuppression was also observed but was not dose limiting. Only 3 instances of grade 3 or 4 hematologic toxicity occurred and in each case were in subjects with the most severe mucositis and skin rash. The nadir counts characteristically occurred between days 8 and 11 and recovery was seen by days 15–18.

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 diffuse with a predilection for the face, neck, and upper trunk, 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 became confluent and occasionally resulted in denuded areas of skin with weeping of serosanguinous fluid. The pruritus was poorly relieved by diphenhydramine or other antihistamines. It was occasionally relieved by the use of topical hydrocortisone cream and several subjects used this method prophylactically to prevent a recurrence on subsequent courses with apparent success. In any given single subject the severity of the rash tended to vary from course to course even when the dose remained the same. The most severe toxicity was usually seen on the first course of therapy at a given dose. Pathologically, the skin was grossly edematous with infiltration of the dermis by inflammatory cells. The pattern was 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

**Table 3 Toxicity at four highest doses**

<table>
<thead>
<tr>
<th>Dose (mg/m²)</th>
<th>Number of courses</th>
<th>Grade (Eastern Cooperative Oncology Group Criteria)</th>
<th>Nausea, vomiting</th>
<th>Mucositis</th>
<th>Rash</th>
<th>Leukopenia</th>
<th>Thrombocytopenia</th>
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<tbody>
<tr>
<td>170</td>
<td>19</td>
<td>0</td>
<td>16</td>
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<td>15</td>
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<td>0</td>
<td>12</td>
<td>4</td>
<td>10</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

**Table 4 Mean day 1 pharmacokinetic parameter values at each dose level**

<table>
<thead>
<tr>
<th>Dose level (mg/m²)</th>
<th>Evaluable subjects</th>
<th>$V_C$ (liters/m²)</th>
<th>$V_m$ (liters/m²)</th>
<th>AUC (µg·h/ml)</th>
<th>$\alpha$ half-life (h)</th>
<th>$\beta$ half-life (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>3</td>
<td>3.23</td>
<td>7.48</td>
<td>20.7</td>
<td>0.55</td>
<td>4.3</td>
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<tr>
<td>48</td>
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<td>48.5</td>
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<td>64</td>
<td>4</td>
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<td>45.0</td>
<td>0.71</td>
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<tr>
<td>85</td>
<td>5</td>
<td>3.49</td>
<td>8.72</td>
<td>68.9</td>
<td>0.81</td>
<td>7.2</td>
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<tr>
<td>110</td>
<td>5</td>
<td>2.85</td>
<td>8.51</td>
<td>149</td>
<td>0.93</td>
<td>12</td>
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<tr>
<td>135</td>
<td>5</td>
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<td>7.79</td>
<td>111</td>
<td>0.77</td>
<td>6.6</td>
</tr>
<tr>
<td>170</td>
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<td>2.80</td>
<td>7.68</td>
<td>169</td>
<td>0.63</td>
<td>6.8</td>
</tr>
<tr>
<td>210</td>
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<td>250</td>
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<td>3.19</td>
<td>7.88</td>
<td>347</td>
<td>0.80</td>
<td>9.6</td>
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<tr>
<td>300</td>
<td>6</td>
<td>2.65</td>
<td>6.20</td>
<td>386</td>
<td>0.70</td>
<td>6.8</td>
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</table>
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 best-fit Michaelis parameter values and the same model with linear elimination of drug at a rate set equal to $\frac{V_{\text{max}}}{K_m}$. The $V_{\text{max}}$ values for the respective model fits are $V_{\text{max}} = 30 \text{ (ug/ml)}/h$ and $K_m = 127 \text{ ug/ml}$. 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.

Predictions of the individual plasma drug concentrations for day 5, based on 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 $V_{\text{max}}$ and $V_{\text{max}}$ which are similar to the day 1 values [day 1: $3.05 \pm 0.72$ (SD) liter/m$^2$, day 5: $3.04 \pm 0.87$ liter/m$^2$ for $V_{\text{max}}$; day 1: $7.91 \pm 2.35$ liter/m$^2$, day 5: $8.51 \pm 2.44$ liter/m$^2$ for $V_{\text{max}}$]. 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 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 $V_{\text{max}} = 30 \text{ (ug/ml)}/h$ and $K_m = 127 \text{ ug/ml}$. 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.

**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 µg/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 $V_{max}$ 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.

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**REFERENCES**


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**Table 5** Correlation analysis of drug toxicity (NCI grading scheme) and day 1 kinetic parameter values

<table>
<thead>
<tr>
<th>Kinetic parameter</th>
<th>Nausea, vomiting</th>
<th>Mucositis</th>
<th>Rash</th>
<th>Leukopenia</th>
<th>Thrombocytopenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>NS</td>
<td>0.57*</td>
<td>0.41*</td>
<td>0.43*</td>
<td>NS</td>
</tr>
<tr>
<td>AUC</td>
<td>NS</td>
<td>0.62*</td>
<td>0.40*</td>
<td>0.47*</td>
<td>NS</td>
</tr>
<tr>
<td>Peak drug concen</td>
<td>NS</td>
<td>0.58*</td>
<td>0.39*</td>
<td>0.45*</td>
<td>NS</td>
</tr>
<tr>
<td>Beta elimination</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.38*</td>
</tr>
</tbody>
</table>

* $P < 0.002$, the criterion for statistical significance using the Bonferroni procedure for multiple tests.

$0.05 > P > 0.002$.

$N_S$, not significant.
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