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Unpredictable Serum Levels After Oral Methotrexate in Children with Acute Lymphoblastic Leukaemia

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Summary. *Serum methotrexate levels were measured for 5 h after oral intake in 11 children with acute lymphoblastic leukaemia. The curves obtained with the child's regular dose of methotrexate varied widely, and were independent of the doses used. Peak levels were found in samples taken up to 3 h after ingestion, and ranged from 300 to 1250 ng/ml. In the doses used, methotrexate toxicity was present in one of the eleven children, and was associated with a delayed peak and a high 5-h methotrexate level. Individual drug metabolism could be an important factor in the response to treatment, and needs to be evaluated in the assessment of protocols.*

Introduction

Significant advances in the management of children with acute lymphoblastic leukaemia (ALL) were made with the introduction of combination chemotherapy and separate treatment of the central nervous system [12, 15]. Since then a bewildering variety of protocols have been reported [7, 13, 18, 19], using as many as ten cytotoxic agents, in different combinations, doses and schedules, given by both enteral and parenteral routes. Despite these variations, the overall results are surprisingly uniform, and the clinical and haematological findings at diagnosis [8] remain the best predictors of long-term response to treatment. The presenting features have been related to particular subtypes of ALL [4], and it is clear that these subtypes are a much more important determinant of prognosis than the endless permutations and combinations of therapy, provided protocols adhere to certain generally accepted principles of treatment [14].

Response to treatment may be influenced by another variable – individual drug metabolism – which has been largely ignored in the assessment of protocols. Drugs have been recommended in standard doses based on the child's weight or surface area; but the doses are modified by individual toxicity – and in general combination chemotherapy is titrated against its effect on the white cell count. This style of treatment assumes that toxicity is directly related to efficacy. Studies with high-dose methotrexate (MTX) have shattered this illusion [6], and indicate that efficacy and toxicity can be separated by using high serum levels for 24–48 h, which are then neutralised with folinic acid. Without folinic acid rescue the dose of MTX is limited by toxicity; and the relationship between that dose of MTX and its effect on the neoplasm is probably determined by individual pharmacokinetics. Because individual pharmacokinetics may be an important variable, we measured the changes in the serum MTX of 11 children with ALL after their regular, low-dose MTX.

Patients and Methods

During 1975 and 1976, 25 children were admitted to this hospital with ALL, and 19 were treated according to a protocol based on the St. Jude Children's Research Hospital total V study [18], differing only insofar as reinduction courses were not given and the sole maintenance treatment consisted of daily mercaptopurine or thioguanine and weekly MTX, both taken by mouth. When the present study was initiated, three of the 19 children had died after haematological relapse and three were off treatment, leaving 13 children in complete, continuous remission who could be considered for the study. The procedure involved only a slight variation from their routine outpatient visit, as blood was taken via an indwelling butterfly needle instead of a finger prick, and the children were admitted to a day ward for the 5- to 6-h duration of the study. Twelve of the 13 families (parents and children) agreed to the investigation, one child being omitted because of a strong preference for capillary blood tests. One patient was excluded because of intermittent pancytopoenia and consequent irregular doses of MTX.

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Table 1. Clinical details, biochemistry, individual MTX doses and results

Patients	AC	JD	PF	CH	TH	DH	NM	AS	SM	LL	GS
Age (decimal years)/sex	6.42/M	11.46/F	7.99/M	1.44/F	7.46/F	14.45/F	6.56/M	2.18/M	4.23/F	4.09/F	8.87/M
Time (decimal years) on treatment	1.30	1.63	1.30	2.05	2.41	0.87	2.79	1.77	1.40	1.51	2.27
MTX dose (mg/m^2)	22.2	11.7	17.1	24.5	17.9	16.9	9.1	17.9	18.5	20.9	16.0
Serum MTX (ng/ml) 0 min	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
Serum MTX 60 min	550	450	250	120	125	110	820	600	195	1250	170
Serum MTX 120 min	450	530	420	300	(260)[a]	460	530	500	400	680	680
Serum MTX 180 min	180	100	370	200	(136)[a]	730	230	(500)[a]	380	480	360
Serum MTX 300 min	114	90	260	190	(33)[a]	450	58	(200)[a]	190	131	150
Creatinine (μmol/litre)	2	42	–	38	50	54	50	50	37	42	56
Urea (mmol/litre)	3.3	4.0	3.8	2.4	–	3.1	2.7	5.2	2.1	2.0	–
Serum bilirubin (μmol/litre)	13	16	20	14	–	31	8	29	51	14	13
Aspartate amino transferase (IU/litre)	16	40	12	36	–	13	–	17	11	18	19

[a] Levels in brackets were recorded after a child had vomited

The children fasted overnight and their regular dose of MTX was given at zero time (0 min). The recommended dose of MTX was 20 mg/m^2/week, but this had been altered according to tolerance, and in the children studied the regular dose ranged from 9.1 to 24.5 mg/m^2/week (Table 1). MTX was taken as commercially available tablets (Lederle) with a drink of water, except by one child who routinely took her oral MTX in liquid form, which is commercially available as a parenteral preparation (Lederle). Blood samples were taken through the same indwelling butterfly needle at 0 min, 60 min, 120 min, 180 min, and 300 min. The children remained fasting until after the 60-min sample was taken, and then a light breakfast was allowed. Other scheduled drugs were not given until after the study was completed.

The serum was separated and stored at −4° C on the day of the investigation, and the MTX was later measured in two batches by a radioimmunoassay (Diagnostic Biochemistry Inc., San Diego; U. K. Distributor: Uniscience Ltd., 8 Jesus Lane, Cambridge CB5 8BA). The assay's accuracy was poor below an MTX concentration of 10 ng/ml (2.2 × 10^{-8} *M*), and the SE of 42 control sera with a mean of 500 ng/ml was 7.7 ng/ml.

Plasma creatinine levels and/or urea levels were also taken at 0 min. Liver function tests were noted when estimated within 3 months of the investigation.

Results

All 11 children had a trace of MTX present in the zero sample, reflecting the previous week's dose, but the level was too low to be assayed accurately. A graph of the mean values ± 1 SD at 60 min, 120 min, 180 min, and 300 min is shown in Figure 1. No correlation was found between the doses used and subsequent blood levels at 60 min, 120 min, 180 min, and 300 min. This is illustrated by the scatter diagram (Fig. 2) of the values taken

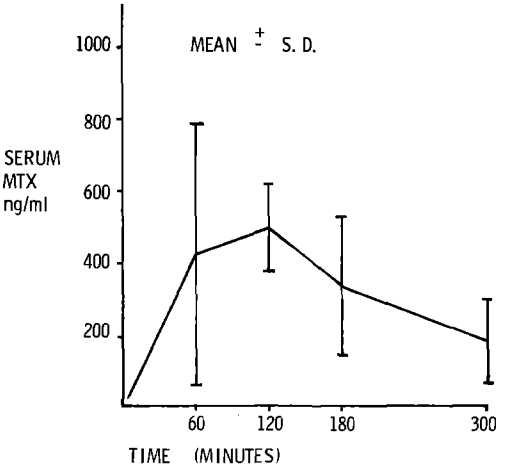

Fig. 1. Serum methotrexate curve: mean of 11 children

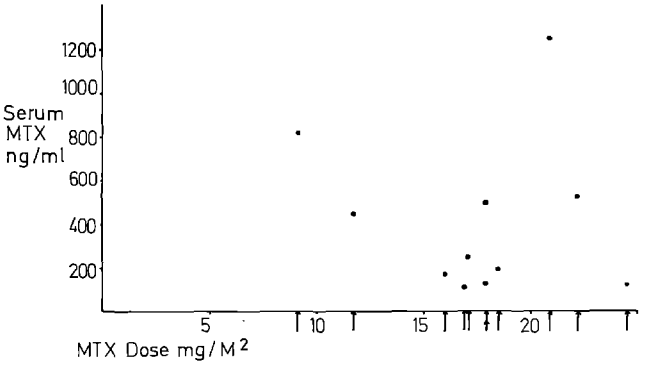

Fig. 2. Scatter diagram of individual serum methotrexate levels at 60 min. There is no correlation between the dose of MTX taken and the serum MTX values

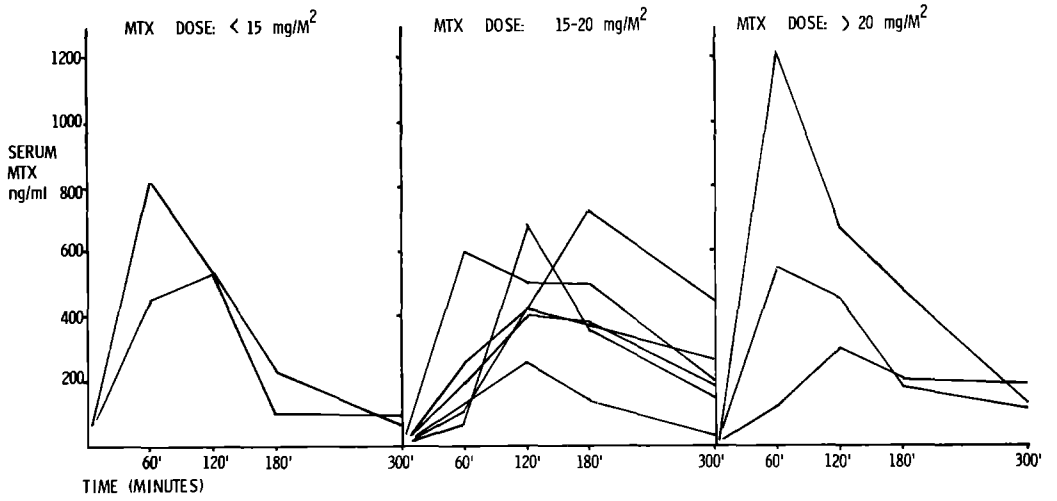

Fig. 3. Individual serum MTX curves with three dosage ranges

at 60 min. The individual MTX tolerance curves of three different MTX dosage ranges are seen in Figure 3, which shows the variation in both the peak levels and the shape of the curves, independent of the dose. No relationship of levels was observed to the sex of the patient or time of treatment. Two children vomited, one at 70 and one at 120 min; and all the individual values are shown in Table 1. The peak values were at 60 min in four children, at 120 min in six children, and in one child at 180 min, and ranged from 300 to 1250 ng/ml, except in one child, who vomited at 70 min (Table 1). One of the 11 children had mouth ulcers due to MTX around the time of the study. Her serum MTX curve (DH in Table 1) differed from the others in that the peak level was not reached until the 180-min sample and the 300-min level was the highest in the group, being 2 SD above the mean for the 300-min sample.

Plasma creatinine and/or urea levels were within normal ranges; some of the liver function tests gave abnormal results, showing slight elevations of the bilirubin and enzyme levels, but the albumin levels were all normal. These values and clinical details of the children are shown in Table 1.

Discussion

Oral absorption of MTX is essentially complete following doses below 30 mg/m² [20]. Our results demonstrate the wide individual variation in serum levels found after low-dose oral MTX in 11 children with ALL. Both the timing and the level of the peak MTX concentrations were unrelated to the dose. Above very low levels of MTX (2×10^{-8} *M*) [21], toxicity is directly related to the duration of exposure longer than 36 h [6]. The doses given were related to haematological (WBC: $2.5–3.5 \times 10^9$/litre) and clinical toxicity; so it is possible that a more accurate MTX assay might have detected a correlation between a 48-h postingestion MTX level and the doses used in the different children. Clinical toxicity was present in only one of the eleven children; it is of interest that the curve obtained in that child showed a delayed peak and a slow excretion of the drug.

From these results it can be inferred that uniform doses of oral MTX are unlikely to produce predictable serum MTX levels; and this is supported by another radioimmunoassay study of three patients with ALL, who had dissimilar absorption curves following 20 mg MTX orally [1]. This unpredictability has many possible sources. Methotrexate disappearance from the plasma is triphasic after IV injection, reflecting distribution, renal clearance and an enterohepatic circulation [11]. Individual MTX absorption is another factor likely to contribute to these variables after oral ingestion. Furthermore, malabsorption in children with ALL has been related to the cumulative dose of MTX [5], suggesting that individual MTX curves may vary with length of treatment. Interaction with other drugs such as cephalothin and hydrocortisone can influence MTX uptake by leukaemia cells [2], and antibiotics may interfere with the enterohepatic circulation. Albumin levels, which were normal in our children, should be known as up to 70% of the drug is protein-bound [11]. Normal renal function is critical, for the majority of the drug is excreted unchanged in the urine; creatinine and urea levels were normal in the 11 children. Thus the difficulties involved in proper evaluation of a single cytotoxic agent are considerable.

Resistance to chemotherapy leading to haematological relapse is now a major factor in terminating response to treatment. Although this can be predicted to some degree by the clinical and haematological findings at

diagnosis, many exceptions occur in both good- and poor-risk groups. Clearly, other factors are operating – and individual pharmacokinetics may explain some of these unpredictable responses. Plasma concentration and the duration of exposure are two functions of a single dose of MTX that can be easily measured in vivo, and their relationship to toxicity is well defined [6, 21]. The effects of a given dose of MTX on a neoplasm are much less clear, especially when MTX is used in remission. Drug doses affect the length of remission [16], but the role of plasma concentration and duration of drug exposure is not known. Available studies suggest that a neoplasm's growth fraction increases when the cancer cell population is reduced [17], e.g., after remission induction. Phase-specific agents such as MTX are recommended after remission has been achieved [10], as they are more efficient against actively proliferating cells; but their efficacy cannot be measured unless the leukaemia relapses. Acquired resistance to MTx is associated with impaired transport of the drug into the cell [9]. In some children, haematological relapse may be due to doses that are suboptimal because of individual pharmacokinetics; thus cell lines with a decreased MTX uptake can have a selective survival advantage.

There is a need now to assess protocols based on the blood level of drugs, rather than recommended doses based on anthropometry or the limits of clinical toxicity. The assessment of MTX in this way has advantages as it can be easily measured and patients can be rescued from toxicity by folinic acid [6] or asparaginase [3]. The knowledge gained may help to explain the unpredictability of haematological relapse and lead to the development of more effective treatment.

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