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A phase I study of trimetrexate (NSC 352122) administered by 5-day continuous intravenous infusion*

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Summary. Trimetrexate (TMTX) is a potent inhibitor of dihydrofolate reductase that circumvents the transport resistance seen with methotrexate and has a wide spectrum of preclinical activity. A total of 18 patients with advanced cancer were treated in a clinical and pharmacological phase I trial with TMTX given as a continuous 5-day intravenous infusion. Neutropenia, thrombocytopenia and stomatitis were the dose-limiting toxicities at the maximum tolerated dose of 50 mg/m^2 per 120 h (10 mg/m^2 per day for 5 days). There was one septic death associated with neutropenia. Other toxicities were mild rash, mild nausea and transiently raised serum transminase levels. Significant relationships between the dose given and the AUC of plasma TMTX and the steady-state plasma level were established. Significant, although weak, relationships between the percentage of change in neutrophils and platelets and both the AUC and steady-state plasma level of TMTX were also observed. No objective tumour responses were seen, although six patients had stable disease. The recommended phase II dose for a continuous infusion of trimetrexate is 40 mg/m^2 per 120 h.

Introduction

Methotrexate is a drug with a wide spectrum of antitumour activity and use in clinical oncology; however, its usefulness has been limited by initial or acquired tumour resistance [3, 4]. Trimetrexate glucuronate (TMTX; NSC 352122) is a 2,4-diaminoquinazoline derivative that may overcome tumour resistance, at least in some tumours. TMTX has substantial activity in vitro against a number, of cell lines, and in vivo against i.p. implanted B16 melanoma, colon 26, L1210 and P388 leukemia and s.c. implanted CD8F$_1$ mammary tumour [2, 10, 13]. In contrast to that of methotrexate, the uptake of TMTX into methotrexate-transport-resistant L1210 leukemia is proportional to the extra-cellular drug concentration of TMTX [9]. TMTX thus concentrates in human leukemia cells to a greater extent than methotrexate [2], which may enable it to circumvent resistance to methotrexate caused by impaired transport. Indeed, TMTX is effective against methotrexate-transport-resistant human T-cell leukemia and osteosarcoma cell lines [5].

There is evidence for schedule dependency for both i.p. and i.v. routes, with the drug being more effective when given in repeated daily doses than as intermittent bolus therapy [13]. Pharmacokinetics in dogs indicate that a first-order elimination phase predominates, with a half-life of 3.5 h after a single i.v. bolus of 3 mg/kg [16].

Preclinical animal toxicology was carried out in CF-1 mice and beagle dogs using daily $\times 1$ and daily $\times 5$ i.v. schedules [8, 16]. In mice, toxicity included convulsions, ataxia, depression, minor increases in serum blood urea nitrogen and moderate increases in serum creatinine phospokinase (CPK) levels. In dogs, the major toxicity was gastrointestinal, including anorexia, emesis, salivation and gastrointestinal hemorrhage, and minimal to moderate decreases in hemoglobin and leucocytes were noted.

Phase I evaluation of TMTX was initiated by the United States National Cancer Institute, with schedules including daily i.v. bolus and continuous i.v. infusion. We evaluated a 5-day continuous i.v. schedule to define the maximum tolerated dose (MTD), document all drug-related toxicities, define a recommended phase II dose, note any tumour responses and define the clinical pharmacology of the drug.

Materials and methods

Patient eligibility. Patients eligible for this study had histologically proven metastatic or loco-regionally recurrent solid malignancies for which there was no conventional therapy of proven benefit. Patients also had an expected life span of more than 8 weeks and thus had the potential of receiving two courses of therapy. Pre-treatment neutrophil counts of $>2\times10^9$/l, platelet counts of $>100\times10^9$/l, serum creatinine values of <0.12 mmol/l and normal serum uric acid levels were required. Other requirements included no chemotherapy in the prior 3 weeks (6 weeks for nitrosoureas or mitomycin C) and radiotherapy to $<50\%$ of bone marrow-bearing areas, with no radiation within 6 weeks prior to study entry. All patients were >18 years of age, had no significant non-malignant condition, were not lactating or pregnant, received no other cytotoxic therapy during the trial and had no significant effusions or other potential third spaces. The protocol was written to conform to guidelines of the National Health and Medical Re-

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search Council of Australia and was approved by the Institutional Ethics Committees. Written informed consent was obtained from all patients.

Drug delivery. TMTX was supplied by the Cancer Therapy Evaluation Program of the National Cancer Institute of the United States as the glucuronate salt in 50 mg vials and was stored at 4° C. The vial contents were diluted to 0.1 mg/ml in 5% dextrose-water for infusion. The drug was given as a 5-day (120-h) continuous i.v. infusion via an infusion pump.

Treatment plan. Cohorts of three patients were treated at each dose level. Planned dose levels were 5 mg/m^2 per 120 h (1 mg/m^2 per day for 5 days) and 20, 30, 40, 50 and 60 mg/m^2 per 120 h, with further dose escalation dependent on results. The treatment was repeated every 21 days if appropriate. The dose level of 10 mg/m^2 per 120 h was not studied because data from a parallel study suggested that this dose level was associated with a lack of toxicity. No dose escalation occurred in any individual patient, although dose reduction was permitted for defined toxicity. The dose of TMTX was not escalated in a new cohort of patients until all side effects were fully documented within the cohort on the last dose level. Routine antiemetics were not given unless severe nausea and vomiting occurred. All patients were hospitalized for the duration of the infusion and for at least 24 h after cessation of the infusion.

Study parameters. On study entry, all patients had their clinical history documented, a physical examination, documentation of Eastern Cooperative Oncology Group (ECOG) performance status, full blood examination, determination of serum urea and electrolytes, liver function tests and electrocardiograph as well as investigations, such as X-rays, radionucleotide scans or computerized axial tomographic (CAT) scans, necessary to measure tumour masses. Patients were examined daily while on the 5-day infusion, then weekly between treatments. Full blood examination, serum electrolyte determination and liver function tests were carried out every other day during the infusion, then weekly between treatments. World Health Organization (WHO) criteria for toxicity and response were used [12].

Clinical pharmacological studies. The specific high-performance liquid chromatographic assay of Ackerly et al. [1] was used for the quantitation of plasma and urinary levels as previously described [14]. Lower detection limits were attained by evaporating the eluate from the Bond Elut column extraction procedure and reconstituting it in a small volume of mobile phase. In addition, the AUC, terminal half-life ($t_{½}$), mean residence time (MRT, a measure of the time the drug stayed in the body), percentage of the dose excreted unchanged (%E), and steady-state plasma level (C_{ss}) used in correlations with toxicity were determined by non-compartmental methods. The frequency of sampling and the pharmacological methods and analyses of pharmacological parameters have previously been reported [14]. In addition, the percentage of change in platelets was determined after each TMTX course from the following:

$$\% \text{ change} = 100 \times \frac{\text{pre-treatment platelet count} - \text{platelet nadir}}{\text{pre-treatment platelet count}}$$

Table 1. Patient characteristics

Patients studied		18
Sex:	Male	9
	Female	9
Median age (range)		52 years (28–73)
ECOG performance status:		
	0	7
	1	7
	2	2
	3	2
Diagnosis:		
	Small-cell lung cancer	5
	Non-small-cell lung cancer	4
	Breast cancer	2
	Soft tissue sarcoma	2
	Pancreatic cancer	1
	Melanoma	1
	Adenoid cystic cancer parotid	1
	Renal cancer	1
	Adeno-carcinoma, primary unknown	1
Prior chemotherapy regimens:		
	None	4
	1	7
	2	3
	3	4
Prior radiotherapy:		
	No	6
	Yes	12

The same method was used to calculate the percentage of change in neutrophils. The percentages of change in platelets and neutrophils were correlated with the TMTX AUC, C_{ss}, MRT, dose, %E and $t_{½}$.

Results

A total of 18 patients entered this study. In general, they had been heavily pre-treated but had a good performance status; 9 of 18 had a diagnosis of lung cancer (Table 1). Three patients were treated at each of the dose levels 5, 20, 30 and 40 mg/m^2 per 120 h. Five were treated at 50 mg/m^2 per 120 h, and one, at 60 mg/m^2 per 120 h; the latter patient received a second course of 30 mg/m^2 per 120 h. All patients treated at 50 and 60 mg/m^2 per 120 h had been pre-treated, although one had received radiotherapy only.

Toxicity

The major toxicities encountered were neutropenia, thrombocytopenia and stomatitis (see Table 2). Major toxicity was seen in one of three patients at 40 mg/m^2 per 120 h, in four of five patients at 50 mg/m^2 per 120 h and in one patient at 60 mg/m^2 per 120 h. Cytopenia was first noted on days 7–9 after starting chemotherapy, recovery took place by day 14, with the nadir neutrophil or platelet count occurring at a median of day 10.

Neutropenia. WHO grade 3 neutropenia ($0.5–0.9 \times 10^9$ cells/l) was seen in one previously untreated patient at 40 mg/m^2 per 120 h. Grade 3 or 4 neutropenia ($< 1.0 \times 10^9$ cells/l) was seen in three of five patients at 50 mg/m^2 per 120 h. The duration of grade 4 neutropenia was 5 days in one patient, who developed a fever of > 38° C, with nega-

Table 2. Number of patients with dose-limiting TMTX toxicity ($n = 18$)

	WHO grade[a]	x10^9/l	Dose (mg/m^2 per 120 h)					
			5	20	30	40	50	60
Neutrophilis	0–1	>1.5	3	3	3	2	2	1
	2	1–1.49	0	0	0	0	0	0
	3	0.5–0.99	0	0	0	1	1	0
	4	<0.5	0	0	0	0	2	0
Platelets	0	>100	3	3	3	2	1	0
	1	75–99	0	0	0	1	0	0
	2	50–74	0	0	0	0	0	0
	3	25–49	0	0	0	0	1	1
	4	<25	0	0	0	0	3	0
Stomatitis	0		3	3	3	2	2	0
	1		0	0	0	0	1	0
	2		0	0	0	1	0	0
	3		0	0	0	0	2	1
			$n = 3$	$n = 3$	$n = 3$	$n = 3$	$n = 5$	$n = 1$

[a] See [12]

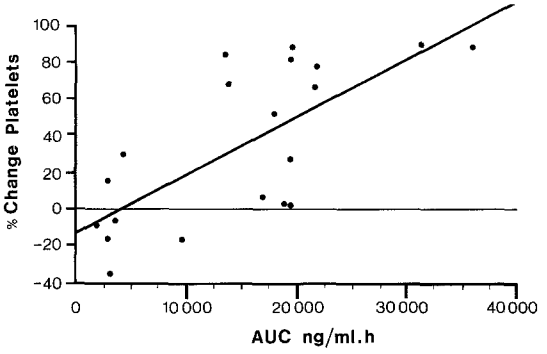

Fig. 1. Relationship between the percentage of change in platelet count and the AUC of TMTX ($r^2 = 0.51$; $P = 0.0004$)

tive cultures, and recovered without sequelae following i.v. antibiotics and the return of a normal neutrophil count. The other patient with grade 4 neutropenia died of progressive disease. Grade 3 neutropenia lasted 2–4 days.

Thrombocytopenia. WHO grade 4 thrombocytopenia ($<25 \times 10^9$ platelets/l) was seen in three of five patients at 50 mg/m^2 per 120 h. The duration of WHO grade 4 thrombocytopenia could not be precisely determined because both patients who survived received platelet transfusions. However, platelet support was no longer required after a recovery in platelet count to $>50 \times 10^9$/l in <4 days in both cases.

Stomatitis. WHO grade 3 stomatitis, with mouth ulcers and the inability to eat, was seen in two of five patients given 50 mg/m^2 per 120 h and in the patient receiving 60 mg/m^2 per 120 h; the duration was 7 days. WHO grade 2 stomatitis lasted for 5 days.

Nausea and vomiting. Nausea and vomiting was generally mild. WHO grade 2 nausea and vomiting, controllable by antiemetics, was seen only at doses of 60 mg/m^2 per 120 h. Only one course was associated with grade 1 nausea at each of the dose levels 5, 20, 30 and 50 mg/m^2 per 120 h. No nausea was seen at 40 mg/m^2 per 120 h.

Other toxicities. One patient died who developed neutropenia, fever, thrombocytopenia and progressive disease after receiving TMTX at a dose of 50 mg/m^2 per 120 h. In this patient, active support therapy for cytopenia was withdrawn at the patient's request in the presence of progressive disease.

A maculopapular rash was seen in two patients, one of whom received 50 mg/m^2 per 120 h and the other, 60 mg/m^2 per 120 h. The rash was widely distributed over the trunk, limbs, palms and soles and lasted for approximately 7 days, with associated dry desquamation, burning sensation and itching. Anorexia was noted in four patients, although one case occurred in the context of progressive disease. Diarrhoea lasting 24–48 h was observed in two patients.

Three patients had a transient rise in serum aspartate transaminase (AST) levels after TMTX administration. At 5 days after starting TMTX, two patients receiving 50 mg/m^2 per 120 h had elevations in AST that were 2–5 times the normal level. AST values in the patient receiving 60 mg/m^2 per 120 h were elevated to >3 times the normal level within 3 days after the beginning of treatment. Rechallenge with TMTX at 30 mg/m^2 per 120 h in this patient resulted in a further transient rise in AST levels to >1.5 times the normal value. Two other patients had shown elevations in both AST and alkaline phosphatase levels prior to starting TMTX; these values did not increase substantially during TMTX treatment.

There were significant linear relationships between the percentage of change in platelets and the TMTX AUC ($r^2 = 0.51$; $P = 0.0004$) (Fig. 1), C_{ss} ($r^2 = 0.43$; $P = 0.0016$), MRT ($r^2 = 0.57$; $P = 0.0001$) and dose ($r^2 = 0.55$; $P = 0.0002$) after 20 courses in 11 of the patients in whom these measurements were made. Similarly, there were significant linear relationships between the percentage of change in neutrophils and the TMTX AUC ($r^2 = 0.44$; $P = 0.0004$) (Fig. 2), C_{ss} ($r^2 = 0.35$; $P = 0.0017$), and dose ($r^2 = 0.58$; $P = 0.0001$) but not MRT ($r^2 = 0.07$; $P = 0.21$). To assess overall toxicity, a major toxicity score was arbitarily generated using the sum of the toxicity grades for hemoglobin, white cells, neutrophils, platelets, rash, nausea and vomiting and stomatitis. In measurements made after 29 courses

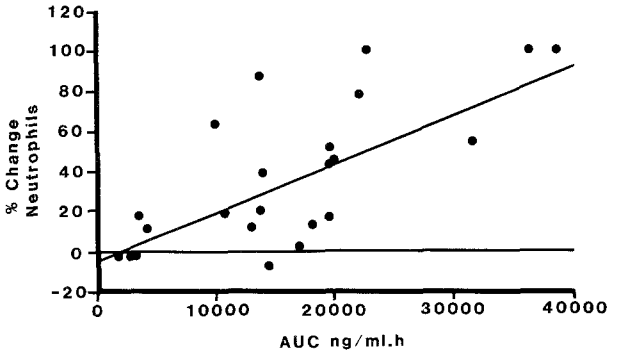

Fig. 2. Relationship between the percentage change in neutrophil count and the AUC of TMTX ($r^2 = 0.44$; $P = 0.0004$)

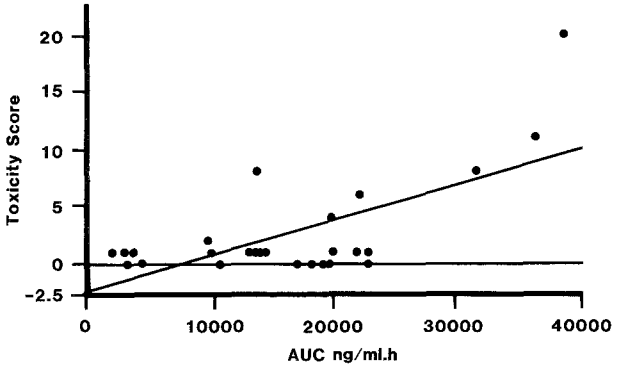

Fig. 3. Relationship between the major toxicity score and the AUC of plasma TMTX ($r^2 = 0.44$; $P = 0.0001$)

in 16 patients, there were significant, although weak, linear relationships between this major toxicity score and the TMTX AUC ($r^2 = 0.44$; $P = 0.0001$) (Fig. 3), C_{ss} ($r^2 = 0.32$; $P = 0.0014$), MRT ($r^2 = 0.16$; $P = 0.03$) and dose ($r^2 = 0.35$; $P = 0.0008$). There were no significant relationships between %E or $t_{½}$ and toxicity.

Responses

No objective responses were seen. In all, 6 patients (non-small-cell lung cancer, 2; small-cell lung cancer, 2; adenoid cystic carcinoma parotid, 1; and breast cancer, 1 had stable disease for at least 4 weeks following the completion of therapy, 11 had progressive disease and 1 was not evaluable for response.

Survival

A total of 14 patients died and 4 are still alive, with a median survival for all patients of 19 weeks (range, 1.5–52 weeks).

Recommended phase II dose

The MTD of TMTX was 50 mg/m^2 per 120 h. The recommended phase II dose for previously treated patients is 40 mg/m^2 per 120 h given as a continuous infusion over 5 days. Insufficient numbers of previously untreated patients were studied to enable the recommendation of a phase II dose for such patients. However, one previously untreated patient who received 40 mg/m^2 per 120 h experienced grade 3 neutropenia and grade 2 stomatitis.

Discussion

In this phase I study, the dose-limiting toxicities of TMTX were neutropenia, thrombocytopenia and stomatitis. The MTD for TMTX as a continuous i.v. infusion was 50 mg/m^2 per 120 h, with the recommended phase II dose being 40 mg/m^2 per 120 h. However, the duration of grade 4 neutropenia and thrombocytopenia was only 4–5 days. Severe stomatitis was seen in two of five patients at 50 mg/m^2 per 120 h and in one patient at 60 mg/m^2 per 120 h. The duration of stomatitis was longer, with grade 3 stomatitis lasting for 7 days and grade 2 stomatitis, for 5 days. In one patient, stomatitis provided the probable portal of entry for infection. All patients with severe toxicities (grade 4 neutropenia or thrombocytopenia or grade 3 stomatitis) had been pre-treated, although one had received limited prior radiotherapy only. Although the MTD was well defined in this study, two patients had no neutropenia and two had no stomatitis at 50 mg/m^2 per 120 h.

The MTD defined in this study is similar to that previously defined by Rosen et al. [15], who recommended 34 mg/m^2 per 120 h as the phase II dose. In that study, two of eight patients developed WHO grade 4 leukopenia and thrombocytopenia at that dose. The MTD in these two infusion studies was substantially lower than that achieved with other schedules. Lin et al. [11] reported that the MTD for TMTX given i.v. over 1 h every 2 weeks was 120 mg/m^2, and that reported by Donehower et al. [6] was >140 mg/m^2 i.v. every 3 weeks. The MTD reported by Weir et al. [16] was 100 mg/m^2 for i.v. TMTX given weekly [16]. As in our study, the dose-limiting toxicity reported by these authors was hematological. However, stomatitis may be a more important side effect with continuous infusion than with intermittent schedules.

Other toxicities encountered were mild. WHO grade 2 nausea and vomiting (controlled by antiemetics) was seen only at a dose of 60 mg/m^2 per 120 h; nausea only was seen at four other dose levels. A rash appeared in two patients receiving 50 and 60 mg/m^2 per 120 h but was of little clinical importance. Two patients given 50 mg/m^2 per 120 h and the one receiving 60 mg/m^2 per 120 h had a transient elevation in serum asparate transaminase levels that was temporarly related to the TMTX infusion. Rechallenge of the latter patient with 30 mg/m^2 per 120 h resulted in a further rise in transaminase, suggesting a causal relationship.

Weak correlations between toxicity and the AUC or C_{ss} of TMTX were apparent in this study. Correlations were also obtained between toxicity and the dose given. This was consistent with our previously reported observations of excellent linear relationships between the TMTX AUC, C_{ss} and dose [14]. Therefore, knowledge of the TMTX C_{ss} did not provide any additional advantage over dose in predicting toxicity in this study. However, these relationships require reassessment in phase II studies in which larger numbers of patients receive the same dose. Lin et al. [11] have reported a correlation between the 24-h plasma concentration and toxicity after a 1-h infusion of TMTX. Further studies may therefore establish a role for plasma level monitoring in the clinical use of TMTX.

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