Cancer Chemother Pharmacol (1982) 10: 36–39

Cancer Chemotherapy and Pharmacology

Pharmacokinetics of Low-Dose Methotrexate in Children Receiving Maintenance Therapy for Acute Lymphoblastic Leukaemia

C. R. Pinkerton[1], S. G. Welshman[2], J. G. Kelly[3], R. G. Shanks[3], and J. M. Bridges[4]

[1] The Royal Belfast Hospital for Sick Children, Belfast,
Departments of [1] Child Health, [3] Therapeutics and Pharmacology and [4] Haematology, Queens University of Belfast, Belfast
[2] Department of Clinical Biochemistry of the Belfast City Hospital, Belfast, Northern Ireland

Summary. *Serum methotrexate (MTX) concentrations were measured by immunoassay in 28 children receiving maintenance therapy for acute lymphoblastic leukaemia. Patients were studied either after a single weekly dose or on the first day of a 5-day course of treatment. A standard dose (15 mg/m²) was given PO and/or IV.*

After PO dose the peak serum MTX concentration and its timing varied widely between cases and there was a significant positive correlation between the rate of serum concentration rise and the area under the concentration curve up to 4 h. The absorption rate constant showed a biphasic distribution and correlated less closely with early serum concentrations.

After IV and PO administration drug disposition was triphasic with an initial rapid distribution phase, an intermediate phase, and a prolonged terminal elimination phase. The intermediate phase half-life was significantly longer after PO than after an IV dose. The MTX clearance rate was consistently lower than the glomerular filtration rate and there was no significant correlation between the two.

The mean bioavailability (PO/IV) was 42%, but bioavailability was as low as 6% in some cases due to prolonged high serum concentrations after an IV dose, which was not seen with the equivalent PO dose.

Introductioin

The folic acid antagonist methotrexate (MTX) is used in most current treatment protocols for children with acute lymphoblastic leukaemia (ALL). It is usually given as a moderately low oral or parenteral dose (15–20 mg/m²), either weekly or as a 5-day course every 3–4 weeks [16]. MTX pharmacokinetics have been extensively studied in adults following high-dose IV therapy [22, 23] and in a small number of children with solid tumours [18, 24]. There is, however, less information relating to the lower doses used in children with ALL. Pharmacokinetic details may be of value in the design of effective schedules for cytotoxic drugs [25] and this may be of particular importance in children with ALL, as it has been suggested that disease relapse may be related to the pattern of drug absorption [6]. In this study serum MTX concentrations were measured after PO and IV doses in such patients.

Reprint requests should be addressed to C. R. Pinkerton, Dept of Child Health, Institute of Child Health, Guilford St, London WC1N 1EH

Patients and Methods

Twenty-eight children with ALL and aged 3–16 years were studied. All were being managed according to the United Kingdom Acute Lymphoblastic Leukaemia (UKALL) protocols of the Medical Research Council Working Party on Leukaemia in Childhood [16]. The duration of therapy ranged from 1 to 27 months (mean 10.4). All were in remission from their disease with satisfactory blood counts and were clinically well.

The PO route of administration was studied in all cases and in 18 patients serum concentrations were also determined after IV administration of the same dose of MTX. In eight cases blood samples were taken for at least 20 h after either PO or IV doses; two were studied for such a period after both routes of administration. Patients had not received MTX for at least 6 days prior to study.

Following an overnight fast a venous cannula was inserted for routine blood tests and a sample taken for basal MTX concentration. A standard single dose of MTX (15 mg/m²) was given either in tablet form (2.5 mg) with water or as an IV bolus given over 30 s (25 mg/ml parenteral solution diluted in 5 ml saline). Further blood samples were taken at 20, 40, 60, 90, and 120 min and hourly up to 4–8 h. After an IV dose a 10-min sample was also taken. In the prolonged studies samples were taken at intervals up to between 20 and 24 h after administration.

Serum was separated within 6 h of sampling and stored at −20° C. Samples were analysed using enzyme-linked immunoassay (EMIT MTX Assay, Silva, Maidenhead). Concentrations below 0.2 μmol/l, which could not be accurately estimated by this method [7], were measured by radioimmunoassay (^{125}I-MTX RIA, Uniscience, Cambridge).

Serum MTX profiles were analysed both to calculate basic pharmacokinetic parameters and to study the components of the early PO profile. The latter is of particular importance in children with leukaemia, as it is likely that the response to therapy is related to drug concentrations achieved during this early phase [6].

The following were calculated:

1. Absorption rate constant (Ka) by the method of residuals [9], using PO and IV profiles in the same individuals.

2. 'Rate of absorption', derived from the gradient of the first part of the PO serum concentration curve beyond the initial lag phase (Fig. 1). From this profile the peak concentration, time to peak concentration, and area under the curve

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up to 4 h (AUC_4) were estimated. The latter was calculated using the trapezoid method.

3. Elimination half-lives were calculated for both PO and IV profiles using linear regression analysis of the log concentration-time curves. Bioavailability was estimated using data-obtained between 1 and 10 h after drug administration in ten cases. (Bioavailability = $AUC_{PO}/AUC_{IV} \times 100$.)

4. Glomerular filtration rate (GFR) was estimated in 11 cases using the plasma creatinine and the patients' height [19]: GFR (ml/min · 1.73 m^2) = 0.55 × height (cm)/creatinine (mg · dl). In these patients MTX clearance was estimated from the intermediate phase half-life and the volume of distribution ($C_{MTX} = 0.693/t_{\nu 2} \times V$).

Statistical analysis was carried out using Student's *t*-test and linear regression where the data were normally distributed; otherwise the Mann-Whitney U-test and rank correlation were used.

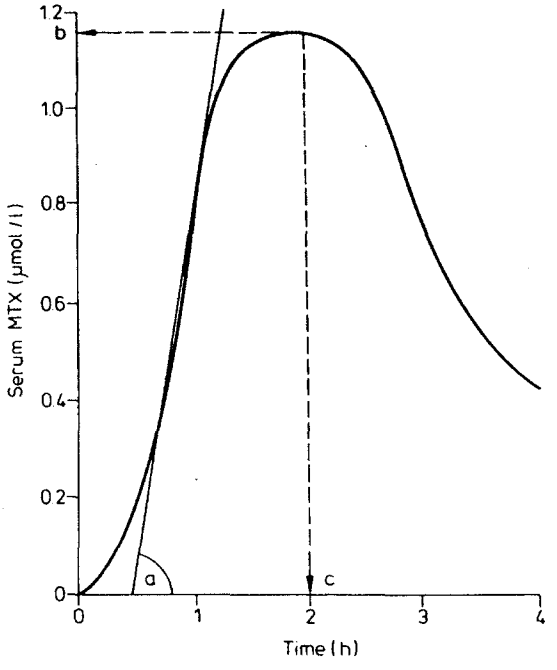

Fig. 1. Derivation of parameters from early serum MTX profile after PO administration, α = rate of absorption; b = peak MTX concentration; c = peak time

Results

The mean serum concentrations up to 20 h after PO and IV administration are shown in Table 1. After a PO dose the peak serum concentration ranged from 0.4 to 1.6 μmol/l and its timing from 40 min to 4 h (Fig. 2). The rate of absortion ranged from 0.08 to 2.0 μmol/l · h (Fig. 2) and showed a significant correlation with the peak concentration ($r = +0.59$, $P < 0.002$) and the AUC_4 ($r = +0.66$, $P < 0.01$). The absorption rate constant calculated in those cases with both PO and IV profiles ranged from 0.01 to 0.15 min^{-1} (Fig. 2). By contrast with the rate of absorption the Ka did not correlate closely with either the peak concentration or the AUC_4. Moreover, unlike the other parameters derived from the early serum profile, the Ka values appeared to fall into two distinct groups (Fig. 2): over 0.01 min^{-1} and below 0.05 min^{-1}. These groups were significantly distinct from one another ($P < 0.001$, Mann-Whitney U-test).

No significant correlation was evident between either Ka or rate of absorption and patient's age, sex, duration of therapy or treatment schedule.

With the IV route serum MTX concentrations fell rapidly during an initial distribution (α) phase. The half-life of this phase ranged from 0.12 to 0.65 h (mean 0.3 ± 0.05 h). By 2 h after administration there was no significant difference between serum MTX concentration following IV administration and that following PO administration. It was evident from the serum profiles of cases studied beyond 8 h that after the initial phase the elimination of MTX was of a biphasic character – an intermediate β phase was followed by a prolonged γ terminal phase. The mean half-life of the β phase, between 3 and 8 h after either PO or IV doses, was 3.4 ± 0.4 h, which was significantly shorter than the terminal phase beyond 8 h, 6.6 ± 0.7 h ($P < 0.001$).

Comparison of the β-phase half-life after PO and IV doses showed a significant prolongation with the PO route, 4.3 ± 0.5 h compared with 2.8 ± 0.3 h ($P < 0.05$).

MTX clearance and GFR values are shown in Table 2. MTX clearance ranged from 26 to 97 ml/min (mean 63 ± 6.5 ml/min) and the GFR from 68 to 121 ml/min · 1.73 m^2. There was no significant correlation between these two estimates.

Bioavailability was calculated in 15 cases where data were recorded up to 10 h after both PO and IV administration (Table 3). The AUC_{10} after MTX PO ranged from 1.8 to 5.1 μmol/l · h (mean 3.3 ± 0.3 μmol/l · h). By contrast, the AUC_{10} after MTX IV varied more widely with a range from 3.4 to

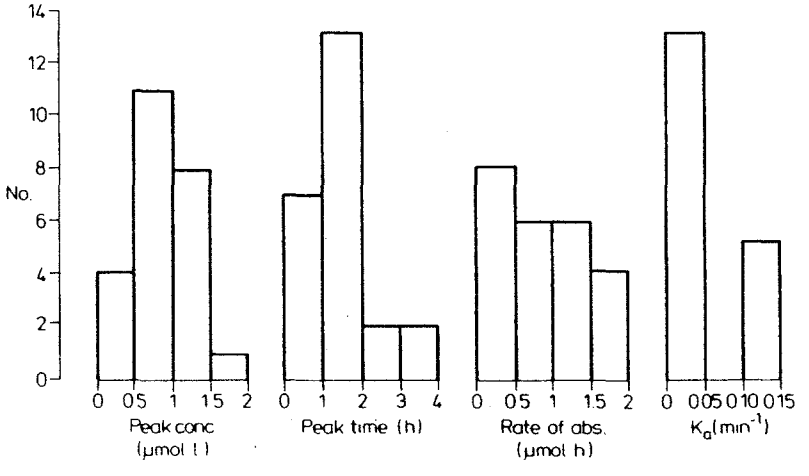

Fig. 2. Distribution of parameters derived from the early serum MTX profile after PO administration

Table 1. Serum methotrexate concentrations after PO and IV administration (15 mg/m^2) in children with acute lymphoblastic leukaemia.

			Time after methotrexate												
			(min)					(h)							
			10	20	40	60	90	2	3	4	5	6	8	12	20
PO administration	Serum MTX (μmol · l)	Mean	–	0.18	0.49	0.67	0.72	0.69	0.46	0.30	0.21	0.13	0.12	0.05	0.03
		SE	–	0.05	0.07	0.07	0.08	0.08	0.04	0.04	0.02	0.01	0.02	0.01	0.01
		n	–	23	23	23	23	23	23	23	16	10	8	7	5
IV administration	Serum MTX (μmol · l)	Mean	29.7	21.9	3.90	1.71	–	0.93	0.56	0.37	0.28	0.19	0.12	0.04	0.02
		SE	8.7	8.0	1.2	0.23	–	0.14	0.08	0.04	0.03	0.01	0.01	0.01	0.005
		n	18	18	18	18	–	18	18	18	16	16	16	6	5

Table 2. MTX clearance after IV administration, contrasted with the glomerular filtration rate

MTX clearance (ml/min)	Glomerular filtration rate (ml/min/1.73 m^2)
46	78
48	79
56	89
84	97
39	88
97	68
26	121
66	120
70	106
74	121
83	91

Table 3. MTX bioavailability between 1 and 10 h after administration by the PO and IV routes

Area under serum concentration curve (μmol/l · h)		Bioavailability (%)
IV	PO	
5.9	3.5	59
6.3	4.2	66
4.3	3.9	91
3.4	1.8	53
32	2.8	9
52	3.0	6
3.6	2.6	72
14	4.4	31
11	5.1	46
8.2	4.2	51
15	2.6	17
4.4	3.7	84
21	3.8	18
27	3.1	12
9.5	2.8	29
7.0	1.9	27

52 μmol/l · h (mean 14.4 ± 4 μmol/l · h). This was the result of persistently high serum MTX concentrations at 1 and 2 h in a small number of cases. In one case the 2-h MTX level was 1.2 μmol/l. This delay in drug distribution and excretion led to low bioavailability values although the PO MTX profiles in such cases compared favourably with those in other cases with higher bioavailability values.

Discussion

Previous studies of MTX absorption in children with ALL have emphasised interpatient differences with regard to the serum MTX concentration at 1 h after administration [6, 8]. This parameter has been correlated with both the drug's effectiveness and its toxicity. Such an estimate may be an inaccurate guide to the overall serum profile, however, due to delayed absorption where normal peak levels are achieved after an initial lag period [17]. In the present study the 'rate of absorption' was contrasted with other parameters of the serum profile, including the absorption rate constant. The latter is a more accurate estimate of intestinal uptake of MTX but requires an extended profile for its derivation [9]. This is a practical problem in terms of prolonged blood sampling in children. In vitro studies indicate that the antileukaemic effect of MTX is maximal at serum concentrations in the region of $10^{-6}M$ [11], and it is likely that the drug concentrations in the early period after administration are therefore of importance with regard to therapeutic effectiveness. The rate of absorption appears to be a useful indicator of these early drug concentrations, and it correlated closely with both peak concentration and AUC_4.

The distinct groups of 'fast' and 'slow' absorbers described by Freeman Narrod on the basis of 1-h MTX concentrations were not evident when the rate of absorption was considered. However, in the case of the Ka there did appear to be two separate groups (Fig. 2). Although the number of patients was small this observation does support the hypothesis that there are homogeneous groups differing in their ability to transport MTX across the small intestinal mucosa. The transport mechanism for MTX in the proximal small gut is similar to that in a number of other cell membranes [10], and it is possible that there is a comparable variability in MTX transport into other tissues. This might be of importance for both cytotoxic effect and drug penetration into sanctuary sites.

MTX was rapidly distributed after both PO and IV doses, and an intermediate phase of drug disposition was evident within 1–2 h of an IV dose and 3–4 h of PO ingestion. A striking feature in the case of both routes was the brevity for which MTX concentrations were maintained in the region of $10^{-6}M$. In most cases drug levels fell to below $10^{-7}M$ by 4–6 h. It is likely that for maximum cytotoxic effect adequate extracellular concentrations must be maintained for a prolonged period [3], and the effectiveness of current single-dose regimens may therefore be limited by the rapidity of drug elimination. Alternative schedules with subdivision of doses might be more effective without increasing toxicity [21].

The intermediate β phase is similar to the 'excretion' phase described in adults [22, 23]. This phase largely reflects renal elimination of MTX and up to 85% of a single dose of MTX has been recovered in the urine by 8 h after administration [12]. With the PO route there may be prolonged residence of drug in the small gut and this may have been the cause of the longer phase compared with the IV route.

By contrast with some adult data [14], no correlation was seen between GFR and either the β-phase half-life or MTX clearance. MTX clearance was also invariably lower than the GFR. This is consistent with some degree of tubular reabsorption of MTX at low serum concentrations, by contrast with tubular secretion seen at high serum concentrations [13].

The bioavailability of MTX PO was similar to that previously reported after intermediate doses [15]. The very low bioavailability in a few cases reflected unusual IV profiles rather than drug malabsorption.

The third, terminal or γ phase has also been reported in adults after a wide range of oral and parenteral doses [1, 22]. This phase is a composite of renal elimination, enterohepatic recycling, and drug metabolism [4, 5]. There may also be a 'deep pool' where drug is bound to dihydrofolate reductase for a prolonged period or present in slowly equilibrating tissue such as bone marrow [20].

As with most studies of MTX pharmacokinetics, there are limitations in interpretation due to possible assay non-specificity. Both EMIT and RIA may cross react with intestinal or hepatic metabolites [7], but this is probably of more importance after high-dose schedules where metabolite concentrations are high.

In view of the time and concentration thresholds for mucosal toxicity – 48 h and $10^{-8}M$ [4] – it is likely that the terminal phase is of major importance in the development of oral and small intestinal toxicity. Oral ulceration is rarely seen after a single dose of MTX at 15 mg/m^2, and such toxicity could not therefore be correlated with features of the MTX serum profile. After a 5-day course of MTX up to 75% of patients develop mouth ulcers as both time and concentration thresholds are exceeded with this schedule. It has recently been suggested that increased enterohepatic recycling may lead to severe small intestinal toxicity due to both systemic and local effects of the drug [2].

To achieve serum drug concentrations that are likely to be optimally effective in ALL further studies are required in relation to both drug dose and timing of administration. Furthermore, the elucidation of the factors contributing to the terminal phase of disposition may assist in the control and prevention of toxicity.

Acknowledgements. We thank Mr H. Mackey and Mr M. McMaster, Belfast City Hospital, and the staff of the haematology and biochemistry laboratories of the Royal Belfast Hospital for Sick Children for technical help.

CRP was in receipt of a clinical research fellowship from the Royal Belfast Hospital for Sick Children.

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Received March 26/Accepted September 1, 1982