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Prognostic importance of systemic clearance of methotrexate in childhood acute lymphoblastic leukemia

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Summary. Pharmacokinetic studies of methotrexate have been carried out in 21 children with acute lymphoblastic leukemia diagnosed in 1981. Children were treated with intermediate dose (500 mg/m^2) methotrexate in keeping with the 1981 ALL treatment Protocol of the Hungarian Childhood Leukemia Working Group. Of the 21 children, 8 relapsed, and 13 are in continuous complete remission. In the relapsed patients significantly increased systemic clearance of methotrexate was observed at the time of the second methotrexate treatment cycle compared with the calculated value after the first administration of the drug. No such change in the clearance was found in patients who are still in remission. There was no difference between children who relapsed or who are in remission in the elimination half-time of the drug. Age, sex, WBC at diagnosis, and systemic clearance of methotrexate were found to be connected with the probability of relapse in the patients studied. The possible reasons for the prognostic role of systemic methotrexate clearance are discussed.

Introduction

Methotrexate (MTX) has an important role in the treatment of acute lymphoblastic leukemia (ALL) of childhood. The importance of the dose level of MTX was first demonstrated by the CALGB in childhood leukemia. [19]. The introduction of intermediate-dose (500 mg/m^2) MTX therapy with folinic acid rescue resulted in an increase in the relapse-free survival rate for patients with standard-risk ALL [9, 14]. The therapeutic results with doses of 6–8 g/m^2 are even more encouraging (P. J. Moe 1986, personal communication).

The 1981 treatment protocol of the Hungarian Childhood Leukemia Working Group included two cycles of intermediate-dose methotrexate (500 mg/m^2) with folinic acid rescue after the induction phase of the treatment and before cranial irradiation [15] (Fig. 1). Between 1 January 1981 and 30 June 1983 a total of 173 children received therapy according to this protocol. On 15 January 1986, the event-free survival was 57.23% (99 patients) for the children treated according to the 1981 Treatment Protocol.

Results with this protocol have been promising from two aspects: the relapse-free survival rate of our patients has increased [16] compared with the outcome of therapy with our previous protocols; and during the follow-up period only a low frequency of central nervous system side effects has been detected [18].

Safe administration of intermediate- and high-dose treatment with MTX requires measurement of the concentration of the drug in the serum. However, when the method used for the determination of MTX levels was introduced, one of our research objectives was to study the possible relationships between individual pharmacokinetic parameters and the clinical course of the disease [1].

Patients and methods

Of the children with acute lymphoblastic leukemia diagnosed in 1981, a group of 21 took part in the study. All the patients participating in this study had non-B non-T cell disease. The age of the children was 5.3 ± 4.2 years (mean ± SD; range: 1.2 years to − 13.6 years) at the time of diagnosis. The male : female ratio was 13 : 8. Renal function (serum electrolytes serum creatinine, BUN) and liver function (serum bilirubin, SGOT, SGPT, gamma-GT) tests were performed for all patients the day before and the 2 days after MTX infusions were started. All the patients had normal renal and liver functions at time of MTX therapy.

The observation period at the time of writing is 58.6 + 3.8 months (range 54–65 months). With the exception of one patient, all the children reported here entered hematologic remission after the induction phase of the treatment, confirmed by bone marrow examination. As consolidation therapy, in keeping with the protocol, two cycles of MTX (500 mg/m^2) were given with a treatment-free interval of 2 weeks. The drug was administered i. v.: after a push of one-fifth of the total dose, four-fifths of the dose was given in an infusion over 24 h. Just before the start of the MTX infusion MTX was also given intrathecally in a dose of 12 mg/m^2. Alkalinization was carried out with sodium bicarbonate administered p. o. or i. v. to maintain the pH of the urine above pH 7. Folinic acid rescue was given in two doses of 12 mg/m^2 i. m. at 42 and 48 h after the start of the MTX infusion.

No serious toxicity of MTX has been observed with the above mode of administration of the drug, except in one child with Down's syndrome, who developed mucositis

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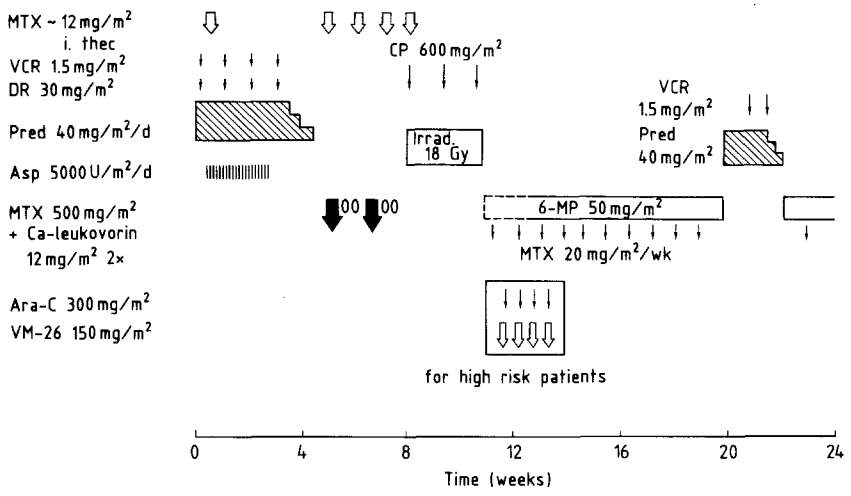

Fig. 1. Treatment protocol for patients with acute lymphoblastic leukemia (ALL)

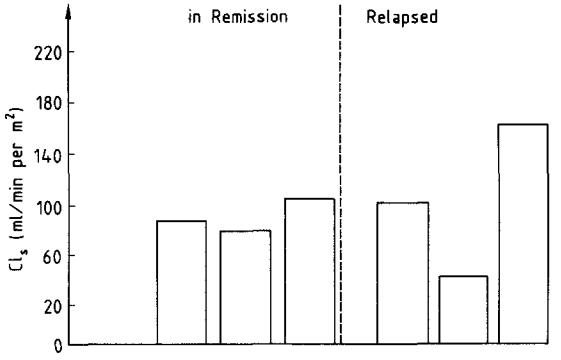

Fig. 2. Systemic clearence of methotrexate (MTX) in children with ALL in remission or in relapse. In patients who relapsed Cl_s of MTX was significantly increased at the time of the second MTX treatment cycle:

	Total	1st	2nd	Total	1st	2nd
Mean	85.6	82.8	104.1	102.6	43.2	162.2
2SD	13.4	20.9	17.3	29.3	12.6	48.8
n	26	13	13	16	8	8
Cl_s 1st : 2nd	0.84 ± 0.23*			0.42 ± 0.15*		

*$P < 0.01$

and bone marrow suppression on day 3 after the start of MTX. Her symptoms lasted for 4 days, during which time she received additional folinic acid rescue 12 mg/m² in every 3 h.

Venous blood samples for the determination of MTX concentrations were drawn 24 h and 48 h after the start of the MTX infusion. Samples were centrifuged and taken for determination immediately. Concentrations of MTX were measured by means of an enzyme inhibition assay [2] with a lower limit of sensitivity of 2×10^{-9} *M*. Systemic clearance of MTX (Cl_s) was calculated according to the equation: $Cl_s = k_o / C_{pss}$, where k_o is the zero-order infusion rate constant (mg/min per m²) and C_{pss} is the steady-state concentration of MTX in the serum (mg/ml) measured at the completion of the MTX infusion. The systemic clearance of MTX characterizes all the processes that take place in the elimination of the drug from the serum. About 65% of it corresponds to the renal excretion and 35%, to the non-renal elimination processes (hepatobiliary-gastrointestinal excretion, metabolism, distribution in the various body compartments) [6]. The elimination half life ($t_{1/2}$) during the first 24 h of the post-infusion period was calculated as $t_{1/2} = \ln 2 / k_e$, where k_e (apparent elimination rate constant) is the negative slope of the natural logarithm of serum concentration-versus-time curve [17].

In this context, Cl_s is an index parameter of the elimination processes during the administration of the drug and $t_{1/2}$ is an index parameter of the elimination processes after the drug-infusion has been stopped. Student's *t*-test was used to test for significant differences between mean pharmacokinetic parameters of grouped data.

Results

In all, 8 of the 21 children relapsed during the observation period, 2 with CNS, 1 with testicular, and 5 with bone marrow relapses. CNS and testicular relapses were followed by recurrence in the bone marrow as well. The times from the date of the diagnosis to relapse were as follows: 4 months for 1 child, 12–18 months for 5 children, 29 months for 1 child, and 36 months for 1 child. There have been 6 deaths among the group, and 2 of the 8 children who relapsed are in second remission.

When the distribution of relapsed and remission cases was examined in arbitrary age groups, (Table 1) the well known fact that very young and older children are at greater risk for relapse was confirmed. Children who relapsed had significantly higher white blood cell counts at diagnosis than those who have continued in remission (44.8 ± 21.3 vs 24.7 ± 7.8 g/l; $p < 0.05$).

The male to female ratio was 7 : 6 in CCR (continuous complete remission) cases and 6 : 2 in the cases with relapse: that is, 6 of 13 boys relapsed and only 2 of 8 girls.

Table 1. Number of relapses/continous complete remissions in different age groups

Age (years)	0< <2	2< <10	>10
Relapsed	2	2	4
In CCR	1	11	1
n	3	13	5

Table 2. Elimination half-time of MTX in the first 24 h of post-infusion period

Clinical status	in CCR	Relapsed
Number of patients	13	8
t ½ (total)	5.89 ± 0.78 h*	5.68 ± 0.55 h
t ½ after 1st MTX treatment	5.44 ± 0.94 h*	5.10 ± 0.77 h
t ½ after 2nd MTX treatment	6.34 ± 1.23 h*	6.30 ± 0.71 h
1st t ½ to 2nd t ½	0.95 ± 0.12*	0.74 ± 0.11

* $P > 0.05$

The comparison of systemic clearances of MTX in the CCR group and the relapse group revealed that although there was no statistically significant difference between the total values, children with relapse had markedly increased Cl_s for MTX at the time of the second treatment cycle; that is to say, a significantly lower ratio of the first to the second values of Cl_s characterized the relapse cases (Fig. 2)

Those children who had a more than two fold increase in Cl_s for MTX at the second MTX treatment had a significantly shorter relapse-free survival time than those whose Cl_s had not changed to such an extent (23.1 ± 9.6 months vs 56.7 ± 3.8 months; $p < 0.01$). There were 11 children who had such an increase of Cl_s of MTX and all the 8 children who experienced relapse belonged to this group. However, 3 of the 11 still continue in remission.

Comparison of the elimination half-time of MTX failed to show any difference between the relapse and the CCR groups (Table 2).

Discussion

Our observations indicate – although the number of the patients studied is relatively small – that the individual pharmacokinetics of MTX has influenced the relapse-free survival rate of children treated according to protocol 81 of the Hungarian Childhood Leukemia Working Group. According to a study conducted in St Jude Children's Research Hospital [7] the probability of relapse for children with standard risk ALL is influenced by the following factors: white cell count and hemoglobin at diagnosis, and the mean value of Cl_s for MTX, calculated after 15 cycles of MTX treatment at a dose of 1.0 g/m^2. However, the presence or absence of changes in the Cl_s during the consecutive cycles is not mentioned in this report. After repeated analysis of the data the authors conclude that a possible reason for the treatment failure in children with fast Cl_s of MTX was non-achievement of a limit steady-state concentration of 1.6×10^{-5} *M* in these patients [8]. This concentration is one order of magnitude higher than that accepted as cytotoxic. [11] On the other hand, faster Cl_s of MTX characterized relapsed children in a study [3] in which high doses of the drug (up to 8 g/m^2) were used and steady-state concentrations even higher than 1.6×10^{-5} *M* have been achieved. We feel that the question as to whether the biological parameters of the host organism or the biology of the disease itself predisposes to the high Cl_s of MTX should be answered. It could also be suspected that high or increasing systemic clearance of MTX is a sign of resistance to the drug. It has been shown that the metabolism of MTX is characterized by wide intra- and interpatient variations [5]; however, we have failed to find any correlation between steady-state MTX and 7-OH concentrations [4].

The difference in Cl_s and the lack of difference in the elimination half-time of MTX found in our study suggest that a number of processes that take place in the elimination of MTX from the serum are different during the period of administration of the drug and afterwards.

Decreased affinity of carrier systems for MTX in the cell membrane (one reason suggested for resistance to MTX [10]) could provide higher quantities of the drug for the elimination processes, and therefore a faster systemic clearance of MTX could result. The same 'symptom' of fast systemic clearance of MTX could arise if the affinity of dihydrofolate reductase for MTX were decreased. A correlation has been shown between the binding affinity of the target enzyme to MTX and cytotoxic effect of the drug [12]. However, it is also possible that the volume of distribution of MTX is changed differently in different patients by the pretreatment alkalinization [13].

More detailed pharmacokinetic studies are needed to give definitive solutions to the above problems. Such studies could probably also cover the mechanism of action and the mechanism of resistance. However, our results and other similar observations demonstrate the possible importance of individual pharmacokinetics of anticancer agents in the prognosis of malignant diseases.

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