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Kinetics of 7-hydroxy-methotrexate after high-dose methotrexate therapy

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Summary. Kinetics of 7-hydroxy-methotrexate (7-OH-MTX) excretion after high-dose methotrexate (MTX) (12 g/m^2) therapy were monitored in 93 consecutive drug cycles of 19 adolescent patients with osteosarcoma. A reversed-phase HPLC method was used. Serum elimination was found to be monophasic with a mean half-life of 5.5 h. Shortly after the 4-h MTX infusion period 7-OH-MTX levels exceeded those of the parent compound. By 12 h after MTX infusion 7-OH-MTX levels were 16.5 times higher than those of MTX itself.

Autostimulation of MTX metabolism leading to enhanced 7-OH-MTX production after repeated drug cycles was not observed. The production of 7-OH-MTX decreased significantly from the first to the last high-dose MTX cycle of the adjuvant chemotherapy protocol.

Introduction

Monitoring serum levels of methotrexate (MTX) is essential in high-dose MTX therapy. After the introduction of high-performance liquid chromatography (HPLC), metabolism of MTX was found to occur in humans. Metabolic conversion of MTX to 7-hydroxymethotrexate (7-OH-MTX), which is many times less potent as dihydrofolic acid-reductase inhibitor [5], was first observed in humans by Jacobs et al. [6]. An hepatic aldehyde oxidase is assumed to be the converting enzyme. Until now there are few reports describing the 7-OH-MTX elimination kinetics in man after high-dose MTX therapy [1, 2, 7, 8, 11]. The reports cited confirm the early findings of Jacobs that after high-dose MTX therapy 7-OH-MTX levels exceeding those of the parent compound are achieved in plasma. Whether elimination of the metabolite is mono- or biphasic [11] is controversial, as is the question as to whether enzyme induction following repeated doses of MTX leads to enhanced metabolite production [7]. Whether 7-OH-MTX production contributes to the clinical toxicity of high-dose MTX or influences its therapeutic effectiveness is still not clear.

The present paper demonstrates data on 7-OH-MTX kinetics of 19 children and adolescents with osteosarcoma undergoing repeated high-dose (12 g/m^2 body surface area) MTX cycles followed by leukovorin rescue and forced alkaline diuresis. The results of this study, which was a retrospective study, go some way toward shedding light on the questions touched on above.

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Materials and methods

Nineteen patients between 7 and 24 years were treated with high-dose MTX 12 g/m^2 given as 4-h infusions. Alkalinization of urine was started with sodium bicarbonate IV before the beginning of each MTX infusion. In the first 24-h, starting with the MTX infusion 260 mval/m^2 sodium bicarbonate was given IV, followed by a second 24 h period with 150 mval/m^2. Forced diuresis was maintained with 2.5 l fluid per m^2 per 24 h for 2 days. Citrovorum factor rescue was started between 12 and 24 h after MTX infusion, with 15 mg/m^2 PO four times a day per 3 days. Fourteen MTX cycles were included in the adjuvant chemotherapy protocol (COSS-80) for osteosarcoma [12]. Routine monitoring of MTX serum levels was performed according to the MTX-EMIT method (SYVA, Corp.) at 4, 12, 24, and 44 h after the start of the MTX infusion.

For the present study the sera were frozen at −20 °C and analyzed by a reversed-phase HPLC method using a μ-Bondapak $-C_{18}$ column (Waters) as the stationary phase and phosphate buffer pH 7 (1/15 M) acetonitrile (95/5 v/v) as the liquid phase. Detection was performed at 313 nm. The lower limit of detection is 2×10^{-7} mol/l for MTX and 3.3×10^{-6} mol/l for 7-OH-MTX. The interassay coefficient of variation for MTX was found to be 8% (concentration 600 ng/ml = 1.1 μmol/l, $n=25$) and that for 7-OH-MTX, 7% (concentration 6.5 μg/ml = 15.7 μmol/l, $n=18$).

For quantification the external standard method was used (recovery 74.2%±5.2% for MTX and 81.2%±8% for 7-OH-MTX at concentrations of 1.1 μmol/l and 15.7 μmol/l).

Chromatographic purity, tested at various wavelengths (260–370 nm), was found to be 99.3% for 7-OH-MTX. The UV spectrum of 7-OH-MTX was comparable to that described by others [1].

The method is described in detail elsewhere [3].

Results

Elimination kinetics from 93 drug cycles of 19 patients are given in Fig. 1 on the basis of 12-, 24-, and 44-h 7-OH-MTX concentration values. 7-OH-MTX elimination in this time period is monophasic, following first-order kinetics under the conditions described. The half-life of 7-OH-MTX excretion was found to be 5.5 h. The corresponding elimination rate constant is 0.125 h^{-1}. These values were

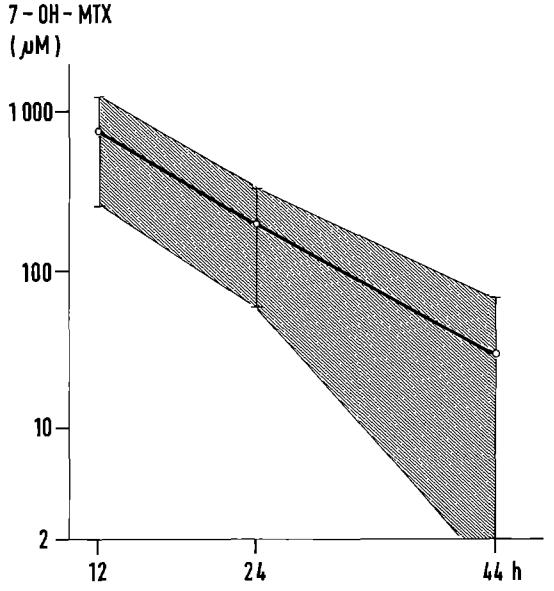

Fig. 1. Elimination of 7-OH-MTX from human plasma. The values given are means ±SD of 93 MTX high-dose drug cycles (12 g/m² b. s.) of 19 patients.

Table 1. 7-OH-MTX maximum levels obtained in each patient

Patient no.	Max. level of 7-OH-MTX (μM)	Time after start of MTX infusion (h)	No. of MTX drug cycle
1	2718	4	7
2	1760	4	6
3	1287	12	5
4	1214	4	1
5	1456	12	7
6	1898	12	1
7	1045	4	1
8	582	4	4
9	915	4	9
10	2091	12	1
11	887	4	1
12	873	12	2
13	1013	4	1
14	1395	12	1
15	1045	12	13
16	1081	4	1
17	698	4	4
18	1172	12	1
19	1301	12	13

determined from the least-squares fit of the log concentration/time curve.

Elimination of 7-OH-MTX is slower than that of the parent compound. T ½ values for MTX under the same conditions in the same patient group were 2.5 h for the first (6.6 h for the second) elimination phase.

As shown by the wide range of standard deviation there is great variability in 7-OH-MTX serum levels between the patients investigated.

In contrast to the findings of Breithaupt et al. [1], the same was observed when repeated drug cycles were considered for each patient.

Serum peak levels of 7-OH-MTX could not be precisely determined. Multiple venipunctures during MTX infusion and the 12-h period immediately after seemed unacceptable for ethical reasons. Nevertheless, in 10 of 19 patients the highest levels of 7-OH-MTX were measured at the end of the 4-h MTX infusion period, whereas in 9 patients the levels at 12 h exceeded those at 4 h. Table 1 shows the highest 7-OH-MTX levels in each patient, ranging from 582 to 2718 μ*M*.

Therefore, by 12 h after the start of MTX infusion at the latest, 7-OH-MTX accounted for most of the MTX administered exceeding the serum level of the parent compound by 16.5 times. After 24 h the ratio of 7-OH-MTX to MTX in the serum was 43.4. These values are the means of all 93 drug cycles described.

Enzyme induction by repeated MTX administration leading to enhanced metabolite production was assumed by Lankelma et al. [7]. To evaluate this assumption 7-OH-MTX: MTX ratios 12 h after MTX infusion were compared in each patient between the first and the last (14th) drug cycles of our protocol. As shown in Fig. 2a, 7-OH-MTX : MTX ratios dropped significantly from 28.7 to 13.0 (Wilcoxon's test for paired values, P=0.01). As corresponding MTX values did not differ the same is true for absolute 7-OH-MTX serum levels (Fig. 2b).

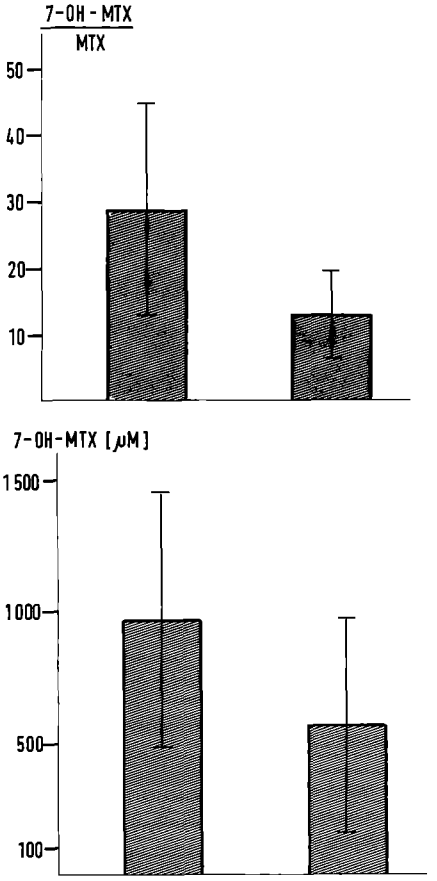

Fig. 2a, b. The molar quotient 7-OH-MTX/MTX 12 h after MTX infusion *(a)* is compared for the first and last MTX cycle. Values given are means ±SD of 19 patients. The difference is significant according to the Wilcoxon's test (P=0.01). The same result is obtained when the concentrations of 7-OH-MTX alone are considered *(b)*

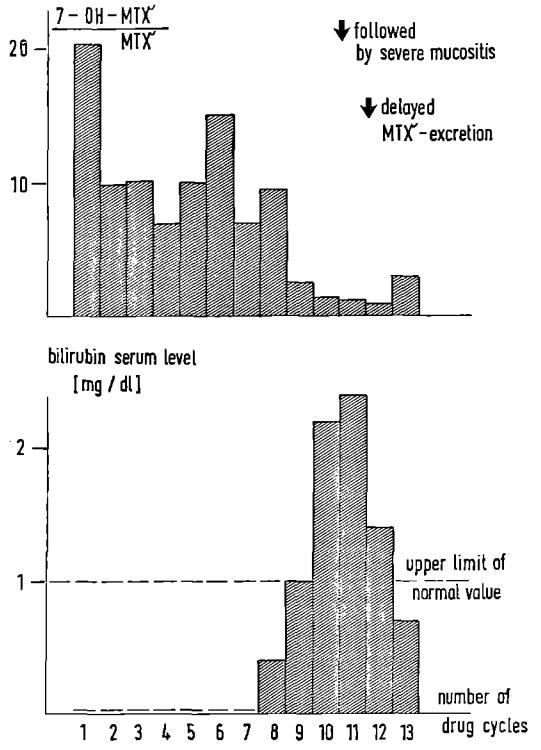

Fig. 3. 7-OH-MTX/MTX ratio in consecutive high-dose MTX cycles of one patient showing severe toxicity. From the ninth drug cycle 7-OH-MTX production falls, which correlates with a rising serum bilirubin level with severe mucositis and bone marrow aplasia

No correlation was observed between 7-OH-MTX production measured as the 12-h 7-OH-MTX : MTX ratio and age, sex, leukocyte nadir, or serum creatinine levels. Each high-dose MTX treatment course was followed by a rise in serum transaminases (SGOT from 28 ± 30 to 116 ± 180 and SGPT from 51 ± 76 to 160 ± 210 units/l 24 h after infusion). There was no positive or negative correlation between serum transaminase levels and 7-OH-MTX production. Among all 93 drug cycles investigated, severe clinical toxicity leading to stomatitis and long-lasting neutropenia was observed only in two cycles in the same patient. It should be mentioned that these drug cycles were associated with hyperbilirubinemia. Only in the second of these two cycles was an elevated 48-h MTX level found; this indicated that the patient was at risk for requiring enhanced and prolonged leukovorin rescue. As shown in Fig. 3, in this patient 7-OH-MTX production was markedly decreased, as indicated by a 7-OH-MTX : MTX ratio of about 1 during the drug cycles followed by clinical toxicity. In the patient group investigated there was no other observation of such an impairment of 7-OH-MTX production.

Discussion

When the reports covering the kinetics of 7-OH-MTX in humans are considered together it is obvious that 7-OH-MTX is the main metabolite following high-dose MTX therapy [6, 11]. Its level exceeds that of the parent compound in serum shortly after the end of an MTX infusion. As recently shown by Milano et al. [8] in a group of 13 patients treated with different doses of MTX, there is a marked dose-dependence of maximum 7-OH-MTX levels. Moreover, a comparison of the data reported on 7-OH-MTX elimination in humans [1, 2, 6–8, 11] reveals the same close relation between 7-OH-MTX peak levels, ranging from 1 to 560 μM after an MTX dose of 40–350 mg/m^2. Therefore it is not surprising that the maximum 7-OH-MTX levels observed in the 19 patients in our group were even higher, because we used a much higher dosage (12 g/m^2). It may be concluded that even at this dosage the converting enzyme system in humans is not saturated.

Although our patient group was very homogeneous regarding morbidity, age, and MTX dosage, there was marked interpatient variability in 7-OH-MTX production, confirming the data reported by Breithaupt et al. [1] in a series of seven adult patients with osteosarcoma. In contrast to this author, we also found marked intrapatient variability. In general, 7-OH-MTX serum levels declined with increasing numbers of drug cycles in the same patient. This may be due to cumulative hepatic toxicity of our chemotherapy protocol, which included 14 drug cycles of high-dose MTX. In all high-dose MTX cycles investigated elevated SGOT and SGPT were observed. On the other hand, there was no correlation between transaminase level and decreased 7-OH-MTX production. Our results – in contrast to the findings of Lankelma et al [7] – indicate that there is no autostimulation of MTX metabolism in the conditions described. It should be mentioned that our regimen includes repeated MTX cycles at times in weekly intervals. On the other hand, with other drugs self-induction of metabolism is observed at low dosages. Therefore, the discrepancy between our results and those reported by Lankelma et al. [7] may reflect the pronounced difference in the MTX doses given (12 g/m^2 versus 240 mg/m^2).

All authors reporting clinical observations on 7-OH-MTX speculate on the question as to whether this compound contributes to the nephrotoxicity of high-dose MTX therapy. This discussion started with the publication of Jacobs et al. [6], who found deposits of 7-OH-MTX crystals in monkey kidney after the administration of MTX, since the aqueous solubility of 7-OH-MTX is much lower than that of MTX itself at physiological urine pH. However, our patient collective also provides no evidence of correlation between 7-OH-MTX kinetics and nephrotoxicity in humans: such data could only be obtained in prospective clinical studies including routine monitoring of MTX metabolites. Obviously, if alkaline forced diuresis is provided, keeping the urine pH value above 7.5, even high amounts of 7-OH-MTX (up to 1.2 g, taking as a basis a renal excretion of about 10% of the parent compound [1] are safely removed by the kidneys.

It may be assumed that conversion of MTX to 7-OH-MTX contributes to the detoxication of MTX high-dose therapy. Redetzki et al. [10] showed rabbits to be highly resistant to MTX, because they metabolize high amounts of the substance. The resulting metabolite was found to be 7-OH-MTX. It has 200-fold less inhibitory activity for dihydrofolic acid reductase than MTX itself [5]. Moreover, 7-OH-MTX interferes with MTX accumulation in Ehrlich ascites tumor cells, as shown by Lankelma et al. [7], and also with MTX polyglutamate formation in human lymphoblastic leukemia cells in vitro [4].

Therefore, from a theoretical point of view, enhanced production of 7-OH-MTX should result in reduced MTX antitumor activity. In turn, reduced 7-OH-MTX formation may lead both to improved clinical effectiveness and to higher clinical toxicity. The observed association of reduced 7-OH-MTX production with severe toxicity and hyperbilirubinemia in two MTX cycles in one of our patients may support this suggestion. Moreover, one might ask whether decreased 7-OH-MTX production following repeated high-dose MTX cycles, as shown in our patient group, could explain the observation of Perez et al. [9], who found increasing clinical toxicity in patients who had more than ten drug cycles. Our data, which were collected retrospectively, do not answer this question. Therefore, prospective studies are needed, which should correlate not only MTX serum levels but also those of its metabolites both with the observed clinical toxicity and with the clinical effectiveness of MTX high-dose therapy.

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