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Phase I clinical and pharmacologic trial of trimetrexate in combination with 5-fluorouracil

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Summary. Based on the synergy of sequential methotrexate (MTX) and 5-fluorouracil (5-FU) in vitro and in vivo and the superior antitumor activity of trimetrexate (TMTX) compared with MTX in preclinical models, we carried out a phase I trial of TMTX and 5-FU (fixed dose, 400 mg/m^2 per day), both given as 10-min i.v. infusions daily $\times 5$ days, every 28 days. The TMTX dose was escalated from 3.0 to 14.0 mg/m^2 per day. In all, 92 evaluable courses were given to 34 patients, half of whom were heavily pretreated with radiation or cytotoxics. Myelosuppression and mucositis were the dose-limiting toxicities but were not different in heavily or minimally pretreated patients; there were five episodes of moderate to severe mucositis. Rash, fatigue, and diarrhea were mild toxicities. Plasma TMTX elimination was biexponential, with a mean $t_{1/2\alpha}$ of 0.23 h and a $t_{1/2\beta}$ of 16.7 h. The area under the plasma TMTX concentration versus time curve increased linearly with dose, suggesting first-order elimination. Total plasma TMTX clearance (mean $\pm$ SD) was 14.3 ± 5.9 ml/min per m^2. Renal clearance accounted for approximately 7% of total clearance, indicating biotransformation as the major route of elimination. TMTX was highly protein-bound (97%). Thus, TMTX can be given with 5-FU (400 mg/m^2) on a daily $\times$ 5-day bolus schedule at the 12 mg/m^2 per day dose level, which was the recommended dose of TMTX as a single agent for phase II studies using the 5-day bolus schedule.

Introduction

Trimetrexate (TMTX) is a 2,4-diaminoquinazoline antifolate being evaluated in phase II trials. Although its intracellular target, dihydrofolate reductase, is the same as that for methotrexate (MTX) [10], TMTX is more lipid-soluble, is not transported into cells via the reduced folate carrier used by the parent antifol [11], and does not undergo polyglutamylation. The antitumor activity of TMTX was superior to that of MTX in preclinical models [12, 17]. Responses were observed in early phase I trials of TMTX in patients with lung, breast, and colorectal tumors.

The antipurine effect of MTX results in elevation of intracellular phosphoribosylpyrophosphate (PRPP) pools; thus, pretreatment with this agent may increase the anabolism of 5-FU to its active metabolites. Cadman et al. showed that methotrexate pretreatment of L1210 [6] and HCT-8 cells [4] resulted in increased incorporation of 5-FU into RNA and synergistic tumor cell kill, in parallel with changes in PRPP levels. In vivo confirmation was obtained by Mulder et al. [16], who showed that this synergistic interaction was highly schedule-dependent; an 18 to 24-h interval between MTX and 5-FU gave the best results. Studies in man have usually disregarded the scheduling requirement, but in those with an interval of at least 18 h, response rates have usually been $\geqslant 30\%$ in colorectal cancer (for review see [14]).

In vivo synergy with 5-FU has also been demonstrated for TMTX by repetitive dosing in P388 leukemia [13]. To study the efficacy and safety of this combination in humans, we carried out phase I and II studies of TMTX given with 5-FU. We report the results of our phase I study, including the pharmacokinetics of TMTX in this combination.

Materials and methods

Patients entered in this study had histologically confirmed, metastatic solid tumors either refractory to conventional therapy or for which there was no conventional therapy; a performance status (ECOG) of 2 or less and a life expectancy of at least 2 months; recovery from prior chemotherapy- or radiation therapy-induced toxicity; adequate bone marrow function as evidenced by an absolute granulocyte count $>1{,}500/\text{mm}^3$ and platelet count $>100{,}000/\text{mm}^3$; adequate renal function as defined by serum creatinine levels of <1.5 mg/dl or creatinine clearance $\geqslant 60$ ml/min; serum bilirubin levels of $\leqslant 2.4$ mg/dl; and no prior treatment with phase I or II investigational cytotoxic agents. The protocol for this study was approved by the Human Investigation Review Board at the Fox Chase Cancer Center, and written informed consent was obtained from each patient.

A history, physical examination, chest X-ray, ECG, complete blood count (CBC), platelet count, chemistry profile, urinanalysis, tumor measurement, and determination of performance status were obtained for each patient within 1 week of study entry. Other studies [e.g., computerized axial tomographic (CAT) and radionuclide scans) necessary for assessing tumor status were obtained prior to and during treatment as required in individual

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cases. A physical examination, toxicity assessment, tumor measurement, and chemical profile were carried out at least every 4 weeks and usually every 2 weeks during the initial two treatment courses. CBC, differential, and platelet counts were monitored weekly. Trimetrexate (NSC 352122; TMTX) was supplied by Warner-Lambert/Parke Davis as vials containing 10 mg preservative-free trimetrexate glucuronate for injection.

Each treatment course consisted of the daily administration of TMTX by i.v. infusion over 10 min, immediately followed by 5-FU (400 mg/m^2) given by i.v. infusion over 10 min, for 5 consecutive days. Treatment courses were repeated at 4-week intervals if toxicity was acceptable and the disease had not progressed. The starting dose of TMTX was 3 mg/m^2 per day. Dose escalation proceeded according to a modified Fibonacci schema, at subsequent dose levels of 4.5, 6.0, 8.0, 10.0, 12.0 and 14.0 mg/m^2 per day. The dose of 5-FU was fixed at 400 mg/m^2 per day. A minimum of three patients were treated at each level. Additional patients were treated at a given dose level if grade 3 or 4 toxicity was encountered in any patient at that level. Doses in patients who had no toxicity at a given dose level were escalated to the next highest level.

Toxicity criteria were those of the World Health Organization [23]. Patients were evaluable if they completed one treatment course of five consecutive daily doses of TMTX and 5-FU; they were reassessed in 4 weeks unless treatment was stopped for drug-related toxicities. The maximum tolerated dose (MTD) of TMTX in this combination and schedule was defined as that dose producing dose-limiting toxicity in 50% or more of the patients initially treated at that dose level. Dose-limiting toxicity was defined as (a) a granulocyte nadir of $\leq 500/mm^3$ or a platelet nadir of $\leq 50{,}000/mm^3$; (b) failure of the granulocyte and platelet counts to recover to levels of $1{,}500/mm^3$ and $100{,}000/mm^3$, respectively, by day 42 of the treatment cycle; (c) nonhematologic toxicity more severe than grade 1; and (d) nonhematologic toxicity of grade 1 lasting 6 weeks or longer. Disease status was evaluated using standard response criteria (ECOG) [19].

Selected patients participated in pharmacokinetic studies during their initial treatment courses. Venous blood samples were collected in vacutainer tubes at timed intervals over the 5 days of treatment. Samples were centrifuged at 1,200 *g* for 15 min and the plasma fractions were removed and stored at $-20°$ C until analysis. Urine samples were collected at 6-h intervals for the first 12 h after dosing and for the 12 h before the next administration of TMTX. The total volume of urine collected during each interval was recorded and an aliquot was stored at $-20°$ C until analysis. The analytical method used was essentially that described by Ackerly et al. [1], consisting of the extraction of TMTX from plasma and urinary samples using Bond Elut C18 sample-preparation columns, followed by high-pressure liquid chromatographic (HPLC) quantitation of TMTX.

The HPLC analysis of TMTX was carried out on an HP 1090 liquid chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a diode array detector and an auto sampler. The analytical column was a 100×4.6 mm, 5-μm Spherisorb-ODS, (Hewlett-Packard, Palo Alto, CA) preceded by a 15×3.2 mm, 7-μm Aquapore C18 guard column (Brownlee Labs Inc., Santa Clara, Calif).

TMTX was eluted by a gradient mobile phase at a flow rate of 1.5 ml/min at ambient temperature. The starting mobile phase was 85% HPLC water containing 0.04% phosphoric acid, 0.08% triethylamine, and 15% acetonitrile. The acetonitrile concentration was linearly increased to 40% over 10 min. Each analysis was followed by a 10-min reequilibration period under the starting conditions. Detection of TMTX was done by UV absorbance at 241 nm, and peak height measurements were carried out and reported by an HP 85B computer workstation (Hewlett-Packard, Palo Alto, Calif). The retention time of TMTX was 7.6 min.

A standard curve for TMTX measurement, ranging from 0.1 to 5 μg/ml, was constructed in plasma or urine and extracted daily along with patient samples to be analyzed. Standard curves were linear over this range (plasma $r > 0.999$; urinary $r > 0.998$). Patient samples were diluted when necessary to produce concentrations in the range of the standard curve. The concentration of TMTX in the patient samples was determined by comparing the peak height of TMTX in the unknown sample to the standard curve.

Pharmacokinetic calculations

Semilogarithmic plots of plasma TMTX concentration vs time showed apparent multiexponential elimination that conformed to the general equation

$$\text{plasma TMTX concentration} = \sum_{i=1}^{n} Xe^{-x_i t}$$

Plasma TMTX elimination curves were fitted to both bi- and triexponential equations by the NONLIN84 nonlinear curve-fitting program [20]. The Akaike information criterion [24] was used to determine which equation best fit the data. Initial parameter estimates, required for the NONLIN84 program, were obtained with the ESTRIP exponential curve-stripping program [5]. For all cases the plasma elimination curve was best fitted by a biexponential equation of the form

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t}.$$

The half-life of each elimination phase was calculated by 0.693/rate constant. Total body clearance, Cl_{total}, was calculated from dose/AUC, where AUC is the total area under the plasma concentration vs time curve, calculated from $A/\alpha + B/\beta$. Renal clearance, Cl_{renal}, was calculated from $(\Sigma U/dose)Cl_{total}$, where $\Sigma U/dose$ is the fraction of the dose excreted unchanged in the urine. Metabolic clearance, $Cl_{metabolic}$, was estimated from $Cl_{total} - Cl_{renal}$.

The volume of the central compartment, V_c, equivalent to the initial distribution volume was calculated from dose/A+B. The steady-state volume of distribution, V_{ss}, was calculated from dose · $(AUMC)/(AUC)^2$, where AUMC is the area under the first moment of the plasma concentration vs time curve calculated from $A/\alpha^2 + B/\beta^2$. The pseudo steady-state volume of distribution V_β, which relates plasma concentration to the amount of drug in the body during the terminal elimination phase, was calculated from $Cl_{total/\beta}$[8].

Serum protein binding

Fresh serum was obtained from a healthy male volunteer and spiked with TMTX to produce a final concentration

of 5 μg/ml. After incubation at 37° C for 30 min, protein binding was determined by ultrafiltration through a 30,000 dalton cutoff, centrifree micropartition device (Amicon Corp., Danvers, Mass). The concentration of TMTX in the resultant ultrafiltrate was measured by the direct injection of 100μl ultrafiltrate onto the HPLC column under the previously described conditions, followed by a comparison of the TMTX peak height to a standard curve constructed in serum ultrafiltrate. The nonspecific adsorption of TMTX to the filtration device was determined by filtration of serial 400-μl samples of spiked serum ultrafiltrate (1 ml total volume). The percentage of protein-bound drug was calculated from

$$\frac{\text{serum concentration} - \text{ultrafiltrate concentration}}{\text{serum concentration}} \times 100.$$

Chemicals

Trimetrexate glucuronate (TMTX) was obtained from Warner-Lambert/Parke Davis Pharmaceutical Research (Ann Arbor, Mich). Sodium citrate and sodium acetate trihydrate were purchased from Sigma Chemical Co. (St. Louis, Mo). HPLC-grade water and acetonitrile were purchased from Burdick and Jackson Labs (Muskegon, Mich). Phosphoric acid and triethylamine were obtained from Fisher Scientific Co. (Fair Lawn, NJ) and methyl alcohol, from Mallinckrodt Inc. (Paris, Ky).

Results

A total of 34 patients received 120 courses of TMTX and 5-FU; their characteristics are presented in Table 1. Most patients had previously been treated with cytotoxic chemotherapy (19/34) or radiation (10/34).

The TMTX dose was escalated from 3.0 mg/m^2 to 14 mg/m^2. Table 2 shows the number of patients initially treated at each TMTX dose level. In all, 6 patients (17.6%) received only a single treatment course, 8 (23.5%) received more than one course without an increase or decrease in their initial TMTX dose, and 13 (38.2%) underwent more than one course with escalations of their initial TMTX doses for one or more subsequent treatment courses. Six patients (17.6%) received a total of 25 courses at TMTX doses lower than their starting doses. These downward dose adjustments were made in patients who experienced grade 3 or 4 toxicity at their initial TMTX doses but demonstrated stable disease and were felt to be benefiting from continued treatment. Courses at the lowered doses were not considered to be evaluable in the analysis of toxicity. Three additional treatment courses were nonevaluable due to incomplete hematologic data. Thus, of the 120 courses, 92 were evaluable.

Myelosuppression was the most frequently encountered dose-limiting toxicity. As shown in Tables 2 and 3, sporadic grade 3 or 4 myelosuppression was observed with this combination, even at the lower TMTX dose levels. Neutropenia and thrombocytopenia occurred with equal frequency. Neutrophil nadirs were usually observed on day 8 from the start of treatment courses, whereas platelet nadirs were most often seen on day 15. With one exception, neutrophil and platelet counts recovered by day 21: one patient with hepatocellular carcinoma receiving 14 mg/m^2 died of neutropenic sepsis on day 12, before marrow recovery.

Mucositis occurred in 17/92 (18.4%) courses but was usually mild; in three patients it was severe: at the 3.0, 8.0, and 14.0 mg/m^2 dose levels. Mild to moderate diarrhea was observed in 12/92 (15%) courses and rarely required antidiarrheal medication for control. Three patients developed maculopapular rashes during five treatment courses at the 8.0 and 12.0 mg/m^2 TMTX levels; in two patients the rash was limited to the anterior chest and neck, whereas the third patient developed a generalized rash without desquamation. In each case the rash resolved within 1 week.

Table 1. Patient characteristics

Number of patients	34
Men/Women	25/9
Age (years):	
Median	59
Range	31–80
Performance status:	
Median	1
Range	0–2
Prior treatment	Patients (*n*):
Chemotherapy alone	13
Immunotherapy alone	2
Radiotherapy alone	6
Chemotherapy plus radiotherapy	4
Chemotherapy plus immunotherapy	2
None	7
Tumor type:	
Colorectal	17
Lung (non-small-cell)	6
Pancreatic	4
Other	7

Table 2. Myelotoxicity, first course of treatment

TMTX dose (mg/m^2)	Patients evaluable (*n*)	Neutropenia				Thrombocytopenia			
		1	2	3	4	1	2	3	4
3.0	5	1	0	1	0	1	1	0	0
4.5	5	0	0	0	0	0	0	0	0
6.0	5	0	0	1	0	1	0	0	0
8.0	6	1	0	1	1	0	2	0	1
10.0	6	0	0	0	1	1	0	0	1
12.0	5	1	0	0	0	1	0	0	0
14.0	2	0	1	0	1	1	0	1	0

Table 3. Myelotoxicity for all evaluable courses

TMTX dose (mg/m^2)	Patients/ Evaluable courses	Courses with toxicity (*n*)							
		Neutropenia (grade)				Thrombocytopenia (grade)			
		1	2	3	4	1	2	3	4
3.0	5/8	1	0	1	0	1	1	0	0
4.5	7/10	0	0	0	0	2	0	0	0
6.0	10/17	1	0	4	0	1	0	0	0
8.0	12/15	1	0	1	1	1	2	0	1
10.0	12/18	2	0	1	1	3	0	0	1
12.0	11/21	4	0	2	0	4	1	1	0
14.0	3/3	0	1	0	1	2	0	1	0

Hepatic toxicity was seen in two patients. A patient who was initially treated at the 8.0 mg/m^2 TMTX level developed an elevation in serum glutamic oxaloacetic transaminase (SGOT) to 10 times the normal level, with only a slight elevation in lactic dehydrogenase (LDH) and alkaline phosphatase. His TMTX dose was reduced to 4.0 mg/m^2 for five subsequent courses due to grade 4 myelosuppression and mucositis, and the SGOT elevation occurred after the fifth course; 2 months after chemotherapy was stopped his SGOT decreased to 3 times the normal level. The radionuclide liver scan remained normal throughout. Irreversible cholestatic jaundice without evidence of tumor progression developed in a second patient after one treatment course at the 10 mg/m^2 level. This patient had had reversible cholestatic jaundice during previous intrahepatic FUDR infusion.

Other toxicities included mild nausea in six courses and partial alopecia in two patients. Five patients (13 courses) noted mild to moderate fatigue lasting 3–5 days, beginning just after or midway through the 5 days of drug administration. Cerebeller ataxia developed in one patient, a 65-year-old woman, during her third course at the 4.5 mg/m^2 level; no evidence of brain metastasis was detected by cranial CAT scanning. 5-FU was omitted from her fourth treatment course and the ataxia slowly resolved.

There were no objective responses. Two patients with metastatic colon carcinoma had minor regressions. One treatment-related death due to neutropenic sepsis and pneumonia occurred at the 14 mg/m^2 level; this patient, a 50-year-old man with advanced hepatocellular carcinoma, received only four of the five daily doses of TMTX and 5-FU due to mucositis observed on day 5.

Table 4 summarizes the dose-limiting toxicities observed in patients initially treated at each dose level. Although dose-limiting toxicity, as defined above, was observed in four of the initial six patients treated at the 10 mg/m^2 dose level, none of six other patients who were escalated up to this level from lower initial doses experienced dose-limiting toxicity. Dose-limiting toxicity was not observed in the five good-risk patients initially treated at the next higher (12 mg/m^2) level. Two patients were initially treated at the 14 mg/m^2 level, one of whom had grade 4 granulocytopenia, thrombocytopenia, and mucositis. A third patient initially treated at 12 mg/m^2 was also treated at this dose and had grade 2 granulocytopenia. No additional patients were treated above 12 mg/m^2, this being the recommended phase II dose for single-agent studies of TMTX on the 5-day schedule in previously nontreated patients with good performance status.

Pharmacokinetic studies

A representative plasma TMTX concentration vs time curve after a 12 mg/m^2 dose is shown in Fig. 1. Following a 10-min infusion, the elimination of TMTX was biexponential. The distribution half-life ranged from 0.15 to 0.57 h (mean ± SD, 0.33 ± 0.20 h), and the mean terminal elimination half-life was 16.7 ± 5.7 h. These and other pharmacokinetic parameters for the seven patients studied

Table 4. Dose-limiting toxicity (DLT)

Dose level	Patients initially treated (*n*)	Patients (%) with DLT	Type of toxicity (grade)
3.0	5	2 (40)	Mucositis (2) Mucositis (3)
4.5	5	0 (0)	
6.0	4	0 (0)	
8.0	6	1 (16)	Thromb (4), Gran (4), Mucositis (4)[a]
10.0	6	4 (67)	Thromb (4), Gran (4)[a] Mucositis (2) Diarrhea (2)[b] Jaundice (3)
12.0	5	0 (0)	
14.0	3	1 (33)	Thromb (3), Gran (4), Mucositis (3)[a]

[a] One patient had multiple toxicities
[b] Previous cholestatic jaundice with FUDR
Thromb, thrombocytopenia; Gran, granulocytopenia

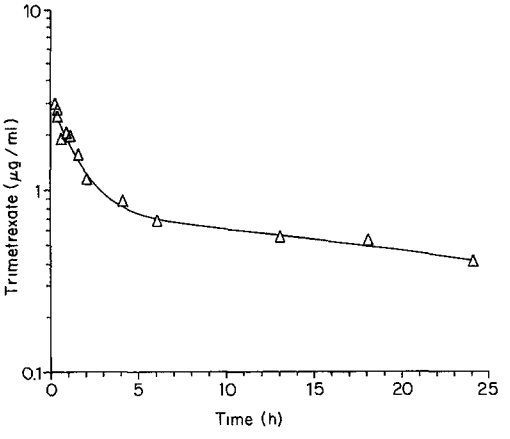

Fig. 1. Time course of plasma TMTX disappearance in a representative patient. The *solid line* represents the computer-simulated curve resulting from parameters obtained from nonlinear regression analysis.

Table 5. Trimetrexate pharmacokinetic parameters[a]

Dose (mg/m^2)	(*n*)	Half-lives (h)		AUC (μg · h/ml)	V_d ss (l/m^2)	Clearance (ml/min per m^2)		Urinary clearance (%)
		α	β			renal	total	
8	1	0.15	12.3	8.86	15.3	1.2	15.1	7.9
10	3	0.18 ± 0.03	16.8 ± 5.0	11.8 ± 5.7	20.1 ± 3.0	0.65 ± 0.37	16.6 ± 8.4	3.6
12	2	0.55 ± 0.02	22.0 ± 5.4	21.7 ± 8.4	17.9 ± 10.0	0.54 ± 0.58	10.0 ± 3.8	5.0
14	1	0.51	10.3	15.3	12.2	2.7	15.2	17.7

[a] Values represent the mean ± SD when $n > 1$

are summarized in Table 5. There was a dose-related linear increase in the AUC and a plot of plasma TMTX concentration/dose for each dosage level was superimposable, consistent with first-order elimination (data not shown).

Protein binding study

The nonspecific binding of TMTX to the centrifree device was evaluated at three concentrations: 0.1, 1.0, and 5.0 μg/ml. the percentage of recovery of TMTX after filtration of the first 400-μl aliquot ranged from 75% to 84%. After filtration of the second 400-μl aliquot, 95% of the TMTX was recovered. For the determination of protein binding, the initial 400 μl ultrafiltrate was discarded and the second 400 μl, collected and analyzed. When serum spiked with TMTX (5 μg/ml) was tested, the TMTX concentration in the ultrafiltrate uncorrected for nonspecific binding was 0.144 ± 0.003 μg/ml (mean ± SD; $n = 3$). After correction for 95% recovery, the serum ultrafiltrate concentration was 0.151 ± 0.003 μg/ml, indicating that TMTX is 97% protein-bound.

Discussion

The primary goal of this study was to determine the phase II dose of TMTX that could be given in combination with 5-FU, both as 10-min infusions, on a 5-day schedule. The 5-day bolus schedule was chosen based on murine studies of (a) TMTX alone, demonstrating maximal antitumor activity with frequent daily doses [18], and (b) TMTX in combination with 5-FU, demonstrating a synergistic antitumor effect with both agents given frequently (TMTX given q3h on days 1, 4, and 9, and 5-FU given as a daily bolus $\times 9$ days) [13]. The phase II dose of TMTX recommended for single-agent studies on this schedule for previously untreated or minimally treated patients was 12 mg/m^2 per day. The present study demonstrated that a standard daily dose of 5-FU (400 mg/m^2 per day) can be combined with the recommended phase-II TMTX dose with acceptable toxicitiy in good-risk patients.

Myelotoxicity and mucositis were the major toxicities in the present TMTX-5FU combination. Severe myelosuppression was sporadic, occurring at TMTX doses of 3.0, 6.0, 8.0, 10.0, 12.0, and 14.0 mg/m^2. At the highest dose studied, 14.0 mg/m^2, two patients had only grade 1 and grade 2 neutropenia, but a third patient developed grade 4 neutropenia and thrombocytopenia and died of gram-negative pneumonia and septicemia. The extent of prior radiation or chemotherapy did not correlate with the degree of myelosuppression, but the number of patients for this analysis was small.

Mucositis on the present regimen was more prominent than that reported for TMTX alone and could be attributed to the addition of 5-FU. Hepatotoxicity consisting of transaminase elevation has been reported with TMTX, and it developed in one patient treated at 4.0 mg/m^2 in the present study. Severe cholestatic jaundice occurred in a second patient, in whom previous FUDR hepatic infusion caused reversible cholestatic jaundice. Other toxicities, mainly nausea, diarrhea, and fatigue, were mild and transient. The skin reactions observed in three patients were similar to those reported in previous phase I studies of TMTX [22].

The elimination of TMTX after a 10-min infusion was best described by a biexponential equation, and half-lives were consistent with values that have previously been reported [3, 7, 9, 15, 21]. The present study confirmed the slow rate of renal clearance of the parent compound previously reported by other investigators. Lin et al. [15] have shown that at single bolus doses below 120 mg/m^2, 1.3% and 0.8% of the dose was excreted in the stool, as determined by the dihydrofolate reductase inhibition assay and by HPLC, respectively. Together, these data support the importance of biotransformation in the elimination of TMTX.

TMTX was found to be highly protein-bound (97%) by the technique of centrifugal ultrafiltration. These results are in general agreement with those of Balis et al. [2], who found 90% protein binding in monkey serum using the equilibrium dialysis method.

No correlation was found between toxicity and any of the above pharmacokinetic parameters in the seven patients in whom TMTX levels were measured by HPLC. The dihydrofolate reductase (DHFR) inhibition assay for TMTX has been shown in one study [15] to yield a better correlation between TMTX plasma levels and toxicity than the HPLC method. The DHFR assay, unlike HPLC, may detect inhibitory metabolites of TMTX that could contribute to toxicity. In conclusion, the recommended phase II dose of TMTX in this combination and on this schedule was found to be the same as that for the single agent. The pharmacokinetics of TMTX in this combination appear similar to those reported by others in single-agent studies.

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