Pediatric Phase I Trial and Pharmacokinetic Study of Trimetrexate


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ABSTRACT

Trimetrexate, a new nonclassical antifolate, was evaluated in a phase I trial in children with refractory cancer including nine with acute leukemia and 21 with solid tumors. The drug was administered as an i.v. bolus injection weekly for three doses, and courses were repeated every 28 days. The dose ranged from 35 to 145 mg/m². Thirty patients who received a total of 33 courses were evaluable for toxicity, including 19 who were evaluable for hematological toxicity. The maximally tolerated dose for patients with a solid tumor and leukemia was 110 mg/m². The dose-limiting toxicities were myelosuppression, mucositis, and a pruritic, diffuse maculopapular rash. Other side effects observed included transient, mild elevations of serum transaminases, mild nausea and vomiting, and a local phlebitis at the site of injection at higher dose levels. A single patient with delayed drug clearance had evidence of renal toxicity with a transient increase in serum creatinine. The pharmacokinetics of trimetrexate were studied in 25 patients over the entire dose range. There was considerable interpatient variability in total drug clearance (range 9.2 to 215 ml/min/m²) and half-life (2.1 to 20 h). There was a suggestion of a correlation between plasma concentration at 24 h and the development of hematological toxicity at the highest dose level. Trimetrexate was cleared primarily by biotransformation with renal clearance accounting for only 10% of total clearance. Two metabolites of trimetrexate which inhibit the enzyme dihydrofolate reductase were identified in the urine. One of these appears to be a glucuronide conjugate.

INTRODUCTION

Trimetrexate, 2,4-diamo-5-methyl-6-[(3,4,5-trimethoxyanilino)methyl]quinazoline) is a new, nonclassical antifolate which, like methotrexate, is a potent inhibitor of the enzyme dihydrofolate reductase (1). However, both the cellular pharmacology and the pharmacokinetics of trimetrexate differ significantly from those of methotrexate. Trimetrexate is more lipophilic and is capable of penetrating cells rapidly, independent of the folate carrier-mediated transport system required by methotrexate (2, 3). In addition, the chemical structure of trimetrexate precludes its intracellular conversion to polyglutamated derivatives, a metabolic step that results in the prolonged intracellular retention of both methotrexate and the naturally occurring folates. There are also significant differences in the route of elimination, rate of clearance, and volume of distribution of trimetrexate when compared to methotrexate (4).

The antitumor spectrum of trimetrexate in preclinical testing with murine tumors included both P388 and L1210 leukemias and several solid tumors (B16 melanoma, colon 26, and CD8F mammary tumor), a wider spectrum than that found with methotrexate (5). The responses observed in P388 leukemia were markedly schedule dependent, with more prolonged exposures producing a greater effect (5). However, the toxicology studies in dogs demonstrated that the tolerable dose of trimetrexate administered on a schedule resulting in prolonged exposure of the drug (daily for 5 days) was over 10-fold less than the dose tolerated as a single i.v. bolus.

In this report we present the results of a phase I trial and pharmacokinetic study of trimetrexate, administered by i.v. bolus injection on a weekly schedule, in pediatric patients with refractory cancer. Methotrexate, administered on a weekly schedule, has proved to be effective and is widely used in clinical practice. The choice of this schedule for evaluating trimetrexate will allow for the direct comparison of these agents in future trials.

MATERIALS AND METHODS

Patient Eligibility. Patients between the ages of 1 and 21 years with histologically confirmed cancer refractory to conventional forms of therapy were eligible for this trial. Patients must have recovered from the toxic effects of prior therapy before receiving trimetrexate. Patients with a solid tumor (without bone marrow involvement) were required to have a granulocyte count greater than 1,500/μl and a platelet count greater than 100,000/μl, and all patients had a bilirubin less than 2.0 mg/dl, serum transaminases less than 1.5 times normal, and normal serum creatinine (for age) and electrolytes.

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

Study Design. The primary objective of the phase I trial was to define the toxicities and determine the maximally tolerated dose of trimetrexate in children when administered as an i.v. bolus weekly for three doses. Courses of therapy were repeated every 28 days. A minimum of three patients evaluable for toxicity was treated at each dose level, and at least two at each dose level had to have adequate bone marrow function to evaluate hematological toxicity. The starting dose for this study was 35 mg/m²/dose, which was 70% of the dose that was being tolerated in an adult phase I trial with the same schedule at the time our trial was opened. The dose was then escalated by approximately 30% increments until dose-limiting toxicity was consistently observed. Dose levels studied were 35, 50, 65, 85, 110, and 145 mg/m²/dose.

Patients were monitored with complete blood counts, electrolytes, creatinine, calcium, phosphorus, uric acid, liver function tests, and urinalysis weekly. They were also closely followed for clinical signs of toxicity. In patients with measurable disease, other laboratory or radiological examinations pertinent to tumor response were obtained at the end of each 28 day cycle.

Individual patients were removed from the study if they experienced unacceptable toxicity or if objective disease progression was noted after one or more courses of trimetrexate. The phase I trial was terminated at a dose level at which consistent dose-limiting toxicity was observed.

Drug Formulation and Administration. Trimetrexate was supplied by the Investigational Drug Branch of the National Cancer Institute as a sterile lyophilized powder in 5-ml vials containing 50 mg of the drug as the glucuronate salt. The drug was reconstituted with 1.9 ml of sterile water (25 mg/ml) and injected by i.v. bolus. Because of its insolubility in chloride-containing solutions, trimetrexate could only be injected into i.v. lines containing 5% dextrose in water. Slowing the rate of injection prevented local reactions observed in some patients at higher dose levels.

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Pharmacokinetics. Twenty-five patients (minimum of three at each dose level) were studied after their first dose of trimetrexate. Blood samples were obtained prior to the dose and at 5, 15, 30, and 45 min and 1, 1.5, 2, 4, 6, 8, 12, 24, and 48 h after the dose. The blood samples were collected in heparinized tubes and placed on ice until plasma was separated by centrifugation. Plasma samples were stored frozen at −20°C until assayed. All urine was collected for the 48 h following the dose in 24-h aliquots. Following the collection, the urine was well mixed, the total volume measured, and an aliquot was removed and frozen for later assay. CSF3 was obtained from one patient 2 h after a dose.

Trimetrexate in plasma and urine was measured by a reversed phase HPLC assay previously described (4). Trimethoprim (Sigma Chemical Co., St. Louis, MO) was added to plasma samples prior to extraction as an internal standard. Trimetrexate was extracted and concentrated from plasma using C18 SEP-PAK cartridges (Waters Associates, Milford, MA) and then injected onto a 5-μm C18 Radial-PAK column (Waters Associates). The mobile phase contained 0.02 mM KH2PO4, pH 4.5, and 25% acetonitrile and was pumped at a rate of 2 ml/min. The eluent was monitored with a UV detector at a wavelength of 240 nm. Retention times for trimethoprim and trimetrexate were 2.8 and 5.0 min, respectively. The lower limit of sensitivity for the concentrated samples is 0.05 μM. Urine samples were filtered through a 0.2-μm Millex-FG filter unit (Millipore Corp., Bedford, MA), diluted, and injected directly onto the column. The concentration of trimetrexate in the CSF sample was too low to be detected by HPLC, so the dihydrofolate reductase inhibition assay was used to measure trimetrexate concentration (4, 6).

Metabolites of trimetrexate in the urine samples were detected by HPLC as previously described (4). Metabolites partially purified from urine samples by HPLC were subjected to β-glucuronidase, type VIII (Sigma) and reinjected onto the column to determine if the metabolite(s) were conjugated derivatives.

Pharmacokinetic parameters were determined by model-independent methods. AUC was derived by the trapezoidal method and extrapolated to infinity (7). CIss was calculated by dividing the dose by the AUC, and renal clearance by dividing the amount of parent drug excreted in the urine over 48 h by the AUC. The volume of distribution at steady state was calculated from the area under the moment curve (8). t1/2 was determined by regression analysis.

RESULTS

Phase I Trial. A total of 40 patients were entered on this trial. Thirty were evaluable for toxicity and of these, 19 were evaluable for hematological toxicity (no bone marrow involvement with tumor). The 10 inevaluable patients included (a) three patients with rapidly progressive disease necessitating a change in therapy before completion of a course of trimetrexate (two with acute lymphoblastic leukemia, one with neuroblastoma); (b) three patients withdrawn from the study by their parents before completing a course (glioblastoma multiforme, Wilms' tumor, and acute lymphoblastic leukemia); (c) three patients who died of their underlying disease prior to completing one course (acute nonlymphocytic leukemia, pinealoblastoma, and astrocytoma); and (d) one patient with osteosarcoma whose therapy was discontinued after two doses when he developed an acute intracerebral hemorrhage related to a large calcified subdural metastasis. These 10 inevaluable patients did not experience any unusual or severe toxicities from trimetrexate while on study.

The characteristics of the evaluable patients are listed in Table 1. The vast majority of patients on this trial had been heavily pretreated with multiple chemotherapy regimens and, in many cases, radiation therapy. Table 2 summarizes the

<table>
<thead>
<tr>
<th>Weekly dose (mg/m²)</th>
<th>No. of patients/no. of courses</th>
<th>No. with dose-limiting toxicity</th>
<th>Nature of toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>3/3</td>
<td>0</td>
<td>Thrombocytopenia (1)*</td>
</tr>
<tr>
<td>50</td>
<td>4/5</td>
<td>1</td>
<td>Pancytopenia (2), mucositis (1), renal (1) and hepatic (1) dysfunction</td>
</tr>
<tr>
<td>85</td>
<td>7/7</td>
<td>0</td>
<td>Pancytopenia (2), rash (2), mucositis (1)</td>
</tr>
<tr>
<td>110</td>
<td>6/6</td>
<td>2</td>
<td>Pancytopenia (2), rash (2), mucositis (1)</td>
</tr>
</tbody>
</table>

- Numbers in parentheses, number of patients.
to normal. Serum bilirubin increased from 0.5 (pretreatment) to 5.8 mg/dl on the 10th day. The bilirubin was primarily conjugated and did not return to normal levels. The persistent elevation appeared to be related to the underlying intraabdominal tumor. Pharmacokinetics monitoring performed in this patient revealed a low CI\textsubscript{total} (10.8 ml/min/m\textsuperscript{2}) and prolonged t\textsubscript{1/2} (20 h) compared to other patients. In addition, this patient had the highest plasma concentrations of trimetrexate at 24 and 48 h after the dose (5.36 and 3.07 \mu M, respectively).

The maximally tolerated dose of trimetrexate administered on a weekly schedule for three doses in pediatric patients was 110 mg/m\textsuperscript{2}. While two of the six patients at this level experienced dose-limiting toxicities, in one (the patient described above) the severe toxicity appears to be related to delayed drug clearance and prolonged exposure to micromolar concentrations of the drug.

Other non-dose-limiting toxicities observed included mild, dose-related, transient elevations in serum transaminases (2- to 3-fold higher than baseline levels), mild nausea and vomiting at all dose levels, and local phlebitis at the site of drug injection in three patients receiving doses of 110 mg/m\textsuperscript{2} and above.

No complete or partial responses were observed in the 29 patients evaluable for response to trimetrexate. Two patients with acute lymphoblastic leukemia had transient clearing of peripheral blasts and one patient with medulloblastoma had stable disease on trimetrexate for 3.5 months.

Pharmacokinetics Study. Twenty-five patients were monitored following the first dose of trimetrexate. Pharmacokinetics parameters derived from these plasma levels at the various dose levels are listed in Table 3. There was considerable interpatient variability in drug disposition as illustrated in the wide range of values of CI\textsubscript{total} (9.2 to 215 ml/min/m\textsuperscript{2}) and t\textsubscript{1/2} (2.1 to 20 h). An example of this variability is also shown in Fig. 1 which is a plot of the plasma disappearance curves of two patients treated at the 145-mg/m\textsuperscript{2} dose level. There was no apparent dose dependence of the kinetic parameters over the dose range studied.

A CSF drug level was obtained in one patient, 2 h after the dose and was approximately 1% of the simultaneous plasma drug concentration.

Trimetrexate was cleared primarily by biotransformation. Renal clearance accounted for only 10% of the total trimetrexate clearance. Two chromatographic peaks with retention times and dihydrofolate reductase-inhibiting capacity identical to metabolites previously detected in monkeys (4) were found in the urine of patients. These more polar metabolites accounted for a majority of the enzyme-inhibiting substances in the urine. Following partial purification by HPLC, the metabolites were incubated with \beta-glucuronidase. The chromatographic peak of the major metabolite completely disappeared after glucuronidase digestion indicating that it is a glucuronide conjugate. However, the glucuronidase-digested metabolite was still capable of inhibiting dihydrofolate reductase. The second metabolite could not be tested because of an interfering peak in the \beta-glucuronidase preparation.

Although there were too few patients with hematological toxicity studied to make valid statistical correlations between pharmacokinetics parameters and toxicity, there was a suggestion of a relationship between the plasma concentration of trimetrexate at 24 h and the development of significant hematological toxicity. Of the three patients studied at the 145-mg/m\textsuperscript{2} dose level who were evaluable for hematological toxicity, the two with dose-limiting toxicity had plasma trimetrexate concentrations 24 h after a dose of 3.49 and 2.09 \mu M while a single patient with no hematological toxicity had a plasma trimetrexate concentration 24 h after a dose of less than 0.1 \mu M.

DISCUSSION

In this pediatric phase I trial of trimetrexate, the hematological and nonhematological dose-limiting toxicities (rash and mucositis) appeared to be dose related. The dose recommended for phase II trials using this schedule is 110 mg/m\textsuperscript{2}.

In a preliminary report the maximally tolerated dose in an adult trial utilizing the same schedule was 50 mg/m\textsuperscript{2} (9). In general, hematological toxicity has been the major dose-limiting toxicity in the adult trials regardless of the schedule (9-14). Other toxicities observed in these trials included rash (9-14), mucositis (11, 12, 14), nausea and vomiting (11, 12, 14), and renal dysfunction (9) and are identical to those observed in the pediatric patients on the present trial.

Trimetrexate pharmacokinetics were characterized by considerable interpatient variability, especially in drug clearance. As a result, the duration of exposure to micromolar concentrations of the drug varied significantly. There is a suggestion from our findings that patients with the higher plasma drug concentrations 24 h after the dose were at higher risk to develop hematological toxicity. Although toxicity was dose related, at the highest dose level only the two patients with micromolar trimetrexate concentrations 24 h had myelosuppression. In addition, the patient with the most severe toxicity and delayed clearance had micromolar levels persisting for greater than 48 h. In a recent phase I trial in adults on a single i.v. bolus dose schedule, a similar correlation between the 24-h trimetrexate plasma concentration and toxicity was observed (14). In other

### Table 3: Pharmacokinetics parameters of trimetrexate in children

<table>
<thead>
<tr>
<th>Dose</th>
<th>n</th>
<th>AUC (\mu M x h)</th>
<th>CI\textsubscript{total} (ml/min/m\textsuperscript{2})</th>
<th>Renal clearance (ml/min/m\textsuperscript{2})</th>
<th>Volume of distribution at steady state (l/m\textsuperscript{2})</th>
<th>t\textsubscript{1/2} (h)</th>
<th>Concentration of trimetrexate at 24 h (\mu M)</th>
<th>Urinary excretion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>5</td>
<td>64.2 ± 63.7</td>
<td>47.5 ± 37.7</td>
<td>3.2 ± 3.5</td>
<td>11.8 ± 9.7</td>
<td>5.3 ± 2.5</td>
<td>0.40 ± 0.68</td>
<td>8.6 ± 7.0</td>
</tr>
<tr>
<td>50</td>
<td>5</td>
<td>76.0 ± 33.1</td>
<td>37.3 ± 23.0</td>
<td>8.9 ± 2.5</td>
<td>12.6 ± 7.5</td>
<td>6.7 ± 2.8</td>
<td>0.41 ± 0.27</td>
<td>14.7 ± 7.7</td>
</tr>
<tr>
<td>65</td>
<td>4</td>
<td>116 ± 44</td>
<td>28.8 ± 12.4</td>
<td>3.1 ± 3.7</td>
<td>8.4 ± 2.7</td>
<td>7.6 ± 2.0</td>
<td>0.69 ± 0.75</td>
<td>9.2 ± 9.6</td>
</tr>
<tr>
<td>85</td>
<td>4</td>
<td>176 ± 106</td>
<td>32.9 ± 26.3</td>
<td>2.7 ± 0.9</td>
<td>8.9 ± 2.2</td>
<td>7.7 ± 2.2</td>
<td>0.97 ± 1.0</td>
<td>15.6 ± 1.3</td>
</tr>
<tr>
<td>110</td>
<td>4</td>
<td>309 ± 140</td>
<td>19.1 ± 9.1</td>
<td>4.3 ± 3.0</td>
<td>15.0 ± 3.9</td>
<td>12.0 ± 5.5</td>
<td>3.1 ± 2.0</td>
<td>20 ± 13</td>
</tr>
<tr>
<td>145</td>
<td>3</td>
<td>225 ± 174</td>
<td>85.7 ± 113</td>
<td>4.5 ± 5.1</td>
<td>17.0 ± 8.7</td>
<td>7.1 ± 3.4</td>
<td>2.1 ± 1.5</td>
<td>8.0 ± 3.6</td>
</tr>
</tbody>
</table>

* Only unchanged drug.
* Mean ± SD.
* n = 22; there were three incomplete urine collections, 2 at the 50-mg/m\textsuperscript{2} dose level and one at the 85-mg/m\textsuperscript{2} dose level.
* 0.46 liters/kg (mean).
studies, variability in drug tolerance has been noted (12), which may reflect the variability in drug kinetics. Statistical correlation of the pharmacokinetics parameters with toxicity requires a larger patient population. For this reason appropriate pharmacokinetics studies should be incorporated into the design of future phases I and II trials.

Penetration of trimetrexate into the central nervous system was very limited in the single patient studied in this trial and is consistent with previous studies in animals demonstrating CSF levels of <5% of plasma levels (4). The poor penetration was attributed to extensive protein binding of the drug in plasma.

Previous reports have indicated that renal excretion is the major route of elimination for trimetrexate (12, 15). However, in the present study we used a specific HPLC assay which can separate trimetrexate from its two previously identified metabolites that are capable of interfering with the dihydrofolate reductase inhibition and the competitive protein binding assays (4). In our patients renal clearance was only a minor route of elimination, suggesting that renal dysfunction may not be an important determinant of dose modifications. Further pharmacokinetics studies in patients with renal dysfunction will be required to confirm this. However, this is an important difference between trimetrexate and methotrexate.

REFERENCES

Pediatric Phase I Trial and Pharmacokinetic Study of Trimetrexate

Frank M. Balis, Ramesh Patel, Enrique Luks, et al.


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