Cancer Chemother Pharmacol (1987) 20: 169–172

Cancer Chemotherapy and Pharmacology

A phase I study of trimetrexate, an analog of methotrexate, administered monthly in the form of nine consecutive daily bolus injections

Jacques Jolivet[1]*, Linda Landry[1], Marie-France Pinard[1], John J. McCormack[2], William P. Tong[2], and Elisabeth Eisenhauer[3]

[1] Hôpital Notre-Dame and Institut du Cancer de Montréal 1560 Sherbrooke St. E., Montreal, Quebec H2L 4M1, Canada
[2] Department of Pharmacology, University of Vermont, Burlington, Vermont, USA
[3] National Cancer Institute of Canada Clinical Trials Group, Kingston, Canada

Summary. Trimetrexate glucuronate (TMTX) is a methotrexate (MTX) analog that is active against transport-deficient MTX-resistant tumor cells. We performed a phase I study of TMTX administered by daily bolus for 9 consecutive days since this schedule is one of the most active in experimental murine tumor models. The drug was administered in this fashion every 4 weeks for at least two cycles. Fifteen patients with refractory metastatic cancers were studied and all had received prior chemotherapy. The dose-limiting toxicity was a rapidly reversible thrombocytopenia first seen at a daily dose of 4.0 mg/m^2 which occurred 7 days after the end of TMTX administration. There was great inter- and intrapatient variability in the platelet nadirs observed in the six patients treated at 4.0 mg/m^2. One patient died of massive hemoptysis during a platelet nadir at that dose level. Granulocyte counts never dropped below 1500/mm^3. Only one patient had significant non-hematological toxicity: a radiation recall skin toxicity along with a self-limited maculopapular rash. One patient with melanoma and lung metastases treated at 4.0 mg/m^2 had a partial response. TMTX plasma levels were measured by HPLC every 3 days prior to daily dosing in patients receiving 4 mg/m^2 to determine whether drug accumulation occurred during this prolonged administration schedule. Nadir drug levels varied from less than 0.02 to 0.35 μM and did not seem to increase during the 9-day schedule in individual patients. By comparison with other phase I trials, the hematologic toxicity of TMTX seems to be schedule-dependent, with less drug being tolerated and more severe thrombocytopenia observed with more protracted treatment protocols. A firm phase II starting dose for daily bolus $\times 9$ schedules is difficult to recommend in view of the variable toxicity observed in the patients treated at 4.0 mg/m^2 daily, who, in addition, had all been extensively pretreated. A reasonable starting dose might be 3.0 mg/m^2 daily with built-in dosage increases or decreases.

Introduction

Trimetrexate glucuronate (TMTX) is one of a series of compounds developed through research efforts to devise antifolates with greater therapeutic indices than methotrexate (MTX) [9]. TMTX is, like MTX, a potent dihydrofolate reductase inhibitor, but enters cells by a different transport system and can overcome MTX resistance in cells with drug transport defects [4, 10, 13, 16]. Furthermore, the analog is also active in a human breast cancer cell line resistant to MTX secondarily to a greatly decreased metabolism to polyglutamate derivatives [3]. As for other antifolates [7], TMTX activity and toxicity was found to be schedule-dependent in experimental models [14, 15]. Nine successive daily injections of the drug in the i.p.-implanted P388 leukemia model were more effective than other schedules consisting of five successive daily injections, intermittent treatment on days 1, 5 and 9 or a single treatment on day 1 [15]. Consequently, we performed a clinical phase I study to determine whether the toxicity of TMTX is increased when the drug is given in a daily bolus $\times 9$ schedule compared to the less protracted administration schedules used in other phase I TMTX trials. Furthermore, we have examined whether the drug accumulates in the plasma during this repeated daily bolus administration schedule.

Materials and methods

Patient selection and monitoring. Patients eligible for this study had metastatic solid tumors refractory to conventional therapy, were fully recovered from previous radiotherapy or chemotherapy and had a life expectancy of at least 8 weeks. Prior to beginning TMTX, each patient underwent a comprehensive evaluation including complete history, physical examination and assessment of disease by appropriate modality (e.g. X-ray, scan). Pretreatment evaluation also included complete blood count with WBC differential, platelet count, blood smear, urinalysis, creatinine clearance and serum chemistries. Complete blood counts were carried out every 3 days during TMTX administration and weekly between cycles along with serum chemistries. All patients had adequate pretreatment renal function as defined by a creatinine clearance of 60 ml/min or greater, a granulocyte count greater than 1500/mm^3, platelet count greater than 100000/mm^3, normal hepatic function with bilirubin less than 2 mg/dl and serum glutamic-oxaloacetic transaminase less than 100 units/dl (except in cases of documented metastatic involvement of liver). All patients gave written informed consent prior to therapy.

* Supported by the National Cancer Institute of Canada Clinical Trials Group.

Offprint requests to: Jacques Jolivet

Table 1. Patient characteristics

No. of patients in study	15
Men	9
Women	6
Median age (years)	55 (36–70)
Performance status	
ECOG grade 0	2
1	12
2	1
Tumor types	
Breast	3
Colorectal	3
Head and neck	3
Hypernephroma	3
Lung non-oat-cell	2
Melanoma	1

ECOG, Eastern Cooperate Oncology Group

Patient population. Patient characteristics are shown in Table 1. Fifteen patients, nine men and six women, ranging in age from 36 to 70 years, were entered in the study. All patients had pathological confirmation of cancer and had received prior therapy including chemotherapy.

Drug formulation and dosage. TMTX was supplied by the Investigational Drug Branch, National Cancer Institute, Bethesda, MD, in 50-mg vials in the form of the glucuronate salt. The product is prepared as a light-yellow powder in 6-ml vials. When reconstituted as directed the solution exhibits no decomposition for at least 7 days of storage both at room temperature and under refrigeration.

The starting dose (0.125 mg/m^2 in 10 ml 5% dextrose) was 1/20 of the LD_{10} in mice treated for 5 consecutive days with TMTX. The usual starting dose of 1/10 of the LD_{10} was halved because of the longer administration schedule used in this trial. A treatment cycle consisted of nine consecutive daily bolus administrations of the drug and was repeated every 28 days. However, since another TMTX phase I study of a daily bolus $\times 5$ schedule [18] had already shown by the beginning of our trial that much higher doses of TMTX could be safely administered, the TMTX dose escalation schedule for the present study was accelerated after the first dose level had been completed as shown in Table 2.

Three patients were evaluable for toxicity at each dose level before escalation occurred. Six patients were entered at the maximum dose studied. No dose escalation was performed in individual patients. Treatment was given for at least two cycles unless contradicted by progressive disease or unacceptable toxicity.

Toxicity criteria. Toxicity was graded according to standard Eastern Cooperate Oncology Group criteria. Maximum tolerated dose (MTD) was defined as being attained when one of the following criteria was met:
- Two patients with grade 3 hematological toxicity
- One patient with grade 4 hematological toxicity
- Two patients with grade 2 non-hematological toxicity
- One patient with grade 3 non-hematological toxicity

Table 2. Acceleration of dose escalation schedule

Dose (mg/m^2)	No. of patients	% increase
0.125	3	–
1.0	3	700
2.0	3	100
4.0	6	100

Drug level measurements. TMTX plasma levels were measured approximately every 3 days during the 9-day schedule immediately prior to drug administration to determine whether drug accumulation occurred. Drug was assayed by HPLC as previously described [1] except that a large injection loop (200 µl) was used because of low sample concentrations. The assay's limit of sensitivity is approximately 0.02 µ*M*. Insufficient concentrations of drug were present at the lower dose levels to permit reliable measurements, however, and results will be presented only for patients receiving 4.0 mg/m^2.

Results

Toxicity

Fifteen patients were treated with TMTX during the study for a total of 52 cycles of therapy. Two patients had tumour progression before the second cycle of therapy could be given. Dose-limiting toxicity consisted of reversible thrombocytopenia which was first observed at 4.0 mg/m^2 daily bolus $\times 9$. The platelet nadir occurred regularly 7 days after the end of TMTX administration and was always transient, lasting no more than 2 days. The characteristics of the six patients treated at this dose level are shown in Table 3. There was great variability in the platelet nadirs observed among the different patients and between different courses in each individual patient. The first grade 4 (<25000) thrombocytopenia was seen in the fifth patient entered at 4 mg/m^2. Prior to that episode, one grade 3 and two grade 2 thrombocytopenias had been observed, suggesting that the MTD was near and prompting us to enter a sixth patient at the same dose level we now felt was the MTD. Within 2 months, however, patients 12 and 13 had two other episodes of grade 4 thrombocytopenia. Patient 14 had transient mild rectal bleeding while thrombocytopenic which stopped after platelet transfusions. He had a large pleural effusion which had progressed rapidly on therapy, however, and he was taken off TMTX. The source of rectal bleeding was not investigated because of rapid clinical deterioration. Patient no. 13 received six courses of therapy despite the observed thrombocytopenia because of stabilisation of his pulmonary metastases. During the sixth course, however, the patient developed a necrotic abscess in one of his metastases and died of massive hemoptysis at a time after therapy when he should have reached his platelet nadir. The patient expired before a blood count could be done. Other episodes of thrombocytopenia did not lead to complications. The degree of thrombocytopenia acheived was not clearly related to the extent of the pretreatment in the six patients entered at 4.0 mg/m^2 (Table 3). Despite the numerous episodes of thrombocytopenia, granulocyte counts never dropped below 1500/mm^3 in any patient.

Other toxicities included one case of mild mucositis at the 2 mg/m^2 dose level and, in patient 14, a radiation recall skin reaction over a chest radiotherapy port (4000 rads administered 10 months prior to TMTX) which was fol-

Table 3. Characteristics of patients treated at 4.0 mg/m^2

Patient (study no.)	On study date	Neoplasm	Previous chemotherapy and radiotherapy (RoRx)	Platelet nadirs[a]	TMTX nadir plasma levels on course 1 (μM)[b]
10	9/9/85	Non-oat-cell lung	P-VDS: 8 courses to 8/85 RoRx: 5000 rads to mediastinum to 10/84 2500 rads to lumbar spine to 5/85 2400 rads to left femur to 5/85	58, 30	ND (day 4); 0.19 (day 6); 0.17 (day 9)
11	9/9/85	Colon	5-FU-MMC: 20 courses to 8/85 RoRx: none	214, 193, 256, 188, 232, 245, 277	ND (day 4); 0.02 (day 6); 0.03 (day 9)
12	10/7/85	Renal cell	VBL-CCNU: 7 courses to 5/83 RoRx: none	195, 53, 20	0.18 (day 4); 0.14 (day 6); 0.15 (day 9)
13	11/4/85	Pharynx	BL-CTX-MTX-5-FU: 7 courses to 3/83 MTX-5-FU-LV: 4 courses to 2/84 DXR-VBL: 9 courses to 1/85 P-5-FU: 5 courses to 9/85 RoRx: 6600 rads to pharynx and neck up to 7/82	97, 13, 49, 27, 29	IS (day 5); 0.10 (day 9)
14	11/4/85	Non-oat-cell lung	CTX-DXR-P: 1 course in 5/85 P-VDS: 1 course in 7/85 VP-16-VBL: 3 courses to 10/85 RoRx: 3750 rads to mediastinum to 12/84	5	IS (day 3); IS (day 5); 0.35 (day 9)
15	12/9/85	Melanoma	BCNU-HU-DTIC: 6 courses to 6/83 RoRx: 6000 rads to neck to 3/83	124, 70, 67, 91, 53, 97, 109, 177	0.18 (day 3); 0.12 (day 5); 0.09 (day 9)

Abbreviations: BCNU, Carmustine; BL, bleomycin; CCNU, lomustine; CTX, cyclophosphamide; DTIC, dacarbazine; DXR, doxorubicin; HU, hydroxyurea; LV, leucovorin; MMC, mitomycin C; MTX, methotrexate; P, cis-platinum; VBL, vinblastine; VDS, vindesine; VP-16, etoposide; 5-FU, 5-fluorouracil; ND, not detected; IS, insufficient sample size for analysis

[a] Platelet nadirs (mm^3) observed during consecutive courses of TMTX

[b] TMTX levels measured by HPLC during the first course of TMTX prior to drug administration on the days mentioned.

Table 4. Phase I trials of TMTX

Schedule	Recommended starting dose for phase II studies	Reference
Bolus, every 2–3 weeks	200 mg/m^2	11
One-hour infusion, every 2 weeks	120 mg/m^2	12
Bolus, every 3 weeks	Not stated	5
Three consecutive weekly boluses, every 5 weeks	50 mg/m^2	6
Five-day infusion	34 mg/m^2/120 h	17
Daily bolus × 5, every 3 weeks	8 mg/m^2	18
		Grillo-Lopez AJ, personal communication

lowed in the next few days by a generalised maculopapular skin rash. The skin reaction rapidly abated and disappeared within a week.

Responses

One patient with melanoma (patient 15) treated at 4.0 mg/m^2 had a 50% reduction in the size of two lung metastases which became evident after four cycles of therapy and was maintained on treatment for an additional 4 months. He had received adjuvant dacarbazine, BCNU and hydroxyurea for 4 months 3 years prior to developing lung metastases and being treated with TMTX. Treatment was stopped after eight courses while the patient was still in a partial response. One month later, however, the patient presented with brain metastases, and he expired within 2 months despite palliative radiotherapy.

Nadir drug levels

Plasma TMTX concentrations were measured prior to drug administration by HPLC approximately every 3 days during the first course of the 9-day schedule in patients receiving 4.0 mg/m^2. The results are shown in Table 3. The limited amount of data available does not allow correlations to be drawn, although the lowest nadir levels measured occurred in patient 11, who had no thrombocytopenia, while the highest day 9 through level was observed in patient 14, who suffered the most profound drop in platelet count.

Discussion

Trimetrexate has been extensively studied in a number of phase I clinical trials examining the variety of treatment schedules detailed in Table 4 [5, 6, 11, 12, 17, 18]. These studies have shown that the drug's toxicity is schedule-dependent: considerably more drug can be safely administered by bolus every 2–3 weeks [11, 12] than during 5-day schedules [17, 18]. The objective of our trial was to study the toxicity of an even more protracted 9-day administration schedule, as murine experimental tumor models had shown that TMTX has maximum antitumor activity when

administered in such schedules [15] Our study, however, underlined an observation already made by other investigators: the great variability in observed toxicity among different patients at the same dose range [11, 12]. Furthermore, platelet nadirs varied markedly within individual patients, with ranges of 20–195, 13–97, and 53–177 in three different individuals. The patient with the lowest platelet nadir (5000/mm^3) had a large pleural effusion, a factor possibly predisposing to greater toxicity [5]. There was, however, no obvious correlation between the extent to previous chemotherapy, radiotherapy and drug tolerance. The great inter- and intrapatient variability in toxicity observed at the highest drug dosage examined (4.0 mg/m^2) makes it difficult to recommend a firm phase II starting dose using the schedule of a daily dose on 9 consecutive days, but if one excludes patients with malignant effusions from TMTX trials, a 3.0 mg/m^2 $\times 9$ starting dose with built-in dosage increases or decreases would seem reasonable. Another point emphasised by our trial was the fact that thrombocytopenia becomes more prominent and leucopenia less severe as the administration schedule is prolonged: the dose-limiting toxicity in phase I trials examining bi- or triweekly bolus injections was leucopenia with only mild thrombocytopenia [5, 11, 12], while 5-day schedules have reported both of these hematological toxicities [17, 18] and no granulocyte drop to less than 1500/mm^3 was observed in our study with the blood sampling schedule used and despite profound platelet nadirs at 4.0 mg/m^2. The radiation recall reaction and skin rash observed in one of our patients have been described previously in patients receiving this drug [19]. It is noteworthy that no patient experienced alopecia, nausea or vomiting and almost all felt that TMTX was the least "toxic" chemotherapy they had received.

The limited number of plasma levels measured suggest that TMTX did not accumulate in plasma during the 9-day administration schedule at 4.0 mg/m^2 despite the prolonged terminal elimination half-lives reported in primates [2] and in other phase I studies [8, 17, 18]. Further studies will be needed to determine whether nadir TMTX plasma levels can be useful in predicting drug toxicity or activity.

TMTX was found to be an easily administered chemotherapeutic agent in the 9-day treatment schedule used, with little non-hematological toxicity and schedule-dependent hematological toxicity. Phase I studies, however, cannot determine whether different treatment schedules also have different antitumor activities: this is a question to be resolved by phase II trials. The partial response observed in this study, however, confirms that TMTX is an interesting drug for phase II testing in various schedules.

References

1. Ackerly CC, Harthshorn J, Tong WP, McCormack JJ (1985) A rapid and sensitive method for determination of trimetrexate from biological fluids. J Liquid Chromatogr 8: 125–134
2. Balis FM, Lester CM, Poplack DG (1986) Pharmacokinetics of trimetrexate in monkeys. Cancer Res 46: 169–174
3. Cowan KH, Jolivet J (1984) A methotrexate-resistant human breast cancer cell line with multiple defects, including diminished formation of methotrexate polyglutamates. J Biol Chem 259: 10793–10800
4. Diddens H, Niethammer D, Jackson RC (1983) Patterns of cross-resistance to the antifolate drugs trimetrexate, metoprine, homofolate and CB 3717 in human lymphoma and osteosarcoma cells resistant to methotrexate. Cancer Res 43: 5286–5292
5. Donehower RC, Graham ML, Thompson GE, Dole GB, Ettinger DS (1985) Phase I and pharmacokinetic study of trimetrexate in patients with advanced cancer. Proc Am Soc Clin Oncol 4: 32 (abstract C-118)
6. Fanucchi M, Fleisher M, Vidal P, Williams L, Bauer T, Cassidy C, Chou T-C, Young C (1985) Phase I and pharmacologic study of trimetrexate. Proc Am Ass Cancer Res 26: 179 (abstract 710)
7. Goldin A, Mantel N, Greenhouse SW, Venditti JM, Humphreys SR (1954) Factors influencing the specificity of activity of an antileukemic agent (aminopterin). Time of treatment and dosage schedule. Cancer Res 14: 311–314
8. Ho DHN, Covington WP, Legha S, Newman RA, Krakoff IH (1986) Clinical pharmacology of trimetrexate. Proc Am Ass Cancer Res 27: 173 (abstract 687)
9. Jackson RC, Fry DW, Boritzki TJ, Besserer JA, Leopold WR, Sloan BJ, Elslager EF (1984) Biochemical pharmacology of the lipophilic antifolate trimetrexate. Adv Enzyme Regul 22: 187–206
10. Kamen BA, Eibl B, Cashmore A, Bertino J (1984) Uptake and efficacy of trimetrexate, a non-classical antifolate in methotrexate-resistant leukemia cells in vitro. Biochem Pharmacol. 33: 1697–1699
11. Legha S, Tenney D, Ho DH, Krakoff IH (1985) Phase I clinical and pharmacology study of trimetrexate. Proc Am Soc Clin Oncol 4: 48 (abstract C-183)
12. Lin JT, Cashmore AR, Baker M, Dreyer RN, Ernstoff M, Marsh JC, Bertino JR, Whitfield LR, Delap R, Grillo-Lopez AJ (1987) Phase I studies with trimetrexate: clinical pharmacology, analytical methodology and pharmacokinetics. Cancer Res 47: 609–616
13. Mini E, Moroson BA, Franco CT, Bertino JR (1985) Cytotoxic effects of folate antagonists against methotrexate-resistant human leukemic lymphoblast CCRF-CEM cell lines. Cancer Res 45: 325–330
14. National Cancer Institute (U. S.) (1983) Clinical brochure of trimetrexate glucuronate (NSC 352122). November
15. O'Dwyer PJ, Shoemaker DD, Plowman J, Cradock J, Grillo-Lopez AJ, Leyland-Jones B (1985) Trimetrexate: a new antifolate entering clinical trials. Invest. New Drugs 3: 71–75
16. Ohnoshi T, Ohnuma J, Takahashi I, Scanlon K, Kamen BA, Holland JF (1982) Establishment of methotrexate-resistant human acute lymphoblastic leukemia cells in culture and effects of folate antagonists. Cancer Res 42: 1655–1660
17. Rosen M, Ohnuma A, Zimet V, Coffey V, Zhang W, Holland JF (1986) Phase I study of trimetrexate glucuronate in a 5-day infusion schedule. Proc Am Ass Cancer Res 27: 172 (abstract 681)
18. Stewart JA, McCormack JJ, Tong W, Delap RJ, Grillo-Lopez AJ (1985) A phase I study of trimetrexate. Proc Am Ass Cancer Res 26: 159 (abstract 631)
19. Weiss RB, James WD, Major WB, Porter MB, Allegra CJ, Curt GA (1986) Skin reactions induced by trimetrexate, an analog of methotrexate. Invest New Drugs 4: 159–163

Received January 27, 1987/Accepted May 19, 1987