Cancer Chemother Pharmacol (1983) 11: 29–32

Cancer Chemotherapy and Pharmacology

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Plasma Levels of 7-Hydroxymethotrexate after High-Dose Methotrexate Treatment

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Summary. *Thirteen patients with cancer being treated with high-dose methotrexate (MTX) chemotherapy (350–5,000 mg/6 h IV were entered in this study. Plasma levels of MTX and 7-OHMTX, its main circulating metabolite, were measured by an HPLC technique. 7-OHMTX appears rapidly in the blood, reaching a maximum 6–12 h after the beginning of treatment.*

The elimination of 7-OHMTX is slower than that of MTX, but the elimination half-lives (24–48 h) are not significantly different: 25.2 h for 7-OHMTX versus 20.3 h for MTX. In all cases, 24 h after starting infusion plasma levels of 7-OHMTX exceeded those of MTX. There was a positive and significant correlation between the dose administered and peak plasma 7-OHMTX. Finally, 7-OHMTX formation was shown to be relatively stable throughout the treatment.

Introduction

Methotrexate (MTX) is the oldest of the cytotoxic agents, and complete data are available on the cellular pharmacology and clinical applications of this product [1]. On a metabolic level, numerous recent studies have dealt with cellular transformation of MTX in polyglutamate derivatives [11, 12, 14]. Following the work of Jacobs et al. [6], we know that MTX is transformed in the liver to a hydroxylated metabolite, 7-OHMTX, which has been found in the urine of cancer patients. Few authors have investigated this field of MTX metabolism [2, 4, 7].

Using an HPLC technique, we have been able to separate MTX from 7-OHMTX and thus follow the blood concentrations of MTX and 7-OHMTX during cycles of high-dose MTX therapy in 13 patients with cancer.

Materials and Methods

Patients. Characteristics of the patients entered on the study are presented in Table 1.

MTX Perfusions. MTX was administered by continuous IV infusion over 6 h in 5% glucose. Six doses of folinic acid (25 mg PO) were given at 6-h intervals, beginning 6 h after completion of the MTX infusion.

Blood Samples. Blood samples (5 ml) were collected in EDTA tubes. Depending on vein condition and patient compliance, a maximum of eight samples were taken: 1 h, 4 h, 6 h, 12 h, 24 h, 30 h, and 48 h after the beginning of perfusion.

Table 1. Patients

Pat.	Age, sex	Localization	Cycle number	Dose (mg)	Associated drugs[a]
1	34, F	Breast	3	1,000	Vindesine
2	74, F	Breast	6	1,000	0
3	28, F	Osteosarcoma	4	3,000	Vincristine
4	17, F	Osteosarcoma	1	3,000	Vincristine
5	17, M	Osteosarcoma	3	350	Vincristine, mitomycin
6	35, M	Lymphosarcoma	2	2,000	0
7	50, M	Lymphosarcoma	4	2,000	0
8	36, M	Schwannosarcoma	5	5,000	0
9	46, M	Fibrosarcoma	1	3,000	0
10	68, M	Colon	2	1,000	0
11	40, M	Cavum	1	1,000	Vincristine
12	51, M	Testis	4	5,000	0
13	61, M	Unknown	1	750	Vincristine, mitomycin

[a] Taking time at which MTX was administered as zero, other drugs were given as follows: mitomycin, 10 mg/m^2 on day −1; vincristine, 1.5 mg/m^2 + vindesine, 3 mg/m^2 on day 0, 3 h before MTX

Biochemistry. Purified 7-OHMTX was obtained from Dr Lankelma (Het Nederlands Kanker Instituut, Amsterdam, The Netherlands) and MTX, from Lederle Laboratories (Oullins, France) (batch no 37859).

For analyses the method of Nelson et al. [9] was used, with modifications: the chromatographic system (HPLC) included a 6000 A pump (Waters Assoc., Milford, MA, USA), a U6K injector (Waters), a Lichrosorb Hibar column, RP 18 (7 μm), 250 mm × 4 mm (Merck, Darmstadt, FRG), an M440 UV detector fitted with a 313 nm interferential filter (Waters) and a Data Module Integrator (Waters).

Aliquots (500 μl) of plasma from patients or blank plasma spiked with MTX and 7-OHMTX were deproteineized with 50 μl 30% trichloroacetic acid in aqueous solution. After centrifugation, 50–75 μl supernatant was injected in the chromatograph.

Separation was performed with 0.1 *M* Tris-phosphate buffer (pH 6.7) containing 20% methanol. The flow rate was adjusted to 1.5 ml/min. Figure 1 shows a chromatographic profile for a patient treated with high-dose MTX. Reproducibility (coefficient of variation) was assessed using five

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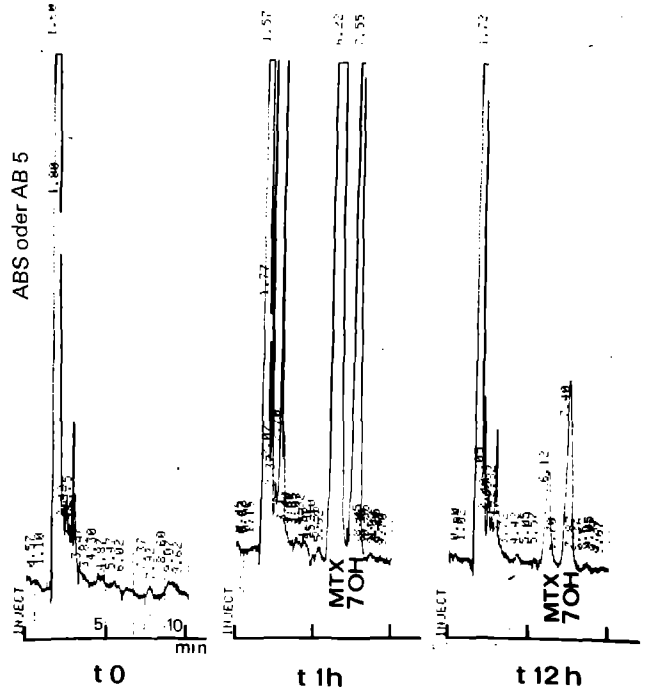

Fig. 1. Chromatographic profile of methotrexate (MTX) and 70H methotrexate (70H) in a patient during infusion of 1,000 mg methotrexate

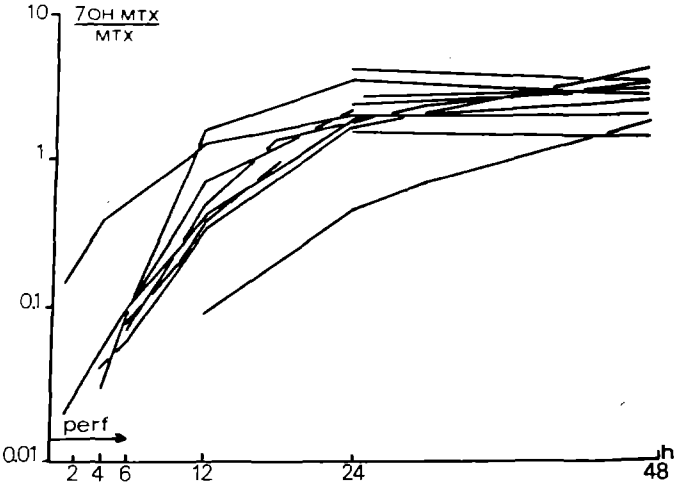

Fig. 3. Course of the 7-OH MTX : MTX ratio over time

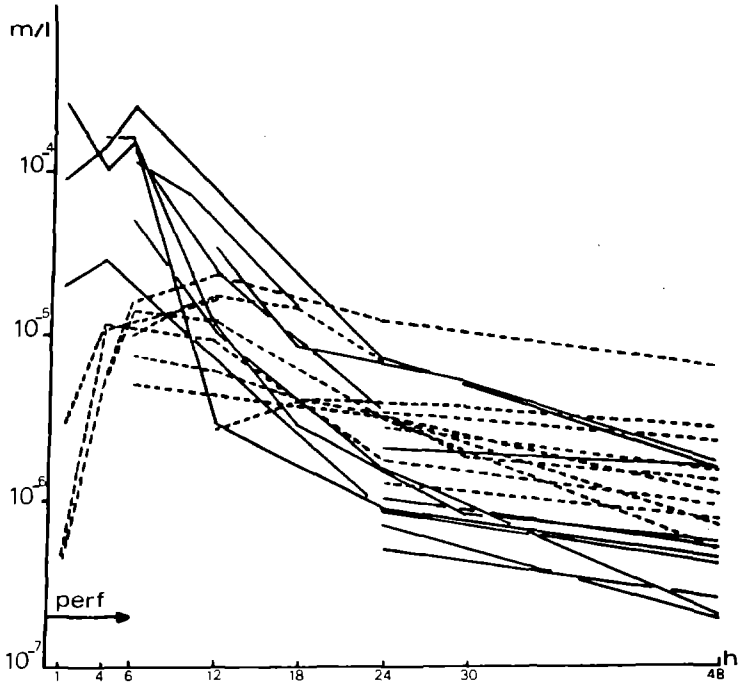

Fig. 2. Course of MTX *(solid line)* and 7-OH MTX *(dashed line)* concentrations over time

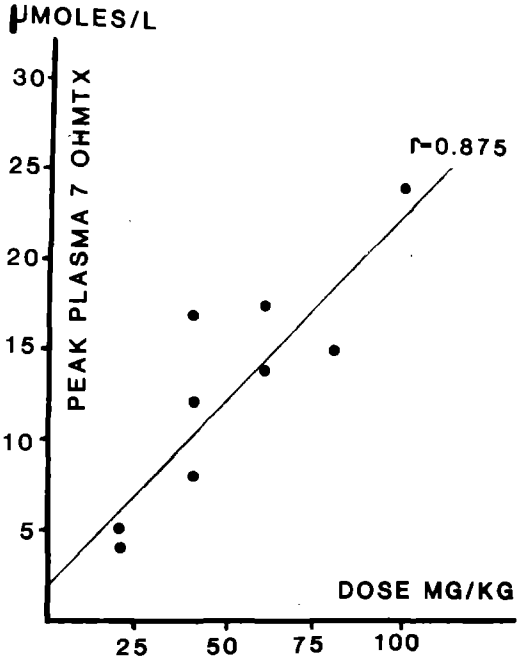

Fig. 4. Relationship between the MTX dose administered and peak plasma 7-OHMTX in nine study patients

different aliquots of the same spiked plasma before deproteinization: at a concentration of $5 \cdot 10^{-6}$ *M* it was 4.9% for MTX and 3.6% for 7-OHMTX; at a concentration of $5 \cdot 10^{-7}$ *M* it was 7.2% for MTX and 5.4% for 7-OHMTX. The limit of sensitivity for MTX and 7-OHMTX was $1 \cdot 10^{-7}$ *M* in plasma.

Computations. Concentrations were calculated on the basis of the peak height, which was automatically integrated by the Data Module Integrator (Waters).

Elimination half-lives were calculated using the formula $t_{1/2}$ (h) = $0.693/\beta$ [10] where β is the slope of the elimination (semi-logarithmic plot) between 24 h and 48 h.

Table 2. Peak plasma 7-OHMTX during successive chemotherapy cycles

Patient	Age, sex	Localization	Cycle number[a]	Dose (mg)	Associated drugs[b]	Peak plasma 7-OHMTX (μmol/liter)
F.S.	40, M	Cavum	1	1,000	Vincristine	3.8
			2	1,000	0	3.2
			3	1,000	0	4.6
			4	1,000	0	6.7
M.A.	68, M	Colon	1	1,000	0	4.8
			2	1,000	0	5.0
			3	1,000	0	4.1
S.M.S.	28, F	Osteosarcoma	4	3,000	Vincristine	14.3
			5	3,000	Vincristine	19.1
			10	3,000	Vincristine	9.5
G.F.	50, M	Lymphosarcoma	4	4,000	0	15.6
			5	4,000	0	12.4

[a] Interval between cycles was 1 month
[b] Vincristine 1.5 mg/m^2 was given 3 h before administration of MTX

Results

Figure 2 shows the blood kinetics of MTX and 7-OHMTX. 7-OHMTX appears rapidly in the bloodstream, with a miximum plateau 6–12 h after the beginning of the infusion. Elimination half-lives (24–48 h) are 20.3 ± 11.1 h and 25.2 ± 11.7 h for MTX and 7-OHMTX, respectively. 7-OHMTX is eliminated more slowly than MTX but the difference is not significant. Figure 3 completes this analysis by showing the course of 7-OHMTX : MTX ratios over time. Thus, from the 24th h onward after the beginning of perfusion, 7-OHMTX is the major product. At 48 h, 7-OHMTX : MTX ratios ranged between 1.75 and 4.13. 7-OHMTX production was not modified by the presence of associated drugs.

Figure 4 shows the evolution of peak plasma 7-OHMTX as a function of the dose administered. There is a positive and significant correlation between these two parameters, with a regression line $y = 0.215\ X + 1.997$ ($r = 0.875$, $P < 0.01$). In an attempt to test for the possibility of enzymatic auto-induction for MTX hydroxylation, serial blood measurements of 7-OHMTX were performed in four patients during monthly chemotherapy courses. Table 2 shows that there was a relative stability of peak plasma 7-OHMTX.

Discussion

This work has shown that during high-dose MTX chemotherapy as 6-h infusions 7-OHMTX appears rapidly in blood circulation, peak plasma values being observed at 6–12 h. The 7-OHMTX formed in this way is eliminated more slowly than MTX itself. Moreover, 24 h after the beginning of infusion 7-OHMTX becomes the main circulating product, with 7-OHMTX : MTX ratios varying around 2. These data are in agreement with results from previous studies, which mention that plasma concentrations of 7-OHMTX fall less rapidly than those of MTX [2, 7]. Chan et al. [4] indicated that for certain patients the metabolite levels at 24 h were more than twice those of MTX, even following conventional or low doses. Metabolic transformation of MTX to 7-OHMTX is probably due to an aldehyde oxidase of mainly hepatic origin [6]. We have shown that there was a positive and significant correlation between the peak plasma value of 7-OHMTX and the dose administered. Although these data were obtained in different patients, it can be assumed that hepatic hydroxylation of MTX is not saturated at these high doses. Consequently, a pharmacokinetic phenomenon of MTX dose-dependency cannot be the result of saturable hepatic metabolism, as suggested by Monjavel et al. [8], who proposed an elegant pharmacokinetic protocol for individualization of MTX dose schedules.

To evaluate the possible auto-induction of metabolic conversion of MTX to 7-OHMTX, peak plasma 7-OHMTX values were compared during spaced chemotherapy courses in four patients. An increase in this parameter was not a constant finding; thus, for the interval of time considered (2–5 months), enzymatic activity was not stimulated during successive monthly chemotherapy courses. In contrast, for shorter time intervals between chemotherapy courses (2–10 days), other authors [7] noticed a steplike rise in plasma concentrations of 7-OHMTX during three consecutive MTX infusions. To explain this, they proposed stimulation of the hydroxylating enzyme system.

It thus seems that with MTX chemotherapy intervals of 1 month hepatic hydroxylating activity of MTX returns to a normal level, whereas with shorter intervals (2–10 days) there is still auto-stimulation of MTX metabolism. This notion needs to be confirmed in a larger population, but if true would warrant reconsideration of the frequency of administration of MTX.

These data necessitate further clinical investigations. Since 7-OHMTX is known to be much less soluble at neutral or acid pH levels than MTX [6], obviously tubular precipitation of 7-OHMTX may occur during renal elimination. Metabolite formation could thus contribute to the nephrotoxicity associated with chemotherapy by MTX [1]. Furthermore, this induced renal failure may lead to delayed drug clearance and excretion, and finally to more or less severe myelosuppression due to drug accumulation in the body [3]. It is also possible, as suggested by Christophidis et al. [5], that 7-OHMTX competes with MTX for active tubular secretion [13] and thus contributes to MTX accumulation. However, it must be kept in mind that total elimination of 7-OHMTX in urine only accounts for 1%–10% of the MTX administered [2, 13].

Nevertheless, it would be interesting to check on the possible association between the degree of metabolic trans-

formation of MTX to 7-OHMTX and the presence of toxic manifestations. Furthermore, the study of the influence of 7-OHMTX on the cellular transport of MTX [7] warrants investigation.

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Received January 24, 1983 / Accepted May 5, 1983

Acknowledgments: The authors wish to thank F. Fein and N. Rameau for their assistance in translating and preparing the manuscript.