Phase I and Clinical Pharmacology Study of Trimetrexate Administered Weekly for Three Weeks

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ABSTRACT

Trimetrexate, a new antifolate compound, was administered by 30-min infusions weekly for 3 weeks to 29 patients with solid tumors in a Phase I study. Thrombocytopenia was dose limiting, but highly variable among patients at a given dose level; other toxicity was mild and uncommon. Twenty-three patients participated in pharmacokinetic studies and five patients participated in a study of the effects of trimetrexate on [6-3H]-deoxyuridine incorporation into hematopoietic cell DNA. The median total body clearance of trimetrexate for each dose level was independent of dose but the total body clearance varied widely among patients at a given dose level. The magnitude of the fall in platelet count in individual patients correlated well with the amount of exposure to trimetrexate, but not with the extent of prior therapy. The amount of [6-3H]deoxyuridine incorporation into hematopoietic cell DNA at 72 h after drug administration correlated with the total body clearance of trimetrexate. The total body clearance of trimetrexate was reduced in patients with impaired hepatic synthetic function, as judged by low pretreatment serum albumin concentrations. The recommended Phase II starting dose on this schedule is 130 mg/m² weekly for 3 weeks; patients with hypoalbuminemia should be treated at lower doses.

INTRODUCTION

TMTX is a competitive inhibitor of dihydrofolate reductase with oncolytic activity against murine and human cell lines and against primary cultures of human tumors (1-4). Unlike the classical antifolates, TMTX enters cells by passive diffusion and does not undergo intracellular polyglutamylation; however, it concentrates in leukemic cells to a greater extent than methotrexate (5). Cells which are resistant to methotrexate due to loss of the ability to transport folates remain sensitive to TMTX; partial sensitivity to TMTX is preserved in those cell lines which overproduce dihydrofolate reductase (4, 6, 7).

This paper presents a Phase I study of TMTX administered by 30-min infusions weekly for 3 weeks to 29 patients with solid tumors. This schedule of administration was evaluated because of the efficacy of methotrexate when given by a weekly schedule and because of the suggestion that TMTX was more efficacious in in vivo murine systems when administered intermittently (4). Twenty-three of the patients participated in a clinical pharmacology study; in these patients the observed toxicity was correlated with the 24-h plasma concentration of TMTX and with selected pharmacokinetic parameters. The inclusion of patients with varying hepatic and renal function permitted an analysis of the influence of these factors on the clearance of TMTX. In addition, the effect of TMTX on [6-3H]deoxyuridine incorporation into DNA from hematopoietic cells was studied in five treated patients.

MATERIALS AND METHODS

Patient Selection and Characteristics. Twenty-nine patients with histologically proven solid tumors refractory to standard therapy and a life expectancy of at least 8 weeks were entered into the study; the patient characteristics are summarized in Table 1. All patients had a Karnofsky performance status of ≥50%, and 79% of the patients had a performance status of 70% or better. No patient had received chemotherapy or radiotherapy within 4 weeks of this study (6 weeks in the case of nitrosoureas or mitomycin). All patients had the following pretreatment hematological and biochemical parameters: WBC > 4000/mm³, platelet count > 150,000/mm³, serum total bilirubin and serum creatinine < 1.5 mg/dl. Patients were allowed to receive acetaminophen and narcotics for pain control and benzodiazepines or antihistamines for sleep.

The extent of prior therapy was quantified for each patient by calculating a prior therapy score, defined as: prior therapy score = (weeks of chemotherapy × number of drugs given concurrently) + (weeks of radiotherapy × number of sites treated concurrently). The mean prior therapy score for the 29 patients was 66 (range, 0-200).

Treatment and Dose Escalation. TMTX, supplied by the Division of Cancer Treatment, National Cancer Institute, was dissolved in 5% dextrose in water and administered by constant infusion over 30 min. One cycle of treatment consisted of a weekly injection of TMTX for 3 weeks followed by a 2-week observation period. The starting dose was 50 mg/m²/week × 3 weeks, which was based on the results of ongoing single dose Phase I studies (8, 9). The proposed dose escalation schedule utilized the following modified Fibonacci sequence: 100, 160, 240, and 300 mg/m²/week × 3 weeks.

History and physical examination were obtained weekly and a complete blood count and biochemical screening profile were obtained twice weekly. Standard toxicity criteria were used (10). Tumor response was assessed every 5 weeks by physical examination and/or X-ray examination. Standard response criteria were used (10). Escalation to a higher dose level was allowed in individual patients who had stable disease and no toxicity at a given dose level. Responding patients and stable patients with Grade 1 or 2 toxicity were retreated at the same dose level.

Pharmacokinetic Studies. The elimination of the initial dose of TMTX was studied in 23 patients. Heparinized blood samples were obtained pretreatment, at end of infusion, and 2, 4, 10, 15, 20, 30, 45, 60, 90, 120 min, and 3, 4, 6, 8, 12, 24, 32, 48, 56, 72, 80, 96 h after the infusion. Urine was collected in 4-h aliquots for the first 24 h, then in 12-h aliquots through 96 h. Blood samples were collected on ice, spun down, and the plasma was stored at -70°C. Urine volumes were recorded and aliquots stored at -70°C.

Analytical Methodology. TMTX was extracted from samples of urine and plasma by elution from bonded phase extraction columns as described in (11), except that the internal standard was 6-[3,4,5-trime-
Table 1  Patient characteristics

<table>
<thead>
<tr>
<th>Performance status (Karnofsky)</th>
<th>No. of patients</th>
<th>% of total patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>2</td>
<td>7</td>
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<tr>
<td>60%</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>70%</td>
<td>11</td>
<td>38</td>
</tr>
<tr>
<td>80%</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td>90%</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td>100%</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

Prior therapy

None 1 3
Chemotherapy 8 28
Radiation therapy 1 3
Chemo and radiation therapy 19 66

* Prior therapy score: range, 0–200; mean, 66.

Thiophenylamino[methyl]-2,4-quinazolinediamine monooacetate (JB-7), obtained from Warner-Lambert, Ann Arbor, MI. The Internal standard and TMTX were measured by reversed-phase HPLC with UV detection, also described in Ref. 11, except that the 4.6-mm x 10-cm analytical column contained 5 μm spherical particles (RIPI-18; Brownlee Co., Menlo Park, CA) and the mobile phase consisted of 56% 0.01 M KH₂PO₄, pH 7.0, and 44% acetonitrile at a flow rate of 2 ml/min. The retention time of the internal standard was 2.5 min and of the TMTX was 4.0 min. The linear range of the assay was 0.04 to 50.0 μg/ml with a coefficient of variation of <5% for a 1.0 μg/ml standard.

Plasma samples were also analyzed for TMTX by DHFR inhibition utilizing an automated centrifugal analyzer (Cobas-Bio; Roche Diagnostics, Nutley, NJ), as described in Ref. 12. The range of the assay was 0.002–2.0 μg/ml with a coefficient of variation of <10% for a 0.8 μg/ml standard.

Urine samples of some treated patients were screened for metabolites by HPLC as described above, except that the concentration of acetonitrile in the mobile phase was adjusted from 3 to 30% at a constant rate over 30 min and then was maintained at 30%. The retention time of TMTX with this mobile phase was 45 min. One-mI fractions of eluant were collected, evaporated to dryness, resuspended in H₂O, and tested for inhibition of DHFR.

Binding to proteins in plasma taken from a normal volunteer was measured at TMTX concentrations of 0.1, 1.0 and 10 μg/ml by ultrafiltration through 10,000 molecular weight exclusion membranes (Centricon System, Amicon Co., Danvers, MA). The concentrations of TMTX in the plasma and in the ultrafiltrates were measured by HPLC.

Pharmacokinetic Calculations. The plasma concentration (C) versus time (t) data measured by HPLC were fit to the equation

\[
C_t = \sum \frac{\partial}{\lambda_i} e^{-\lambda_i t}
\]

where N is the number of exponential terms, using PCNONLIN (Statistical Consultants, Inc., Lexington, KY), an iteratively reweighted nonlinear least squares regression program (13). The elimination half-lives were calculated from the equation \( t_{1/2} = \ln 2 / \lambda_i \). The values for \( \lambda_i \) were corrected for the 30-min infusion time (14). The area under the concentration versus time curve from beginning of administration to infinity \((AUC_{0-\infty})\) was calculated using the equation

\[
AUC_{0-\infty} = \sum \frac{\partial_i}{\lambda_i}
\]

and the area-under-the-moment curve from beginning of administration to infinity \((AUMC_{0-\infty})\) was calculated using the equation

\[
AUMC_{0-\infty} = \sum \frac{\partial_i}{\lambda_i^2}
\]

The total body clearance \((Cl_b)\), the renal clearance \((Cl_R)\), the nonrenal clearance \((Cl_{nr})\), and the volume of distribution at steady state \((V_{dss})\) were calculated from the \(AUC_{0-\infty}\) and \(AUMC_{0-\infty}\) using the following equations (14):

\[
Cl_b = \frac{\text{dose}}{AUC_{0-\infty}}
\]

\[
V_{dss} = \frac{\text{dose}(AUMC_{0-\infty})}{(AUC_{0-\infty})^2}
\]

\[
Cl_R = Cl_b - Cl_{nr}
\]

\[
Cl_{nr} = X/AUC_{0-\infty}
\]

where X is the total amount of TMTX eliminated in urine.

Correlations between Clinical Parameters and Drug Exposure. The data for the 29 patients were examined for a correlation between the prior therapy score and indices of hematopoietic toxicity. Pair-wise correlations between selected indices of drug exposure and indices of hematopoietic toxicity were investigated for each of the 23 patients who participated in the pharmacokinetic study. The indices of drug exposure were the observed 24-h plasma TMTX concentration, the \(AUC_{0-\infty}\) terminal phase \(t_{1/2}\), and the \(Cl_b\). Because pretreatment WBC and platelet counts were highly variable among the patients, the parameters-percent change in WBC and percent change in platelet count were used as indices of hematopoietic toxicity. These parameters were defined by: percent change = 100 \( \times \) (initial count – nadir count)/initial count. The nadir counts were the lowest counts observed at any time during the first cycle of treatment.

The inclusion of patients in this study with variable hepatic and renal function permitted an analysis of pair-wise correlations between the indices of drug exposure and pretreatment biochemical parameters. The biochemical parameters were the serum albumin and SGOT concentrations and the creatinine clearance.

Since the distributions of the parameters were highly nonnormal, the nonparametric Spearman rank order correlation was employed for the statistical analysis (15). The significance level was adjusted for the 18 pair-wise correlations examined, and correlations were judged significant when \( P < 0.002 \).

Incorporation of [6-3H]Deoxyuridine into Hematopoietic Cell DNA. The effect of TMTX on incorporation of [6-3H]deoxyuridine into hematopoietic cell DNA was examined in five patients receiving doses of 50 or 75 mg/m². Samples of bone marrow were aspirated from the posterior iliac crest under local anesthesia into heparinized syringes immediately before treatment and 24 and 72 h after treatment. Thirty μl of [6-3H]deoxyuridine, specific activity 1.0 mCi/μg (New England Nuclear, Boston, MA), were added to 1.5 ml of bone marrow aspirate and incubated at 37°C for 20 min. The reaction was stopped and the red cells lysed by the addition of 7.5 ml of ice-cold 3% acetic acid. The nucleated cells were pelleted by centrifugation then lysed by freeze thawing. The radioactivity in the perchloric acid insoluble DNA fraction was measured by scintillation spectrophotometry as described in (16). The amount of DNA in each sample was quantified by reaction with 3,5-diaminobenzic acid, as described in Ref. 17. Differences in incorporation of radioactivity between pretreatment and 24- and 72-h samples were analyzed using Friedman’s test (18).

RESULTS

Dose Escalation, Toxicity, and Responses. Table 2 summarizes the dose escalation and observed hematological toxicity. Grade 3 hematological toxicity occurred in two patients at the

<p>| Table 2  Hematopoietic toxicity |
|--------------------------|----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Dose (mg/ m²/week ×3)</th>
<th>No. of patients</th>
<th>No. of courses</th>
<th>WBC nadir (range)</th>
<th>Platelet count (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>7</td>
<td>13</td>
<td>2.9 (2.1–3.9)</td>
<td>111 (49–347)</td>
</tr>
<tr>
<td>100</td>
<td>4</td>
<td>6</td>
<td>2.4 (1.3–3.6)</td>
<td>144 (30–314)</td>
</tr>
<tr>
<td>75</td>
<td>8</td>
<td>8</td>
<td>3.6 (0.9–9.7)</td>
<td>132 (11–358)</td>
</tr>
<tr>
<td>90</td>
<td>9</td>
<td>9</td>
<td>4.0 (3.0–7.1)</td>
<td>163 (89–217)</td>
</tr>
<tr>
<td>110</td>
<td>3</td>
<td>3</td>
<td>4.6 (4.5–7.9)</td>
<td>250 (182–296)</td>
</tr>
<tr>
<td>130</td>
<td>7</td>
<td>14</td>
<td>5.3 (1.1–7.0)</td>
<td>64 (9–235)</td>
</tr>
<tr>
<td>155</td>
<td>2</td>
<td>3</td>
<td>4.9 (4.5–5.3)</td>
<td>102 (93–165)</td>
</tr>
</tbody>
</table>

* Median nadir (range) × 1000/mm³.
* Both patients previously treated at 130 mg/m².
second dose level (100 mg/m²). This led to a reduction in dosage level to 75 mg/m² for the next cohort of patients; subsequent dose levels were escalated by 20% increments.

Myelosuppression was observed at all dose levels and was dose limiting. The time required for marrow recovery was approximately 1 week. Thrombocytopenia occurred in excess of neutropenia, but considerable variability was observed in the severity of myelosuppression among patients at a given dose level. Twelve patients were unable to complete the initial cycle of treatments as scheduled due to Grade 2 or higher hematological toxicity. However, five of six patients at 90 mg/m², all the patients at 110 mg/m², and five of the seven patients at 130 mg/m² completed the treatments as scheduled. Eight patients were escalated to a higher dose level.

There were two treatment-related deaths, both occurring in association with thrombocytopenia. The first patient was a 63-year-old with adenocarcinoma of the lung and a prior therapy score of 64. She was hospitalized with fever and myelosuppression (WBC, 900/mm³; platelet count, 11,000/mm³) 7 days after a single dose of 75 mg/m² of TMTX. Despite platelet transfusions, she experienced a sudden massive gastrointestinal hemorrhage with hypotension and cardiac arrest; there was no prior history of gastrointestinal disease. This patient participated in the pharmacokinetic study and in the [6-3H]-deoxyuridine incorporation study and is listed as patient 9 in Tables 4 and 5. The second patient was a 49-year-old woman with pulmonary and pleural metastasis from cutaneous angiosarcoma and a prior therapy score of 55. She had sudden onset of respiratory distress after two doses of TMTX at 100 mg/m² due to a large hemorhagic pleural effusion in association with a platelet count of 30,000/mm³. She continued to bleed heavily from a chest tube despite platelet transfusion and died several days later. Both patients had low pretreatment serum albumin concentrations; i.e., 3.3 and 3.6 gm/dl, respectively, (normal range, 4.0–5.2 gm/dl).

Except for myelosuppression, little toxicity was observed (Table 3). Eight patients who received multiple courses of treatment had mild generalized cutaneous hyperpigmentation. Four patients had acute maculopapular rashes with scaly desquamation; skin biopsy in one patient revealed leucocytoclastic angiitis. The hepatic toxicity consisted of a rise in serum transaminases and the renal toxicity consisted of a rise in serum creatinine; both parameters returned to base line within 2 weeks of treatment.

Twenty-three patients were evaluable for response. A partial response of 2.5 months’ duration was observed in a patient with squamous carcinoma of the esophagus and pulmonary metastasis. Minor responses were observed in three heavily pretreated patients.
patients with adenocarcinoma of the breast and in one patient with adenocarcinoma of the lung. The last patient also had a marked improvement in his chronic generalized psoriasis while receiving TMTX.

Elimination of TMTX. The concentrations of TMTX in plasma obtained over 96 h after a dose of 50 mg/m² to patient 3 (Table 4), measured by HPLC and by DHFR inhibition, and after a dose of 130 mg/m² to patient 22 (Table 4), measured by HPLC, are summarized in Fig. 1. TMTX declined 2 logs in concentration over 48 h and was no longer detectable by HPLC beyond 72 h. The concentrations measured by DHFR inhibition were substantially higher than the concentrations measured by the specific HPLC assay, particularly at the latter time points.

The elimination data, as determined by HPLC, were best fit by a triexponential equation (N = 3) with the sum of squares interactions weighted by 1/(concentration)²; the pharmacokinetic parameters are summarized in Table 4. There was considerable variation in the elimination of TMTX among patients at a given dose level, as shown by a comparison of the observed 24-h plasma concentration, AUC₀₋₂₄ and Clₚ. The median Clₚ for each dose level was independent of TMTX dose (rₛ = 0.11, P = 0.630). TMTX was eliminated from the plasma primarily by nonrenal clearance; the Cl of unchanged drug accounted for a mean of 33% of the Clₚ. Chromatograms of urine revealed the presence of two unidentified early eluting peaks (Fig. 2); fractions obtained from each peak strongly inhibited DHFR.

The binding of TMTX at concentrations of 0.1, 1.0, and 10 μg/ml to plasma proteins of a normal volunteer was >98%.

Correlation between Clinical and Pharmacokinetic Parameters. There was no correlation between the prior therapy score and the percent change in platelet count (rₛ = -0.23, P = 0.20) or the percent change in WBC count (rₛ = 0.17, P = 0.28). The percent change in platelet count was strongly correlated with the observed 24-h plasma concentration of TMTX (rₛ = 0.66, P = 0.0007; Fig. 3) and with AUC₀₋₂₄(rₛ = 0.68, P = 0.0005). There was a strong negative correlation between percent change in platelet count and the Clₚ (rₛ = -0.70, P = 0.0003; Fig. 4). A correlation between percent change in WBC and 24-h plasma concentration of TMTX was suggested (rₛ = 0.51, P = 0.01); however, there was no correlation between percent change in WBC and Clₚ. The terminal phase tₙ could not be correlated with the percent change in platelet count or the percent change in WBC.

The Clₚ was strongly correlated with pretreatment serum albumin concentration (rₛ = 0.66, P = 0.0005; Fig. 5) but not with pretreatment SGOT or creatinine clearance. A negative
correlation between the terminal phase $t_n$ and albumin concentration was suggested ($r = -0.49$, $P = 0.02$); there was no correlation between the terminal phase $t_n$ and SGOT or creatinine clearance. The 24-h concentration of TMTX did not correlate with pretreatment albumin, SGOT, or creatinine clearance.

Incorporation of [6-3H]Deoxyuridine into Hematopoietic Cell DNA. The incorporation of [6-3H]deoxyuridine into hematopoietic cell DNA was inhibited to nearly undetectable levels in all 5 patients at 24 h after drug administration, compared to pretreatment levels (Table 5; $P = 0.0008$). There was a trend towards continued inhibition at 72 h after drug administration ($P = 0.06$), but the amount of incorporation varied among the five patients. There was a strong linear correlation between the amount of incorporation at 72 h and the $Cl_a$ for each patient ($r^2 = 0.938$, $P < 0.005$).

**DISCUSSION**

The dose-limiting toxicity of TMTX given on a weekly $\times 3$ schedule is myelosuppression, with thrombocytopenia in excess of neutropenia. The magnitude of the drop in platelet count was highly variable among patients at a given dose level and correlated well with the amount of exposure to TMTX but not with the extent of prior therapy. Variability in the $Cl_a$ value of TMTX was correlated with pretreatment serum albumin.

TMTX was eliminated from the plasma primarily by nonrenal clearance. The mechanism for the nonrenal clearance is biotransformation and elimination in the liver. We and others have detected the presence of two metabolites in plasma and urine (19). Tong et al. have provided evidence that the first metabolite is formed by demethylation at the 4-position of the phenoxyethyl ring and the second by conjugation with glucuronic acid at this site (19). These investigators have also shown that both metabolites were secreted in bile, together with small amounts of the parent drug, by perfused rat liver preparations (20). We and others have found that both metabolites are capable of inhibiting DHFR (19) but the cytotoxic activity of these compounds is unknown.

There can be wide variability among patients in the clearance rates for drugs which are predominantly eliminated by the liver (21). Hepatic blood flow, binding to plasma proteins and the intrinsic ability of the liver to clear a drug from the blood (in the absence of flow limitations) are primary determinants of hepatic clearance (21). Thus, hepatic clearance can be affected by the age, sex, and race of the patients, and by the presence of primary hepatic disease or systemic illnesses. Patients with liver dysfunction may have reduced clearance compared with normal subjects, but there is often considerable overlap (22). However, there is now evidence that when hepatic synthetic functions are markedly diminished, as judged by serum albumin $<3.0–3.5$ g/dl, drug clearance by the liver can be markedly impaired (23).

The incorporation of [6-3H]deoxyuridine into DNA provides a measure of the intracellular activity of thymidylate synthetase which is the rate-limiting enzyme in the conversion of deoxyuridine monophosphate into thymidine triphosphate and its subsequent incorporation into DNA. The results in this study must be interpreted in light of the fact that TMTX-induced changes in deoxyuridine monophosphate pools will result in dilution of the isotope (4). Nevertheless, the inhibition of [6-3H]deoxyuridine incorporation provides relative information on the degree of depletion of tetrahydrofolate cofactors in hematopoietic cells by TMTX and on the time course with which TMTX exerts its effects. The marked suppression of [6-3H] deoxyuridine incorporation at 24 h after drug administration is consistent with rapid diffusion of TMTX from the plasma into cells and its known potent inhibition of DHFR (4), whereas the variability observed at 72 h reflected the ability of individual patients to eliminate the drug.

Based on the observed toxicity, we recommend a Phase II starting dose for TMTX of 130 mg/m$^2$/week for 3 weeks. However, patients with a low serum albumin will have a reduced
clearance and are more likely to have thrombocytopenia; these patients should be initially treated at lower doses.

REFERENCES

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