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# Bioavailability of Methotrexate: Implications for Clinical Use

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***Summary.*** *The absorption of oral methotrexate in syrup form has been compared in six patients with that of an identical IV dose (50 mg/m²). There was variable absorption amongst the group with respect to maximum levels achieved and the time taken to reach those levels. The area under the time-concentration curve was always smaller when the drug was given orally than after IV administration. A total of 33 patients receiving methotrexate for a variety of tumour types were followed for response to treatment and toxicity. A significantly longer methotrexate half-life ($t^1/_2$) was found in nine partial responders (9.2 ± 1.6 h) than in the nonresponders (3.8 ± 0.7 h). Severe methotrexate toxicity was not seen, though occasional mucositis, conjunctivitis, and diarrhoea occurred in seven patients. The side effects could not be predicted from the dose, the bioavailability data, or the serum creatinine. Measurements of serum and urine methotrexate levels are useful in the assessment of absorption and bioavailability of the drug and the prediction of tumour response.*

## Introduction

Methotrexate (MTX) has been given by mouth for the maintenance of remission in childhood leukaemia for many years. The drug is provided in tablets of 2.5 mg, which is convenient for low-dose maintenance chemotherapy schedules. Intermittent high-dose MTX therapy has been evolved recently in the treatment of solid tumours and lymphomas. In an attempt to save patients taking up to 40 or 50 tablets by mouth or being admitted to hospital for infusions of MTX, an alternative formulation of the drug has been sought and a syrup evolved. The aim of the first part of this study was to assess the extent of absorption of oral MTX in syrup form compared with absorption of an IV reference solution in six patients.

A further 27 patients were then studied in an attempt to assess the value of pharmacokinetic data in predicting toxicity and response. All but seven of these patients were treated with doses of MTX similar to those used in the initial study. The seven exceptions were given higher doses as part of a clinical protocol.

## Materials and Methods

Methotrexate syrup was formulated as follows:

1. Sodium bicarbonate 20 g
2. Injection MTX liquid B.P. 80 ml (25 mg/ml)
3. Syrup B.P. 250 ml
4. Chloroform-water B.P. 1 litre, giving a final concentration of 10 mg/5 ml.

The syrup preparation was found to be stable for periods of up to 1 month in a variety of storage conditions, including clear and brown glass bottles at room temperature and at 4° C. For the first part of the study, six patients who were about to receive chemotherapy for a variety of solid tumours were each given a dose of MTX of 50 mg/m², either as the syrup or as an IV bolus, and received the same dose in the other form 1 week later, the order being determined on a random basis. A further 20 patients received similar doses of MTX and were studied at least once and usually twice, according to the appropriate research protocol. The results of these studies appear in detail elsewhere [6, 7]. Escalated doses were given to seven patients as follows: two patients received 250 mg, two were given 500 mg, two were given 1 g, and one patient received 2 g. These high doses were given in 12-h infusions and were followed by rescue at 24 h with citrovorum factor.

Pretreatment assessment in all patients included measurement of serum creatinine, albumin, and electrolytes and liver function tests. The patient's performance status was derived from the ECOG rating and measurements of patients' disease were made in the usual way.

After an overnight fast, a heparinised polyethylene catheter was introduced into the left antecubital vein under local anaesthesia. A pretreatment blood sample was taken and the patient was instructed to empty the bladder. Methotrexate was then administered either by mouth or IV into the free arm and 5-ml blood samples (a minimum of 11) were taken at appropriate time intervals up to 12 h, and on

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occasion up to 48 h. After centrifugation, serum was removed and was frozen at −20° C for batch assay. Urine samples were collected in batches covering 6-h periods, volumes measured, and aliquots deep-frozen at −20° C for later assay. Concentrations of MTX in the serum and urine were measured by a specific sensitive double-antibody radioimmunoassay [4] using MTX antiserum raised in rabbits and conjugated to bovine serum albumin, and a $^{75}$Se-labelled derivative of MTX supplied by Radiochemicals Ltd., Amersham. Methotrexate and calcium folinate were obtained from Lederle Laboratories, Gosport, Hampshire; folinic acid, dihydrofolic acid, tetrahydrofolic acid, and *N*-5 tetrahydrofolic acid were supplied by Sigma, London. The antibody did not cross-react with 7-OH MTX.

Patients were monitored for side effects, with particular emphasis on renal toxicity.

**Table 1.** Pharmacokinetics of oral MTX in six patients

| Primary tumour site | Oral peak concentration | | Time to oral peak (h) |
|---|---|---|---|
| | μg/ml | % dose/l | |
| 1. Bronchus | 0.42 | 0.6 | 2.5 |
| 2. Breast and colon | 0.72 | 1.44 | 2.0 |
| 3. Head and neck | 0.54 | 0.7 | 1.5 |
| 4. Bronchus | 0.62 | 0.6 | 1.8 |
| 5. Kidney | 0.74 | 0.9 | 1.2 |
| 6. Breast | 1.25 | 1.8 | 1.5 |
| Mean ± SD | 0.71 ± 0.29 | 1.0 ± 0.5 | 1.7 ± 0.5 |

**Table 2.** Areas under concentration-time curves (AUC) for oral and IV MTX

| Patient | % Dose · l$^{-1}$ · h | | Ratio of $\frac{\text{AUC oral}}{\text{AUC IV}}$ % |
|---|---|---|---|
| | AUC oral 0–8 h | AUC IV 0–8 h | |
| 1 | 2.26 | 10.62 | 21 |
| 2 | 5.12 | 9.42 | 54 |
| 3 | 2.22 | 11.52 | 19 |
| 4 | 2.35 | 13.18 | 18 |
| 5 | 3.0 | 19.83 | 15 |
| 6 | 7.94 | 26.46 | 30 |

## Results

Oral MTX was tolerated extremely well [1], and only one patient in the first study complained of diarrhoea after the second dose of the drug. The sites of tumour in each of these six patients are shown in Table 1. Patient 4 was found some weeks later at autopsy to have metastases involving the kidney, and patient 5 had already had a nephrectomy for hypernephroma but had residual tumour. Patient 6 became progressively debilitated within a short time of starting the study, due to a combination of generalised weakness, dehydration, and intercurrent infection. This patient went into renal failure and died 2 weeks after the last dose of MTX.

Table 1 also shows the oral data. There was a threefold variation in peak MTX concentration, and the time taken to reach these peaks varied from 60 to 180 min. The area under the oral concentration-time curve also varied considerably (Table 2), with a range of 2.2% to 7.9% of the dose · l$^{-1}$ · h. In each case the area under the oral curve compared unfavourably with the area under the IV curve. At best, the ratio was 54%; at worst it was 15%.

Renal MTX clearance was calculated for the first 8-h period from the relationship

$$\text{renal clearance} = \frac{\text{amount excreted in urine}_{0-8\text{ h}}}{\text{AUC (IV)}_{0-8\text{ h}}}.$$

Values shown in Table 3 are compared with serum creatinine levels, but the data are insufficient to show any obvious relationship between these parameters.

In the total group of patients, there were nine partial responses (five patients had breast cancer, two had lung cancer, one had cervical cancer, and one had laryngeal cancer). Partial responses were defined in the usual way, as shrinkage of tumour by over 50% in two diameters of measurable disease with no new lesions. Six of these patients received doses of drug within the 50–100 mg range. One response recurred with 250-mg infusions,

**Table 3.** Patient characteristics including serum creatinine compared with MTX clearance and side effects

| Patient | Age | Sex | Performance status | Serum creatinine (mg/ml) | Dose (mg) | MTX renal clearance (ml/min) | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | 44 | M | 0 | 77 | 70 | 136 | No side effects |
| 2 | 62 | F | 0 | 85 | 50 | 130 | No side effects |
| 3 | 75 | M | 0 | 67 | 80 | 126 | No side effects |
| 4 | 62 | M | 1 | 136 | 95 | 85 | Diarrhoea, renal metastases |
| 5 | 57 | M | 1 | 87 | 80 | 61 | Previous nephrectomy |
| 6 | 76 | F | 3 | 87 | 70 | 25 | Renal failure |

one with 500-mg infusions, and one with 1-g infusions. A significantly longer MTX terminal half-life ($t_{1/2}$) was found in the partial responders (9.2 ± 1.6 h) than in non-responders (3.8 ± 0.7 h), $P < 0.01$. In none of the responders was there any evidence of pleural effusion, ascites, renal failure, or liver failure that could account for longer $t_{1/2}$ values. Patients with low serum albumin levels (below 35 g/l) tended to have longer half-lives, but in the nine responding patients only one had a serum albumin of less than 35 g/l.

No severe MTX toxicity was observed. In particular, there was no measurable effect on liver or renal function or on haematological indices. Mucositis and/or conjunctivitis occurred in six patients. The dose did not affect the degree of toxicity, nor was the serum creatinine of any value in predicting side effects. Table 3 shows the details of the six patients in the initial part of the study to emphasise this discrepancy.

## Discussion

Methotrexate given in the syrup form described earlier has been shown to be absorbed in a fairly uniform manner, although peak levels vary between patients and the time taken to reach these peak levels is also variable. The syrup is palatable, but the obvious drawback appears to be inferior bioavailability compared with that of an identical dose of the drug given IV. Clearly, a higher dose of oral MTX might be given without accompanying toxicity and, hopefully, with attainment of higher and more sustained plasma levels. The theoretical problem with increasing each single oral dose is that it seems likely that carrier mediation is required for transport of MTX across gut mucosa [5]. If absorption of MTX is a passive process, however, the portal levels should rise with increasing doses. We have carried out further studies with incremental doses and divided-dosage regimens to establish a regimen that would lead to optimum absorption. The results of such a divided dose study are considered later [6]. A divided-dose schedule might mimic the high-dose infusion technique, which has been shown to be successful in a limited number of solid tumours, e.g., those of the head and neck, and osteosarcomas. Whereas there have been several studies of the pharmacokinetics of MTX given in small doses, viz 15 mg/m$^2$ [2] and 30 mg/m$^2$ [3], and numerous studies of the effect of giving doses of 1 g upwards [8], there are few studies with the present dose, 50 mg/m$^2$, for comparison of plasma levels. This is surprising, because this dose is common to many current solid tumour protocols. The Medical Research Council advanced ovarian cancer trial, for instance, employs an oral dose of 50 mg MTX twice a month. Although this is a convenient route of administration the present paper raises the serious problem of bioavailability.

Whereas it is clear that MTX serum levels are of considerable assistance in planning citrovorum factor rescue for high-dose MTX infusions, in the low-dose situation they may be of more value for study and assessment of absorption and bioavailability. As there were no instances of serious toxicity in the present study, little comment can be made on their value in this respect. A quarter of the patients studied, however, did achieve a partial response to therapy with an essentially low-dose regimen, and it is of interest that in these patients response was associated with a relatively prolonged MTX terminal half-life. It is clear that the way in which MTX is given orally may have an important bearing on bioavailability, and hence on response and toxicity. A degree of uncertainty will exist if large single doses are given, and as has been shown in the accompanying paper [6], this drawback may be overcome by subdividing an oral dose so as to achieve a more predictable and reproducible long-term exposure of tumour to MTX.

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