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Pharmacokinetics of oral and intramuscular methotrexate in children with acute lymphoblastic leukaemia

Andrew D. J. Pearson[1], Steven Mills[2], Helen A. Amineddine[1], David R. Long[1], Alan W. Craft[1], and Judith M. Chessells[2]

[1] Department of Child Health, University of Newcastle upon Tyne, and
[2] Hospital for Sick Children, Great Ormond Street, London, England

Summary. Repeated methotrexate absorption studies were performed under standard conditions in 127 children receiving either oral or intramuscular methotrexate for acute lymphoblastic leukaemia. There was marked variability in peak concentration, area under the serum concentration curve and clearance both between patients and in repeated studies on the same patient. Although the intramuscular route produced higher serum concentrations and AUC than the oral route, variability within and between patients was considerable and was most marked at higher concentrations. Neither age or sex could account for variation in methotrexate absorption or clearance.

Intramuscular methotrexate, although producing higher serum concentrations and AUC, does not reduce the variability observed with oral administration. Prediction of subsequent methotrexate concentrations from the knowledge of one absorption profile is not possible.

Introduction

Methotrexate (MTX) is one of the main drugs used in continuing (maintenance) therapy of childhood acute lymphoblastic leukaemia (ALL). However, there is uncertainty as to whether the risk of relapse in ALL may be influenced by patterns of MTX absorption. Variability in oral MTX absorption with resultant unpredictable serum levels [1, 5, 9, 12, 16] has been described in small numbers of patients with ALL. After oral administration, higher 1-h MTX concentrations have been associated with a greater relapse-free survival rate than lower levels [5], and a higher rate of leukaemia relapse has been claimed in patients with faster systemic clearance and lower steady-state serum concentrations after high-dose MTX (1 g/m^2) [7, 8].

In view of the marked variability in oral MTX absorption, it has been postulated that intramuscular (i.m.) administration may produce less variability and more predictable serum concentrations. Damage to the intestinal mucosa occurs during the treatment of childhood ALL [13], and it has been suggested that this may be a reason for the observed variability in oral drug absorption and may lead to a progressive decline in MTX absorption occurring during therapy.

Offprint requests to: A. D. J. Pearson, Department of Child Health, University of Newcastle upon Tyne, The Medical School, Framlington Place, Newcastle upon Tyne, NE2 4HH, England

Differences in MTX absorption between individuals have been investigated, but variation in absorption in the same individual has not previously been assessed. This is important if prediction of subsequent MTX absorption is to be attempted from one MTX absorption profile.

To determine the extent of inter- and intraindividual variability following low-dose (20 mg/m^2) MTX administration and to determine whether i.m. administration produces less variability than oral dosage, a group of 127 children receiving MTX for maintenance therapy of ALL had repeated MTX absorption studies performed.

Patients and methods

164 children with non-T, non-B ALL were treated at the Hospital for Sick Children, Great Ormond Street, between 1979 and 1982 on the PLOD protocol as shown in Fig. 1 [3]. Induction therapy comprised vincristine, prednisolone, daunorubicin and late L-asparaginase. CNS prophylaxis comprised six doses of intrathecal MTX and cranial irradiation (24 Gy until 1981, 18 Gy after 1981).

Treatment was continued with vincristine and prednisolone every 6 weeks together with MTX and 6-mercaptopurine. MTX was administered weekly, with the aim of giving a constant dose of 20 mg/m^2, either by mouth (fasting in the morning), or by a single i.m. injection. Intram-

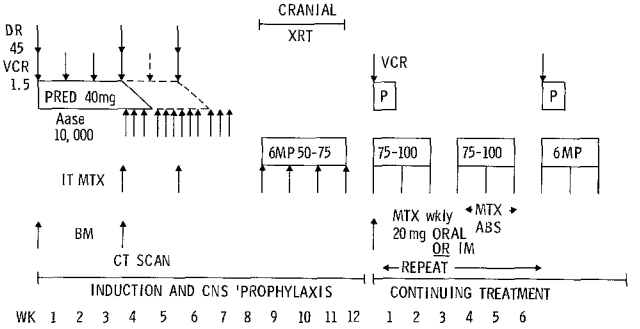

Fig. 1. PLOD protocol for childhood. non-T, non-B ALL (Great Ormond Street Hospital, 1979). *DR*, daunorubicin; *VCR*, vincristine; *PRED, P*, prednisolone; *Aase*, late L-asparaginase; *IT*, intrathecal; *MTX*, methotrexate; *6-MP*, 6-mercaptopurine; *ABS*, absorption studies; *BM*bone marrow examination; Dose in mg/m^2 except Aase u/m^2

uscular MTX was administered at a concentration of 25 mg/ml to the nearest 2.5 mg. 6-Mercaptopurine was given daily for 2 weeks out of 3 at a dose of 70–100 mg/m^2, adjusted, if necessary, to maintain the neutrophil count above 1.0×10^9/l.

MTX absorption studies were performed at the beginning of continuing therapy and at 12 and 18 months. Children received their weekly MTX under standardised, fasting conditions. Blood samples were obtained at 0, 0.5, 1, 2, 3, 5, 8, 12, and 24 h. Serum MTX was measured by an EMIT assay [10], which not only measures MTX itself but may also cross-react with the two metabolites, 4-amino-4-deoxy-N^{10}-methyl pteroic acid and to a lesser extent 7-hydroxymethotrexate.

A total of 164 children entered into the study of whom 158 achieved remission. One hundred and forty-four were randomised to receive oral or i.m. MTX, and of these 127 had MTX studies performed, 63 receiving i.m. MTX and 64 receiving oral MTX. 32 i.m. and 41 oral children had three studies performed, 14 i.m. and 11 oral had two studies and 17 i.m. and 12 oral had only one study performed.

MTX absorption curves were analysed in patients who had complete data using analysis of variance with repeated measurements incorporating F tests to detect intra- and interpatient group differences. The analyses are based on patients who had three studies performed with nine blood samples in each study; 18 for oral and 20 i.m. As a number of patients had samples missing at one or more of the nine sampling times and not all patients had three studies performed, the X^2 and the Kolmogorov-Smirnov tests [6] were used to test for differences in patient characteristics between those with and without missing data.

Comparisons of interindividual variability were then performed by Levene's test [6] and the stability of variation considered by diagnostic plots.

MTX clearance (l/h/m^2) in patients receiving i.m. MTX was calculated from dose ($\mu M/m^2$)/ area under the serum concentration curve $AUC_{(24)}$ (μM/h). The Kolmogorov-Smirnov test [6] was used to compare differences in clearances between the sexes and Spearman correlation to study the effect of age. Intrapatient variability in clearances was assessed using the paired Wilcoxon test.

To assess intraindividual variability, coefficients of variation and the maximum percentage change of peak MTX concentration, $AUC_{(24)}$ and i.m. clearance were calculated in those patients with data for all three studies at each of the nine times over 24 h.

Permission for the study was given by the Joint Committee on Ethical Practice of the Hospital for Sick Children and the Institute of Child Health and informed parental consent for randomization was obtained for each child.

Results

Methotrexate absorption measurements

Patients with or without missing data were found to have entirely similar characteristics in terms of sex ($P = 0.3$), age ($P = 0.3$), route of MTX administration ($P = 0.3$) and white blood cell count at presentation ($P = 0.06$).

The dose of MTX administered, peak MTX concentrations and $AUC_{(24)}$ are shown in Table 1. The time of the peak concentration varied from 0.5 to 3 h. The dose of MTX administered at the commencement of continuing therapy was significantly higher than that administered at 12 months ($P = 0.006$) and 18 months ($P = 0.016$), although there was no significant difference between the doses at 12 and 18 months ($P = 0.4$).

There was no significant correlation between the dose of MTX administered and peak serum concentration ($r = -0.09$) or $AUC_{(24)}$ ($r = -0.13$) at the first assessment. These results were similar at 12 ($P = 0.2$), and 18 ($P = 0.1$) months.

Comparison of intramuscular with oral methotrexate

Patients receiving oral MTX received significantly higher doses of MTX initially ($P = 0.009$) and at 12 months ($P = 0.06$) and 18 months ($P = 0.03$) than those receiving MTX via the i.m. route.

There was a significant difference in MTX absorption during the 24-h period in patients receiving i.m. rather than oral MTX ($F = 153$; $P < 0.0001$). Intramuscular MTX produced significantly higher peak concentrations ($P < 0.0001$) and $AUC_{(24)}$ ($P < 0.001$) than oral MTX at 0, 12 and 18 months. Comparisons by analysis of variance showed that the pattern of MTX absorption was significantly different in i.m. and oral MTX ($F = 111$; $P < 0.0001$) (Fig. 2). After i.m. MTX the serum concentration rose rapidly to a peak at 30 min, but with oral MTX the peak was not only lower but occurred later.

There was increasing variability at higher serum MTX concentrations (Fig. 2). The 95% confidence intervals increase at higher serum MTX concentrations, indicating a greater interpatient variability in MTX absorption in i.m. patients. However, this was present only at the peak absorption times of 30 min ($F = 73$; $P < 0.0001$), 1 h ($F = 37$; $P < 0.0001$), and 2 h ($F = 93$; $P = 0.003$). Oral patients

Table 1. MTX levels (mean and range) in 127 children receiving oral or i.m. MTX

	Dose (mg/m^2)	Peak MTX concentration (μM)	$AUC_{(24)}$ (μM/h)	Clearance (l/h/m^2)
MTX oral				
0 months	19.6 (16.7–23.8)	0.70 (0.09–2.50)	3.29 (0.35– 6.55)	–
12 months	19.1 (14.3–24.0)	0.76 (0.23–1.36)	3.31 (0.97– 5.40)	–
18 months	19.2 (15.6–22.7)	0.77 (0.20–1.20)	3.36 (0.71– 5.96)	–
MTX i.m.				
0 months	18.8 (15.6–21.6)	2.39 (0.04–4.14)	6.48 (4.02– 9.52)	6.66 (4.26–10.74)
12 months	18.0 (11.8–21.3)	2.22 (1.02–3.96)	5.87 (3.10–10.28)	7.19 (3.17–15.28)
18 months	18.0 (10.0–20.8)	2.20 (1.04–4.24)	5.43 (3.42– 7.54)	7.57 (5.36–12.53)

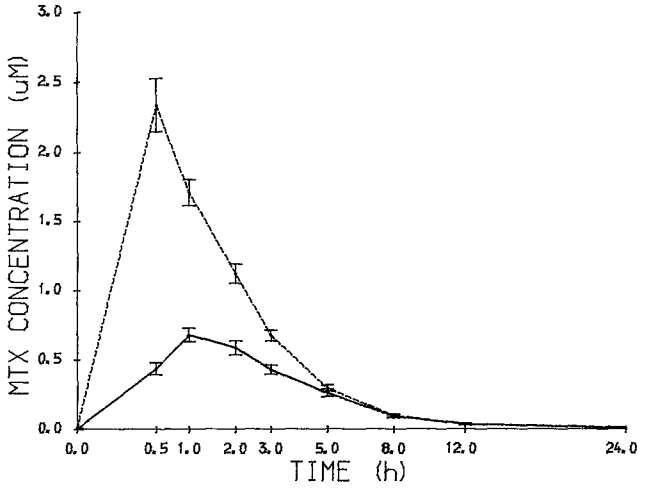

Fig. 2. MTX absorption profile in patients receiving MTX methotrexate via the oral (———) or the i.m. (----) route (mean ± SEM)

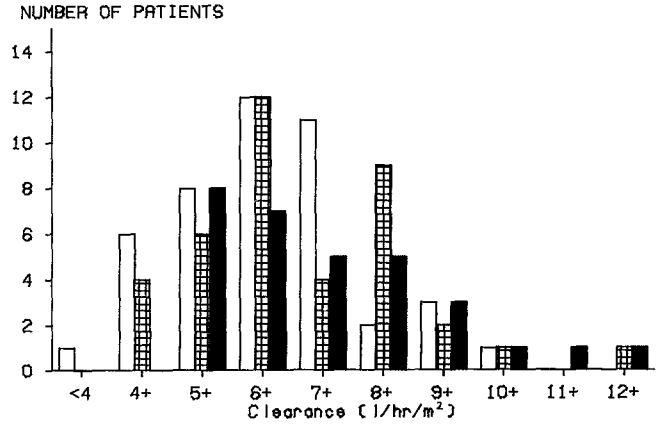

Fig. 3. Distribution of MTX clearance (l/h/m^2) in those patients receiving the drug via the i.m. route. Results shown for 0 (□), 12 (⊞), and 18 (■) months after start

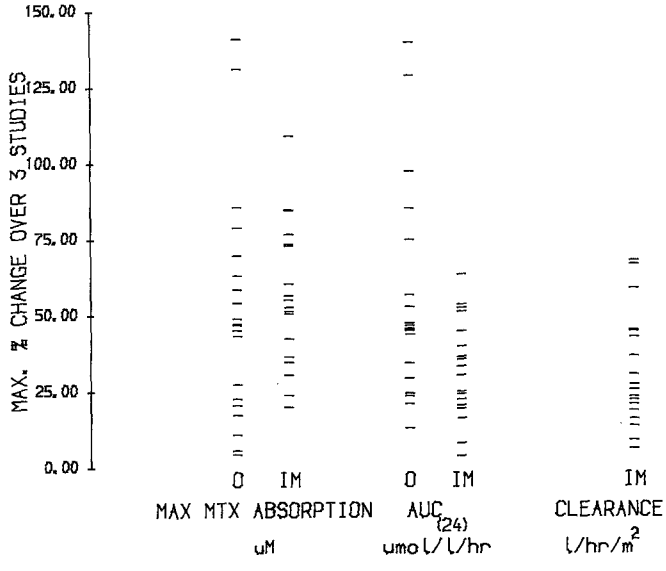

Fig. 4. Maximum percentage change of MTX peak concentration (μM), $AUC_{(24)}$ (μM/h) and clearance (l/h/m^2) between the three studies at commencement of continuing therapy, 12 months and 18 months

had relatively greater variation than i.m. patients when adjustment was made for mean concentration.

The correlation between the peak MTX concentration or $AUC_{(24)}$ with the dose of MTX administered was poor for patients who received either oral or i.m. MTX.

Effect of age and sex on methotrexate absorption

In neither oral nor i.m. patients was there any significant correlation between age and peak MTX concentration or $AUC_{(24)}$ ($P = 0.48$, $P = 0.44$ respectively), nor were there any differences between males and females ($P = 0.31$, $P = 0.32$, respectively).

Clearance

Intramuscular MTX clearance varied from 3 to 15 l/h/m^2 with a mean of 7 l/h/m^2 (Fig. 3). In patients studied sequentially there was no significant change in MTX clearance during therapy ($P = 0.97$ and $P = 0.6$) at 12 and 18 months compared to 0 months. The rate of MTX clearance did not differ between males and females at each of the three time points ($P > 0.1$), nor did age correlate significantly with MTX clearance ($P > 0.1$).

Intraindividual variability

There was no significant difference between the peak MTX concentration and $AUC_{(24)}$, either for oral or i.m. MTX, at the three time points of 0, 12 and 18 months and there was no significant change in MTX absorption during therapy ($F = 0.49$; $P = 0.62$).

Coefficients of variation for the peak MTX concentration, $AUC_{(24)}$ and i.m. clearance of MTX are shown in Table 2. Patients receiving oral MTX had a significantly

Table 2. Coefficients of variation (%) for repeat values of peak MTX concentration, $AUC_{(24)}$ and clearance in those patients who had three studies performed (mean and range)

	Peak MTX concentration (μM)	$AUC_{(24)}$ (μM/h)	Clearance (l/h/m^2)
Oral	27 (2.4–79)	28 (6.4–70)	–
i.m.	30 (11–55)	17 (2–35)	17 (3.2–39)

Table 3. Correlation of MTX measurements at 0, 12 and 18 months in 127 children receiving i.m. or oral MTX (r value/P)

	Correlation		
	0 vs 12 months	0 vs 18 months	12 vs 18 months
Peak concentration (μM)			
Oral	0.41/0.07	−0.1 /0.26	0.13/0.2
i.m.	0.1 /0.7	0.24/0.1	0.3 /0.06
$AUC_{(24)}$ (μM/h)			
Oral	0.25/0.2	0.26/0.1	0.18/0.2
i.m.	0.68/0.02	0.16/0.26	0.38/0.04
Clearance (l/h/ml)			
i.m. only	0.68/0.002	0.16/0.27	0.4 /0.03

higher coefficient of variation for $AUC_{(24)}$ than those receiving MTX via the i.m. route ($P < 0.01$). There was no significant relationship between age nor sex with any coefficients of variation.

Correlations between peak concentrations, $AUC_{(24)}$ and clearances at 0, 12 and 18 months are shown in Table 3. The only significant correlations were for patients receiving i.m. MTX where that between the $AUC_{(24)}$ at 0 and 12 months and 12 and 18 months ($P = 0.02$ and $P = 0.04$) and the clearance for the same time periods ($P = 0.002$ and $P = 0.03$) were significant.

The maximum percentage change in peak concentration, $AUC_{(24)}$ and clearance at 12 and 18 months from the initial value are shown in Fig. 4. Some children had values greater than 100%. There was no significant difference in the maximum percentage change in peak concentrations between oral and i.m. patients.

Discussion

Although MTX has been used in the treatment of childhood ALL for more than 30 years the optimal dose, frequency, route and time of day [20] of administration have yet to be determined. Doses have varied from 15 mg to 6 g/m^2, with "conventional low-dose MTX" (15–20 mg/m^2) being administered weekly or on 5 consecutive days with a gap of 2 weeks. In early studies MTX was administered daily, but this resulted in significant toxicity. Oral, i.m. and i.v. routes have been employed for administration of the drug.

This study has confirmed, in a large group of patients, that there is significant interindividual variability in oral MTX absorption and serum levels are unpredictable. There was no correlation between the administered dose of MTX and either the peak MTX concentration or $AUC_{(24)}$. Concurrent administration of food [17] can alter MTX absorption. However, even when oral MTX is administered in a fasting state under standardised conditions there is variability. Intestinal transit time [14] has been shown to affect absorption, but the role of intestinal function is less well defined [18].

Gastrointestinal damage is known to be caused by cytotoxic drugs in experimental animals [25], and in man both morphological [26] and functional [4, 15] changes have been demonstrated. One study suggested a progressive decline in intestinal function throughout treatment for ALL [4], and therefore it has been hypothesised that this may impair MTX absorption. However, there was no significant decline in MTX absorption during therapy in patients receiving the drug by the oral route; therefore, this does not support changing the route of MTX administration towards the end of continuing therapy.

Intramuscular MTX produced higher concentrations and $AUC_{(24)}$ than oral MTX, with an earlier peak concentration [19]. It has been suggested that administration of MTX by the i.m. route would produce less variability and more constant serum concentrations. Variability following i.m. administration was not less than with oral administration.

Previous studies of MTX absorption have concentrated on variations between individuals [1, 5, 9, 12, 16]. From this study it can be seen that there is substantial variation in MTX absorption within the same patient. Intraindividual variability has been shown with other drugs [21, 27], and very similar coefficients of variation have been found with the cytotoxic agent, VP16 [11]. This intraindividual variability is not reduced when MTX is given by the i.m. route. Also, no subgroup of patients was identified where i.m. MTX was associated with less variability.

The initial values for peak concentration, AUC and clearance could not be used to predict subsequent values, as there was no correlation between measurements at these times. This is of importance in that some studies have suggested that pharmacokinetic measurements of MTX absorption performed at the beginning of continuing therapy may be of prognostic significance [5] and have suggested manoeuvres to alter absorption [14]. Any prognostic classification based upon one value at the beginning of continuing therapy is ill-founded, and modifications of therapy would also be unwarranted.

Intraindividual variability after i.m. administration must be related to differences in clearance. MTX is eliminated both by renal and non-renal routes, and which component is responsible for the variation is at present not understood.

Interindividual variability in MTX clearance has also been shown by Evans et al. with intravenous high-dose MTX [7, 8]. Some investigators have demonstrated an age effect in MTX clearance [2] but in the limited age range of this study (1–14 years) no such effect was apparent.

MTX was measured by EMIT assay, which cross-reacts with two of the major metabolites of the drug. These may be produced in greater quantities after prolonged treatment. However, this is unlikely to have affected the findings of this study, in particular the peak levels and earlier time points.

There have been recent doubts about the compliance of children, and in particular adolescents, with prescribed cancer chemotherapy [22]. Administration by the i.m. route ensures patient compliance, though physician compliance with the leukaemia treatment protocol cannot be guaranteed. Intramuscular MTX produces no greater predictability in serum concentrations either between patients or in the same patient. The only advantage in administration of MTX via the i.m. route is that higher concentrations are obtained. There is some evidence that administration of larger doses of MTX via the oral route would result in incomplete absorption as there would be saturation of the MTX transport system [23]. However, reports have described higher levels when the dose of oral MTX is divided and administered over a longer time period [24]. Higher peak MTX concentrations may be associated with greater neurotoxicity. Seizures and abnormal brain CAT scans have been seen more frequently in those patients under the age of 2 years receiving i.m. MTX and exclusively in those receiving it by this route in those over the age of 2 years [3].

Thus, it appears that if variability in MTX absorption is of prognostic significance, then changing to the i.m. route may not substantially improve the prognosis in these children whilst causing increased patient discomfort and potentially increased CNS toxicity.

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