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ORIGINAL ARTICLE

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Final results of a phase I and pharmacokinetic study of γ-methylene-10-deazaaminopterin (MDAM) administered intravenously daily for five consecutive days in patients with solid tumors

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Abstract *Purpose*: To determine the maximum tolerated dose (MTD) of γ-methylene-10-deazaaminopterin (MDAM), a unique antifolate structurally similar to methotrexate (MTX), in the treatment of patients with solid tumors and to characterize toxicity and pharmacokinetic profiles of MDAM administered intravenously for five consecutive days repeated every 21 days. *Methods*: A group of 18 patients with treatment-refractory colorectal cancer (CRC) were given MDAM at increasing dose levels from 80 to 300 mg/m^2 per day intravenously for 5 days every 3 weeks. *Results*: A total of 18 patients were entered into the study. Grade 2 or less nausea, vomiting, diarrhea, anorexia and fatigue were observed at doses ≥160 mg/m^2 per day. Both patients enrolled at 300 mg/m^2 per day experienced grade 3 stomatitis and one patient had grade 4 granulocytopenia. At 270 mg/m^2 per day, grade 3 stomatitis ($n=2$), thrombocytopenia ($n=1$) and hyperbilirubinemia ($n=1$) were observed. All toxicities were relatively brief in duration and reversible. Leucovorin rescue was not required. Of 17 evaluable patients, no complete or partial responses were observed, and 3 patients demonstrated stable disease. Pharmacokinetic analyses were performed in 16 of the 18 patients receiving MDAM at doses of 80, 160, 240, 270 and 300 mg/m^2. Normalized clearance of MDAM was approximately 1.5 times that reported for MTX (125 vs 80 ml/min per m^2) in adults. *Conclusion*: MDAM is a novel antifolate with potential pharmacokinetic and safety advantages over MTX. Based on the results of this phase I study, stomatitis emerged as the dose-limiting toxicity and the recommended starting dose for phase II trials using this schedule and route of administration is 240 mg/m^2 per day.

Keywords MDAM · γ-Methylene-10-deazaaminopterin · Phase I · Solid tumors · Cancer · Pharmacokinetics

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Introduction

Folate antagonists disrupt folic acid-mediated coenzyme pathways through inhibition of the enzyme dihydrofolate reductase (DHFR) [1]. This action interferes with regeneration of the reduced tetrahydrofolate pool required in purine base biosynthesis. Farber et al. were the first to demonstrate in 1948 that the antimetabolite aminopterin (4-amino-4-deoxypteroylglutamic acid) could achieve clinical remission in patients with acute leukemia [2]. Subsequently, methotrexate (4-amino-4-deoxy-10-methylpteroylglutamic acid, MTX) has become the prototype for this chemotherapy class, and has been used widely in the treatment of neoplastic diseases ranging from hematologic malignancies (leukemia and lymphoma) to solid tumors (head, neck, lung, breast, bone, gastrointestinal and genitourinary) [3, 4, 5, 6].

Both toxicities and drug resistance mechanisms have limited further therapeutic advancement of MTX use [7]. Interest has evolved, therefore, in the development of novel antifolates that exploit unique pharmacologic mechanisms to modify toxicity and/or avert classic mechanisms of MTX drug resistance [8, 9]. MDAM is similar in molecular structure to MTX, but unlike MTX

it does not undergo polyglutamylation, and therefore potentially would show less host toxicity and more consistent DHFR specificity, while offering more specific antitumor action (Fig. 1) [10].

MDAM has previously demonstrated activity against human tumors in preclinical studies, although it has not been extensively investigated in human subjects [11, 12, 13]. The aim of this study was to determine the qualitative and quantitative toxicity of MDAM, to investigate its clinical pharmacology, to determine its maximum tolerated dose (MTD) in the treatment of patients with solid tumors and to document antitumor activity.

Material and methods

Patients

Patients with histologically (or cytologically) confirmed solid tumors (excluding primary CNS neoplasms) refractory to conventional therapy or for which no conventional treatment existed were eligible for the study. Detailed eligibility criteria also included age ≥18 years; ECOG performance status (PS) of 2 or better; life expectancy greater than 3 months; adequate hematologic, hepatic, and renal functions determined by an absolute neutrophil count $\geq 1.5\times10^9$/l, platelets $\geq 100\times10^9$/l, total bilirubin not more than 1.5 times normal, SGOT and SGPT not more than 2.0 times normal (or not more than 5.0 times normal with hepatic metastases), and serum creatinine ≤ 2.0 mg/dl. Measurable or evaluable disease was required. Signed informed consent was obtained prior to enrollment.

Pretreatment evaluation included a detailed medical history and physical examination, complete blood count, serum chemistry, liver function tests, prothrombin time, urinalysis, ECG, chest radiograph, and staging studies as clinically indicated to define the extent of metastatic disease.

Treatment plan

Treatment consisted of outpatient intravenous administration of MDAM over 60 min for five consecutive days every 3 weeks. The starting dose was 80 mg/m^2 per day (dose level 0) with subsequent interpatient dose escalations to 160, 240, and 300 mg/m^2 per day (dose levels 1, 2, and 3, respectively) as permitted by toxicity and response criteria. After a dose-limiting toxicity (DLT) occurred at 300 mg/m^2 per day, an additional dose level was added (270 mg/m^2 per day). Patients with evidence of progressive disease were withdrawn from the study and managed at the discretion of the treating physician.

Pharmacokinetic evaluation

A total of 25 blood samples were obtained from each patient. On days 1 and 5 samples were collected prior to drug administration and at 15, 30 and 45 min, and 1, 2, 3, 4, 6, 8 and 24 h after the start of the infusion. On days 2 through 4, samples were collected before and at the end of the MDAM infusion. These samples were collected on ice in heparinized Vacutainers and centrifuged at 1000 *g* for 10 min at 4°C. Plasma was transferred to polypropylene tubes and stored at −70°C until analyzed. Following the start of the MDAM infusion, urine was collected over 6-h intervals for a 24-h period during day 1 only. Total urine volumes were recorded and a 30-ml aliquot from each collection was frozen at −70°C for analysis.

The content of MDAM and its hydroxylated metabolite, MDAM-OH, in plasma and urine samples was quantified by HPLC with fluorescence detection. At the time of analysis, MDAM and MDAM-OH were isolated by solid-phase extraction (SPE). The samples were first allowed to thaw at room temperature while the SPE column (tC18; Waters, Milford, Mass.) was conditioned with 1 ml methanol followed by 1 ml HPLC-grade water. The plasma or urine sample (250 µl) was then applied, washed with 1 ml water and eluted with 1 ml methanol. The eluent was dried under a nitrogen stream and reconstituted in 250 µl mobile phase. Then 100 µl of the eluent was injected on-column and separated by HPLC via gradient elution using a C18 analytical column (150×4.6 mm I.D. ODS, 3 µm particle size; Phenomenex, Torrance, Calif.). Gradient elution was performed at a fixed flow rate of 0.8 ml/min with solvent A (10 m*M* potassium phosphate buffer containing 5 m*M* tetrabutylammonium bromide, pH 6, ranging from 68% to 84%), and an organic phase consisting of 8% to 20% solvent B (acetonitrile) and 8% to 12% solvent C (methanol). Excitation and emission wavelengths were 375 and 460 nm, respectively. Retention times in extracts from human plasma and urine were 24.1 min and 19.6 min for MDAM and 25.6 min and 22.2 min for MDAM-OH, respectively. Absolute drug concentrations were determined using external standardization. The linear dynamic range for this method was 0.005 to 10 µ*M* for MDAM and 0.005 to 1 µ*M* for MDAM-OH in both plasma and urine, with intra- and interday coefficients of variance of $<6\%$. Samples containing MDAM concentrations above the linear dynamic range were diluted with mobile phase and the analysis repeated.

Pharmacokinetic parameters for MDAM were determined by fitting various compartmental models to each subject's plasma concentration-time data using ADAPT II (Biomedical Simulations Resource, USC, Los Angeles, Calif.) [14]. Model selection was based on goodness of fit, Akaike's information criterion, and visual inspection. The ratio of parent to metabolite concentrations in plasma was determined. In addition, the cumulative amounts of MDAM and MDAM-OH excreted over time were calculated as a percentage of the administered dose.

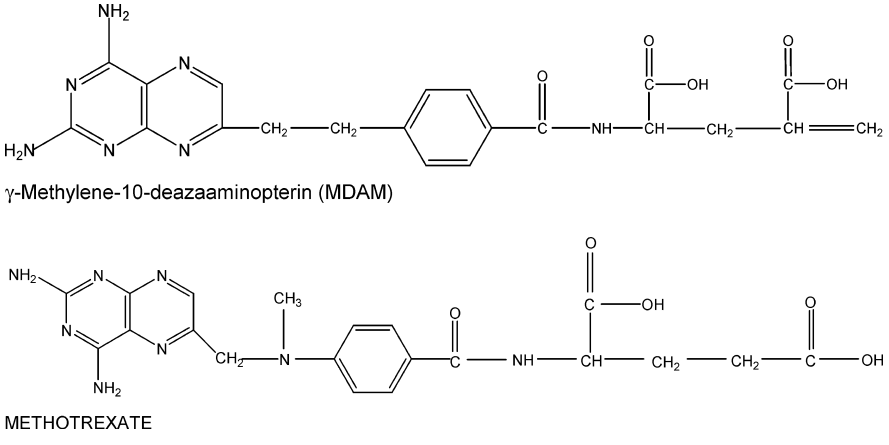

Fig. 1 γ-Methylene-10-deazaaminopterin (MDAM) and MTX

Toxicity evaluation

Serum chemistry was monitored weekly for each patient along with complete blood counts. Clinical re-evaluations were performed every 3 weeks including documentation of treatment-related toxicity, physical examination and complete laboratory evaluation. Restaging of disease was performed after every two cycles to assess tumor response. Treatment-related toxicity was scored using the NCI-CTC grading criteria.

Starting at 80 mg/m^2 per day (dose level 0), patients were enrolled into cohorts of three per dose level as permitted by toxicity. The dose was escalated by 100% until biologic activity (other than mild nausea, vomiting, fatigue or alopecia) was observed. If grade 1 toxicity (excluding alopecia, nausea or vomiting) was observed, then subsequent dose escalation was made in 50% increments. Once grade 2 or higher toxicity was observed, dose escalation was adjusted by increments of 25% in subsequent patients.

DLT was defined as a grade 3 or higher nonhematologic toxicity, grade 4 hematologic toxicity, or unresolved toxicity warranting a 2-week treatment delay at a specific dose level. With observation of a DLT, three additional patients were enrolled at the same dose level or an intermediate dose level. If no further instances of a DLT were observed, then dose escalation continued. If two or more patients experienced a DLT at a given dose level, further entry at that dose level was terminated and the MTD recommended for future phase II trials would be the next lower dose level.

Response evaluation

Antitumor activity was assessed on evaluable lesions (up to six representative lesions) for each patient every two treatment cycles (6 weeks). Complete response (CR) was defined as disappearance of all known disease as determined by two observations not less than 4 weeks apart. Partial response (PR) was considered a 50% or greater decrease in the sum of the products of the largest perpendicular diameters of measurable lesions as determined by two observations not less than 4 weeks apart. For evaluable nonmeasurable disease (having a largest diameter below the cutoff defined for measurable lesions), an estimated decrease in tumor size by 50% or more was required. Otherwise, no lesion should have progressed and no new lesion should have appeared to be considered a response. Stable disease (SD) was defined as a less than 50% decrease or a less than 25% increase in the sum of the products of the largest perpendicular diameters of measurable lesions (or estimate thereof in the case of nonmeasurable disease) at least 6 weeks after treatment initiation. Progressive disease (PD) was defined as a greater than 25% increase in the size of at least one evaluable lesion, the appearance of a new lesion, or the development of a pleural effusion or ascites.

Results

From December 1997 to June 1999, 18 patients with refractory adenocarcinoma of the colon or rectum were enrolled on this phase I study. Patient demographics are listed in Table 1. Dose levels at study entry are displayed in Table 2. All patients were evaluated for toxicity, and 17 patients were evaluated for response. The median patient age was 61 years (36–77 years); 13 patients were male, and all had a Zubrod/ECOG PS of 0–1. All patients had received prior surgery and chemotherapy, with 11 patients having been treated with three or more different chemotherapy regimens previously.

The toxicities observed are shown in Table 3. No DLT was observed in the first three patients treated at dose levels 0 (80 mg/m^2 per day), 1 (160 mg/m^2 per day) and 2 (240 mg/m^2 per day). Grade 1 and 2 stomatitis, nausea, vomiting, diarrhea, anorexia, fatigue and muscle weakness (asthenia) were noted at doses ≥160 mg/m^2 per day. Grade 2 granulocytopenia was noted at dose level 2 (240 mg/m^2 per day). Both patients enrolled at dose level 3 (300 mg/m^2 per day) developed grade 3 stomatitis and one also had grade 4 granulocytopenia. At the intermediate dose level (270 mg/m^2 per day), three of six patients developed a grade 3 nonhematologic toxicity. These toxicities were grade 3 stomatitis in two of six patients, one with concomitant grade 3 thrombocytopenia. An additional patient at this dose level developed grade 3 hyperbilirubinemia. One patient who received 300 mg/m^2 and a second who received 270 mg/m^2 for one cycle each had their dose reduced to 240 mg/m^2 for subsequent cycles. No DLTs were observed in these two patients following administration of 240 mg/m^2 or in the four patients originally enrolled at 240 mg/m^2. Therefore, the MTD was determined to be 240 mg/m^2 per day using the 5-day schedule.

Table 1 Patient demographics (values are number of patients, except age in years)

No. of patients	18
Gender (M/F)	13/5
Age (years)	
Median	61
Range	36–77
Performance status	
0	10
1	8
Previous therapy	
Chemotherapy	18
Immunotherapy	1
Radiation therapy	1
Surgery	18
Prior chemotherapy regimens	
One	2
Two	5
Three	7
More than three	4
Prior number of agents	
Two	4
Three	7
More than three	7
Disease	
Colon adenocarcinoma	15
Rectal adenocarcinoma	3
Other major sites of disease	
Liver	16
Lung	8

Table 2 MDAM dose levels

Dose level	Dosage (mg/m^2 per day, days 1–5)	No. of patients
0	80	3
1	160	3
2	240	4
3	300	2
4	270	6

Table 3 Occurrence of adverse events by patient (highest NCI toxicity grade)

	MDAM dose level																	
	0			1			2				3		4					
Patient number	01	02	03	04	05	06	07	08	09	10	11	12[a]	13	14	15	16	17	18[a]
Cycles received	2	2	2	2	4	2	6	3[b]	2	2	1	2	2	2	2	4	2	2
Granulocytopenia								2		2	4	2						2
Thrombocytopenia											2							3
Hyperbilirubinemia						3[c]							3					
Diarrhea					1							2		1	1			
Anorexia						1				1								
Nausea				2			2		1	2			1	1	2	1		
Vomiting				2			1		1	1			2		1	1		
Skin reaction												2			2			
Stomatitis							2	2	2	1	3	3	1	1	2	3	1	3
Fatigue				2		1	2	1	2	1		2		1	2		2	1

[a]Dose was reduced to 240 mg/m^2 in these patients following cycle 1. Following administration of 240 mg/m^2 in cycle 2, patient 12 had grade 2 diarrhea, skin reaction, stomatitis and granulocytopenia, and patient 18 had grade 3 thrombocytopenia and grade 1 stomatitis and granulocytopenia
[b]Patient received only partial course on cycle 1
[c]Not considered a DLT since the patient was grade 2 at baseline

Table 4 MDAM plasma pharmacokinetic parameters following intravenous administration presented as means ± SD

Dose level	Dose (mg/m^2/day)	*n*	C_{max} (μM)[a]	V_c (l/m^2)	V_{ss} (l/m^2)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	Cl_T (ml/min/m^2)	AUC (μM·h)
0	80	3	13.0 ± 1.6	8.0 ± 4.1	20.6 ± 13.9	0.76 ± 0.58	10.7 ± 7.7	99.9 ± 8.6	30 ± 3
1	160	2	42.7	6.2	18.8	0.74	16.0	72.7	83
2	240	4	27.7 ± 10.6	13.8 ± 3.7	36.3 ± 17.5	0.56 ± 0.44	8.7 ± 7.8	209.2 ± 122.5	52 ± 22
4	270	5	40.3 ± 19.8	13.8 ± 14.3	35.9 ± 47.2	0.49 ± 0.38	5.1 ± 1.8	129.0 ± 117.4	163 ± 132
3	300	2	60.1	6.6	14.7	0.39	3.3	32.9	190
All patients		16		10.9 ± 8.6	28.3 ± 28.2	0.6 ± 0.4	8.2 ± 8.1	124.6 ± 101.1	103 ± 99

[a]Average C_{max} over days 1–5 calculated for each patient

Of 18 patients enrolled, 17 were evaluated for response. One patient enrolled at 300 mg/m^2 per day who received only one course was not eligible for this evaluation. No CRs or PRs were observed, but three patients demonstrated SD, one patient each at dose levels 160, 240 and 270 mg/m^2 per day following four, six and two cycles of MDAM, respectively.

Pharmacokinetics

Pharmacokinetic evaluations were performed in 16 of 18 patients enrolled in this trial. Patients 6 and 17 were excluded from the analysis due to insufficient sample collection. Plasma drug concentration versus time data were best described by a two-compartment model. MDAM pharmacokinetic parameters and variances for each dose level are listed in Table 4. The mean MDAM C_{max} over 5 days of dosing ranged from 13.0 ± 1.6 μM at dose level 0 to 60.1 μM at dose level 3. The mean population plasma clearance for MDAM was 125 ml/min per m^2, or 7.5 l/h per m^2, but clearance varied greatly within and between each dose level. Figure 2a demonstrates the high degree of interpatient variability in MDAM plasma clearance without apparent dose dependency observed in this limited population. The mean central and steady-state distribution volumes for MDAM were 10.9 l/m^2 (0.25 l/kg) and 28.3 l/m^2 (0.65 l/kg), respectively, and the mean terminal half-life was 8.2 h. Increasing MDAM exposure was observed with dose escalation. Figure 2b depicts the expected incremental increase in mean AUC as MDAM dose is escalated (with the exception of dose level 240 mg/m^2 per day), while at the same time demonstrating substantial overlap in drug exposure over the dose escalation range.

Mean day-1 plasma MDAM-OH concentrations reached a peak of 1.61 ± 1.0 μM within 4 h (0.26–4.45 μM). By 1 h after infusion, mean MDAM-OH plasma concentrations were 7.8% of parent drug plasma concentrations, with an interquartile range (25–75) of 2.75% to 8.5%. By hour 8, the parent-to-metabolite ratio was typically one or less, with a median MDAM plasma concentration of 0.8 μM. A representative plot of plasma concentration–time data for MDAM and the hydroxylated metabolite is shown in Fig. 3. No significant accumulation of metabolite was observed on this daily ×5 schedule.

Mean renal MDAM clearance was 74.1 ± 42.3 ml/min per m^2 measured over the first 24 h. During this interval, 31.3 ± 18.4% of the parent compound was excreted in the urine, with 18% excreted in the first 6 h. Less than 2% of the total dose administered was excreted as the hydroxylated metabolite over the first 24 h.

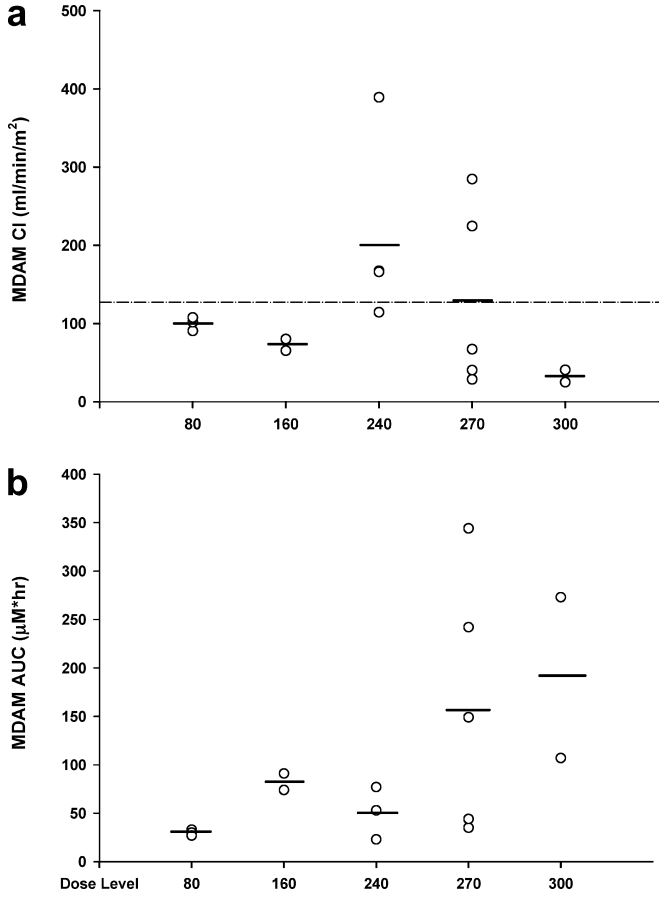

Fig. 2 **a** MDAM clearance by dose level. A high degree of interpatient variability in MDAM plasma clearance was observed at each dose level (—— population mean, — dose level mean). **b** Mean AUC increased as MDAM dose was escalated with substantial overlap in drug exposure over the dose escalation range (— dose level means)

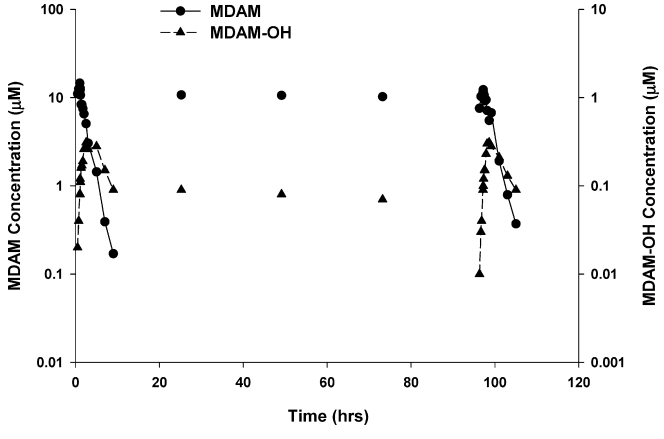

Fig. 3 Plasma concentrations of MDAM and its hydroxylated metabolite, MDAM-OH, in a patient receiving 160 mg/m^2 per day ×5

Discussion

MTX has been considered an effective chemotherapy agent for the treatment of an array of tumor types for decades. It has a well-defined biochemical mechanism of action and well-known toxicity profile. An analogue of folinic acid, it enters the cell by active transport through a specific transmembrane protein called the reduced folate transporter or RFT [15, 16]. It then undergoes polyglutamylation (the progressive serial addition of glutamic acid residues) facilitating intracellular retention and more effective inhibition of DHFR (versus native MTX) [15, 17]. The process of polyglutamylation is not without cost, however, as it eventually leads to loss of specificity for DHFR activity (compromising antitumor effect) as well as delay of cellular drug efflux (resulting in protracted exposure to normal host tissues and thus toxicity) [15, 17, 18].

Furthermore, multiple mechanisms of resistance to MTX have been described including increased DHFR gene amplification in tumor cells, decreased MTX uptake by tumor cells (either decreased influx or impaired polyglutamylation), and alteration of DHFR structure with less affinity for MTX binding [18, 19].

To overcome problems associated with cellular uptake, strategies have been developed, such as the administration of MTX at high doses (HDMTX), thereby relying on mass transfer to deliver increased MTX to the cell. These approaches rely on the use of hyperhydration, urinary alkalinization, and folate rescue for safe administration. The pharmacokinetics of MTX at these dosages becomes nonlinear, requiring a significant amount of clinical intervention. Patients must be continually monitored, resulting in increased costs of hospitalization. Even in patients who are the best candidates for HDMTX (normal renal and hepatic function, no ascites, normal folate status), adverse events occur [20]. In most cases, these patients require continued follow-up for weeks after drug administration to prevent toxicity.

In summary, there are significant obstacles to the use of MTX. Response rates are limited and toxicities are vast including bone marrow suppression, mucositis, renal toxicity, liver dysfunction, lymphocytic pulmonary infiltrates and encephalopathy [21, 22, 23, 24]. A new antifolate agent with a better toxicity profile, greater antitumor activity, and more predictable pharmacokinetics than MTX would be an important advance in the treatment of multiple tumor types.

Other agents being explored in this setting include trimetrexate (TMTX). This agent is also a DHFR inhibitor, but unlike MTX it enters the cell largely by passive diffusion, it does not require enzymatic activation, and it does not undergo intracellular polyglutamation [25, 26]. Clinical trials of TMTX treatment in patients with colorectal cancer (CRC) have shown antitumor activity [27, 28]. MDAM also does not undergo polyglutamylation, and therefore potentially shows less host toxicity and more consistent DHFR specificity while offering more specific antitumor action [10]. Results have shown that its major metabolite, 7-hydroxy-MDAM, is also unable to undergo polyglutamylation which may be an additional therapeutic advantage.

The antifolate class of antineoplastics has been evaluated in the treatment of CRC. MTX has been evaluated in the treatment of metastatic disease, demonstrating activity in combination with 5-fluorouracil (5-FU), although appearing equivalent to the combination of 5-FU and leucovorin [29, 30]. Likewise, TMTX has been investigated in combination with 5-FU/leucovorin in previously treated unresectable or metastatic CRC. These studies suggested that this combination provides no overall survival benefit and little increase in progression-free survival in patients with CRC [26, 27]. In the current study, response was assessed in 17 of the 18 MDAM-treated patients. In our heavily pretreated population, no responses were observed, but three patients demonstrated SD. All trial participants had a tissue diagnosis of adenocarcinoma of the colon or rectum, and all had failed several prior treatments including the combination of 5-FU and leucovorin.

This phase I trial demonstrated that MDAM is reasonably well-tolerated, even in heavily pretreated patients. The MTD was reached at 240 mg/m^2 per day when given daily for 5 days every 3 weeks, which is the recommended starting dose for phase II trials. The two major DLTs were hematopoietic (granulocytopenia and thrombocytopenia) and gastrointestinal (mucositis). These toxicities were of relatively short duration and completely reversible without the need for leucovorin rescue. No serious adverse events occurred over the duration of the study.

Pharmacokinetic evaluation demonstrated a somewhat higher MDAM clearance compared to that of MTX, although this was highly variable between patients in this study. Higher clearance of MDAM could be related to less host toxicity and an improved therapeutic index compared to other drugs in this class. The mean initial and steady-state distribution volumes for MDAM of 0.25 l/kg and 0.65 l/kg are similar to reported values for MTX, with the steady-state distribution volume approximating total body water [18, 31]. The mean terminal half-life, 8.2 h, also approximated that reported as the $T_{1/2\beta}$ for MTX. The relatively low amount of active metabolite formed and the absence of observed accumulation with daily dosing may also relate to lower potential for host toxicity. In contrast to MTX, only one-third of the compound was recovered in the urine, suggesting significant extrarenal elimination.

A total of 46 cancer patients have now been treated with MDAM in phase I clinical trials completed at The Johns Hopkins Oncology Center and M. D. Anderson Cancer Center. Phase I trials were conducted using racemic MDAM containing the L-MDAM and D-MDAM forms. It has been demonstrated in preclinical studies that L-MDAM is largely responsible for the antitumor activity and that L-MDAM potentially could be administered orally at reduced doses relative to racemic MDAM. Oral L-MDAM has shown similar antitumor activity relative to intravenously administered racemic MDAM in preclinical studies. Evaluation of the possibility of phase I trial with L-MDAM to determine its safety and potential efficacy is currently ongoing.

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