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Pharmacokinetics of erythrocyte methotrexate in children with acute lymphoblastic leukemia during maintenance treatment

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Summary.

1. The concentration of methotrexate (MTX) in erythrocytes (E-MTX) was measured in 47 children with acute lymphoblastic leukemia during maintenance treatment with MTX 1.7–21.6 mg/m^2/week and 6-mercaptopurine 25–75 mg/m^2/day.
2. At the time of measurement the plasma MTX concentration was less than 2 nmol/l. The steady state E-MTX varied between 51 and 202 nmol/l erythrocytes.
3. Alterations in the E-MTX took place over 8–12 weeks after a change in dosage.
4. A significant correlation was found between the E-MTX and the weekly dose of MTX administered.
5. Noncompliance was revealed in two patients.
6. A very low E-MTX was found in one patients, probably caused by inhibition of erythropoiesis.
7. No correlation was found between E-MTX and the total amount of MTX administered or the length of treatment.
8. A terminal half-life of 2–5 weeks after discontinuation of the drug showed that the erythrocytes functioned as a slow-changing compartment for MTX.
9. Unexpectedly low E-MTX could mean noncompliance, impaired erythropoiesis, altered metabolism, or poor drug absorption.

Introduction

The introduction of maintenance therapy with daily 6-mercaptopurine (6-MP) and weekly methotrexate (MTX) PO has contributed to the improved survival of childen with acute lymphoblastic leukemia (ALL).

Leukemia still recurs in about half these children, often during maintenance therapy. This may be due to development of resistance to treatment in the leukemic cells, but other factors such as poor absorption, altered metabolism of drugs, or noncompliance may also play a role.

In children with ALL receiving weekly MTX treatment PO, MTX has been demonstrated in erythrocytes when the concentration of the drug in the plasma is below 1–2 nmol/l. The clinical significance of these findings is not yet clear [4, 7, 11, 12].

Long-term low-dose weekly MTX therapy (5–20 mg/m^2) may be hepatotoxic, judging by elevated aspartate amino transferase (ASAT) and histologically confirmed fibrosis and cirrhosis [15, 16, 22]. In children with ALL who are receiving maintenance treatment with 6-MP and MTX the doses often have to be reduced because of elevated ASAT or leukopenia.

As there is evidence that MTX is incorporated in the red cell precursors in the bone marrow [4, 14, 19, 21], the erythrocyte MTX concentration (E-MTX) may reflect the impact of MTX on bone marrow cells and other reticuloendothelial tissues. The purpose of this paper is to describe the pharmacokinetics of MTX in erythrocytes in children receiving maintenance treatment with weekly MTX and daily 6-MP for ALL.

Materials and methods

We have examined 47 children with ALL, each of whom is or was being treated at one of the three major departments of pediatric oncology in Denmark. Induction and consolidation treatment depended on the initial clinical parameters, so different schedules have been used. All children were treated with 20 mg MTX/m^2 weekly PO and 75 mg 6-MP/m^2 daily PO. Leukopenia and elevated hepatic transaminases caused the actual weekly dose of MTX to vary from 1.7 to 21.6 mg/m^2 and the daily dose of 6-MP, from 25 to 75 mg/m^2.

All children were in complete remission on the day of investigation, as judged by clinical and hematological examinations. Children who relapsed within 3 months of the day of investigation were excluded from the study. All patients had received maintenance treatment with 6-MP and MTX at unchanged doses for at least 8 weeks before the study (steady state [8]), and had received no IV MTX infusions, no IT MTX injections, and no vincristine prednisone reinduction pulses for the last 8 weeks.

The time of the erythrocyte MTX analysis was from 2 to 7 days after the latest dose of MTX. At the same time the complete hematologic status was examined, and S-ASAT or S-ALAT and S-creatinine or S-carbamide were measured. Specimens (10 ml) of EDTA blood were mailed in Saarstedt transport containers from Odense and Copenhagen to Aarhus. This system kept the blood samples cooled below 10 °C for 16 h, and no decline in MTX concentration was observed when the blood was prepared within 24 h. Blood samples arriving 2 days or more after sampling were discarded and new blood samples were then requested.

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Table 1.

	Patients (no.)	Mean E-MTX ±SD (nmol/l)	MTX dose ±SD (mg/m²/week)
Copenhagen	17[b]	120 ±41	15.8 ±5.1
Aarhus	16[a]	106 ±37	13.1 ±4.9
Odense	14	81 ±23	13.0 ±2.4

[a] Noncompliance in two children, who were therefore regarded as ineligible for evaluation

[b] Transient inhibition of erythropoiesis probable in one child, who was also considered ineligible for evaluation

In one institution E-MTX was measured when the children paid their monthly visits to the clinic.

Preparation of blood. The EDTA blood was centrifuged, after which the plasma was kept at −20 °C until assay, and the erythrocytes were washed twice in cold 0.15 *M* NaCl and hemolyzed in three volumes of H_2O, boiled for 7 min, and centrifuged at 9000 *g* for 15 min. The clear supernatant was stored at −20 °C for no longer than 4 weeks before assay.

MTX assay. The MTX concentrations of the supernatants were measured in duplets with a modified enzyme inhibition assay with a range of 10–60 nmol/l and a detection limit of 3 nmol/l. With this assay there was a recovery of 85%–115% of MTX added to hemolyzed erythrocytes in concentrations from 10 to 60 nmol/l. The within-run and between-run precision was 7.4% and 13.5%, respectively, for control 10 nmol/l, and 1.2% and 3.2% respectively, for control 50 nmol/l. The assay has been described in detail elsewhere [20].

MTX concentrations below 10 nmol/l in erythrocyte supernatants and in plasma were determined with a more sensitive radioligand-binding assay [10] with a detection limit of 1 nmol/l. In our laboratory we found a satisfactory correlation between the two assays [20]. The MTX concentrations measured were corrected for the dilution and for the erythrocyte volume fraction after the washing procedure. The erythrocyte MTX concentrations were expressed as nanomoles per liter of erythrocytes.

Results

The erythrocyte MTX concentrations (E-MTX) varied from 51 to 202 nmol/l among 44 patients studied in steady state and in whom noncompliance was not suspected. Table 1 shows the mean E-MTX and the mean dose of MTX in children from the three participating institutions. Se-MTX concentrations were all below 2 nmol/l.

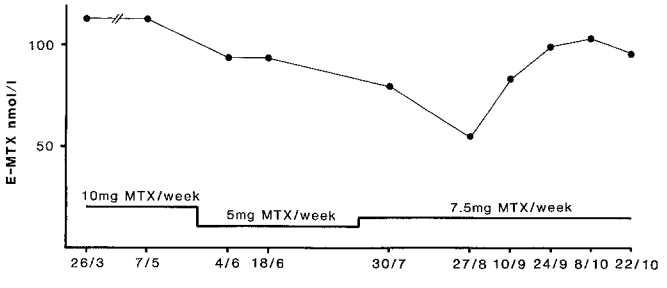

Fig. 1. Longitudinal E-MTX monitoring over 7 months in a 4-year-old girl

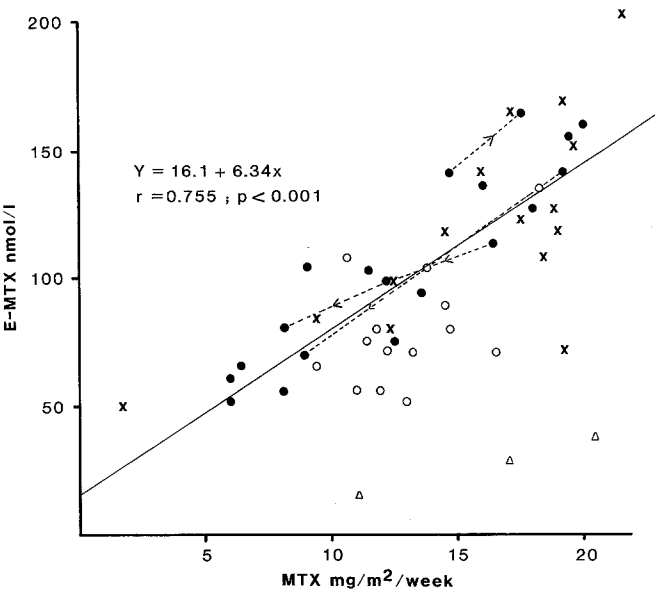

Fig. 2. Steady state E-MTX in relation to the weekly MTX dose in 47 children with ALL at three institutions. *Dotted lines* combine new steady state levels after alteration of drug dosage. ● Aarhus, × Copenhagen, ○ Odense

Figure 1 shows the change in the E-MTX in relation to changes in the weekly dose of MTX in one patient over 7 months. The lowest E-MTX was reached about 12 weeks after a 50% reduction in dosage. During the last 4 weeks the E-MTX continued to fall in spite of an augmentation of dosage. But after 8 weeks of unaltered treatment at the same (7.5 mg MTX/week) a new steady state was reached.

Figure 2 shows the correlation between the steady state E-MTX and the weekly dose of MTX/m² in the 47 children included in the study. Besides the new steady state, E-MTX is shown for three children after change in the weekly dose (dotted lines). The new steady state concentration can be seen to parallel the regression line in two of the patients. In the third patient studied at three different dose levels an almost linear relationship was observed between the different doses and the E-MTX. On linear regression analysis we found a significant correlation ($r = 0.755$; $P < 0.001$) between the steady state E-MTX and the weekly dose of MTX in the 44 evaluable children. The E-MTX of two patients who admitted noncompliance and one child who probably had transient inhibition of the erythropoiesis are included in the data for Fig. 2 (△), but their data have not been included in the statistical calculations.

Figure 2 also shows a considerable difference in the E-MTX in children apparently receiving the same weekly dose. These differences were unrelated to the time that had elapsed since the last dose of MTX.

There was no correlation between the total dose of MTX or the length of treatment and the E-MTX, as shown in Fig. 3. Again, the mean E-MTX correlated to the mean MTX dose.

The E-MTX was followed in three patients discontinuing maintenance treatment after 3 years of continuous complete remission (Fig. 4). In two of the children the E-

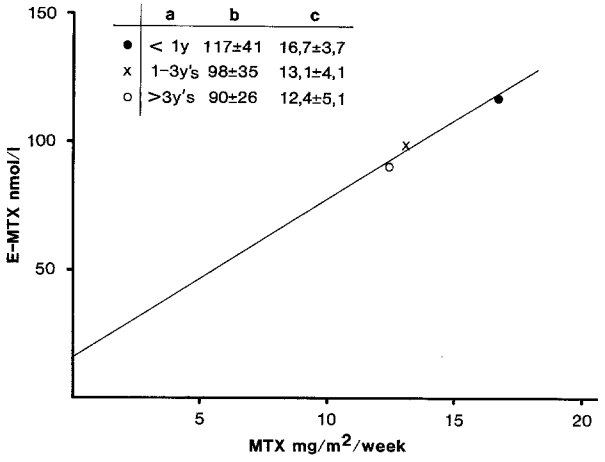

Fig. 3. Length of treatment **(a)** in relation to mean E-MTX **(b)** and mean MTX dose mg/m^2/week **(c)**

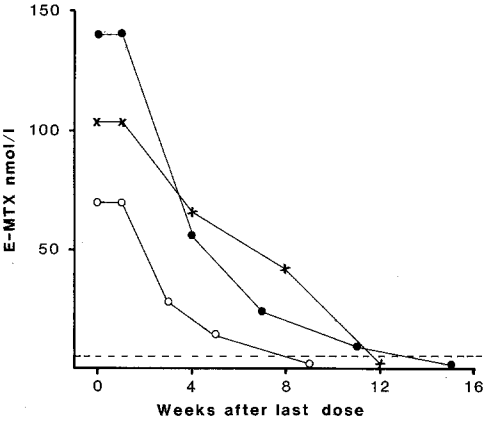

Fig. 4. Disappearance curves of E-MTX in three patients after discontinuation of MTX therapy. – – – –, detection limit of the radio ligand-binding assay

MTX declined in an exponential manner, the terminal half-life being 2–2½ weeks. In the third patient the E-MTX evidently declined more linearly, the half-life being about 5 weeks. The E-MTX was below the detection limit of the assay 2–3 months after the drug had been discontinued.

Discussion

Methotrexate accumulation in eryhtrocytes has been described sporadically in the literature [4, 7, 11, 12], and theories as to a relation between the E-MTX and toxicity towards the drug have been proposed [11, 21]. Before such theories can be investigated more closely, however, the pharmacokinetics of MTX in erythrocytes in patients receiving low-dose MTX treatment PO has to be described.

After ingestion of 10–30 mg MTX PO a plasma peak concentration of 500–1000 nmol/l is reached after 1–3 h [3, 5, 8]. Simultaneously a similar concentration profile is seen in the erythrocytes, although considerably lower [8]. This may reflect passive diffusion or a carrier-mediated transport of MTX over the red cell membrane during the plasma peak concentration. After 24 h the MTX concentrations in both plasma and eryhtrocytes are below 10 nmol/l. At 4–6 days after the first MTX dose the drug reappears in the eryhtrocytes although the plasma concentration is below 1 nmol/l [4, 8].

In psoriasis patients treated with MTX the E-MTX reached a steady state after 4–6 weeks of weekly MTX medication PO. This level was maintained for at least 6 months with unchanged MTX dosage [8].

We found the erythrocytes were a slow-changing compartment, as 8–12 weeks might elapse before a new steady state concentration was reached after a change of dosage (Fig. 1). Therefore, we have considered the E-MTX to be in steady state after at least 8 weeks of unchanged drug dosage.

Preliminary results of sequential E-MTX in five children receiving MTX at an unaltered dosage for 4–7 months, who were studied at monthly or bimonthly intervals, showed a coefficient of variation of 6%–18% (to be published). No increase in the E-MTX was observed after steady state had been reached. This fact was also supported by the lack of correlation of the E-MTX to the total amount of MTX administered and to the duration of treatment.

After discontinuation of therapy the terminal half-life of MTX in erythrocytes was 2–5 weeks, which is in accordance with observations of terminal half-life after high-dose MTX infusions [18, 19]. The present terminal disappearance curves of E-MTX showed that the decline of E-MTX apparently was faster than the erythrocyte life-span, assuming that erythrocytes of these patients had a normal life-span of 100–120 days. Information on this aspect is not available in the literature.

The long terminal half-life of the E-MTX might be explained by polyglutamation of MTX [6, 11, 14, 18]. MTX polyglutamates, especially those with 4–6 polyglutamyl derivatives [20, 22], are known to leave the cells at a much slower rate than genuine MTX in the absence of extracellular MTX [1, 2, 9, 19]. Binding to some intracellular protein, perhaps dihydrofolate reductase (DHFR), might also account for the long terminal half-life [4].

Our data confirm previous observations that children with ALL receiving MTX once a week PO retain MTX in erythrocytes even when the MTX concentration in serum is below 2 nmol/l [8, 11, 12]. The range observed (51–202 nmol/l) was no different from that reported by Kamen et al. [12] although different assay methods were used.

The significant correlation between the E-MTX and the dose ingested/prescribed weekly has not been described previously in children with ALL. No such correlation was found in 25 psoriasis patients receiving weekly MTX treatment, possibly because the range of the weekly dose was much smaller than in our study [8]. The correlation between the steady state E-MTX and the weekly dose was further supported by the observation that a change of dose led to a new steady state E-MTX, which was correlated with the new dosage (Fig. 2).

Measurement of E-MTX is useful in children with ALL receiving maintenance treatment with 6-MP and MTX, as a means of detecting noncompliance. We found that the E-MTX was far below the expected concentrations in three patients, two of whom admitted not having taken the drug regularly. In one of these children absorption studies of MTX yielded a normal absorption curve. In the third patient receiving 17 mg MTX/m^2/week noncompli-

ance was very unlikely. Absorption studies were not possible as the patient had his maintenance therapy discontinued. On the day of the E-MTX analysis hemoglobin had dropped from 13.6 to 9.4 g/dl over 7 weeks. If the E-MTX reflects the MTX incorporated in the erythroblasts of the bone marrow, as has been suggested [4, 9, 21], a temporary inhibition of erythropoiesis implies a relatively fast decline of the E-MTX (cf. the E-MTX disappearance curves; Fig. 4).

Although we found a significant correlation between the weekly dose of MTX and E-MTX, Fig. 2 shows that there was a two-fold difference in the E-MTX in children apparently receiving the same dose of MTX. This phenomenon might be caused by unrecognized noncompliance, temporary inhibition of erythropoiesis, interindividual variation in MTX absorption, which is known to vary [3, 5, 17], or different degrees of MTX polyglutamation in the red cell precursors.

A difference in the mean E-MTX was noted among the three centers, although the variation was rather large. No differences in sex, age, length of treatment, or sample handling was apparent among the three institutions.

Longitudinal surveillance with E-MTX measurements every month would be useful as a means of learning more about the intraerythrocytic pharmacokinetics of the drug, judging possible alterations in relation to bone marrow recurrence or clinical signs of toxicity to the drug, and revealing noncompliance. Such studies are in progress. When a low E-MTX is encountered in a patient with unaltered hemoglobin concentration he or she should be questioned about compliance, and absorption studies should be performed. Metabolic studies (i.e., degree of polyglutamation) would be very interesting in these cases.

If no other dose-limiting factors are present (e.g. low WBC, grossly elevated hepatic transaminases, or stomatitis) the MTX dose should be augmented under close clinical surveillance in patients with low E-MTX.

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