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Short communication

7-Hydroxymethotrexate concentrations in serum and cerebrospinal fluid of children with acute lymphoblastic leukemia

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Summary. Concentrations of methotrexate (MTX) and 7-hydroxymethotrexate (7-OH-MTX) were determined by HPLC in the serum and cerebrospinal fluid (CSF) of 29 children with acute lymphoblastic leukemia. CSF and serum samples were obtained at the end of 104 infusions of MTX given in a dose range of 0.5–8.0 g/m^2. Concentrations, distribution ratios in serum and CSF for MTX and 7-OH-MTX, and the metabolic index were analyzed with regard to the MTX dose, age and clinical state of the patients. A wide inter-patient (2- to 12-fold) but narrower (1.1- to 3.5-fold) intra-patient variability of the concentrations was observed. A dose-proportional increase in the metabolite concentration was found in serum. On the other hand, the elevation of the level of metabolite in CSF was less than porportional to the dose. The CSF/serum distribution data suggest the existence of a saturable carrier system for MTX and 7-OH-MTX between serum and CSF that has lower affinity for 7-OH-MTX. No correlation was found between concentrations of MTX and 7-OH-MTX in the serum of patients receiving the same dose of MTX. No significant difference was observed in the values for metabolic index between relapsed patients and those who were in continuous complete remission. A significant correlation was found between age and metabolic index: the younger the patient, the higher the metabolite concentration measured in serum.

Introduction

The wide intra- and inter-patient variations in the pharmacokinetics of methotrexate (MTX) have been extensively discussed in the literature. Moreover, the individual pharmacokinetics – especially the systemic clearance – of MTX may influence the outcome of acute lymphoblastic leukemia (ALL) in children [2, 3, 7, 8, 20]. The major metabolite of MTX biotransformation is 7-hydroxy-MTX (7-OH-MTX), which plays a role in the development of nephrotoxicity following intermediate- or high-dose MTX therapy [12]. At a cellular level, the presence of 7-OH-MTX creates the potential for a number of competitive interactions between the parent drug and the metabolite [11], which may have consequences on the therapeutic or toxic effect of MTX folinic-acid rescue therapy.

The aim of the present study was to investigate the effect of dose and age on the formation of the metabolite and to determine its role in the well-known variability of steady-state MTX concentrations as well as in the prognosis of ALL in children.

Patients and methods

Data obtained from the administration of 104 infusions of MTX to 29 patients (median, 3 infusions/subject; range, 2–8 infusions/patient) were analyzed. The mean age of the patients was 5.2±2.9 years (range, 1–12 years; median, 4 years). All children participating in the study were treated for ALL. In all, 11 achieved a continuous complete remission (CCR); 2 relapsed in the CNS and 6, in the bone marrow; and 10 had a combined form of relapse. None of the patients had renal or hepatic impairment at the time of the MTX treatments.

The mean observation period of this study was 6.45±2.9 years (median, 6 years).

The dose of MTX was in the range of 0.5–8 g/m^2. MTX was given in 24-h continuous i.v. infusions, with adequate (3,000 ml/m^2) hydration. All MTX treatments were given as a part of the Norwegian National Treatment Protocol and Pilot Studies for the Therapy of ALL in Childhood. The exact schedule and the regimen for the administration of folinic acid rescue are described in detail elsewhere [17, 18, 21, 22].

Venous blood and cerebrospinal fluid (CSF) samples were drawn at the end of the MTX infusions. CSF samples were taken by routine lumbar puncture, carried out for the purpose of intrathecal MTX administration. Thus, the concentrations of MTX and 7-OH-MTX found in serum and CSF resulted exclusively from the i.v. administration of MTX. All samples were centrifuged and subsequently frozen at –20° C until analysis.

Determination of MTX and 7-OH-MTX concentrations. Samples of serum and CSF were deproteinized by the addition of 200 µl 2 *M* perchloric acid to 500 µl sample. After centrifugation at 10,000 *g* for 5 min

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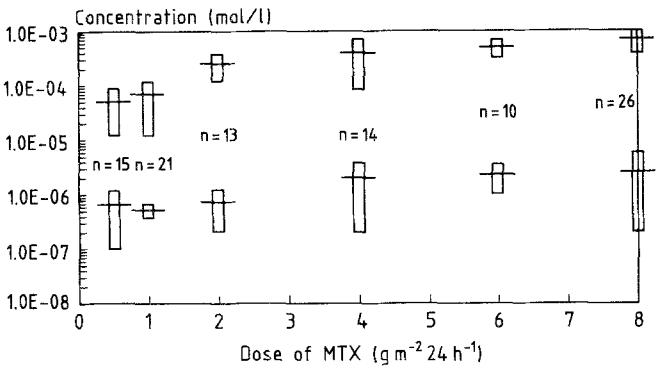

Fig. 1. Concentration of 7-hydroxy-MTX in serum and cerebrospinal fluid (CSF) at the end of the MTX infusion. The 16-fold increase in the dose of MTX from 0.5 to 8 g/m^2 resulted in a 14-fold increase in 7-OH-MTX levels in serum (*top*) but in only a 3.7-fold elevation of the metabolite levels in CSF (*bottom*). ⊣⊢, + 2 SD

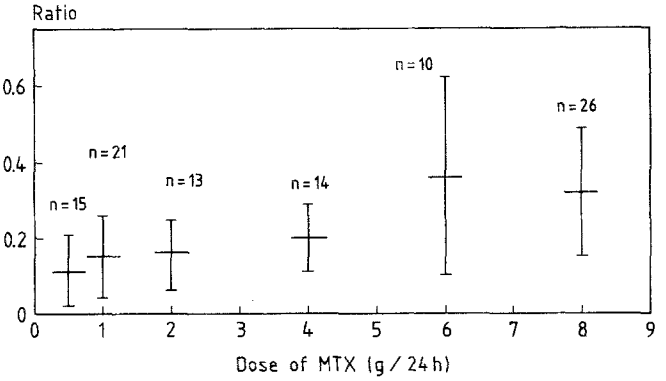

Fig. 2. Ratio of MTX/7-OH-MTX concentrations in serum at the end of the infusion. This ratio increased 3-fold with an increase in the MTX dose from 0.5 to 8 g/m^2, demonstrating that the elevation of MTX levels was more pronounced than that of the metabolite (see [1]). ∓, +2 SD

and appropriate dilution of the supernatant, MTX and 7-OH-MTX concentrations were determined by high-performance liquid chromatography [15].

Pharmacokinetic calculations. The systemic clearance of MTX was calculated according to the following equation: $Cl_sMTX = K_o/Cp_{ss}$, where K_o = the zero-order infusion-rate constant (mg min m^{-2}) and Cp_{ss} = the steady-state concentration of MTX in serum (mg/ml). The metabolic index (MI) was calculated as $MI = K_o/C_{sst}$, where K_o = the zero-order infusion rate of MTX (mg min m^{-2}) and C_{sst} = the concentration of 7-OH-MTX at the end of the infusion. Values for the different distribution ratios of MTX and 7-OH-MTX were calculated by simple methods [19].

Statistical calculations. Student's *t*-test was used to test significant differences between the mean values for grouped data. Analysis of variance and calculation of coefficients of correlation were performed by standard methods. Statistical calculations were carried out using the Statmed PC program, kindly provided by Nycomed Scandinavia [13].

Results

Concentrations of 7-OH-MTX in serum and CSF at the end of the MTX infusion are shown in Fig. 1. The 16-fold increase in the MTX dose resulted in a 14-fold rise in the 7-OH-MTX level in serum but in only a 3.7-fold elevation of the metabolite concentration in CSF. Wide variability of the concentration values was characteristic in both the serum and the CSF compartments. No significant difference in metabolite concentrations was found between the neighbouring dose levels of MTX. Whereas inter-patient differences of as much as 3- to 11-fold were observed, the intra-patient variations were lower: only 1.2- to 2.5-fold differences in the 7-OH-MTX concentrations were detected in the same patients during consecutive treatments.

The difference in the effect of the MTX dose on the 7-OH-MTX concentrations in serum and CSF was reflected in the distribution ratio for 7-OH-MTX between CSF and serum: it decreased from 1.3% to 0.3% as the dose was increased from 0.5 to 8 g/m^2. The ratio of the concentrations of MTX and 7-OH-MTX in serum at the end of the MTX infusion increased from 0.11 to 0.32 when the delivered doses of MTX were 0.5 and 8 g/m^2, respectively (P <0.01; Fig. 2). No significant differences (P >0.01) were found in the ratio of MTX:7-OH-MTX concentrations in CSF, regardless of the dose (data not shown).

Linear regression analysis was performed to examine a possible relationship between MTX and 7-OH-MTX concentrations in the serum of patients receiving the same dose of MTX and between the systemic clearance of MTX and the metabolic index of 7-OH-MTX. However, no significant correlations were found (coefficients of correlation; 0.09 and 0.22; P = 0.2 and 0.7, respectively).

As a mass-descriptive parameter, the metabolic index (MI) was used to examine the effect of the age and clinical state of the patients on the formation of the metabolite. The MI was found to be 19.7 ± 22.2 and 19.5 ± 13.6 ml min^{-1} m^{-2} at an MTX dose of 0.5 and 8 g/m^2, respectively (P >0.05). No significant difference was found between the MI values of relapsed patients and of those who achieved a continuous complete remission (CCR): 15.8 ± 10.5 vs 22.4 ± 18.4 ml min^{-1} m^{-2}, respectively (P >0.01).

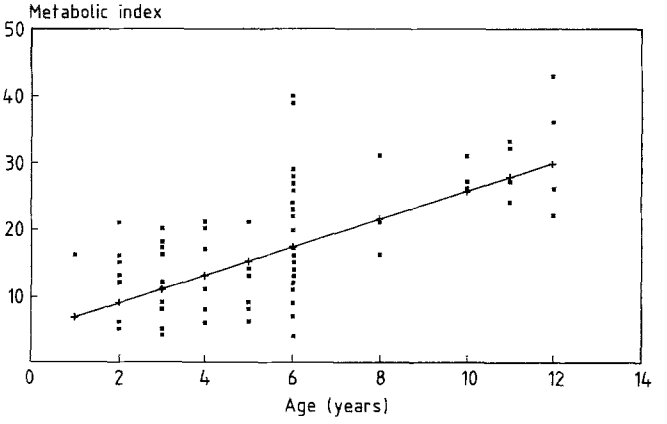

Fig. 3. Effect of age on the metabolic index (ml min^{-1} m^{-2}) of methotrexate. Younger patients had a lower metabolic index in the dose range of 0.5–8 g/m^2 MTX, representing exposure to higher concentrations of 7-OH-MTX. r^2 = 0.3; P <0.01.

A significant correlation was observed between the MI values and the age of the patients ($r^2 = 0.3$; $P < 0.01$); the younger the child, the lower the MI value (higher concentration of the metabolite in the serum) found (Fig. 3). Age had no influence on the concentration of the metabolite in CSF ($r^2 = 0.1$; $P = 0.22$).

Discussion

The exact role of 7-OH-MTX in the mechanism of action of MTX has not been clearly established. In other studies as well as in ours [5, 14], it has been shown that levels of 7-OH-MTX exceed those of MTX at 24 h after the start of the MTX infusion. The concentrations of 7-OH-MTX in serum reported in the present study are in good agreement with data presented by others [5] but are markedly higher than those reported by Slørdal et al. [22] for a dose range of 6–8 g/m^2. This dissimilarity may be due to the difference in the numbers of patients studied (5 vs 29 subjects) and could be explained by the wide inter-patient but relatively narrow intra-patient variability of the serum concentration of the metabolite.

An almost parallel increase in 7-OH-MTX concentrations in serum with increasing MTX dose could be demonstrated in our subjects. Under different experimental conditions (dose, infusion time), a significant correlation between the dose and the serum concentrations of 7-OH-MTX was also reported by Milano et al. [16]. Whereas the formation of 7-OH-MTX was proportional to the MTX dose, steady-state MTX concentrations increased in a non-linear fashion. An almost 50-fold increase in the steady-state concentrations of MTX (data shown in [1]) was accompanied by a 14-fold increase in the 7-OH-MTX levels in serum with a 16-fold increase in the dose of the parent drug. This is reflected in the change of the ratio of serum MTX:7-OH-MTX concentrations (Fig. 2) from 0.1 to 0.3 for MTX doses of 0.5 and 8 g/m^2, respectively. These data and the lack of correlation between serum MTX and 7-OH-MTX levels in the same dose range suggest that metabolism of MTX to 7-OH-MTX does not explain the wide inter- and intra-patient variability of steady-state concentrations (systemic clearance) of MTX [1, 4, 6].

A dose-dependent increase in the concentrations of 7-OH-MTX in CSF was shown; however, this increase was lower than that observed in serum. No further significant increase could be observed when the dose was elevated from 4 to 8 g/m^2. We have described a similar tendency for the diffusion of MTX to the CSF as well [1]. However, whereas the CSF/serum distribution ratio for MTX was $4.6\% \pm 1.7\%$ at a dose of 0.5 g/m^2 and $1.2\% \pm 0.6\%$ at a higher dose, the corresponding values for 7-OH-MTX were $1.3\% \pm 1.4\%$ and $0.5\% \pm 0.4\%$, respectively. Thus, it can be presumed that MTX and 7-OH-MTX share the transport mechanism from serum to CSF, which is saturated for both MTX and 7-OH-MTX at a serum concentration of $1-2 \times 10^{-4}$ mol/l. The difference in the CSF/serum distribution ratios for MTX and 7-OH-MTX could mean either that the metabolite has a lower affinity for the carrier or that passive diffusion is less pronounced for 7-OH-MTX.

Possible pitfalls of the interpretations of our concentration data should be mentioned. As the drug and metabolite levels were determined in a single sample taken at the end of the infusion, it may be possible that for a certain, undefined interval, 7-OH-MTX levels further increased in serum and/or CSF after the termination of the MTX infusion. If this is the case, the lack of equilibrium between CSF and serum may, at least to some extent, explain the reduction in CSF/serum ratios of the metabolite with increasing dose.

In the present study, concentrations of 7-OH-MTX at 24 h after the start of the MTX infusion did not differ significantly between children who relapsed and those who remained in CCR. This is of special interest, since systemic clearance of MTX has been found to have prognostic importance in a similar cohort [2].

The age dependency shown for metabolism in this study adds to a series of other observations on the age-dependent pharmacokinetics of cytostatics and may be of clinical value. Although the clinical significance of 7-OH-MTX was not proved in the present study, effects of 7-OH-MTX on the mechanism of action of MTX cannot be excluded [9–11, 14]. Thus, the consequences of the fact that younger children are exposed to relatively higher levels of 7-OH-MTX require further investigation. Studies on the connection between MTX metabolism and changes in liver enzyme activities are under way in our institute.

Studies on the pharmacokinetics of 7-OH-MTX may additionally improve our understanding of the mode of action of MTX, which remains one of the most important chemotherapeutic agents against ALL in childhood. Considering the quantity of 7-OH-MTX formed in patients receiving high-dose MTX and the sites [9, 10] of competitive interactions between MTX and 7-OH-MTX (membrane transport, dihydrofolate reductase, folylpolyglutamate synthetase), the role of 7-OH-MTX could be more important than previously supposed. However, further studies are needed to elucidate the clinical role of this metabolite.

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