

Pharmacokinetics of Trimetrexate Administered by Five-Day Continuous Infusion to Patients with Advanced Cancer

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ABSTRACT

The disposition of the methotrexate analogue trimetrexate (TMTX, NSC 352122; 2,4-diamino-5-methyl-6-[(3,4,5-trimethoxyanilino)methyl]quinazoline) was determined in a Phase I study in 16 patients with refractory or relapsing cancer. The drug was administered by continuous 5-day infusion at doses of 5 to 60 mg/m²/120 h (1–12 mg/m² daily for 5 days). Plasma and urine collections were made during and after infusion and TMTX levels were quantitated by a specific and sensitive high-performance liquid chromatographic assay. Estimates of pharmacokinetic parameters were similar when determined by either compartmental or noncompartmental methods. There were no significant differences in parameters between the first and second courses of treatment to 10 of the patients. Significant linear relations between TMTX dose and the area under curve of plasma TMTX ($r^2 = 0.858$, $P = 0.0001$) and the steady-state TMTX plasma level ($r^2 = 0.764$, $P = 0.0001$) were established. Total TMTX clearance was 30.4 ± 7.6 (SD) ml/min/m², renal clearance 7.80 ± 3.9 ml/min/m², nonrenal clearance 23.2 ± 7.1 ml/min/m², volume of distribution 32.8 ± 16.6 liters/m², and terminal half-life 13.4 ± 7.0 h. The percentage of the trimetrexate dose excreted unchanged in urine ranged from 8.4 to 40.7% (mean, $24.9 \pm 9.2\%$) and was related to creatinine clearance ($r^2 = 0.312$, $P = 0.010$). Trimetrexate renal clearance was also related to urine flow ($r^2 = 0.330$, $P = 0.008$). Trimetrexate pharmacokinetics was linear over the dose range 5 to 60 mg/m² when given by 5-day continuous infusion to patients but there was evidence of urine flow-dependent renal clearance which requires further examination.

INTRODUCTION

TMTX¹ is a potent dihydrofolate reductase inhibitor with activity against several tumor cell lines (1–3). TMTX does not undergo polyglutamation and appears to have a broader range of antitumor activity than methotrexate (3). Differences in cell uptake mechanisms for TMTX appear to account for its activity in a number of methotrexate resistant cell lines (2–4). Preclinical studies have been reported in mice (5), dogs (6), and monkeys (7), and there have been several preliminary reports of Phase I trials (8–10).

Pharmacological studies have been reported in animals (5, 7), in patients (8–10), and in the isolated perfused rat liver (11). The drug is metabolized by oxidative demethylation (6, 7, 11) and conjugation with glucuronic acid (12). The glucuronide metabolite was excreted into bile and accounted for 75% of the dose in the isolated perfused rat liver (12). Renal clearance of TMTX was reported to be only 4% of total clearance in monkeys (12), suggesting that this was a relatively unimportant route of elimination for the drug.

Hook *et al.* (13) demonstrated that TMTX cytotoxicity was cell cycle specific and that the drug was most effective with frequent administration to mice. Phase I studies of a 9-day consecutive low-dose schedule (9) and 5-day continuous infu-

sion schedule (10) of the drug have therefore been undertaken in order to prolong plasma levels, minimize toxicity, and maximize antitumor effect. We report here the disposition of TMTX in the plasma and urine of patients with advanced cancer in a Phase I trial of 5-day continuous infusion over the dose range 5–60 mg/m²/120 h (1–12 mg/m² daily for 5 days). Plasma and urinary TMTX levels were measured specifically by high-performance liquid chromatography (14) in order to avoid interference by metabolites with dihydrofolate reductase inhibitory activity (7). The renal clearance of TMTX has not been adequately described in patients and the present study was designed to provide information on this route of elimination. Potential changes in disposition of the drug with repeated courses were also examined.

MATERIALS AND METHODS

Patients. All patients studied had proved solid tumor malignancy which was either refractory or recurrent. Patients had recovered from prior chemotherapy or radiotherapy and had not received chemotherapy for at least 3 or 6 weeks, respectively, in the case of nitrosoureas and mitomycin C. Life expectancy of patients was in excess of 8 weeks. All patients were more than 18 years of age. Liver and renal function tests and serum uric acid were within normal limits prior to entry into the study. Other antineoplastic drugs were not administered during the study period. Pregnant women were excluded from the study and all patients gave written informed consent before proceeding with the study. Comprehensive biochemical, hematological, and clinical profiles were obtained for all patients prior to treatment and at regular intervals throughout treatment. Sixteen patients were studied over a dose range of 5 to 60 mg/m²/120 h (1–12 mg/m² daily for 5 days) (Table 1).

Drug Administration. TMTX as the glucuronate salt was provided by the National Cancer Institute, Bethesda, MD, in vials containing 50 mg for injection. The intact vials were stored at 2–8°C prior to use. The contents of the vial were diluted to 0.1 mg/ml in 5% dextrose-water for infusion. TMTX dosage was calculated on the basis of body surface area and administered by constant rate, continuous i.v. infusion using an infusion pump over 5 days (120 h). This course was repeated every 3 weeks. The starting dose was 5 mg/m²/120 h (1 mg/m² daily for 5 days) and dosage escalation was determined by a modified Fibonacci scheme. No individual patient received a dose escalation. Calcium leucovorin rescue was not used in this study. All patients remained hospitalized during the infusion of the drug and for a minimum of 24 h after completion of the infusion.

Specimen Collection. Pharmacokinetic studies were performed in patients for the first course of therapy at each dose level. The studies were repeated for the second course of therapy in 11 patients (Table 1, Patients 2–6, 8, 9–11, and 16) and for the third course of therapy in 3 patients (Patients 2, 5, and 8). Patient 16 received 60 mg/m²/120 h in the first course and 30 mg/m²/120 h in the second course. Blood samples (5 ml) were collected into heparinized tubes immediately prior to the start of the TMTX infusion and 24, 48, 72, 96, and 120 h during the infusion. Additional samples were collected 0.5, 1, 1.5, 2, 4, 6, 12, and 24 h after the infusion. Blood was centrifuged and plasma was stored at –70°C to await analysis. Twenty-four-h urine collections were made during the infusion, the total volume was noted, and a 10-ml aliquot was stored at –70°C. Urine was collected as voided postinfusion, the volume was noted, and a 10-ml aliquot was stored at –70°C. Urine collections were not made in Patients 4, 5, and 16 (Course 1). All samples were transported in dry ice.

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¹ The abbreviations used are: TMTX, trimetrexate (NSC 352122; 2,4-diamino-5-methyl-6-[(3,4,5-trimethoxyanilino)methyl]quinazoline); AUC, area under the curve; HPLC, high-performance liquid chromatography.

Table 1 Patient characteristics and estimates of pharmacokinetic parameters determined by noncompartmental methods for the first course of therapy

Patient	Sex	Age (yr)	Surface area (m ²)	Dose (mg/m ² /120 h)	Creatinine clearance (ml/min)	AUC (ng/ml/h)	C _{ss} (ng/ml)	Cl(AUC) (ml/min/m ²)	Cl(C _{ss}) (ml/min/m ²)	Cl _r (ml/min/m ²)	Cl _{nr} (ml/min/m ²)	t _{1/2} (h)	% of dose excreted in urine
1	F	69	1.7	5	66.5	3,561	34.0	23.4	20.4	6.46	16.9	13.4	27.6
2	F	62	1.7	5	184	4,311	36.0	19.3	19.3	4.75	14.6	7.25	24.6
3	F	52	1.7	5	83.8	3,140	28.5	26.5	24.4	5.64	21.9	7.25	21.3
4	M	45	1.8	20		14,412	173	23.1	16.1			9.82	
5	M	67	1.7	20		9,982	101	33.4	27.6			12.7	
6	M	58	1.7	30	96.4	16,974	169	29.5	24.7	5.25	24.2	14.3	17.8
7	F	63	1.5	30	40.2	9,622	91.9	52.0	45.3	5.67	46.3	12.4	10.9
8	M	35	1.6	30	46.2	19,494	239	25.6	17.4	5.73	19.9	10.5	22.4
9	M	73	1.8	40	115	21,968	175	30.3	31.7	9.61	20.7	20.8	31.7
10	F	29	1.6	40	105	19,554	211	34.1	26.3	8.63	25.5	12.3	25.3
11	M	60	1.8	40	227	21,836	188	30.5	29.6	12.4	18.1	38.9	40.7
12	M	62	1.8	50	102	31,498	260	26.5	21.4	2.23	24.3	24.6	8.4
13	F	68	1.7	50		22,616	202	27.6	26.4			6.42	
14	F	51	1.7	50			200		34.7				
15	F	50	1.6	50	51.0	38,600	361	21.6	19.2	3.65	17.9	11.9	16.9
16	F	68	1.6	60	103	36,244	316	27.6	26.4			15.4	
Mean		56	1.7		102			29.3	26.1	6.37	22.7	14.5	22.5
SD		12	0.1		55			8.0	7.7	2.9	8.5	8.3	9.2

^a AUC, total area under the plasma level-time curve.
^b C_{ss} steady-state plasma level; Cl(AUC), clearance determined from the total AUC, i.e., dose/AUC; Cl(C_{ss}), clearance determined from the steady-state plasma level, C_{ss}, i.e., infusion rate/C_{ss}; Cl_r, renal clearance; Cl_{nr}, nonrenal clearance; t_{1/2}, terminal half-life.

Analytical Methods. The high-performance liquid chromatographic assay of Ackerley *et al.* (14) was used for the specific quantitation of plasma and urinary levels of trimetrexate with the following modification. The eluate from the Bond Elut column extraction procedure was evaporated and reconstituted in mobile phase. This allowed concentration of the extract and lowered the detection limits of the assay. Chromatography was carried out on 250- x 4.6-mm 5-μm C₁₈ column (Brownlee Associates, Santa Clara, CA) at a column temperature of 50°C. Urine specimens were sonicated and warmed to 37°C to ensure dissolution of any precipitate present prior to analysis. Reduced recovery of TMTX from urine was observed in specimens containing undissolved precipitate.

Pharmacokinetic Analyses. Plasma concentrations of TMTX were analyzed by both compartmental and noncompartmental methods and the results were compared. The former involved use of either a one- or a two-compartmental model where both during and postinfusion data were fitted simultaneously. This approach has been shown to provide more accurate estimates of equation parameters than the use of either during-infusion or postinfusion data separately (15). Either Equation A or Equation B shown below was used to model the data and final parameter estimates were obtained using the BMDP nonlinear least squares program "Nonlinear."

$$C = C' * (1 - e^{-Kt}) * e^{-Kt} \tag{A}$$

$$C = A' * (1 - e^{-\alpha t}) * e^{-\alpha t} + B' * (1 - e^{-\beta t}) * e^{-\beta t} \tag{B}$$

In these equations, *K* was the first order rate constant for elimination; *T* was the infusion time; *t* was the time after start of the infusion; *C'*, *A'*, and *B'* were constants; and α and β were complex rate constants for distribution and elimination, respectively. *C* was the predicted plasma concentration of TMTX based on the model. Estimates of clearance (*Cl*), volume of distribution at steady state (*V_{ss}*), and terminal half-life (*T_{1/2}*) were determined from the coefficients of these equations.

The noncompartmental approach involved determination of the total AUC and area under the first moment curve as described by Gibaldi and Perrier (16). Clearance was also calculated from the mean steady-state plasma level (*C_{ss}*) obtained 72, 96, and 120 h after the start of the infusion. The terminal half-life of the drug was determined by regression analysis of the terminal plasma concentration data points.

TMTX renal clearance (*Cl_r*) was determined from the amount of TMTX excreted over the entire 6-day study period divided by the corresponding area under the curve of plasma TMTX. Renal clearance determined separately for each 24-h period during the infusion did not indicate that there were any time-dependent changes occurring in renal excretion. The percentage of TMTX dose excreted unchanged over the duration of the 6-day study was determined for each patient. Nonrenal clearance (*Cl_{nr}*) was determined from the difference between total and renal clearance.

Creatinine clearance was determined by standard colorimetric methods for each 24-h period of the 6-day study. Mean values were then obtained in each patient for each course.

The percentage change in platelets was determined after each TMTX course from the relationship

$$\% \text{ of change} = 100 \times \frac{\text{pretreatment platelet count} - \text{platelet nadir}}{\text{pretreatment platelet count}}$$

RESULTS

Mean plasma levels of TMTX in patients 1–15 for all courses at 5, 20, 30, 40, and 50 mg/m²/120 h are shown in Fig. 1. Steady state was attained approximately 72 h after the start of the infusion in all patients. Postinfusion levels then declined biexponentially. There were insufficient data points immediately postinfusion to accurately describe the distribution phase in all patients. However, this had a negligible effect on calculations of total clearance and renal clearance by either the compartmental or noncompartmental methods since either the total plasma area under curve both during and postinfusion or steady-state levels were used in these calculations.

Pharmacokinetic parameters determined by noncompartmental methods for the first course of therapy in the 16 patients are shown in Table 1. There was no significant difference in total TMTX clearance when determined from either the AUC or steady-state plasma level. There was also no significant difference between estimates of total clearance and terminal

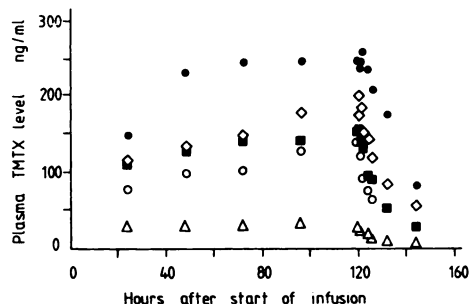


Fig. 1. Mean plasma levels of TMTX in Patients 1–15 for all courses at 5 (Δ), 20 (○), 30 (■), 40 (◇), and 50 (●) mg/m²/120 h. The drug was infused at a constant rate at doses of 1, 4, 6, 8, and 10 mg/m² daily for 5 days.

half-life determined by compartmental and noncompartmental data analysis methods (Table 2). Mean renal clearance was 25% of total clearance; *i.e.*, the percentage of total dose excreted in urine unchanged was 25%. However, urinary recovery of unchanged TMTX was unusually low in Patient 16 (8.4%). Mean nonrenal clearance was calculated to be 75% of total clearance although this did not allow for possible biliary excretion of the drug.

There was a highly significant correlation between dose and the total AUC of TMTX (Fig. 2) ($r^2 = 0.858$, $P = 0.0001$). AUC increased approximately linearly with dose over the range 5–60 mg/m²/120 h. A similar correlation was observed between dose and steady-state TMTX plasma level (Fig. 3) ($r^2 = 0.764$, $P = 0.0001$).

There were no significant differences in pharmacokinetic parameters determined for the first and second courses of therapy to 10 patients. Three patients (Patients 2, 5, and 8) also received a third course of TMTX treatment. There was no evidence of any changes in renal function or pharmacokinetic parameters when comparing this course with the first. Mean clearance (calculated from the AUC) in all patients for all courses was 30.4 ± 7.6 ml/min/m², renal clearance 7.80 ± 3.9 ml/min/m², volume of distribution at steady-state 32.8 ± 16.6 liters/m², half-life 13.4 ± 7.0 min, and the percentage of the dose excreted unchanged in urine $24.9 \pm 9.2\%$.

The percentage of the total TMTX dose excreted unchanged in urine over the duration of study was significantly related to creatinine clearance (Fig. 4) ($r^2 = 0.312$, $P = 0.010$). TMTX renal clearance was also related to creatinine clearance but the

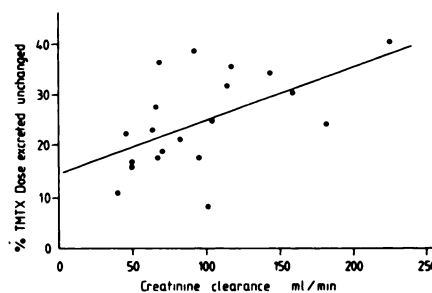


Fig. 4. Relationship between the percentage of the total TMTX dose excreted unchanged in urine and creatinine clearance in all patients for all courses ($r^2 = 0.312$, $P = 0.010$).

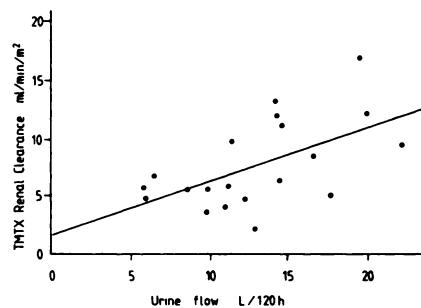


Fig. 5. Relationship between TMTX renal clearance and urine flow in all patients for all courses ($r^2 = 0.330$, $P = 0.008$).

Table 2 Comparison of compartmental and noncompartmental methods of parameter estimation, first course only (mean \pm SD)

	Cl (ml/min/m ²)	<i>t</i> _{1/2} (h)
Compartmental	29.3 \pm 5.4	15.5 \pm 8.6
Noncompartmental	29.3 \pm 8.0 ^a	14.5 \pm 8.3
<i>P</i>	NS ^b	NS

^a Clearance determined from the AUC.
^b NS, not significant.

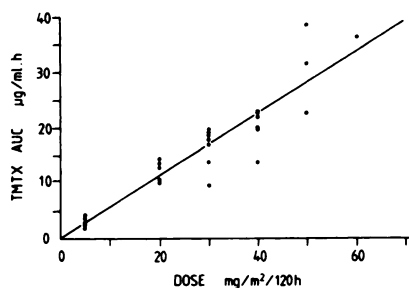


Fig. 2. Relationship between TMTX dose and area under curve of plasma TMTX in all patients for all courses ($r^2 = 0.858$, $P = 0.0001$).

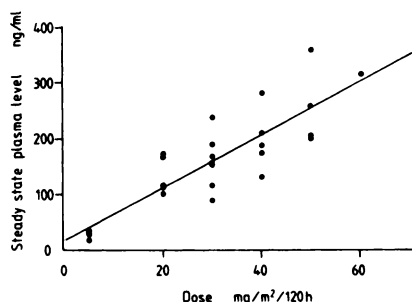


Fig. 3. Relationship between TMTX dose and steady-state plasma level of TMTX in all patients for all courses ($r^2 = 0.764$, $P = 0.0001$).

relationship was not as strong ($r^2 = 0.201$, $P = 0.047$). Urine flow in all patients ranged from 6 to 22 liters/120 h. There was a significant correlation between TMTX renal clearance and urine flow (Fig. 5) ($r^2 = 0.330$, $P = 0.008$).

The percentage of change in platelets in all patients for all courses was significantly related to TMTX AUC ($r^2 = 0.51$, $P = 0.0004$), steady-state plasma level ($r^2 = 0.43$, $P = 0.0016$), and dose ($r^2 = 0.55$, $P = 0.0002$).

DISCUSSION

Comparison of estimates of pharmacokinetic parameters in this study with literature values was possible only where specific assay methods were used in the published studies. Total clearance would be underestimated and renal clearance overestimated in studies using methods which did not distinguish the parent drug from metabolites with dihydrofolate reductase inhibitory activity. For example, using a dihydrofolate reductase assay (8), TMTX renal clearance was recently found to be 25 ± 17 ml/min in patients, compared with a total clearance of 28 ± 17 ml/min. Renal clearance was only 25% of total clearance using a specific assay in the present study. Balis *et al.* (7) found that the TMTX half-life was 199 min in monkeys when determined by HPLC but was 248 min using the dihydrofolate reductase assay. Total clearance was 124 ml/min/m² by HPLC and 73 ml/min/m² using the enzyme assay. Renal clearance was only 5 ml/min/m² when HPLC was used for the analyses. Volume of distribution was 17.8 liters/m² by HPLC and 23.7 liters/m² by the enzyme assay.

Rosen *et al.* (7) recently reported preliminary results from a Phase I study of 5-day continuous infusion where plasma levels were measured by HPLC. Plasma levels decayed biexponentially with a terminal half-life of 44 ± 32 h. This was considerably longer than the half-life found in both the present study (13.4 ± 7.0 h) and in the study of Hook *et al.* (13) (15.2 ± 5.7 h). The method of pharmacokinetic analysis was unlikely to be a contributing factor to such differences since similar parameter

estimates were obtained using either compartmental or non-compartmental data analysis techniques in the present study (Table 2). There also appeared to be no significant differences in half-life between different courses to the same patient.

TMTX clearance in patients given the drug by continuous 5-day infusion varied over almost a 3-fold range. Similar variability has been reported for methotrexate clearance when given by 24-h infusion to patients with acute lymphocytic leukemia (17). Much of the variability in methotrexate disposition could probably be attributed to interpatient differences in renal handling of the drug (18). Methotrexate renal clearance is approximately 50% of total drug clearance. TMTX renal clearance was proportionally less, but highly variable, ranging from 8.4 to 40.7% of total clearance. Urinary excretion of unchanged TMTX in humans is therefore considerably higher than in monkeys (7).

Prior treatment with nephrotoxic drugs such as cisplatin may have affected the renal elimination of TMTX in three of the patients in this study. Patients 3, 8, and 12 had received at least two courses of cisplatin prior to the study. Patient 12 had received the last of two courses of cisplatin 3 weeks immediately prior to the TMTX study. Cisplatin is known to reduce both its own renal elimination (19) and that of methotrexate (20) often without a large change in creatinine clearance (19), probably by interfering with active tubular secretory mechanisms. The extent of the effect is related to cumulative cisplatin dosage and to the time elapsed since cisplatin treatment (20). Patient 12, who eliminated only 8.4% of the TMTX dose via the kidneys in this study, may have had impaired renal tubular secretion of TMTX induced by the cisplatin, despite the fact that creatinine clearance was apparently normal.

Methotrexate has been shown to undergo concentration dependent renal clearance in humans (18). At low doses and low plasma levels, methotrexate renal clearance was found to be linear. At higher doses and higher plasma levels, renal clearance was reduced; this was attributed to saturation of renal tubular reabsorption of the drug. At still higher doses, renal tubular secretion was saturated and renal clearance was decreased, eventually to fall below the level at low doses. There was no evidence in the present study of dose dependent renal clearance of TMTX. However, it is possible that TMTX may be reabsorbed in the kidney since renal clearance was urine flow dependent (Fig. 5). The extent and linearity of TMTX plasma protein binding have not been reported in humans, although it is $90 \pm 1\%$ bound in the monkey (7). Assuming that the free fraction was 10% in the present study, *i.e.*, $f_u = 0.1$, then the product $f_u \cdot \text{GFR}$ was 5.8 ml/min/m². This was less than the mean renal clearance in these patients (7.8 ml/min/m²) and was tentative evidence that TMTX was actively secreted in the kidney. Clearly, the mechanism of TMTX renal clearance requires study once TMTX plasma protein binding characteristics have been established.

Regardless of the mechanism or differences in the renal handling of the drug between patients, TMTX pharmacokinetics was approximately linear over the dose range 5 to 60 mg/m²/120 h; *i.e.*, there was no large systematic change in total clearance or volume of distribution, and the AUC and steady-state plasma level of TMTX increased approximately linearly with dose. However, the design of the Phase I study precluded sequential dose increases within patients in order to confirm this observation.

Recently Evans *et al.* (17) reported that for the same dose, there was a relationship between methotrexate clearance or

steady-state methotrexate plasma levels and the clinical effect of the drug. They suggested that patients with high clearance and corresponding low steady-state plasma levels should receive a dose increase. Since TMTX clearance varied over the same approximate range as methotrexate clearance, potential relationships between clinical effect and pharmacokinetics may be equally relevant for TMTX and require evaluation in more extended trials of the drug. Relationships between the degree of myelosuppression and the AUC or steady-state plasma level of the drug were apparent in the present study.

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