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A phase I trial of trimetrexate glucuronate (NSC 352122) given every 3 weeks: clinical pharmacology and pharmacodynamics*

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Summary. Trimetrexate glucuronate (TMTX), a non-classic folate antagonist, has been evaluated clinically on several schedules. We studied TMTX given as an i.v. bolus over 5–30 min every 3 weeks in 44 patients with advanced solid tumors; it was given at doses ranging from 20 to 275 mg/m^2. The maximal tolerated dose (MTD) on this schedule is 220 mg/m^2, which we also recommend as a starting dose for phase II studies in patients without extensive prior therapy. Because of wide individual differences in drug tolerance, dose escalation in 25% increments is recommended for non-toxic patients. The principal dose-limiting toxicity was myelosuppression, although in some patients a flu-like syndrome precluded dose escalation. Significant rash and mucositis also frequently occurred in toxic patients. TMTX plasma concentrations were measured after the first dose and the data were fit by two- or three-compartment mammillary pharmacokinetic models. The TMTX clearance rate was 36.5 ± 21 ml/min per m^2 and did not change with dose; non-linearities with increasing dose were apparent in the steady-state volume of distribution (V_{SS}) and in the terminal disposition half-life ($t_{½}$). The difference between pre-treatment and nadir leucocyte counts was correlated with TMTX dose ($r = 0.58$; $P = 0.0006$) and with the area under the concentration vs time curve (AUC) ($r = 0.41$; $P = 0.02$). Pre-treatment plasma albumin concentrations correlated weakly with the nadir white blood count ($r = -0.36$; $P = 0.047$). Optimal schedules for the administration of TMTX have not been established and phase II trials using both bolus and daily × 5 schedules are under way.

Introduction

Trimetrexate glucuronate [(6-(3,4,5-trimethoxyphenyl)-aminomethyl)-5-methyl,2,4-quinazoline diamine; TMTX] is one of a series of 2,4-diamino quinazoline antifolates synthesized by Elslager and his colleagues at Parke-Davis. These compounds are potent inhibitors of dihydrofolate reductase and have been explored in vitro and in murine models by Bertino et al. [2, 3] and Jackson et al. [17]. TMTX has several other unique pharmacologic properties that stimulated our interest in further investigating its clinical role. This drug does not enter cells using the reduced folate transport system and is effective in tumor lines exhibiting resistance to methotrexate because of decreased transmembrane transport [5, 20, 22]. As TMTX does not have a glutamic acid moiety, intracellular polyglutamates are not formed, but it is retained intracellularly at concentrations higher than MTX at comparable extracellular concentrations [3, 9, 25]. This suggests that TMTX might also overcome MTX resistance that occurs as a result of low-level amplification of dihydrofolate reductase (DHFR). TMTX is highly protein-bound [1, 24] and its elimination is predominantly non-renal.

Phase I studies of TMTX have examined its pharmacology on bolus weekly [8], daily × 5 [12, 28], and other schedules [14, 18, 23, 27]. This report presents the results of a phase I study of TMTX given as a short i.v. infusion every 3 weeks. The major goals of this study were: (1) to determine the maximally tolerated dose (MTD) of TMTX given as a short i.v. infusion every 3 weeks; (2) to describe and quantitate the clinical toxicities of TMTX on this schedule; (3) to evaluate the disposition of TMTX; (4) to define the pharmacodynamics of drug-induced toxicity; and (5) to seek preliminary evidence of therapeutic activity in patients with advanced solid tumors.

Patients and methods

Patient population. Patients with histologically documented solid tumors refractory to conventional treatment or for which there is no known effective therapy were eligible for this study. Eligibility criteria included: (1) age of > 18 years; (2) performance status (Eastern Cooperative Oncology Group, ECOG [26] of 3 or better; (3) life expectancy of at least 6 weeks; (4) no major surgery within 14 days and no radiation therapy or chemotherapy within 28 days of the beginning of treatment (6 weeks for nitrosoureas or mitomycin C); (5) adequate bone marrow function (white cell count of > 4,000/μl, platelet count of > 100,000/μl), hepatic function (bilirubin concentration of < 2.0 mg/dl) and renal function (creatinine concentration of < 1.5 mg/dl); and (6) no co-existing medical problem sufficiently severe to prevent compliance with the study or to expose the patient to untoward risk.

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All patients were evaluated by one of the investigators. A complete history was taken and a physical examination including an ECG was carried out. Height, weight, performance status and clinical tumor measurements (when available) were recorded. Laboratory data including a complete blood count, white cell differential and platelet count, determination of serum electrolytes, urea nitrogen, creatinine, glucose, total protein, albumin, calcium, phosphate, uric acid, alkaline phosphatase, total and direct bilirubin, ALT and AST as well as prothrombin time and urinalysis were obtained. A chest radiograph and appropriate radiologic evaluations of tumor extent were also carried out. All of the patients gave written informed consent according to federal, state, and institutional guidelines.

Dosage and formulation. The starting dose of TMTX was 20 mg/m^2 every 21 days. The dose was escalated to 40, 90, 112, 140, 175, 220 and 275 mg/m^2 in successive groups of patients. Dose escalation was permitted for previously treated patients with toxicity grade ≤ 2 after three other patients had been treated at the next dose. TMTX (manufactured by Warner-Lambert Co., Ann Arbor, Mich) was supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, Md); it was prepared in sterile water to a final concentration of 10 mg/ml and given i.v. over 5 min at the lowest doses. Infusion time was increased to 15 min at 140 mg/m^2 and to 30 min at 220 mg/m^2 because of concerns about adverse CNS effects seen in murine models after rapid i.v. injection of large doses of TMTX.

Subsequent evaluation. Patients were seen weekly and interim history, physical and laboratory findings were recorded. Toxicity was graded weekly using ECOG criteria [26]. Where available, tumor measurements were obtained every 6 weeks (two cycles).

Pharmacologic studies. Heparinized blood samples (5 ml) were collected at 1, 5, 10 and 30 min and at 1, 2, 4, 8, 24 and 48 h after the first drug dose. TMTX concentrations in plasma were measured using the method of Drake et al. [6], a competitive ligand-binding radioassay using dihydrofolate reductase (DHFR). DHFR also binds some metabolites of TMTX, leading to systematic overestimation of TMTX concentration when its concentrations are low and metabolite concentrations are high [1].

The plasma TMTX concentrations for each subject were fit by two- and three-compartment mamillary pharmacokinetic models using nonlinear regression analysis (PCNONLIN; Statistical Consultants, Lexington, Ky). The AUCs were also calculated using the trapezoidal rule [11]. Statistical analyses were carried out using standard parametric techniques (CSS; Statsoft, Tulsa, Okla).

Results

A total of 44 patients received 1–18 courses of TMTX in this study. The patients' ages ranged from 22 to 73 years (median, 54 years). The performance status, prior treatment and malignancies represented are listed in Table 1. A total of 152 courses of TMTX were given at doses of 20–275 mg/m^2 (Table 2); all but 3 were evaluable for toxicity. In all, 33 patients received more than one course of therapy and 13 received four or more courses. The 6 most extensively treated patients received 7, 8, 8, 8, 11 and 18 courses, respectively; no cumulative toxicity was apparent in these patients. A total of 13 patients were treated at more than one dose level, and 1 subject was treated at three different doses. Six patients' doses were increased; in eight other cases the dose was reduced as a result of toxicity. One patient with metastatic rectal adenocarcinoma had a partial response that was sustained for five cycles of treatment.

Table 1. Patient characteristics

Patients (*n*)	44		
Courses (*n*)	152 (149 evaluable)		
Sex (M:F)	25:19		
Median age (range)	54 years (22–73 years)		
Performance status	0	–	8
	1	–	26
	2	–	10
Prior therapy	CHEMO	–	12
	CHEMO + RT	–	27
	None	–	1
	RT	–	4
	Prior MTX	–	10
Disease:			
Colon/rectum	– 15	Stomach	– 2
Head/neck	– 7	Adenocarcinoma unknown origin	– 2
Lung	– 7	Pancreas	– 1
Breast	– 4	Mesothelioma	– 1
Cervix	– 4	Kidney	– 1

CHEMO, chemotherapy; RT, radiotherapy; MTX, methotrexate

Table 2. TMTX dose escalation

Dose (mg/m^2)	Number of patients (evaluable)	Number escalated	Number decreased	Number of courses (evaluable)
20	3 (3)	–	–	8 (8)
40	6 (6)	–	–	9 (9)
60	4 (4)	2	1	5 (5)
90	5 (5)	–	1	9 (9)
112	7 (7)	2	–	21 (20)
140	5 (5)	–	–	16 (16)
175	10 (10)	1	4	23 (23)
220	11 (11)	–	2	39 (38)
275	7 (7)	1	–	22 (21)

Hematopoietic toxicity. Leucopenia was the principal dose-limiting toxicity observed on this schedule. Thrombocytopenia occured much less frequently than leucopenia and was much milder. Although leucopenia was observed at all doses, there was a trend towards increasing severity and frequency of leucopenia as the maximally tolerated dose (MTD) was approached. The hematologic toxicities associated with TMTX at the five highest doses are shown in Table 3. There was an unexplained high frequency of leucopenia at 112 mg/m^2 (11/20 courses at grade 2 or 3). However, from 175 to 275 mg/m^2, the frequency of grade ≥ 2 leucopenia increased progressively: 6 of 23 courses at 175 mg/m^2, 15 of 38 at 220 mg/m^2 and 11 of 21 at 275 mg/m^2.

Table 3. The occurrence (per course) of various toxicities at the five highest doses of TMTX

Dose (mg/m^2)	Courses (*n*)	ECOG grade	Leuco	Thromb	N/V	Malaise	Skin	Mucositis
112	20	0	7	14	12	16	12	17
		1	2	3	4	4	8	3
		2	6	1	4	0	0	0
		3	5	1	0	0	0	0
		4	0	1	–	–	–	–
140	16	0	8	14	16	15	13	16
		1	8	1	0	1	1	0
		2	0	1	0	0	2	0
		3	0	0	0	0	0	0
		4	0	0	–	–	–	–
175	23	0	9	21	22	18	19	21
		1	8	2	1	3	4	0
		2	3	0	0	2	0	2
		3	3	0	0	0	0	0
		4	0	0	–	–	–	–
220	38	0	9	26	32	29	31	37
		1	14	10	3	5	6	1
		2	11	1	2	3	1	0
		3	2	1	1	1	0	0
		4	2	0	–	–	–	–
275	21	0	7	17	18	17	9	19
		1	3	4	3	3	11	1
		2	6	0	0	1	1	1
		3	5	0	0	0	0	0
		4	0	0	–	–	–	–

LEUCO, leucopenia, THROMB, thrombocytopenia; N/V, nausea and vomiting

Sporadic instances of myelotoxicity occurred at even lower doses. At 40 mg/m^2, grade 2 leucopenia occurred during 2 of 9 courses. At 60 mg/m^2, grade 2 leucopenia occurred during three courses and grade 3 leucopenia, during two courses; two of these patients also had grade 2 thrombocytopenia. At 90 mg/m^2, grade 2 leucopenia occurred in three courses and grade 3 leucopenia, in one patient; grade 3 thrombocytopenia was seen in two of these courses. Maximal granulocytopenia was documented on day 8, with complete recovery in most patients on day 15. In contrast, the nadir platelet counts were recorded on day 15 but had resolved by day 21 in all patients.

We arbitrarily defined "extensively treated" patients as those who had received radiation to a significant portion of their marrow-bearing bones (>30%) or had received chemotherapy with mitomycin C or nitrosoureas. Table 4 shows the occurrence of leucopenia per patient after TMTX treatment, with previously minimally and extensively treated patients listed separately. Patients whose doses were increased or decreased are listed for courses at each dose level. Extensively treated patients did not appear to be at greater risk of toxicity than minimally treated patients, although the numbers were too small for a definitive conclusion.

Table 4. The occurence of leucopenia after TMTX treatment in patients who had previously undergone minimal or extensive radiotherapy and/or chemotherapy

Dose (mg/m^2)	Prior therapy	Patients (*n*)	Grade (WBC × 10^{-3})				
			0 (>4.0)	1 (3.0–4.0)	2 (2.0–3.0)	3 (1.0–2.0)	4 (<1.0)
112	Minimal	3	1	0	0	2	0
	Extensive	4	1	1	0	2	0
140	Minimal	1	0	1	0	0	0
	Extensive	4	2	2	0	0	0
175	Minimal	6	1	4	0	1	0
	Extensive	4	2	0	1	1	0
220	Minimal	11	1	2	3	3	2
	Extensive	0	0	0	0	0	0
275	Minimal	5	1	0	2	2	0
	Extensive	2	1	0	0	1	0

Mucocutaneous toxicity. Mucositis involving the naso- and oro-pharynx, perineal and stomal mucosa was seen in nine courses (five patients), including one patient receiving 60 mg/m^2. At all dose levels, an erythematous, maculopapular skin rash was observed that was occasionally pruritic and typically involved the neck and upper chest; less frequently, an intertriginous rash developed. Mucositis was an infrequent complication of TMTX given on this schedule. In addition, two patients treated at 220 mg/m^2 reported hair loss that was not cosmetically apparent.

Other toxicity. A number of patients reported the occurrence of or an increase in malaise following doses of TMTX. Again, this was not clearly dose-related; since it is a common symptom of advanced malignancy, the significance is unclear. However, the timing of the onset of the malaise and its recurrence on multiple courses suggests that it was drug-related. In three patients, it took the form of an acute, febrile, flu-like illness, with chills, myalgias, and profound malaise that resolved in 24–36 h. This syndrome prevented two patients from receiving treatment at 220 mg/m^2 and was therefore dose-limiting.

Several other toxicities occurred sporadically. Nausea and vomiting were minor problems during this study and, as shown in Table 3, did not appear to be dose-related. Three patients who received 220 mg/m^2 showed transient increases in serum creatinine to between 1.3 and 4.0 mg/dl, with no apparent etiology other than drug toxicity. Four patients exhibited elevations in aminotransferases following doses of 40 (grade 1), 140 (grade 1), and 220 mg/m^2 (grade 1, grade 2). Two patients receiving 140 and 220 mg/m^2 developed pain, itching or hives at the site of drug injection. The incidence of rash, mucositis, malaise, nausea, alopecia and diarrhea is presented in Table 3.

Pharmacokinetics and pharmacodynamics

Pharmacokinetic samples were obtained from 32 patients. Compartmental models could be fit to the data from 24 of the 26 patients with complete data sets; for 6 of these patients, the plasma drug disposition curves were fit well only by a three-compartment model (Table 5a). A representative drug-disposition curve for these patients is shown in Fig. 1 (subject 19). In the remainder of the patients, the data were well described by a two-compartment model (Table 5b). The drug disposition curve of subject 2 in Fig. 1 is typical for this group of patients.

Plasma TMTX concentrations ranged from 9.5 to 61 μmol/l (mean, 27.1 μmol/l) 1 h after doses of 175–220 mg/m^2 in eight patients. These concentrations ranged from 0.86 to 5.8 μmol/l (mean, 3.7 μmol/l) 24 h later. The mean value for the central volume of distribution (V_C) in patients whose data were fit by the three-com-

Table 5. TMTX pharmacokinetic parameters in patients

Subject number	Dose (mg/m^2)	AUC (μmol · min/m^2)	Clearance rate (ml/min per m^2)	V_c (l/m^2)	V_{ss} (l/m^2)	Half-lives		
						Alpha (min)	Beta (min)	Gamma (h)
A: Drug disposition described by three compartments								
1	20	1,116	48.6	1.0	24.7	1.7	42.8	12.3
8	40	2,272	47.7	0.2	14.7	0.9	36.3	10.2
9	40	4,442	25.6	0.4	22.6	1.1	39.8	16.1
11	90	5,574	43.8	7.1	28.8	5.7	87.1	10.2
19	140	18,631	20.4	0.4	40.1	1.3	72.8	32.5
35	275	33,984	21.9	1.3	44.8	2.1	88.1	29.3
		Mean	34.7	1.7	29.3	2.1	61.2	18.4
		SD	12.4	2.4	10.3	1.6	22.2	9.1
B: Drug disposition described by two compartments								
2	20	1,983	27.3	3.0	23.0		16.9	12.2
6	40	16,823	6.4	2.4	4.4		15.9	8.1
7	40	4,175	26.0	3.6	21.4		39.8	15.0
14	112	8,128	37.3	17.8	58.6		43.0	20.0
17	112	10,915	27.8	7.0	19.8		36.5	9.6
21	140	11,499	33.0	10.6	37.9		56.3	16.5
22	140	21,547	17.6	9.9	23.9		61.9	17.4
24	175	27,023	17.6	8.0	28.3		77.9	23.1
26	175	22,178	21.4	14.1	29.1		131.0	19.0
29	220	17,543	34.0	13.3	46.8		47.5	18.3
30	185	15,208	33.0	6.6	23.2		18.4	9.0
31	220	12,939	46.1	1.7	37.7		2.7	10.5
32	220	16,798	35.5	8.9	46.9		33.8	18.3
37	275	30,764	24.2	7.3	40.6		35.4	22.6
38	275	24,156	30.8	16.8	69.7		77.4	31.2
40	275	21,947	34.0	10.4	50.3		45.3	20.8
42	220	40,037	14.9	0.6	15.6		4.6	14.2
43	275	26,625	28.0	17.1	26.1		56.8	11.3
		Mean	27.5	8.8	33.5		44.5	16.5
		SD	9.2	5.3	15.9		30.0	5.8

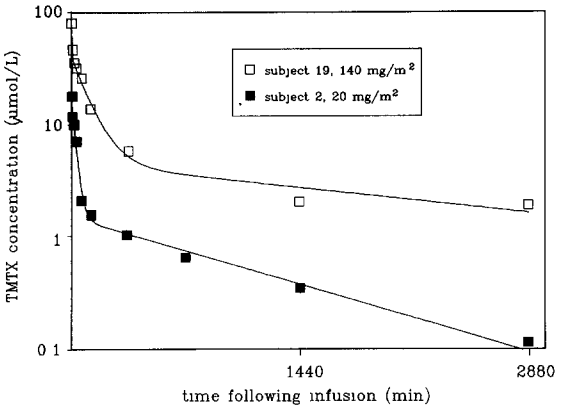

Fig. 1. TMTX plasma clearance in two patients receiving 20 mg/m^2 (■) and 140 mg/m^2 (□). *Symbols* represent measured concentrations and *lines* represent two-compartment (*subject 2*) and three-compartment (*subject 19*) model fits

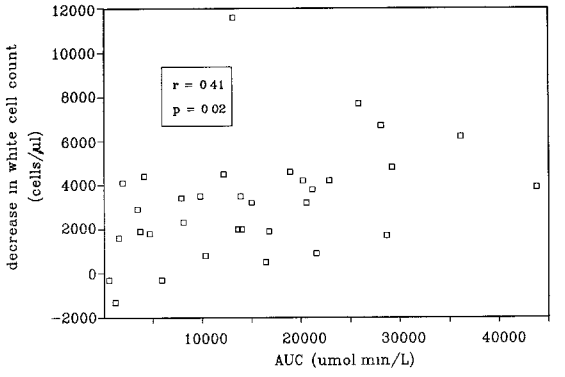

Fig. 2. Decrease in WBC count (pre-treatment to nadir) vs dose of TMTX

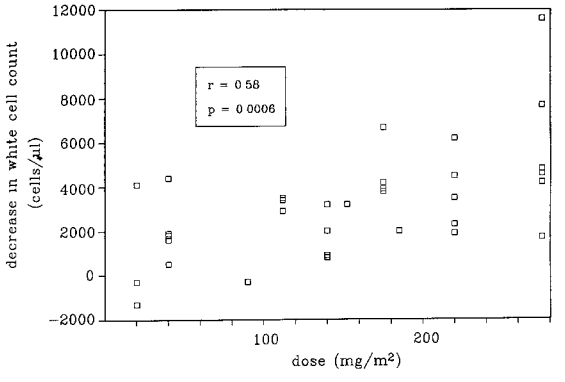

Fig. 3. Decrease in WBC count (pre-treatment to nadir) vs the AUC

partment model was significantly smaller than the mean V_C in those whose data were fit by the two-compartment model ($t = 3.07$, $P = 0.006$). There were no other significant differences in the mean kinetic parametric values for the two groups. The AUCs and clearance rates calculated using the trapezoidal rule were very similar to the values generated by compartmental modeling in patients for whom such modeling was possible ($r = 0.99$ and 0.96, respectively). For all 32 patients evaluated using the trapezoidal rule, the mean TMTX clearance rate (non-compartmental) was 36.5 ml/min per m^2 (SD, 21.0 ml/min per m^2).

The relationship between TMTX dose and AUC was linear, although there was considerable scatter in the data ($r = 0.66$, $P < 0.0001$). This indicates that the elimination of TMTX obeyed linear kinetics over the dose range used in this study. The scatter in the relationship resulted from the wide range of TMTX clearance rates in these patients. Unexpectedly, a significant, positive correlation was found between the steady-state volume of distribution (V_{SS}) and dose ($r = 0.59$, $P = 0.003$). A similar relationship has bean noted by Lloyd Whitfield (personal communication). The physiologic basis for this finding is not known. The gamma, or terminal, half-life was also positively correlated with dose ($r = 0.49$, $P = 0.01$); however, this association was probably secondary to that between V_{SS} and dose, since the terminal half-life is a hybrid kinetic parameter that is directly related to V_{SS}. V_C was not correlated with TMTX dose.

The magnitude of the decline in WBC count consequent to the administration of TMTX was correlated with dose (Fig. 2) and AUC (Fig. 3). The plasma TMTX concentrations at 1 and 24 h following drug infusion showed modest, although significant, correlations with the decrease in WBC count ($r = 0.46$, $P = 0.009$ and $r = 0.48$, $P = 0.007$, respectively). The plasma albumin concentration, which has been reported to be predictive of hematopoietic toxicity, was only marginally correlated to the WBC count nadir ($r = 0.36$, $P = 0.047$) and did not correlate with the change in WBC count ($r = -0.034$, $P = 0.86$). The TMTX clearance rate also did not correlate with the change in WBC count ($r = 0.043$, $P = 0.843$).

Discussion

TMTX has been evaluated on a variety of schedules, including i.v. bolus treatments every 3 weeks, weekly, daily for 5 and 9 days and continuous infusion schedules [8, 12, 14, 18, 23, 27, 28]. As reported in studies using this and other schedules, there is a wide range in the dose that an individual patient can receive with acceptable toxicity. When the occurrence of leucopenia in the present study is evaluated per patient (Table 4) rather than per course (Table 3), several conclusions are apparent.

Due to the low number of patients at each dose level, there is no clear indication that extensively treated patients are at greater risk for toxicity than minimally treated patients. Since 5 of 11 and 2 of 5 minimally treated patients receiving 220 and 275 mg/m^2 TMTX, respectively encountered grade 3 or 4 toxicity, we recommend 220 mg/m^2 as a suitable dose for subsequent phase II studies in such patients. This study did not clearly define a starting dose for extensively treated patients, for whom we recommend initiating TMTX treatment at 175 mg/m^2, based on other experience with TMTX on bolus and repeated-dose schedules. If minimal toxicity is seen in the first cycle, subsequent courses can be prescribed with 25% dose escalations, since there is no suggestion of cumulative myelotoxicity; some patients receiving 275 mg/m^2 developed no toxicity, and other investigators [21] have been able to treat some patients at doses as high as 400 mg/m^2.

This study did not identify clinical risk factors that are strongly predictive of myelotoxicity and could therefore be used for individualizing dose regimens. There was a significant correlation between dose and AUC. No correlation was demonstrable between plasma albumin concentration and TMTX clearance rate, although Fanucchi et al. [8]

Table 6. Pharmacokinetic parameters from studies using HPLC and DHFR assays

References	Patients (*n*)	Method	Clearance rate (ml/min per m^2)	V_{ss} (l/m^2)	$t_{1/2}$ (h)
[8]	23	HPLC	32.4	36.6	15.1
[27]	16	HPLC	30.4	32.8	13.4
[14]	11	DHFR	14.0	25.0[b]	15.2
[23]	14	HPLC	30.8[a]	25.1[a]	16.4
[28]	19	HPLC	21.0[a]	12.2[a, b]	9.9
Present study	32	DHFR	36.5	32.4	16.5

[a] Converted using 1.73 m^2 = 70 kg
[b] V_d area

Table 7. Schedule-dependent MTD in TMTX clinical trials

Schedule	MTD/course (mg/m^2)	AUC (μmol · min/l)	Plasma concentration		References
			1-h (μmol/l)	24-h (μmol/l)	
Every week (3 of 5)	390	39,000	13.5	0.40–3.40	[8]
Every 2 weeks	240	16,300	7–21	1.20–4.90	[23]
Every 3 weeks	220	16,000	9–61	0.50–2.00	Present study
Every 3 weeks	200	–	27–54	3–8	[14]
Daily × 5 days	60–75	5,000–8,370	0.15– 4.2	0.08–0.25	[12, 28]
Infusion × 5 days	50	5,025	0.69[a]	0.24	[27]
Daily × 9 days	27–36	–	–	0.02–0.35	[18]

[a] Steady-state concentration

have reported a correlation between low albumin levels and TMTX clearance rate ($r = 0.66$). A negative correlation was found between plasma albumin concentration and nadir WBC count in the present series of patients.

Pharmacologic measures were better correlated with myelotoxicity. The best correlation was between dose itself (in mg/m^2) and the decrease in WBC count; the AUC and the TMTX concentration 24 h after the dose were also correlated with the decrease in WBC count. Although some other investigators [18, 28] have not reported statistical correlations between kinetic behavior and toxicity, Fanucchi et al. [8] reported a correlation between the percentage of change in platelet count and the TMTX concentration 24 h after a dose ($r = 0.66$) as well as the AUC ($r = 0.68$). Correlations between the AUC and the percentage of change in platelet count have also been noted by Reece et al. [27] on a 5-day infusion schedule ($r = 0.43$). On a daily × 5 schedule [12], we have also documented a correlation between the decrease in WBC count and the TMTX concentration 1 h after a dose ($r = 0.6$) as well as the AUC ($r = 0.65$). Individual patients with unusual kinetic parameters have also been noted to develop toxicities [14, 28].

In an analysis of the phase I and II trials sponsored by the NCI (United States) and the NCI-Canada, patients with low plasma albumin [7, 11] and those with hepatic metastases [7] were found to be at increased risk of severe toxicity. More episodes of severe toxicity have also been reported in patients treated on the daily × 5 schedule [11]. These authors have recommended dose reductions in the initial course of TMTX treatment in patients with hypoalbuminemia. Despite these precautions, severe toxicity can be expected to occur in 18–33% of patients receiving TMTX [11]. We could not identify a clinical or pharmacologic parameter that reliably identified additional patients at risk for severe toxicity.

The DHFR inhibition assay was used in this study because it would have been a convenient method for the analysis of large numbers of clinical specimens [6]. This assay measures both unchanged TMTX and metabolite(s) that bind DHFR, which may or may not be involved in cytotoxicity. This has been shown to result in overestimates of TMTX plasma concentration at late time points [1, 8, 23], which should cause predictable biases in pharmacokinetic parameters: underestimation of the V_{SS} and overestimation of the terminal half-life and the AUC. If the DHFR-binding metabolite(s) are active, estimates based on DHFR binding might better correlate with the outcome than would those based on the parent compound alone, although Lin et al. [23] could not demonstrate this. Investigators using both DHFR and high-performance liquid chromatographic (HPLC) techniques on the same samples have found 25%–50% concentration differences at 24 and 48 h, respectively, resulting in overestimates of the AUC of up to 66% [1, 8, 23]. However, a comparison of the pharmacokinetic data from studies using HPLC and DHFR detection (Table 6) suggests that the interpatient variability in phase I studies may result in variances so large that the expected systematic differences are not apparent.

TMTX has been clinically evaluated on several schedules because of known effects of schedule in pre-clinical and murine tumor models [3, 16, 24]. Bertino and his colleagues [3] have predicted that low-dose, continuous exposure over at least one generation would be necessary for inhibition of tumor growth. This hypothesis did not hold for all cell lines tested in vitro, however, and the growth of HCT-8, a human colon carcinoma cell line, was markedly inhibited by short-term exposure to moderate concentrations of TMTX [3]. This line has been shown to maintain persistently high intracellular TMTX concentrations. In in vivo murine models, maximal increased life spans (ILS) were observed with doses given every 3 h on days 1, 5 and

9 (ILS, 133%) or as nine successive daily injections (ILS, 108% [24]). Optimal scheduling for ILS in murine models approximated a continuous infusion, which is in accord with the hypothesis that antimetabolite activity in general, and antifolate activity specifically, is associated with prolonged exposures to cytotoxic concentrations [4].

In the completed phase I trials in patients, schedule-dependent toxicity has been documented. The maximally tolerated dose (MTD) for a course of therapy, AUC and concentrations achieved on various schedules are summarized in Table 7. Since equivalent myelotoxicity occurs at different doses, toxicity may possibly be related to the duration of drug exposure above a specific threshold concentration. Support for this possibility is provided by the results of other studies using TMTX over several days [18, 27]. Considerations of dose intensity [15] lend support to the bolus schedule for clinical use.

However, multiple-dose schedules may result in a better therapeutic index for malignancies that are best killed by very prolonged exposures at low concentrations, or in which the time sequence of drug exposure is important. The choice of schedule for TMTX thus depends on drug disposition in patients, the characteristics of the normal hematopoietic precursors and the target malignancy or pathogen (e.g., Pneumocystis carinii). It had been hoped that studies of schedule dependence in murine models would assist in the choice of schedules for further clinical development. Unfortunately, the kinetics of TMTX disposition in the mouse ($t_{1/2}$, 50 min) is very different from that in humans [12, 13], making allometric comparisons even more complicated than usual. In the absence of specific information regarding the cellular pharmacology of TMTX in a specific clinical setting, it may be necessary that phase II trials be carried out on more than one schedule to assess activity.

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