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A phase I trial of trimetrexate (NSC352122) on a daily ×5 schedule in patients with refractory adult leukemia

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Seven adult patients with refractory acute leukemia were administered trimetrexate (TMTX), a non-classical folate antagonist, in a phase I trial. TMTX was administered as an intravenous bolus for five consecutive days at doses of 9–12 mg/m² based on marrow response. The maximum tolerated dose was 12 mg/m². Hepatotoxicity was the dose-limiting toxicity. Initial dosage reductions in patients with liver disease and/or low protein concentrations may be necessary since TMTX is significantly protein bound and cleared primarily by hepatic metabolism. The recommended phase II dose on this dosing schedule is 9 mg/m².

Key words: Dose, hepatotoxicity, leukemia, phase I, trimetraxate.

Introduction

Trimetrexate (TMTX) is a novel, non-classical lipophilic folate antagonist more potent than methotrexate in its ability to inhibit human leukemia cell lines[1] as well as other preclinical models.[2] TMTX also differs from methotrexate pharmacologically in that its cellular uptake is not dependent on reduced folate transport and its metabolism does not require conversion to polyglutamate derivatives intracellularly.[3] Additionally, TMTX produces variable indirect inhibition of purine biosynthesis, resulting in inhibition of both DNA and RNA synthesis.[4]

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Results of phase I and II clinical trials in patients with solid tumors have shown TMTX to have some antineoplastic activity in patients with breast cancer, head and neck cancer, and non-small cell lung cancer.[2,5,6] It has shown no clinical activity in patients with melanoma or sarcoma.[7,8] The phase I dose-limiting toxicity in patients with solid tumors is myelosuppression, although sporadic gastrointestinal, hepatic and dermatologic toxicities have also been observed.[2] Based on results in experimental systems demonstrating antineoplastic activity with TMTX against methotrexate-resistant leukemia cells,[3,9] as well as the results of a phase I trial in pediatric patients with solid tumors and leukemia,[10] we conducted a phase I clinical trial utilizing TMTX in adult patients with refractory leukemia. Pharmacokinetic data were also obtained.

Materials and methods

Patient selection

Patients with histologically documented acute nonlymphocytic leukemia, acute lymphocytic leukemia or chronic myelogenous leukemia in blast crisis with disease refractory to conventional therapy or to prior investigational therapy were eligible for this study. Eligibility criteria included: age >18 years; performance status [Southwestern Oncology Group (SWOG)] criteria <3; life expectancy >4 weeks; no cytotoxic chemotherapy within at least 1 week of beginning therapy; recovery from any toxic effects of prior chemotherapy; and adequate hepatic function (total bilirubin <2.0 mg% and SGOT <3 times normal value) and renal function (serum creatinine <1.5 mg% or creatinine clearance >60 ml min). A complete history was taken and a physical examination was performed.

Laboratory tests acquired before and after drug administration included: CBC, WBC differential, platelet count, reticulocyte count, serum electrolytes and chemistries, partial thromboplastin time, prothrombin time, and urinalysis. A chest radiograph and other appropriate radiographic studies were also performed. All patients underwent a bone marrow biopsy and aspirate with stains for peroxidase, Sudan black, ASD chloroacetate esterase, non-specific esterase and PAS. In addition, cytogenetic studies were performed and terminal deoxynucleotidyl transferase (Tdt) levels were determined for all patients. Informed consent was obtained in all patients according to federal, state and institutional guidelines.

Dosage and formulation

Prior phase I studies in patients with solid tumors established the maximum tolerated dose (MTD) for TMTX to be 7.6–15 mg/m^2/day for 5 days repeated every 21 days, depending on the extent of prior treatment as well as patient response to therapy.[11–13] The starting dose for TMTX in the present study was 9 mg/m^2/day for 5 days as a 20 min infusion. Subsequently, the dosage escalation planned was from 9 to 12, 15, 18 and 21 mg/m^2 based on marrow response. TMTX was supplied by the Division of Cancer Treatment of the National Cancer Institute (Bethesda, MD) as the glucuronide salt. Each 50 mg vial was reconstituted with 1.9 ml of sterile water to provide a solution with a pH of 3–5.

Treatment and subsequent evaluation

All patients received an initial induction treatment for 5 days (D1–5) at the appropriate dose. Twelve days later a bone marrow aspiration and biopsy were performed to determine patient response using the SWOG definition of response for adult leukemias.[14] If the results of the bone marrow showed less than 10% blasts and promyelocytes (A1 marrow), then patients began maintenance therapy starting on day 33. If the marrow biopsy results failed to show an A1 response, then patients received a second induction treatment on day 14 followed by re-examination of the marrow on day 26. If the repeated marrow showed less than 25% promyelocytes and blasts, and less than 50% lymphocytes, then maintenance therapy was started on day 47. Patients were removed from study if the marrow failed to show a response after the second induction regimen. If the marrow response on day 12 was hypocellular (below 25%), then weekly examinations were performed until the marrow demonstrated recovery to greater than 25% cellularity, at which time treatment was instituted according to marrow response.

Maintenance therapy consisted of TMTX given at the same dose as that administered during induction treatment for 3 days and repeated thereafter every 21 days.

Histories and physical examinations were scheduled weekly during the maintenance phase. Laboratory evaluations, including CBC, WBC differential, platelet count, serum electrolytes and chemistries, prothrombin time, partial thromboplastin time, and urinalysis, were performed and recorded weekly. Performance status and toxicity were recorded weekly and graded by SWOG criteria. Bone marrow biopsies and aspirates for response were assessed at least every 6 weeks.

Pharmacologic studies

Blood samples were obtained prior to the dose, at 5, 10, 20 and 40 min, and at 1, 2, 4, 8 and 12 h after the infusion on the first day; and prior to the dose and at 5, 10, 20, 40 and 60 min, and at 2, 4, 8, 12 and 24 h after the infusion on the fifth day. The blood samples were collected in heparinized tubes and placed on ice until plasma was separated by centrifugation. Plasma samples were stored frozen at -20°C until assayed.

TMTX in plasma was measured by a reversed-phase high performance liquid chromatography (HPLC) assay previously described by Balis *et al.*[15] Trimethoprim (Sigma, St Louis, MO) was added to plasma samples prior to extraction as an internal standard. TMTX was extracted and concentrated from plasma using C_{18} Sep-Pak cartridges (Waters Associates Inc., Milford, MA), and then injected onto a 5 μM C_{18} Nova-Pak radial column (Waters Associates). The mobile phase contained 0.02M KH_2PO_4 (pH 4.5) and 25% acetronitrile and was pumped at a rate of 2 ml/min. The eluent was monitored with a UV detector at a wavelength of 240 nm. Retention times for trimethoprim and TMTX were 2.8 and 5.0 min respectively. The lower limit of sensitivity for the concentrated samples was 0.05 μmol/liter.

TMTX metabolites in the urine samples were detected by HPLC as previously described.[15] Metabolites partially purified from urine samples by HPLC were subjected to β-glucuronidase, type VIII

Sigma, and reinjected onto the column to determine if the metabolite(s) were conjugated derivatives.

Pharmacokinetic parameters were determined by model-independent methods. The area under the plasma concentration–time curve (AUC) was derived by the trapezoidal method and extrapolated to infinity.[16] Total body clearance (Cl_{total}) was calculated by dividing the dose by the AUC, and renal clearance was determined by dividing the amount of parent drug excreted in the urine over 48 h by the AUC. The volume of distribution at steady state was calculated from the area under the moment curve.[17] The elimination half-life ($t_{1/2}$) was determined by regression analysis.

Results

Seven eligible patients (four males and three females) entered this trial. Patient characteristics are summarized in Table 1. Three patients received five courses of TMTX at a dosage of 9 mg/m^2 while four patients received five courses of TMTX at a dosage of 12 mg/m^2. All seven patients received one induction treatment and three of these patients received a second induction treatment at the same dose. No dosage reductions were made; however, none of the patients received a maintenance dose. Five of the seven patients developed grade IV (SWOG toxicity criteria[14]) granulocytopenia (<250/mm^3), while six of the seven patients developed grade IV thrombocytopenia ($<25\,000$/mm^3) requiring blood product transfusions. In two patients, the absolute neutrophil count (ANC) recovered by day 11, but in the remaining five patients the ANC never rose above 250/mm^3. Reversible hepatotoxicity was observed at all dose levels and was dose-limiting at a dosage of 12 mg/m^2. Hepatic toxicity, characterized by elevated total bilirubin, was observed in two patients treated at the 9 mg/m^2 dosage level (one grade II, one grade III—SWOG toxicity criteria) and in all patients treated with 12 mg/m^2 (one grade II, three grade III—SWOG toxicity criteria). Other significant toxicities included nausea and vomiting grade I (SWOG toxicity criteria) in four patients at both doses and mucositis grade I–III (SWOG toxicity criteria) in four patients at both doses. There were two treatment-associated deaths due to refractory sepsis.

All patients ($n = 7$) were evaluable for response. Antileukemic effects were limited to two patients with transient marrow hypoplasia at a dose of 12 mg/m^2, with no evidence of a remission in any of the patients.

Pharmacokinetic parameters for TMTX were determined by analyzing blood samples from three of the seven patients participating in this trial. Results are summarized in Table 2. Plasma disappearance of TMTX was bioexponential. Peak TMTX levels 5 min post-infusion on day 5 ranged from 2.24 to 4.35 μmol/l. The terminal $t_{1/2}$ on day 5 ranged from 2 to 14 hrs, with a volume of distribution ranging from 19 to 32 L/m^2 and Cl_{total} ranging from 1.3 to 7.1 L h m^2. Mean AUC

Table 1. Patient characteristics

Parameters	No. of patients
Number of evaluable patients	7
Number of courses	10
Age (years)	
median	38
range	20–57
Performance status	
0	2
1	1
2	2
3	2
Prior therapy	
chemotherapy	7
radiotherapy and chemotherapy	3
allogeneic bone marrow transplant	2
Type of leukemia	
AML	3
ALL	3
CML	1

Table 2. TMTX Pharmacokinetic parameters (daily ×5 schedule)

Patient	Dose (mg/m^2)	Total dose (mg)	Half-lives (h)				Clearance (l/h/m^2)		Volume of distribution				AUC (μM h)	
			$t_{1/2\alpha}$		$t_{1/2\beta}$				V_{dc}		V_{dss}			
			D1	D5	D1	D5	D1	D5	D1	D5	D1	D5	D1	D5
1	9	15.0	—	0.8	—	2	—	7.1	—	6.0	—	19.3	—	3.5
2	12	19.2	0.5	0.5	8	11	1.5	1.3	4.8	7.2	14.7	20.0	22.0	24.2
3[a]	12	22.6	0.2	0.5	11	14	1.9	1.6	11.1	13.4	30.2	32	17.0	19.8

[a]13% renal excretion of dose over 24 h on day 5. renal clearance 6.8 ml/min.

was dose-dependent and was determined to be 22.01 μM h for the 12 mg/m^2 dose.

Discussion

In this phase I trial of TMTX in adult patients with refractory leukemia hepatotoxicity was dose-limiting with a MTD of TMTX of 12 mg/m^2. The recommended phase II dose on this schedule is 9 mg/m^2.

In other phase I studies in patients with solid tumors utilizing the same schedule, the MTD has also been 12 mg/m^2, although myelosuppression was the dose-limiting toxicity.[11] Recommended phase II doses have ranged from 6.8 to 8 mg/m^2/day or a total dose over 5 days of 34–40 mg/m^2. In several phase II trials utilizing a dose of 8 mg/m^2 daily for 5 days in previously untreated patients and 12 mg/m^2 daily for 5 days in previously untreated patients, myelosuppression was also the most common dose-limiting toxicity. However, an increased incidence of severe non-hematologic toxicities, including mucositis, anemia, bleeding, diarrhea and dermatologic changes, was observed in patients with low serum protein levels and metastatic liver disease.[18,19]

Conclusion

TMTX hepatotoxicity has been identified as an additional dose-limiting toxicity. All of the patients that developed hepatotoxicity in the present study manifested pre-treatment albumin concentrations of 3.5 mg/dl or less, although none of these individuals demonstrated clinical evidence of metastatic liver disease. These findings suggest that since TMTX is extensively protein bound and is cleared primarily by hepatic metabolism, alterations in drug protein binding could potentially be responsible for enhanced toxic effects seen with TMTX.[18] Therefore, lower TMTX starting doses may be necessary in patients with metastatic liver disease and/or low serum protein levels.

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