Cancer Chemother Pharmacol (1999) 44: 372–380

ORIGINAL ARTICLE

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A phase I evaluation of multitargeted antifolate (MTA, LY231514), administered every 21 days, utilizing the modified continual reassessment method for dose escalation

Received: 20 October 1998 / Accepted: 30 March 1999

Abstract *Purpose*: To determine toxicities, maximally tolerated dose (MTD), pharmacokinetic profile, and potential antitumor activity of MTA, a novel antifolate compound which inhibits the enzymes thymidylate synthase (TS), glycinamide ribonucleotide formyltransferase (GARFT), and dihydrofolate reductase (DHFR). *Methods*: Patients with advanced solid tumors were given MTA intravenously over 10 min every 21 days. Dose escalation was based on the modified continual reassessment method (MCRM), with one patient treated at each minimally toxic dose level. Pharmacokinetic studies were performed in all patients. *Results*: A total of 37 patients (27 males, 10 females, median age 59 years, median performance status 90%) were treated with 132 courses at nine dose levels, ranging from 50 to 700 mg/m^2. The MTD of MTA was 600 mg/m^2, with neutropenia and thrombocytopenia, and cumulative fatigue as the dose-limiting toxicities. Hematologic toxicity correlated with renal function and mild reversible renal dysfunction was observed in multiple patients. Other nonhematologic toxicities observed included mild to moderate fatigue, anorexia, nausea, diarrhea, mucositis, rash, and reversible hepatic transaminase elevations. Three patients expired due to drug-related complications. Pharmacokinetic analysis during the first course of treatment at the 600 mg/m^2 dose level demonstrated a mean harmonic half-life, maximum plasma concentration (Cpmax), clearance (CL), area under the curve (AUC), and apparent volume of distribution at steady state (Vdss) of 3.08 h, 137 μg/ml, 40.0 ml/min per m^2, 266 μg · h/ml, and 7.0 l/m^2, respectively. An average of 78% of the compound was excreted unchanged in the urine. Partial responses were achieved in two patients with advanced pancreatic cancer and in two patients with advanced colorectal cancer. Minor responses were obtained in six patients with advanced colorectal cancer. *Conclusions*: The MTD and dose for phase II clinical trials of MTA when administered intravenously over 10 min every 21 days was 600 mg/m^2. MTA is a promising new anticancer agent.

Key words Multitargeted · Antifolate · MTA · LY231514

The opinions or assertions herein are the private views of the authors and are not to be construed as reflecting the views of the Department of the Army or the Department of Defense

Supported by a grant from Eli Lilly and Company and NCI grant P30-CA-54174

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Introduction

MTA (*N*-[4-[2-(2-amino-4,7-dihydro-4-oxo-1H-pyrrolo, 3-d]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic acid disodium salt) is a novel compound representative of a new class of folate antimetabolites. It has a pyrrole ring replacing the pyrazine ring in the pterine portion of folic acid, and a methylene group replacing the benzylic nitrogen in the bridge portion (Fig. 1). The antitumor effect of MTA is via inhibition of the enzymes thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltranferase (GARFT). MTA is an excellent substrate for the enzyme folylpolyglutamate synthetase (FPGS), leading to extensive intracellular polyglutamation. This converts the

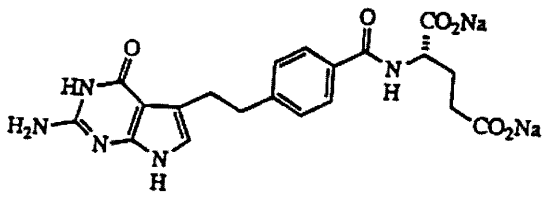

Fig. 1 Multitargeted antifolate (MTA, LY231514)

drug from a form that readily effluxes from the cell to a form that is retained intracellularly for a prolonged period, producing a more sustained drug effect [1–3]. It has demonstrated in vitro activity against human tumor colony-forming units obtained from patients with colon cancer, renal cancer, hepatoma, carcinoid tumor, and both non-small-cell and small-cell lung cancer (D.D. Von Hoff, personal communication, 1996 [4]).

Two schedules of MTA have been evaluated previously in phase I clinical trials, with the compound being administered as a 10-min intravenous infusion in each trial. In one trial MTA was administered weekly for 4 weeks, every 42 days. The maximally tolerated dose (MTD), recommended dose for phase II clinical trials, and doze-limiting toxicity (DLT) were 40 mg/m^2, 30 mg/m^2, and neutropenia, respectively [6]. In the second trial, MTA was administered daily for 5 days, repeated every 21 days. The MTD and recommended dose for phase II clinical trials was 4 mg/m^2 per day with neutropenia and hepatotoxicity being the DLT [7, 8].

Based on the results of the weekly phase I trial, the starting dose for this trial was 50 mg/m^2. The dose escalation format was based on the modified continual reassessment method (MCRM), proposed by Faries [9]. Using this scheme, a single patient is treated at each minimally toxic dose level and more patients are added to a level when significant toxicity is observed. This dose escalation format reduces the number of patients treated with lower, possibly less effective doses, while increasing the proportion treated at dose levels closer to the MTD. The objectives of this study were to determine the qualitative and quantitative toxicities, the MTD, pharmacokinetic profile, and antitumor effect of MTA when administered as a single intravenous dose over 10 min, every 21 days.

Materials and methods

Patient selection

All patients underwent a complete history, physical examination, chest radiography, and laboratory evaluation. Eligibility criteria included: (1) histologic evidence of solid tumors refractory to conventional therapy and other investigational agents of higher priority; (2) at least 18 years of age; (3) WHO performance status ≤ 2; (4) life expectancy ≥ 12 weeks; (5) off previous anticancer therapy for at least 3 weeks (at least 6 weeks for nitrosoureas or mitomycin C); (6) adequate bone marrow function (WBC ≥ 3000/mm^3 or granulocytes ≥ 1500/mm^3, platelets ≥ 100 000/mm^3, Hgb ≥ 9 g%), hepatic function (bilirubin ≤ 1.5 mg%, AST not more than twice the upper limit of normal, albumin ≥ 2.5 g/dl, normal PT/PTT), renal function (creatinine ≤ 1.5 mg/dl or Cl_{creat} ≥ 60 ml/min), cardiac function (no dysrhythmias requiring therapy, no myocardial infarction in the previous 6 months), and metabolic function (electrolytes within normal limits unless due to cancer, blood glucose < 200 mg/dl); and (7) the provision of written, informed consent. Exclusion criteria included: (1) clinical evidence of brain metastases; (2) serious preexisting medical conditions which would prevent full compliance with the study; (3) pregnancy; (4) concomitant anticancer therapy; (5) the use of aspirin; and (6) the presence of pleural or peritoneal effusions. Patients requiring chronic aspirin therapy and those with effusions were excluded due to the potential for excess drug accumulation based on structural similarities of MTA and methotrexate. Methotrexate may be displaced from albumin and its renal secretion may be impaired by the concurrent use of aspirin, thereby increasing its cytotoxic effect [10]. It is also retained in effusions and released slowly into plasma, causing potentially substantial toxicity.

Pharmacokinetic and statistical analysis

Plasma samples and urine collections for pharmacokinetic analyses were planned for all patients during their first treatment course. Immediately prior to MTA administration, an indwelling intravenous catheter was placed in the arm contralateral to the drug infusion. Heparinized blood samples (8 ml) were collected preinfusion, at the end of the 10-min infusion, and at 5, 10, 30 and 45 min, and 1, 2, 4, 5, 9, 18, 24 and 48 h postinfusion. Blood samples were immediately centrifuged (3000 rpm for 15 min), and the plasma removed and stored at −80 °C until analysis. Urine samples (20-ml aliquots) were collected at baseline and every 2 h for the first 4 hours, every 4 h up to 12 h, then from 12 to 24 h and stored at −80 °C until analysis.

The concentrations of MTA in plasma and urine were measured by a modified HPLC method as previously described [6]. Lometrexol (Eli Lilly, Indianapolis, Ind.) was used as the internal standard for the analysis of both plasma and urine samples. Briefly, plasma samples mixed with internal standard were subjected to solid-phase extraction (Bond Elut Certity II, Varian, Harbor City, Calif.). The extraction efficiency of MTA was 60%. Reconstituted and filtered plasma residue (50–150 µl) was injected onto an octadecylsilyl column (YMC basic, 4.6 × 250 mm; YMC, Wilmington, N.C.) preceded by a YMC basic precolumn (4 × 23 mm). Early eluting peaks were removed by the precolumn controlled by a programmed column switching technique. The mobile phase consisted of 14% acetonitrile and 86% (pH 3.0, 50 m*M*) sodium phosphate buffer solution pumped at 0.8 ml/min, and monitored by UV detection at 250 nm. The lower limit of detection was set at 10 ng/ml. Three calibration curves (10–200, 200–4000 ng/ml and 4–80 µg/ml) were used to determine MTA in the patients' samples. The coefficient of variation was 8.2% at 10 ng/ml and 1.3% at 72.7 µg/ml.

Urine samples were mixed with the internal standard, filtered and directly injected onto a C_{18} column (YMC basic, 4.6 × 250 mm, 5 µm). The mobile phase consisted of 16% acetonitrile and 84% pH 3.0 phosphate buffer, pumped at a flow rate of 1.0 ml/min, and monitored by UV detection at 250 nm. The lower limit of quantitation was 2 µg/ml. At the limit of detection (1 µg/ml), the intraassay coefficient of variation was 15%. The remaining validation samples had intraassay coefficients of variation ranging from 1.1% to 6.9%.

The maximum plasma concentrations (Cpmax) were determined by selecting the highest plasma concentration observed directly from the patient's plasma concentration profile following intravenous administration. The pharmacokinetic parameters were calculated using model-independent methods. The terminal rate constant (k) was determined by log-linear regression analysis of the last three terminal data points on the plasma concentration-time curves. The terminal plasma half-life ($t_{1/2}$) were calculated from the equation: $t_{1/2} = 0.693/k$. The area under the plasma concentration-time curve (AUC) was determined using the linear trapezoidal method with extrapolation to infinity. Total body clearance was calculated by dividing the total dose administered by the AUC. The volume of distribution at steady state (Vdss) was estimated from the equation CL × MRT, where CL is the clearance and MRT the mean resi-

dence time. MRT was calculated by dividing the AUMC by AUC where AUMC is the area under the drug concentration-time versus time curve calculated by the trapezoidal method with extrapolation to infinity. Renal clearance of MTA was estimated by dividing the amount of MTA recovered in the urine during the first 24 h by the plasma AUC from 0 to 24 h. Creatinine clearance estimation were performed using the Cockroft and Gault equation [11].

Linearity between doses was tested by performing linear regressions forced through zero between AUC or Cpmax values with the MTA dose normalized per square meter of body surface area.

Pharmacodynamic correlations were attempted between indices of toxicity, total dose (milligrams), and pharmacokinetic parameters (Cpmax and AUC). Several measurements were collected over the dosing period, with the nadir after the first dose and the absolute nadir (nadir after all continuous MTA therapy) used as indices of toxicity. Each of these was correlated on a log-linear basis, with the value of the pharmacokinetic parameters correlated to the logarithm of the toxicity parameters. In addition, the percentage decrements in the ANC and platelet count were calculated as:

$$\% \text{ decrease} = \frac{100 \times (\text{pretreatment counts} - \text{nadir counts})}{\text{pretreatment counts}}$$

These were related to drug exposure using a simple maximum effect (E_{max}) model of drug effect or linear regression analysis [12]. The relationships between drug exposure and the occurrence of grade IV neutropenia during the first course of therapy were explored. Differences in means were tested using the *t*-test and one-way analysis of variance and means comparison was performed using Student's *t*-test for each pair and the Tukey-Kramer HSD for all comparisons. To assess the possibility of cumulative hematologic toxicity, two statistical methods were applied. A Poisson regression was used to analyze the relationship between the dependent variables (ANC, platelet nadir and predose counts) and time. The mixed effects analysis of covariance was used to assess whether there was a significant change in the toxicity parameters from course one. Linearity between doses was tested by performing linear regressions forced through zero between AUC or Cpmax values with the MTA dose normalized per square meter of body surface area.

Drug administration

MTA disodium was supplied as a lyophilized powder in 20- and 100-mg vials, and reconstituted in 10 ml on normal saline. The appropriate dose was then withdrawn and diluted in normal saline to a total volume of 50–200 ml. This was administered intravenously over 10 min every 21 days. Toxicity was assessed according to the WHO Toxicity Criteria. Patients were evaluated by a physician weekly during therapy for signs and symptoms of toxicity. The initial patient treated at each dose level was observed for a minimum of 3 weeks before decisions regarding dose escalation were made. Folinic acid rescue would be considered based on animal data, for grade IV myelosuppression persisting 7 days or for grade III/IV nonhematologic side effects. The planned dosing of folinic acid was 50 mg/m^2 intravenously every 6 h for 2 days, then 40 mg/m^2 intravenously every 6 h for 6 additional days. All serious adverse events were reported to the Institutional Review Board and the study sponsor, Eli Lilly and Company.

Dose escalation

The dose levels to be studied were 50, 75, 100, 150, 225, 350, 525, 700, and 900 mg/m^2. Dose escalation was planned based on the MCRM, with one patient treated at each minimally toxic dose level. Before each new patient was treated, an estimated MTD was calculated based on the toxicity experienced by all previously treated patients. In the original plans for this study, the starting dose level was to be 75 mg/m^2 every 21 days. In the weekly clinical trial of MTA, toxicity was observed at a much lower dose level than expected based on animal toxicity data. Based on these factors and concern for patient safety, 50 mg/m^2 was chosen as the initial dose level for this trial, and the 100 mg/m^2 dose level was also added. The dose level selected for a new patient was based on the following criteria:

- The dose level for a new patient could not be more than one level above the level assigned to the previous patient
- The dose level could not be greater than the estimated MTD
- A minimum of three patients would be treated at a level before dose escalation when moderate reversible toxicity (grade III hematologic or grade II non-hematologic toxicity, excluding nausea, vomiting, and alopecia) occurred
- A minimum of six patients would be treated at a dose level before escalation when unacceptable reversible toxicity (grade IV hematologic or grade III non-hematologic toxicity, excluding nausea, vomiting, and alopecia) occurred

The MTD was defined as that dose level at which 30% of the patient population developed unacceptable reversible toxicity. The recommended dose for phase II clinical trials on this schedule would be the dose that caused moderate reversible toxicity in most patients, with at least ten patients treated at this dose level. Intrapatient dose escalation was allowed if the next higher dose level was completed without unacceptable toxicity.

Efficacy criteria

Disease assessment was performed every one or two courses. Standard response criteria were used. A complete response required disappearance of all evidence of disease for at least 4 weeks. A partial response required a 50% or greater decrease in the sum of the products of the diameters of all measured lesions for at least 4 weeks. There also could be no new lesions or increases in the size of any evaluable lesions. A minor response was defined as a ≥25% reduction in measurable or evaluable disease, but not meeting criteria for a partial response. Progressive disease was defined as greater than 25% increase in the sum of the products of the diameters of the measured lesions or the appearance of any new lesions. Stable disease was defined as not meeting criteria for a response or progressive disease.

Results

A total of 37 patients were enrolled in the study. Their characteristics are listed in Table 1. The majority of the patients participating in the trial had metastatic colon cancer refractory to 5-fluorouracil. A total of 132 courses of MTA were administered at doses from 50 to 700 mg/m^2, with a range of 1 to 12 courses per patient.

Toxicities

The first eight patients were treated at seven dose levels, ranging from 50 to 525 mg/m^2. Two patients were treated at the 150 mg/m^2 dose level. The body surface area of the initial patient at this level was 2.62 m^2. His dose was adjusted for safety based on his ideal weight, rather than his actual weight. A second patient was added at this level before dose escalation. There were no instances of grade III or IV toxicities during the initial courses of therapy in these patients (Table 2). The initial patient treated at the 700 mg/m^2 dose level experienced grade IV neutropenia and grade III thrombocytopenia, and rash. Three patients were then added to the 525 mg/

Table 1 Patient characteristics

Number entered	37
Number evaluable	37
Male/female	27/10
Age (years)	
Median	59
Range	30–74
Performance status (Karnofsky)	
100	16
90	4
80	14
60	3
Number of prior chemotherapy regimens	
0	4
1	8
2	12
3+	13
Prior abdominal/pelvic radiotherapy	8
Tumor type	
Colorectal	25
Pancreas	3
Others (one each)	9

m^2 dose level, based on the updated estimate of the MTD of 527 mg/m^2. One of these patients developed grade III thrombocytopenia, but no grade IV toxicity occurred.

Five patients were sequentially treated at the 700 mg/m^2 dose level. Of the six total patients treated at this level, three developed grade IV neutropenia, with grade III or IV thrombocytopenia also occurring in these patients. Nonhematologic toxicity was also substantial, with grade III side effects in four patients (mucositis in two patients, and fatigue, diarrhea, rash, and anorexia in one patient each), including the last three patients treated at this dose level. This was not considered a tolerable dose level. At this time, patients were being added on a weekly basis, which retrospectively was too soon for maximal toxicity to be defined.

Since toxicity was significant at 700 mg/m^2, but relatively mild at 525 mg/m^2, an intermediate dose level of 600 mg/m^2 was added. The updated estimate of the MTD at this time was 645 mg/m^2. A total of 20 patients were treated at this dose level. Five patients developed grade IV neutropenia during their first course of treatment, with one of these patients also developing grade IV thrombocytopenia. Mild to moderate nonhematologic toxicity also occurred in most patients during their first course of treatment at this dose level (Tables 2 and 3). No patient developed grade III–IV nonhematologic toxicity during the first treatment course. The most common moderate (grade II) nonhematologic toxicity was a pruritic rash which occurred in ten patients, and which was ameliorated with the use of a prophylactic 3-day course of steroids (dexamethasone 4 mg orally twice daily for 3 days starting day 1) around future doses. Six of the 20 patients developed moderate nondermal toxicity at this dose level.

Table 3 Toxicity: course 1 (600 mg/m^2 dose level, $n = 20$)

Toxicity	Grade (WHO)				
	0	I	II	III	IV
Neutropenia	7	3	1	4	5
Thrombocytopenia	12	3	3	1	1
Mucositis	18	0	2	0	0
Dermatitis	8	2	10	0	0
Anemia	11	4	5	0	0
Anorexia	17	2	1	0	0
Diarrhea	14	5	1	0	0
Nausea/emesis	15	3	2	0	0
Fatigue	10	8	2	0	0
Transaminasemia	12	7	1	0	0
Renal	19	1	0	0	0
Conjunctivitis	18	2	0	0	0

After the first 12 patients were treated at the 600 mg/m^2 dose level, there appeared to be evidence of cumulative myelosuppression and fatigue. In order to investigate whether this increase in toxicity was due to a change in the drug's pharmacokinetics, eight patients were added to this dose level with plans for plasma and urine drug levels after both their first and fourth courses of treatment. Eight patients enrolled at the 600 mg/m^2 dose level received four or more total doses of MTA. Hematologic toxicity and fatigue appeared to become more prominent with the latter doses (Table 4). Changes in renal function alone did not appear to account for this apparent cumulative toxicity.

Of the 37 patients enrolled on the trial, 23 were withdrawn due to disease progression. Seven patients were withdrawn due to toxicity, without evidence of disease progression. This was due to fatigue in six patients and thrombocytopenia in one patient. Three patients died during the study related to drug toxicity. A 66-year-old male with metastatic colorectal cancer, developed both grade IV neutropenia and thrombocytopenia after his first course of MTA at 700 mg/m^2. His second course, at 525 mg/m^2, was complicated by grade III neutropenia and grade II thrombocytopenia as his maximal toxicity. A CT scan after his second course revealed a 40% reduction in his measured disease. The

Table 2 Toxicity: course 1 (numbers of patients with maximum WHO grade toxicity)

Dose level (mg/m^2)	Number of patients	Neutropenia					Thrombocytopenia					Nonhematologic				
		0	I	II	III	IV	0	I	II	III	IV	0	I	II	III	IV
50–350	7	4	1	2	0	0	7	0	0	0	0	4	3	1	0	0
525	4	0	1	3	0	0	3	0	0	1	0	1	1	2	0	0
600	20	7	3	1	4	5	12	3	3	1	1	1	6	13	0	0
700	6	0	0	1	2	3	2	1	0	1	2	0	0	2	4	0

Table 4 Cumulative toxicity

Patient ID	Course 1				Course 4			
	Dose (mg/m^2)	Serum creatinine (mg/dl)	Granulocyte nadir (per mm^3)	Platelet nadir (per mm^3)	Dose (mg/m^2)	Serum creatinine (mg/dl)	Granulocyte nadir (per mm^3)	Platelets nadir (per mm^3)
173	600	1.0	912	62	350	1.3	2	5
175	600	0.9	969	45	350	1.0	140	14
176	600	1.0	2067	189	350	0.8	1564	56
177	600	1.1	3456	111	600	1.1	1856	50
182[a]	600	0.6	1722	316	600	0.6	777	263
183[a]	600	0.7	2812	234	600	0.8	1980	143
184[a]	600	0.8	1539	127	450	1.0	1058	168
186[a]	600	1.0	2627	195	600	1.0	1450	183
Mean			2013 ± 903	160 ± 91			1103 ± 748*	110 ± 93**

*$P = 0.046$, **$P = 0.295$, vs course 1, not adjusted for dose reductions for course 4
[a] Pharmacokinetic studies after courses 1 and 4 which were not significantly different in those retreated at 600 mg/m^2

dose for his third course was reduced to 350 mg/m^2, due to concerns about the risk of infection from a persistent anal fissure. This course was complicated by grade IV neutropenia and grade IV thrombocytopenia. He expired due to sepsis on day 16.

A 63-year-old male with metastatic colorectal cancer, developed grade III neutropenia and grade II thrombocytopenia as his maximal toxicity after his first two courses of MTA at 600 mg/m^2. A CT scan after his second course revealed a 39% reduction in his measured disease. His third course, also at 600 mg/m^2, was complicated by both grade IV neutropenia and thrombocytopenia. The dose of his fourth course was reduced to 350 mg/m^2, but was again complicated by grade IV neutropenia and thrombocytopenia. He expired of fungal sepsis on day 24. A 65-year-old female, also with metastatic colorectal cancer, received five courses at 600 mg/m^2 with grade I neutropenia as her maximal toxicity. Her sixth dose, also at 600 mg/m^2, was complicated by grade II neutropenia and grade III mucositis. Her seventh course was reduced to 450 mg/m^2 and complicated only by grade I neutropenia. During her time on-study, her renal function gradually declined. Her baseline serum creatinine was 0.7 mg/dl, increasing to 1.2 mg/dl before her seventh course. Prior to her eighth dose, her serum creatinine was 1.5 mg/dl. Since her seventh course at 450 mg/m^2 was well tolerated and she exhibited a response to her treatment with a 51% reduction in her measured disease and an 87% reduction in her serum carcinoembryonic antigen (CEA) level, the same dose was administered for her eighth course. This was complicated by grade IV neutropenia and grade IV thrombocytopenia. Her neutrophil count had recovered and her platelet count had significantly improved, when she developed acute respiratory distress syndrome (ARDS) and expired of respiratory failure.

Mild reversible renal dysfunction was observed in multiple patients treated at the highest dose levels, and drug toxicity appeared to correlate with renal function. Serum creatinine levels were obtained weekly. Five of 20 patients treated at the 600 mg/m^2 dose level and 2 of 6 patients treated at the 700 mg/m^2 dose level exhibited a maximal serum creatinine that was greater than 50% over baseline. The highest measured serum creatinine level was greater than 2 mg/dl in five patients (2.5, 2.6, 2.6, 3.1 and 3.4 mg/dl, respectively). This nephrotoxicity appeared to be reversible and nonprogressive despite continued treatment in the majority of these patients.

The mean estimated creatinine clearance of those patients at the 600 mg/m^2 dose level developing grade IV neutropenia after their first course was 86 ml/min. This compared to 97 ml/min for those not developing grade IV neutropenia. Two of the three patients with a baseline estimated creatinine clearance less than 70 ml/min developed grade IV toxicity after their first dose at the 600 mg/m^2, compared to one of the nine patients with an estimated creatinine clearance greater than 90 ml/min.

The development of thrombocytopenia appeared to be a marker of sensitivity to MTA. A total of 37 courses were administered to 15 patients who developed grade 0–IV neutropenia with grade 0–II thrombocytopenia during the preceding course. Only three follow-up courses were complicated by grade IV neutropenia, and two of these were also complicated by grade IV thrombocytopenia. Three patients were treated after developing grade IV neutropenia and grade IV thrombocytopenia after the preceding course. One patient had his dose reduced from 700 to 525 mg/m^2. He developed grade III neutropenia and grade II thrombocytopenia after this dose. The remaining two patients had their doses reduced from 600 to 350 mg/m^2, a 42% dose reduction. Despite this reduction, both again developed grade IV neutropenia and thrombocytopenia, with one of these patients expiring of neutropenic sepsis. The baseline serum creatinine levels in these 2 patients were 1.0 and 0.9 mg/dl and before their courses at 350 mg/m^2 were 1.3 and 1.0 mg/dl, respectively. Even though the number of patients treated after developing grade III or IV thrombocytopenia was small, this appeared to be a possible marker of sensitivity to MTA, independent of renal function, necessitating large dose reductions for follow-up courses.

Folinic acid was administered as a rescue, based on animal data [1], to five patients. Two patients, who ultimately died due to drug toxicity, received folinic acid. This was initiated on day 12 in the patient who expired on day 16, and on day 15 in the patient who expired on day 24. Toxicities resolved in the remaining three patients, although it is unclear if the folinic acid potentiated the recovery.

Pharmacokinetic analysis

Blood samples for pharmacokinetic analysis were obtained from 35 of the 37 patients enrolled in the study. The pharmacokinetic parameters for MTA are summarized in Table 5. MTA exhibited a relatively small apparent volume of distribution of 6.8 ± 1.3 l/m^2 (n = 34), a mean CL of 40.5 ± 13.3 ml/min per m^2 (n = 34) and a harmonic mean half-life of 3.01 ± 1.0 h (n = 34). Considering the small number of patients at each dose level, other than the 600 mg/m^2 dose level, there was a fairly good linear relationship between dose (mg/m^2) and AUC (Fig. 2). Urinary excretion of MTA was complete, or nearly complete, within 24 h. An average of 77.6% of unchanged drug was excreted into the urine during the first 24 h of administration. The mean (n = 16) renal CL for MTA was 32.4 ± 13.6 ml/min per m^2. A moderate correlation (r^2 = 0.3, P < 0.02) was found between MTA CL and estimated creatinine clearance.

We examined the effects of gender upon the disposition of MTA from the plasma data available from 26 men and 9 women and urine data from 11 men and 7 women. Gender did not affect the drug's CL (44.3 female vs 40.0 male ml/min per m^2) or Vdss (6.6 female vs 7.1 male l/m^2). A significant difference (P = 0.01) though was found between women and men comparing the percent of drug excreted during the first 24 h: 90.4% vs 68.4% of dose, respectively.

To explore whether the disposition of the drug might have changed after multiple courses, we obtained samples from four patients at the 600 mg/m^2 dose level after their first and fourth courses. The mean plasma concentration vs time profiles for the two courses are displayed in Fig. 3. One patient had a dose reduction to 450 mg/m^2 on course 4 (data not included). Renal function remained stable during these time periods (Table 4). The overall pharmacokinetic parameters were nearly identical between the two courses.

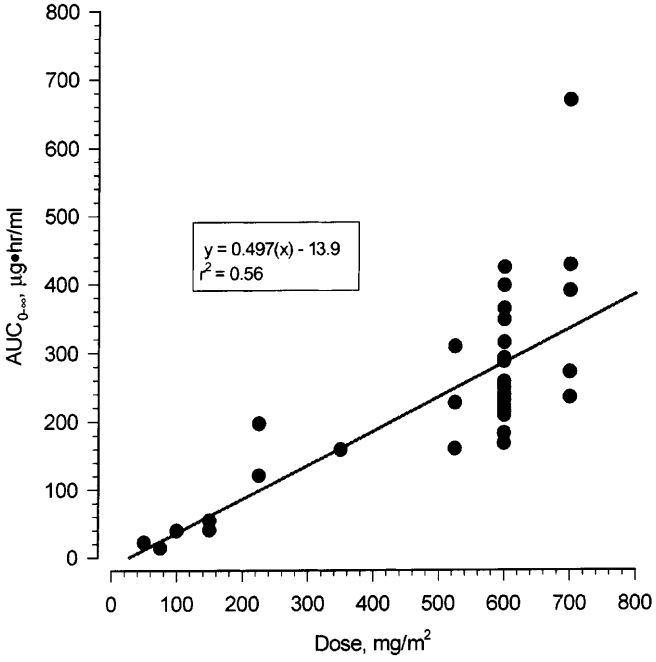

Fig. 2 MTA dose versus AUC

Attempts were made to correlate MTA exposure with toxicity. Correlation with MTA dose allowed a prediction of toxicity with an r^2-value of 0.25 for the ANC nadir after the first dose. A similar correlation was found for Cpmax (r^2 = 0.21). Although these correlations were adequate, the correlation between AUC and ANC nadir provided the best prediction of toxicity with an r^2-value of 0.30 when all treated patients were included. However, when only patients treated the the 600 mg/m^2 dose level were included, this correlation became less significant. Relatively poor correlations (r^2 < 0.2) were present between dose or Cpmax and platelet nadir, whereas the correlation between AUC and the platelet nadir was the strongest (r^2 = 0.32). The relationship

Table 5 Pharmacokinetic parameters of MTA (values are means ± SD)

Dose (mg/m^2)	Patient no.	Cpmax (µg/ml)	AUC (µg · h/ml)	CL (ml/min/m^2)	Vdss (l/m^2)	$t_{1/2}$ (h)	MRT (h)
50	1	11.89	21.85	38.1	6.88	2.48	3.01
75	1	10.01	13.57	92.1	9.58	1.51	1.73
100	1	27.33	39.45	42.2	5.92	2.38	2.34
150	1	39.46	53.92	46.4	5.25	2.23	1.89
225	1	64.40	120.40	31.2	5.50	2.72	2.94
350	1	91.39	158.40	36.8	5.68	2.70	2.57
525	3	121.20 (±15.6)	231.26 (±74.6)	40.7 (±13.3)	6.85 (±0.8)	3.86[a] (±1.0)	2.85[a] (±0.6)
600	20	137.00 (±45.5)	265.76 (±70.4)	40.0 (±9.6)	7.00 (±1.4)	3.08[a] (±0.9)	2.80[a] (±0.9)
700	5	175.46 (±47.6)	397.53 (±171)	33.6 (±12.9)	6.39 (±0.5)	3.69[a] (±1.6)	3.11[a] (±1.5)

[a] Harmonic mean

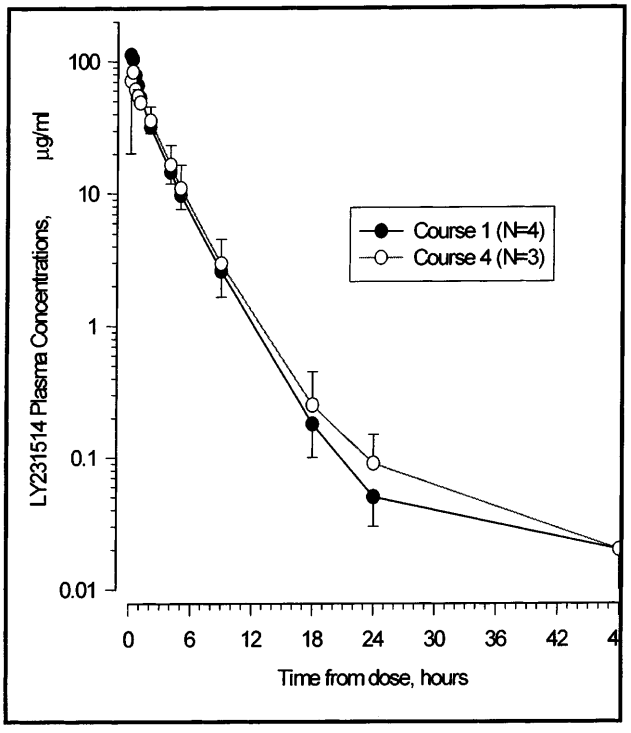

Fig. 3 Plasma concentration versus time, course 1 versus course 4

between AUC and the percentage decrements in ANC were comparable, whether described by a simple Emax model ($r^2 = 0.47$) or by linear correlation analysis ($r^2 = 0.40$). The relationships between AUC and percentage reduction in platelet count were not as strong as for ANC using the simple Emax model ($r^2 = 0.32$) or linear regression ($r^2 = 0.39$). Mean AUC values were significantly higher in those patients who experienced grade IV neutropenia (347 versus 215 µg · h/ml; $P = 0.015$). Dose and Cpmax were not predictive of grade IV neutropenia ($P > 0.1$).

Antitumor activity

Two of three patients with metastatic pancreatic cancer achieved partial responses. Both patients had liver metastases and were treated at the 600 mg/m^2 dose level. One patient with disease progression during treatment with 5-FU before treatment with MTA, achieved a 53% reduction in his measured disease and decrease in his serum CA 19-9 from 22 600 to 262 U/ml. His treatment was stopped after his fourth dose due to fatigue. The second patient, previously untreated, achieved a 76% reduction in his measured disease. His treatment was stopped after his sixth dose due to fatigue.

Two of 25 patients with colorectal cancer also responded to their treatment with MTA. Both patients had liver metastases and were treated at the 600 mg/m^2 dose level. The first patient, previously treated with intrahepatic FUDR, achieved a 60% reduction in his measured disease and a decrease in his serum CEA from 37 to 8 ng/ml. His treatment was stopped after his sixth dose due to fatigue. The second patient, progressing during treatment with Tomudex, achieved a 51% reduction in her measured disease and a decrease in her serum CEA level from 69 to 9 ng/ml. She expired after her eighth course due to ARDS (see details in previous section).

Minor responses were also demonstrated in six patients with advanced colon cancer, of whom five had been previously treated with 5-FU. Unfortunately, several of these patients also developed significant side effects from their treatment. Of the three patients who expired during the study related to drug toxicity, one exhibited a partial response and two had minor responses. Of the remaining seven patients exhibiting a partial or minor response, four were withdrawn from treatment due to fatigue, without evidence of disease progression.

Discussion

MTA is a novel antifolate compound, inhibiting the enzymes TS, GARFT, and DHFR. In preclinical studies, it has demonstrated activity against a wide spectrum of tumor types. Toxicities observed in animal studies have included neutropenia, anemia, anorexia, weight loss, emesis, diarrhea, and mucositis.

Two previous schedules of MTA have been evaluated in phase I clinical trials, with the MTA being administered as a 10-min intravenous infusion in each trial. In the initial phase I clinical trial in San Antonio, MTA was administered weekly for 4 weeks, every 42 days. In this trial, 25 patients with advanced solid tumors received 58 courses, at doses ranging from 10 to 40 mg/m^2 per week. The MTD was 40 mg/m^2 per week, with reversible neutropenia as the DLT. Nonhematologic toxicities observed included mild fatigue, anorexia, and nausea. The recommended dose for phase II clinical trials was 30 mg/m^2 per week. At the 30 mg/m^2 per week dose level, the mean harmonic half-life, Cpmax, CL, and apparent Vdss were 2.11 h, 7.48 µg/ml, 39.6 ml/min per m^2, and 5.63 l/m^2, respectively. No major antitumor responses were observed; however, minor responses were achieved in two patients with advanced previously treated colorectal cancer [6].

In the United Kingdom, MTA was evaluated on a daily ×5 schedule, repeated every 21 days, in 38 patients with advanced solid tumors. A total of 116 courses of MTA were administered at ten doses levels ranging from 0.2 to 5.2 mg/m^2. The MTD and recommended dose for phase II clinical trials using this schedule was 4 mg/m^2 per day, with myelosuppression and liver function abnormalities as the DLT. At 4.0 mg/m^2, one of six patients developed grade III hepatotoxicity (bilirubin), and a second developed grade IV neutropenia and grade III hepatic transaminase elevations. No objective tumor responses to MTA treatment were observed, however three patients (non-small-cell lung cancer, colon cancer, pancreatic cancer) achieved minor responses [7, 8].

In the current trial, with doses administered once every 3 weeks, substantially greater dose intensity was achieved. The MTD and recommended dose for phase II trials was 600 mg/m^2, with neutropenia, thrombocytopenia and cumulative fatigue as the DLTs. MTA demonstrated minimal toxicity at doses up to 525 mg/m^2. At the 700 mg/m^2 dose level, four of six patients developed grade IV hematologic and/or grade III nonhematologic side effects. Moderate toxicity occurred at the 600 mg/m^2 dose level. Five of the 20 patients developed grade IV neutropenia during their first course of treatment, with one of these patients also developing grade IV thrombocytopenia. Nonhematologic toxicity, in contrast to the 700 mg/m^2 dose level, was relatively mild. There were no instances of grade III or IV side effects during the first course of treatment.

Toxicity observed after repetitive doses became more significant, despite dose reductions in several of these cases. One mechanism for this was drug-induced nephrotoxicity and reduced drug clearance. Other structurally similar antifolate compounds such as methotrexate, Lometrexol, and especially CB3717, have also been reported to cause variable degrees of renal dysfunction [13–16]. However, nephrotoxicity and reduced drug clearance did not appear to be the sole mechanism for the cumulative toxicity observed. Eight patients treated at the 600 mg/m^2 dose level received at least four doses of the compound. Hematologic toxicity was more severe after the fourth course even though the pretreatment serum creatinine levels had not changed significantly. Four of these patients also had repeat pharmacokinetic analyses after the fourth course, which did not demonstrate any changes in MTA disposition. Other possible explanations for this progressive toxicity could be increased intracellular polyglutamation of MTA with repetitive doses, an incompletely reversible bone marrow effect, and/or a reduction in renal secretion of MTA or active metabolites.

The development of severe toxicity after treatment with MTA appeared to correlate most strongly with baseline renal function. Patients with estimated creatinine clearance values < 80 ml/min were more likely to develop severe myelosuppression than those with a creatinine clearance > 80 ml/min.

MTA exhibits a relatively small apparent volume of distribution similar to that of plasma protein and a clearance, which is approximately 3/4 of glomerular filtration for functional kidneys. The pharmacokinetic parameters are similar to those we previously reported with MTA doses ranging from 10 to 40 mg/m^2 given on a weekly $\times 4$ schedule [6]. Combining the AUC and Cpmax data from both studies, supports the linear disposition of MTA across a wide dosage range (10–700 mg/m^2, $r^2 = 0.81$). Elimination of MTA is dependent upon renal function, with 78% of the dose recovered unchanged in the urine in the first 24 h. This dependency upon renal function was also demonstrated by the correlation of estimated creatinine clearance and MTA clearance. However, with a half-life of 3–4 h, one could expect as much as 98% of the drug to be recovered, if all drug were excreted unchanged. This suggests that a portion of the drug may be metabolized or held within various tissue compartments.

A correlation was apparent between AUC and both neutrophil and platelet nadirs and the percentage reduction in neutrophil and platelet counts when all patients were included in the analysis. In addition, higher AUC values were observed in patients who experienced grade IV neutropenia. Based on the characteristics of MTA (renal elimination, AUC exposure-toxicity relationship), one might suggest that a strategy of adaptive dosing as currently utilized for carboplatin could eventually be developed for MTA.

A unique aspect of this phase I clinical trial was the utilization of the MCRM for dose escalation. The traditional dose escalation design of a phase I investigational drug clinical trial involves a minimum of three patients at a dose level before dose escalation. One patient is treated and observed for a full course of treatment, then two others are added at the same level. If there is no significant toxicity, dose escalation proceeds. When significant reversible toxicity is observed, more patients are added to that dose level. This proceeds until the MTD is determined. The recommended dose for phase II clinical trials is generally the MTD or the dose level below the MTD, depending on the specific side effects and severity [17, 18]. The CRM, proposed by O'Quigley et al. [19], uses a Bayesian format to estimate the MTD, based on toxicity data from all previously treated patients. Patients are then added at the dose level established at the commencement of the trial that is closest to the estimated MTD. The MCRM, proposed by Faries [9], allows for a more rapid dose escalation. It offers the advantages of reducing the number of patients treated with lower, and possibly less effective doses, and increases the proportion treated at dose levels closer to the MTD. The dose escalation format planned for this trial was based on the MCRM, but to enhance safety, additional patients were treated at a dose level if moderate toxicity was observed, rather than a single patient as planned by the MCRM.

In the initial phase I trial of MTA administered weekly $\times 4$ every 42 days, the MCRM did not have an impact on patient accrual due to toxicity at the early dose levels, necessitating the treatment of additional patients at those dose levels before dose escalation. During the current trial, the MCRM allowed for rapid dose escalation. The first eight patients were entered on the trial over 5 months, and treated at seven dose levels ranging from 50 to 525 mg/m^2. Had the traditional dose escalation format been used, it would have taken a minimum of 9 months to reach the seventh dose level. A total of 37 patients were treated, with 30 (81%) treated within one dose level of the MTD (525 to 700 mg/m^2). This compares with 48 patients and 63% treated within one dose level of the MTD, if a minimum of three patients were treated at each dose level. Had only the planned ten patients, instead of the actual 20 patients, been treated at the recommended phase II

dose level, these figures would have been 74 and 53%, respectively.

The MTD and recommended dose for phase II clinical trials of MTA when administered intravenously over 10 min every 21 days is 600 mg/m^2, with neutropenia, thrombocytopenia, and cumulative fatigue as the DLTs. Nonhematologic toxicity at this dose level of MTA was mild to moderate, with no instances of grade III or IV side effects during the first course of treatment. Cumulative fatigue and myelosuppression became apparent in multiple patients treated repetitively. Mild nephrotoxicity occurred in multiple treated patients and renal function appeared to be the most predictive factor for drug toxicity. Caution should therefore be used in treating patients with even mild degrees of renal dysfunction. Currently, a clinical trial of MTA is ongoing in patients with various degrees of renal dysfunction in order to determine whether initial dosing should be based on body surface area or renal function. Concurrent nonsteroidal antiinflammatory agents should be avoided since they may decrease renal clearance of the compound. The development of severe thrombocytopenia appeared to be a marker of sensitivity to MTA and substantial dose reductions or possibly even discontinuation of MTA treatment may be required in this setting. Partial responses were achieved in two patients with advanced pancreatic cancer and in two patients with advanced, previously treated colorectal cancer. Minor responses were observed in six patients with advanced, previously treated colorectal cancer. MTA is a promising anticancer agent, but dose modifications for toxicity and renal dysfunction will need to be further defined in future clinical trials.

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