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Clinical Pharmacology of Intermediate-dose Oral Methotrexate*

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Summary. *The clinical pharmacology of intermediate-dose oral methotrexate (MTX) was studied in nine patients receiving 18 courses of treatment. Serum and urine MTX concentrations were measured by means of a competitive protein binding assay after oral aqueous solution (3 courses), 50-mg tablets (13 courses) or IV drug (2 courses) had been administered in four doses of 100 mg/m² at 6-h intervals or in four doses of 200 mg/m² at 6-h intervals and followed by citrovorum factor rescue. Levels above 150 ng/ml (3.3×10^{-7} M) were maintained throughout all treatment cycles, with rapid disappearance of drug after the last dose. A 100% increase in administered dose resulted in only a 42% increase in the concentration-time level. Methotrexate was absorbed well from both aqueous solutions and 50-mg tablets, but serum levels after 50-mg tablets were only 20% of those achieved after IV administration.*

We conclude that significant serum MTX concentrations can be achieved for prolonged periods of time after oral administration of intermediate doses, but that the proportion of drug absorbed is much less than is seen with lower doses.

Introduction

In 1951, Burchenal et al. [2] reported that low doses of oral methotrexate (MTX) were completely absorbed. Since then similar results have been obtained in several studies with standard dose (< 80 mg/m²) oral MTX [4, 5, 7, 11, 17]. With the discovery that some tumors thought resistant to MTX may respond to high IV doses, larger oral doses have also been studied, in an attempt to improve response rates over those achieved with standard doses [12, 15]. A significant advantage in cost and convenience to the patient can be achieved in this manner, because treatment is administered on an outpatient basis. Unfortunately, very little is known about the absorption of oral MTX formulations at intermediate doses (80–1000 mg/m²). Several reports have indicated that absorption at these doses is incomplete and unpredictable [8, 16], but these studies were performed on small numbers of patients and with relatively insensitive and nonspecific techniques. We undertook this study, therefore, to clarify the absorption pharmacokinetics of intermediate doses of oral MTX.

Materials and Methods

Only patients with a Karnofsky performance status of at least 40%, a creatinine clearance over 60 cm³/min, and tumors considered incurable by standard treatment were eligible for the study. Patients with known gastrointestinal disorders, prior upper GI surgery, or who had received abdominal radiotherapy that might interfere with MTX absorption were excluded. All patients had completely recovered from any prior therapy, and gave their written, informed consent before entry. Pretreatment studies included CBC, platelet count, bilirubin, SGOT, alkaline phosphatase, albumin, BUN, creatinine clearance, urinalysis and d-xylose absorption test. Most patients had a second d-xylose absorption test performed 24 h after the start of treatment to assess changes in gastrointestinal absorption secondary to MTX. Results of this test were expressed as the percentage of a standard 25-g dose excreted in the urine in 5 h.

All patients were hospitalized during study to facilitate specimen collection. Treatments were repeated at 3-week intervals for three courses. The first course consisted of MTX, four doses of 100 mg/m² PO at 6-h intervals (400 mg/m² total dose). The dose was escalated to four doses of 200 mg/m² at 6-h intervals (800 mg/m² total dose) for the second and third courses. Later patients received their first course at the higher dose.

Absorption of 50-mg MTX tablets[1] was compared with that of IV MTX in two patients. They received IV drug during their second course and oral tablets during their third course. Absorption of an

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1 50 mg MTX tablets supplied by the Investigational Drug Branch, National Cancer Institute

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orally administered aqueous solution (IV formulation) of MTX was compared to that of oral 50-mg tablets in a similar fashion in another two patients, while a third received only aqueous solution in a single course.

To avoid known interference with drug absorption and excretion, no solid food was permitted 2 h before and after each dose of oral MTX. During treatment, only antiemetics or non-aspirin-containing analgesics were allowed in addition to protocol drugs.

Supportive therapy administered with each MTX course consisted of $NaHCO_3$, 8–16 mEq PO every 6 h for 4 days, starting 15 h before the first MTX dose; fluids, 3,000 cm^3 daily minimum for the first 2 days; and citrovorum factor rescue, six doses of 5 mg PO at 6-h intervals in 5-mg tablets[2], starting 36 h after the first dose of MTX.

Serum for MTX levels was collected 30 min, and 1, 2, 4, 6, 8, 12, 14, 16, 20, 24, 28, and 36 h after the first dose of MTX. Samples were obtained at later times (up to 145 h) during six of the treatments. Urine was collected continuously for 72 h after the start of therapy. All samples were refrigerated as they were obtained and frozen at −20° C within 24 h for later assay.

The MTX concentration in serum and urine samples was determined in appropriately diluted samples by means of a competitive protein-binding assay, ^{125}I – MTX being used as a tracer and dihydrofolate reductase as the binding protein [10]. The procedure was modified by deleting standards in the horizontal portion of the curve (0.031, 0.063, and 1.0 ng/ml) and adding intermediate standards at 0.75, 0.375, and 0.1875 ng/ml to improve accuracy. Duplicate standards were used in all assays. At least two, and usually three, separate determinations were performed on each unknown sample. The relative amount of MTX absorbed during each course was determined by measuring the area under the MTX concentration-time (CT) curve in accordance with the "cut and weigh" method [6]. The area under the curve was expressed as milligram-hours per milliliter. Urinary MTX levels were expressed as milligrams of drug excreted in 72 h and also as a percentage of the dose actually administered that was excreted in 72 h. Serum MTX half-lives were estimated from a semilogarithmic plot of drug concentration versus time.

Results

Nine patients were entered on the study. Four completed the planned three cycles of treatment, one patient completed two cycles, and four completed a single cycle. Thus, of the 18 courses, three were given in the form of oral aqueous MTX solution, two with IV MTX, and 13 with 50-mg MTX tablets.

There were three patients with head and neck cancer, and one each with colon cancer, lung cancer, Hodgkin's disease, diffuse poorly differentiated lymphocytic lymphoma, malignant melanoma, and adenocarcinoma of unknown primary. There were eight men and one woman, ranging in age from 35 to 67. All had failed at least one trial with 'standard' chemotherapy and/or radiotherapy, and some had failed to respond to various phase I and II clinical trials with other agents.

Toxicity was minimal. Only two patients complained of nausea, and no significant reductions in peripheral blood counts occurred. One patient's creatinine clearance fell below 60 cm^3/min, resulting in his removal from study (pretreatment creatinine clearance, 66 cm^3/min). Treatment was prematurely discontinued in five patients because of obvious disease progression.

Figure 1 shows MTX CT curves obtained from a single patient given oral aqueous and 50-mg tablet formulations at two dose levels. These curves are similar to those obtained in the other 13 courses studied after oral formulations. Serum levels could be detected in the first (30 min) sample with peaks in serum concentrations occurring 2–4 h after each dose. It was not uncommon for the fourth peak to be attenuated or absent with oral formulations. Levels quickly fell toward zero after the last peak in an apparently biphasic manner, with half-disappearance times of approximately 1 and 11 h as determined by estimation from a semi-log plot. This is in agreement with the half-times of 2 and 10 h reported by Stoller [14].

Serum MTX concentrations were usually below the lower limits of assay sensitivity (5 ng/ml or 1.1×10^{-8} *M*) by 42–48 h and, in contrast to Wan's findings [16], there was no indication of a late rise in subsequent samples. The highest peak after oral administration at the 800 mg/m^2 dose ranged from 676 to 1,504 ng/ml, with a mean of 1,212 ± 363 ng/ml (mean ± SD), and usually occurred after the second (6 h) or third (12 h) dose. Trough levels before the fourth (18 h) dose were above 150 ng/ml (3.3×10^{-7} *M*) in all treatment cycles.

Results from the two patients receiving both aqueous and tablet formulations at equivalent 800 mg/m^2 doses are presented in Table 1. Absorption of drug from 50-mg tablets appeared to be equal to or slightly better than that obtained with the aqueous solution, whether expressed as area under the CT curve or urinary MTX excretion in 72 h.

To obtain an estimate of the percentage of drug absorbed after oral administration, the area under the CT curves for two patients given identical doses (800 mg/m^2)

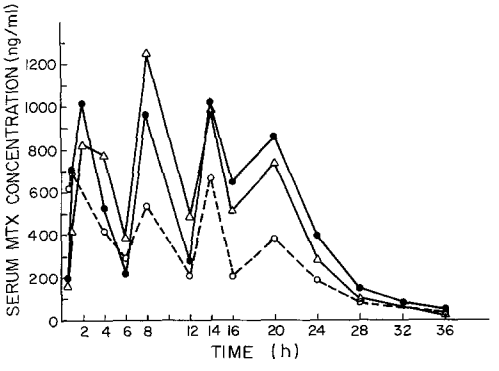

Fig. 1. Concentration × time curves in patient no. 3 after oral MTX. Similar data were obtained on all patients under study. △—△, Course 1; ○ – – ○, Course 2 (aqueous solution); ●—●, Course 3

2 Citrovorum factor 5 mg tablets supplied by Lederle Laboratories

Table 1. Absorption of oral aqueous MTX solution and MTX tablets after four doses of 200 mg/m² at 6-h intervals (800 mg/m² total dose)

Patient	mg MTX excreted in 72 h (% dose)		Area under CT curve (mg-h/ml)	
	Aqueous solution	Tablets	Aqueous solution	Tablets
7	214 (13.4)	150 (9.4)	26.1	27.8
3	77 (4.8)	160 (10.0)	9.9	17.0

Table 2. Absorption of oral MTX tablets compared with IV MTX after four doses of 200 mg/m² at 6-h intervals (800 mg/m² total dose)

Patient	mg MTX excreted in 72 h (% dose)		Area under CT curve (mg-h/ml)	
	IV	Tablets	IV	Tablets (% IV)
2	–	120 (8.6)	117.0	23.8 (20.3)
4	1,046 (65.4)	243 (15.2)	155.6	24.4 (15.7)

of both oral and IV drug were compared (Table 2). In these patients CT values approximately 18% of those observed after IV drug were found. A similar proportion (23.2%) of MTX administered in tablet form was excreted in the urine compared with IV administration.

Of the 13 courses studied after administration of 50 mg MTX tablets, six were at the 400 mg/m² total dose level and seven at the 800 mg/m² dose level. After 400 mg/m² the average highest peak was 1,000 ng/ml, the average concentration-time 15.2 mg/h/ml, and the average 72-h MTX excretion 144 mg (16.9% of administered dose). This compares with values of 1,251 ng/ml, 21.7 mg/h/ml, and 197 mg (13.5%), respectively, at the 800 mg/m² dose level. Thus a 100% dose increase resulted in only a 25% rise in peak level, a 42% rise in concentration-time level, and a 36% rise in urinary excretion.

There was no apparent correlation between d-xylose absorption, nor could any relationship be seen between MTX levels and d-xylose absorption, in patients tested both before and after MTX treatment. Similarly, creatinine clearance did not correlate with serum levels of MTX, but patients with significant renal dysfunction were excluded from the study.

Discussion

Because of advantages in cost and convenience, interest in utilizing oral formulations of MTX has increased. Several investigators have studied oral MTX absorption at intermediate dose levels and discovered a smaller percentage of drug absorbed than had been found with lower doses [8, 16]. Our results confirm these findings. Despite a 100% increase in administered dose, MTX CT levels indicated only a 42% increase. Similarly, the urinary output of drug increased by only 36% and the peak serum level by only 25%. This indicates a decrease in the relative amount of MTX absorbed at the higher dose.

One possible explanation for this phenomenon is the effect of MTX itself on the absorptive capacity of the GI mucosa. An earlier study by Freeman-Narrod [5] indicated that there was a correlation between d-xylose and MTX absorption. The results of d-xylose absorption tests in several of our patients, however, failed to indicate any relationship between drug and sugar uptake, nor was there evidence of decreased d-xylose absorption after treatment. Despite the lack of effect of MTX on d-xylose uptake, we believe drug-induced GI toxicity is still a likely explanation for the inverse relationship between dose and MTX absorption. The observation of an attenuated and sometimes absent fourth peak in serum levels after treatment is consistent with this concept.

Peak serum concentrations in our study ranged from 625 to 1,251 ng/ml at the 400 mg/m² dose level. This compares favorably with peak levels found by Henderson [8] and Robert [12] with tritiated MTX (MTX-^{3}H) and radioimmunoassay techniques after similar oral doses. Kincade [9], on the other hand, using a fluorometric assay, obtained much higher peak concentrations (2,500–3,000 ng/ml) in two patients with normal creatinine clearance. This last study is subject to error, however, since both folic acid and MTX metabolites fluoresce, and may thereby lead to artificially elevated results. The study presented here uses a competitive protein-binding assay which is relatively insensitive to MTX metabolites. This may also explain our failure to observe the late rise in serum MTX levels reported by Wan [16] with the MTX-^{3}H method.

The blood levels achieved in the present study are less than the Michaelis constant for membrane transport of MTX (5.82×10^{-6} *M*) in human leukemic cells [1], and even higher concentrations may be required in some solid tumors. Thus, the present schedule might not overcome resistance based on impaired membrane transport; nevertheless, both peak and trough levels were well above the minimum concentration (10^{-8} *M* or 4.55 ng/ml) found to inhibit tumor DNA synthesis [3].

We conclude that oral intermediate-dose MTX is absorbed well from both 50-mg tablets and aqueous solutions, and provides sustained serum concentrations. After use in conjunction with citrovorum factor, hydration, and sodium bicarbonate, toxicity has been minimal. Peak serum levels, areas under CT curves, and urine recovery of MTX after the tablet formulation, however, are much lower than those achieved after IV adminis-

tration, and the proportion of drug absorbed with this treatment schedule is considerably less than that reported at lower doses [8, 16, 17]. Absorption varies widely from patient to patient and even, to a certain degree, within the same patient. It remains to be determined whether significantly higher levels can be achieved by repeating the doses more often than every 6 h.

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