Pediatric Phase I Trial, Pharmacokinetic Study, and Limited Sampling Strategy for
Piritrexim Administered on a Low-Dose, Intermittent Schedule

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ABSTRACT

Piritrexim, an orally administered, lipid-soluble antifolate, was evaluated in a multi-institutional phase I trial in children. The starting dose was 10 mg/m²/dose administered every 8 h daily for 5 days for 3 consecutive weeks, with dose escalations in increments of 5 mg/m²/dose. Eighteen patients (16 with metastatic sarcoma, 1 with acute lymphoblastic leukemia, and 1 with a brainstem glioma), 3.5–20 years of age, with malignancy refractory to therapy, were entered into the study. The dose-limiting toxicities (DLTs), which were myelosuppression and mucositis, occurred in 4 of 4 patients treated at the 25-mg/m²/dose level but in none of the patients treated at the 15- and 20-mg/m²/dose levels. The recommended dose for phase II trials is 20 mg/m²/dose. Pharmacokinetic monitoring was performed in 15 of the 18 children. The area under the concentration-time curve (AUC) was linearly related to the dose administered. Piritrexim was rapidly absorbed, with the median time to peak level occurring 1.5 h after an oral dose. The terminal half-life of piritrexim ranged from 1.5 to 4.5 h. A limited sampling strategy developed earlier, capable of predicting the AUC based on the plasma concentrations at 3 and 6 h after an oral dose, was prospectively tested in this trial and proved to be highly predictive of the AUC. Pharmacodynamic-pharmacokinetic correlations were obtained after combining data from this and the prior phase I pediatric trial. Trough plasma piritrexim concentration strongly correlated with DLT (r = 0.98, P = 0.0001). Eleven of 15 patients with trough concentrations exceeding this threshold experienced DLTs. Therapeutic drug monitoring may thus play an important role in adjusting the dose and schedule of piritrexim in future trials.

INTRODUCTION

Piritrexim (2,4-diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-d]pyrimidine; BW301U) is an orally administered, lipid-soluble antifolate which, like methotrexate, is a potent inhibitor of dihydrofolate reductase (1). However, it differs from methotrexate in its mechanism of cell entry and intracellular metabolism. Piritrexim appears to rapidly enter cells by passive diffusion (2), in contrast to methotrexate, which is dependent on cell-mediated transport. Because piritrexim lacks the glutamate residue found on methotrexate and naturally occurring folates, it is not polyglutamated intracellularly. Piritrexim, therefore, may be able to circumvent resistance to methotrexate that is based on a transport defect or on decreased polyglutamation.

We previously performed a phase I pediatric trial of piritrexim administered orally every 12 h for 5 consecutive days (3). Recent trials in adult patients with melanoma, however, demonstrated activity when piritrexim was administered on a low-dose schedule over 21 days (4). We therefore performed a phase I trial of piritrexim using a schedule with which an oral dose was administered every 8 h daily for 5 days for 3 consecutive weeks in pediatric patients with refractory cancer. The aim of this study was to identify the optimal dose for phase II trials, to further describe the pharmacokinetics of piritrexim in children, and to attempt to define the relationship between pharmacokinetic parameters and toxicity. In addition, we prospectively tested the limited sampling strategy, developed in our previous trial, that predicted the area under the plasma concentration-time curve based on a plasma drug concentrations obtained 3 and 6 h following an oral dose.

MATERIALS AND METHODS

Patient Eligibility. Pediatric patients between the ages of 5 and 21 years with histologically confirmed cancer refractory to conventional therapy were eligible for this trial. Patients must have recovered from the toxic effects of prior therapy before receiving piritrexim. All patients had adequate hepatic and renal function as defined by a serum bilirubin <1.5 mg/dl, serum transaminases <2-fold normal, and a creatinine <1.5-fold normal for age. Patients with solid tumors (without bone marrow involvement) were required to have a granulocyte count >1500/µl and a platelet count >100,000/µl.

Informed consent was obtained from the patient or his/her parent prior to entry in the study in accordance with individual institutional policies.

Study Design. The primary objective of the phase I trial was to define the toxicities and determine the MTD1 of piritrexim in children receiving an oral dose every 8 h daily for 5 days for 3 consecutive weeks, followed by 1 week of rest. A minimum of 3 patients evaluable for toxicity were treated at each dose level, with at least 2 having adequate bone marrow function to evaluate hematological toxicity. When 2 or more patients exhibited grade 3 or greater toxicity at a dose level, additional patients were treated at that level. This phase I trial defined the MTD as the highest dose level achieved that caused grade 3 or 4 toxicity in <4 of 6 patients. The starting dose for this study was 10 mg/m², with planned dose escalations in 5-mg/m² increments.

Patients were monitored with CBC counts, electrolytes, creatinine, calcium, phosphorus, uric acid, liver function tests, and urinalyses weekly and were also closely observed for clinical signs of toxicity. In patients with measurable disease, other laboratory or radiological examinations pertinent to tumor response were obtained prior to each cycle.

Patients were removed from study if they experienced unacceptable toxicity (grade 3 or 4 according to National Cancer Institute-Cancer Therapy Evaluation Program guidelines) or if objective disease progression was noted after one or more courses of piritrexim.

Drug Formulation and Administration. Piritrexim (isethionate salt) was supplied by Burroughs Wellcome Company (Research Triangle Park, NC) as capsules of 5, 10, or 25 mg potency. The dose was administered at least 1 h prior to and at least 2 h after a meal.

Pharmacokinetic Studies. Fifteen patients were monitored in the third, fourth, or fifth day of the first cycle of piritrexim, when steady-state conditions presumably existed. Blood samples were obtained prior to an oral dose and at 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h after the dose. The blood samples were collected in heparinized tubes and placed on ice.

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1 The abbreviations used are: MTD, maximum tolerated dose; AUC, area under the plasma-concentration time curve; DLT, dose-limiting toxicity; t½, half-life.
The strategy was prospectively tested in this trial by comparing the equation that predicted the AUC based on the plasma concentrations (C) of piritrexim determined 3 and 6 h after an oral dose (in mg/m²): predicted AUC to the measured AUC by linear regression. Statistical analysis was performed on plasma drug concentrations at each time point versus order to define the time points which provide the most predictive reliable estimates of AUC might also be used to assess bioavailability. Dynamic responses (8-11). In the case of an orally administered drug, are derived from plasma drug concentrations obtained at a minimal that a limited sampling strategy, in which reliable estimates of the AUC information about the AUC, the forward multiple regression analysis was performed using stepwise forward multiple regression analysis (3). In order to determine whether the pharmacokinetics of piritrexim were linear over a wide dosage range, pharmacokinetic data from the previous pediatric phase I trial (3), which studied 18 patients treated with divided doses of 70, 100, or 145 mg/m² administered orally for 5 days, were combined with data from this trial. The geometric mean AUC for each dosage cohort was compared to the dose administered using linear regression analysis.

Limited Sampling Analysis. Several recent studies have demonstrated that a limited sampling strategy, in which reliable estimates of the AUC are derived from plasma drug concentrations obtained at a minimal number (2 or 3) of time points, may be useful in predicting pharmacodynamic responses (8-11). In the case of an orally administered drug, reliable estimates of AUC might also be used to assess bioavailability. In our prior phase I trial, we developed a limited sampling strategy for piritrexim using stepwise forward multiple regression analysis (3). In order to define the time points which provide the most predictive information about the AUC, the forward multiple regression analysis was performed on plasma drug concentrations at each time point versus AUC (8, 12). The results of that analysis (3) yielded the following equation that predicted the AUC based on the plasma concentrations (C) of piritrexim determined 3 and 6 h after an oral dose (in mg/m²):

\[
\text{Predicted AUC} = 3.19 \cdot C_3 + 6.54 \cdot C_6 + 0.56 \cdot \left( \frac{\text{dose}}{70} \right)
\]

Fig. 1. Steady-state geometric mean plasma piritrexim concentrations following oral doses of 10 (●, n = 4), 15 (☐, n = 3), 20 (◼, n = 3), and 25 (▲, n = 4) mg/m² are plotted versus time postdosing.

The strategy was prospectively tested in this trial by comparing the the predicted AUC to the measured AUC by linear regression. Statistical significance was determined using an F test.

Pharmacodynamic-Pharmacokinetic Correlation. The AUC and trough plasma concentration of piritrexim in the patients with dose-limiting toxicity were compared to those from patients without dose-limiting toxicity, using the nonparametric Mann-Whitney U test (13). Because of the relatively small numbers of patients who are entered into a phase I trial, a similar analysis was performed after combining toxicity and pharmacokinetic data from both this and the previous pediatric phase I trial (3).

RESULTS

Phase I Trial. A total of 18 patients were entered in the study, 15 of whom were evaluable for toxicity. Two patients, treated at the starting dose level, did not complete the first course of therapy because of progressive disease and were judged inevitably for toxicity. One patient, treated at the starting dose level, was lost to follow-up. One 3.5-year-old child was entered after demonstrating the ability to swallow capsules and was considered evaluable.

The characteristics of the evaluable patients are listed in Table 1. All had been heavily pretreated with multiple chemotherapeutic regimens and, in the majority of cases, radiation therapy.

Both hematological (Table 2) and nonhematological toxicities were dose limiting in this trial. One patient treated at the starting dose level of 10 mg/m²/dose developed grade 4 neutropenia. All 4 patients treated at the 25-mg/m²/dose level developed DLTs; in 3 of these patients, DLT developed prior to the completion of the first cycle. Nadir granulocyte and platelet counts in the patients who experienced DLT occurred at a

Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>No. of patients entered</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>12/6</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>15</td>
</tr>
<tr>
<td>Median Range</td>
<td>3.5-20</td>
</tr>
<tr>
<td>Prior therapy (no. of patients)</td>
<td>2</td>
</tr>
<tr>
<td>Chemotherapy alone</td>
<td>16</td>
</tr>
<tr>
<td>Chemotherapy + radiotherapy</td>
<td>3</td>
</tr>
<tr>
<td>No. of prior regimens</td>
<td>3</td>
</tr>
<tr>
<td>Median Range</td>
<td>1-5</td>
</tr>
</tbody>
</table>

Table 2 Hematological parameters

<table>
<thead>
<tr>
<th>Dose (mg/m²/dose)</th>
<th>No. of patients with DLT/ No. of patients treated</th>
<th>WBC</th>
<th>Granulocytes</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nadir (cells/mm³)</td>
<td>No. of patients with &lt;2000/mm³</td>
<td>Nadir (cells/mm³)</td>
</tr>
<tr>
<td>10</td>
<td>1/4</td>
<td>2213 (600-2950)</td>
<td>1</td>
<td>1140 (342-1608)</td>
</tr>
<tr>
<td>15</td>
<td>0/4</td>
<td>3700 (2900-4500)</td>
<td>0</td>
<td>1703 (1044-2622)</td>
</tr>
<tr>
<td>20</td>
<td>0/3</td>
<td>3600 (3100-4100)</td>
<td>0</td>
<td>2604 (2201-3198)</td>
</tr>
<tr>
<td>25</td>
<td>4/4</td>
<td>1900 (1500-2400)</td>
<td>2</td>
<td>970 (522-1728)</td>
</tr>
</tbody>
</table>

* Dose-limiting toxicity.
* Includes one patient with dose-limiting mucositis.

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LOW-DOSE INTERMITTENT PiritreXim

Table 3 Pharmacokinetics

<table>
<thead>
<tr>
<th>Dose (mg/m²/day)</th>
<th>Half-lifea (h)</th>
<th>Time to peakb (h)</th>
<th>Peak levelc (µM)</th>
<th>Trough levelc (µM)</th>
<th>AUCd (µM·h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>2.2 (1.4-4.5)</td>
<td>1 (1-2)</td>
<td>2.3 ± 1.4</td>
<td>0.4 ± 0.6</td>
<td>9.3 ± 7.4</td>
</tr>
<tr>
<td>15</td>
<td>2.3 (1.8-3.0)</td>
<td>1.25 (1-1.5)</td>
<td>2.3 ± 1.2</td>
<td>0.3 ± 0.04</td>
<td>8.0 ± 3.9</td>
</tr>
<tr>
<td>20</td>
<td>2.1 (1.6-2.9)</td>
<td>1.5 (1-2)</td>
<td>3.3 ± 1.9</td>
<td>0.4 ± 0.4</td>
<td>12.4 ± 8.5</td>
</tr>
<tr>
<td>25</td>
<td>2.1 (1.9-2.5)</td>
<td>1.5 (1-2)</td>
<td>3.3 ± 0.5</td>
<td>0.6 ± 1.0</td>
<td>13.3 ± 5.5</td>
</tr>
</tbody>
</table>

a Harmonic mean (range).
b Median (range).
c Geometric mean ± SD.

dosed AUC was found \( r = 0.98, P = 0.0001 \), confirming the accuracy of the limited sampling strategy.

Pharmacodynamic-Pharmacokinetic Correlation. No statistically significant correlations were found when comparing either the trough plasma piritrexim concentration or the AUC of patients with DLTs to patients without DLTs. Because of the relatively small number of patients with DLTs in this trial, the same analysis was performed after combining the toxicity and pharmacokinetic data from patients entered in both this and the prior pediatric phase I trial (Fig. 4). Trough plasma piritrexim concentration strongly correlated with toxicity, with patients who experienced a DLT having a significantly higher trough piritrexim concentration than patients without toxicity \( P = 0.0016 \). Furthermore, a trough plasma piritrexim concentration >0.5 µM appeared to be predictive of toxicity. Eleven of 15 patients with trough concentrations which exceeded this threshold experienced dose-limiting toxicity.

DISCUSSION

In this pediatric phase I trial, both hematological and non-hematological dose-limiting toxicities appeared to be dose re-

median of 23 and 27 days. Mucositis was dose limiting\(^2\) in the one patient with acute lymphoblastic leukemia treated with 25 mg/m²/dose.

Non-dose-limiting toxicities included transient elevation of serum transaminases (<2-fold of the upper limit of normal) in 2 patients, one treated at the 10-mg/m²/dose level and the other at the 25-mg/m²/dose level. Mild nausea occurred in 2 patients.

The MTD of piritrexim administered on this schedule was 20 mg/m²/dose. None of the 3 patients treated at this dose level experienced dose-limiting toxicity, whereas all 4 patients treated at the next higher dose level (25 mg/m²/dose) exhibited grade 4 toxicity. The recommended dose for phase II trials is, therefore, 20 mg/m²/dose.

No complete or partial responses were observed. One patient with a peripheral neuroepithelioma, treated at the 20-mg/m³/dose level for 4 cycles, had a mixed response, with all but one pulmonary metastasis decreasing in size. One patient with acute lymphoblastic leukemia had a decrease in his peripheral lymphoblast count from 56,000 to 1,400/mm³.

Pharmacokinetics. Pharmacokinetics were studied in 15 patients during their first cycle of piritrexim. Concentration-time curves for the four dose levels studied are shown in Fig. 1. Pharmacokinetic parameters derived from plasma drug concentrations are listed in Table 3. Piritrexim was rapidly absorbed, with the median time to peak levels occurring 1.5 h after an oral dose. The terminal \( t_\alpha \) of piritrexim ranged from 1.5 to 4.5 h.

To determine whether the pharmacokinetics of piritrexim were linear over a wide dosage range, the geometric mean AUCs of the patients treated in both this trial and the prior phase I trial were compared to the dose administered (Fig. 2). A statistically significant correlation was found \( r = 0.96, P = 0.0006 \). Limited Sampling Strategy. The predicted AUC, based on the plasma concentration 3 and 6 h following an oral dose, compared to the measured AUC is plotted in Fig. 3. A strong, statistically significant correlation between the predicted and measured AUC was found \( r = 0.98, P = 0.0001 \), confirming the accuracy of the limited sampling strategy.

Grade 4 is defined as oral ulcerations requiring parenteral support.
lated, with DLT occurring in 4 of 4 patients treated with 25 mg/m²/dose and in none of the patients treated at the 15- and 20-mg/m²/dose levels. The dose-limiting toxicities observed, myelosuppression and mucositis, were identical with those observed in the previous pediatric phase I trial of piritrexim (3). The recommended starting dose for phase II trials of oral piritrexim, administered every 8 h daily for 5 days for 3 consecutive weeks, is 20 mg/m²/dose. This dose is similar to the uniform dose of 25 mg that was used with minimal toxicity in recent adult trials (4, 14). The pharmacokinetic parameters determined from patients studied in this trial were similar to the parameters found in the previous pediatric phase I trial, in which patients were treated with higher doses. Piritrexim was rapidly absorbed, with peak plasma concentrations occurring 1–1.5 h after administration of an oral dose. The mean terminal clearance Cₚₚ ranged from 2.1 to 2.3 h. Although there was interpatient variability, the geometric mean AUC was linearly related to the dose administered over a wide dosage range, from 10 to 145 mg/m²/dose. The linearity of the pharmacokinetics underlies the success of the limited sampling strategy, which was developed from the pharmacokinetic data from patients treated with doses of 70–145 mg/m² and tested in this trial in patients treated with doses of 10–25 mg/m². The limited sampling strategy, using plasma piritrexim concentrations determined 3 and 6 h after an oral dose, accurately predicted the AUC (r = 0.98).

Similar to methotrexate (15), the toxicity of piritrexim appears to be both dose and schedule dependent, with increased toxicity observed in patients maintaining plasma concentrations above a specific threshold concentration. The data from both pediatric phase I trials suggest that this threshold is approximately 0.5 μM. Therapeutic drug monitoring thus may play an important role in future phase II trials of piritrexim. The interpatient variability, probably primarily due to differences in absorption, could be minimized by dose individualization. Determination of piritrexim plasma concentrations just prior to administration of an oral dose (trough) and 3 and 6 h after the dose might allow for accurate dose adjustment.

Piritrexim administered on this schedule was generally well tolerated. It may prove useful not only as an antineoplastic agent but also potentially as a treatment for Pneumocystis carinii infection (16). The pharmacokinetic strategies developed in these phase I trials may benefit future patients treated with this drug.

REFERENCES


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