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Clinical pharmacology of oral intermediate-dose methotrexate with or without probenecid*

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Summary. Serum methotrexate (MTX) levels were measured in 20 patients who received an oral, intermediate-dose MTX regimen preceded by an IV loading dose, with or without probenecid. Plateau serum MTX levels were relatively modest ($\leq 2 \times 10^{-6} M$) during the 24 h of treatment. Pretreatment with probenecid (PBC) led to a doubling of the serum MTX level and a significant increase in the area under the concentration-time curve. Nevertheless, oral therapy is not a suitable means of producing sustained, high (10^{-5} molar) MTX levels, even with the addition of PBC.

Introduction

Studies of the effects of methotrexate (MTX) on malignant cells have emphasized the relatively low free intracellular MTX levels with standard doses [5], reversible inhibition of dihydrofolate reductase [5], and phase-specificity of MTX cytotoxicity. Thus, much attention has been devoted to developing regimens which produce both high and prolonged blood levels of MTX, to overcome these obstacles.

A major shortcoming of the use of oral MTX regimens for such purposes is the incomplete and unpredictable absorption of the drug, which is not altered by dose subdivision or formulation [6, 13, 14]. This group has previously determined the bioavailability of oral intermediate-dose MTX (up to 1000 mg/m^2 per course) and noted much lower serum levels (than with IV dosing) and significant troughs between doses [13]. To increase levels and decrease the fluctuations of serum MTX concentrations, we have studied an oral intermediate-dose MTX regimen including an IV loading dose, with and without probenecid, a competitive inhibitor of tubular MTX secretion [9]. Our goal was to produce stable, high ($\sim 10^5$ molar) MTX levels for 24 h in an ambulatory setting. This paper is a report of our findings.

Methods

Only adults with a Karnofsky performance status of at least 50%, a creatinine clearance over 50 ml/min, and a cancer considered incurable by standard treatment were eligible for the study. One patient (regimen A) had pleural effusions, but none had received abdominal irradiation. 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, BUN, and creatinine clearance. All patients were hospitalized during the study to facilitate specimen collection.

Three oral intermediate-dose regimens were investigated. The first series of patients (group A) received an IV bolus of MTX 50 mg/m^2, then immediately began MTX[1] 100 mg/m^2 PO every 4 h for six doses. A second, identical course was administered 3 weeks later if there was no disease progression or excessive toxicity . A second series (group B) of patients were treated with an IV bolus of MTX 50 mg/m^2, then immediately began MTX 100 mg/m^2 PO every 3 h for eight doses; treatment was repeated 3 weeks later, as described above. A third group (group C) was treated with an IV bolus of MTX 50 mg/m^2 followed immediately by 100 mg/m^2 PO every 3 h for eight doses; 3 weeks later these patients again received this MTX regimen, but were pretreated with probenecid (PBC) 500 mg PO every 6 h for five doses, beginning 12 h before MTX.

No solid food was permitted within 1 h of any oral MTX dose. Supportive care included $NaHCO_3$ 650 mg PO every 6 h for twelve doses, beginning with the start of MTX dosing, 3000 ml fluid during the first 24 h of therapy, and citrovorum factor 5 mg PO every 6 h for six doses, starting 36 h after the start of MTX.

Serum for MTX levels was collected immediately before and 1 h after each oral dose of MTX. Additional samples were obtained in some patients every 6 h between 24 h and 72 h after the start of MTX. Samples were refrigerated as they were obtained, and frozen at −20 °C within 24 h for later assay.

The MTX concentration in serum samples was determined by means of a competitive protein-binding assay which used ^{125}I-MTX as a tracer and dihydrofolate reductase as the binding protein [10]. The areas under the MTX concentration – time (CT) curves were determined by the 'cut and weigh' method [4]. Comparisons were performed with reference to a two-tailed, paired *T*-test for significance.

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

Results

There were 7 evaluable courses of treatment in 5 patients receiving regimen A, and 13 evaluable courses in 11 patients treated according to regimen B. Figure 1 presents serum MTX levels from these groups. Serum levels were generally higher in those patients on 3 h dosing schedule. However, neither regimen was able to maintain a stable serum MTX level comparable to that achieved by the loading IV bolus. With both the 3 h and the 4 h dosing interval MTX levels were relatively modest (approximately 10^{-6} *M*) and gradually declined during the period of MTX administration.

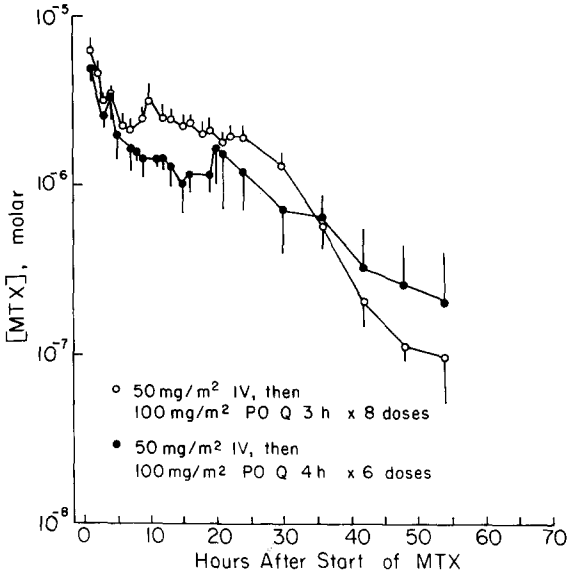

Fig. 1. Serum MTX levels for group A *(solid dot)* and group B *(open dot)* patients. Each *point* is the mean ±SE of at least three patients (average 4.6 and 11.1, respectively)

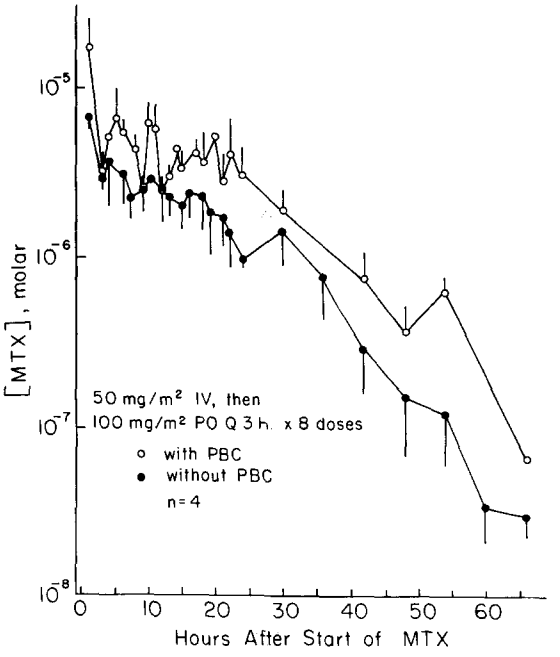

Fig. 2. Serum MTX levels for group C patients with *(open dot)* and without *(solid dot)* PBC. Each *point* is the mean ±SE of at least three patients

Four patients were treated according to regimen C, and their MTX levels are presented in Fig. 2. During PBC administration serum MTX levels were generally about twice those seen with MTX alone. The area under the CT curve in excess of 10^{-7} *M* was significantly greater (25% increase) with PBC than without ($P<0.007$). However, serum MTX levels were still relatively modest. There was no apparent difference in the $T_{1/2}$ of MTX elimination 12 h after the end of PBC pretreatment.

Partial responses were seen in two patients with diffuse lymphomas and in one with a mixed salivary gland carcinoma. Severe myelosuppression (granulocyte count $<500/m^3$ or platetel count $<50\,000/mm^3$) was seen in one patient with pleural effusions, two with extensive bone metastases, and one with circulating tumor cells. One of these patients also developed stomatitis and a skin rash. No other toxicity was noted. No responses or toxicity were seen in the patients receiving probenecid.

Discussion

Probenecid has previously been shown to increase MTX levels in the serum [1] and cerebrospinal fluid [7] of patients. This effect has been attributed to the competition by PBC for transport sites in the renal tubules that would otherwise be used by MTX [9]. The resultant delay in renal clearance produces an increase in serum level of MTX and a prolongation of serum MTX $T_{½}$ of elimination [1, 8]. Since no change in the $T_{½}$ of MTX clearance from human cerebrospinal fluid (CSF) has been noted [7], the increase in CSF levels may be secondary to the higher serum levels. Probenecid has also been shown to modulate influx and efflux of MTX from cells, leading to a net increase in intracellular MTX [11] and its polyglutamate derivatives [3]. These effects are thought to account for the improved therapeutic results seen in some MTX-PBC combination regimens [12].

Our data are consistent with these pharmacologic observations. The lack of effect on the elimination $T_{½}$ in PBC-treated patients is not surprising, since PBC was stopped 12 h before the end of the MTX dosing.

In spite of the potential for enhanced cell kill with the MTX-PBC combination, we noted no responses or toxicity in those patients. The 3-hourly dosing regimen was difficult to complete even in hospitalized patients, and is probably too complex for widespread outpatient use. Furthermore, the serum levels, while significantly higher with PBC than without, were still much lower than the Michaelis constant for membrane transport of MTX (5.82×10^{-6} *M*) of some human tumor cells [2]. We did not think an increase in MTX from 100 mg/m² to 200 mg/m² per dose was warranted, since previous evaluation of these dose levels (without the loading dose) resulted in only a 25% rise in peak serum level and a 42% rise in the CT curve [13]. Thus, oral intermediate-dose MTX regimens do not appear to be practical for production of sustained, high (10^{-5} molar) MTX levels.

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