Br. J. clin. Pharmac. (1993), **35**, 409–412

The pharmacokinetics of methotrexate and its 7-hydroxy metabolite in patients with rheumatoid arthritis

PETER SEIDEMAN[1], OLOF BECK[2], STAFFAN EKSBORG[3] & MONA WENNBERG[2]
Department of Internal Medicine, Division of Rheumatology, Karolinska Institute, Danderyd Hospital[1], Department of Clinical Pharmacology[2], and Karolinska Pharmacy[3], Karolinska Hospital, Stockholm, Sweden

1 The pharmacokinetics of MTX and its 7-hydroxy metabolite (7-OHMTX) were investigated in nine patients with rheumatoid arthritis (RA). Each patient received 15 mg MTX i.v., i.m. and p.o. after an overnight fast in a randomized cross-over design. The plasma concentrations of MTX and 7-OHMTX were measured over 7 days and their urinary excretion over 24 h.

2 Plasma concentrations of MTX were described by a triexponential function after i.v. administration, a triexponential function with zero or first order absorption after oral administration, and a biexponential function with zero or first order absorption after i.m. injection. Plasma concentrations of 7-OHMTX were described by a biexponential function after all three routes of administration. The median terminal elimination half-lives of MTX and 7-OHMTX were 55 h and 116 h, respectively. The area under the plasma concentration-time curve (AUC (0,170 h)) of MTX did not differ between i.m. and oral administration indicating similar bioavailability after these routes of administration. The AUC (0,170 h) values of 7-OHMTX after i.v., oral and i.m. administration were similar. Over 80% of MTX was excreted in urine as intact drug and about 3% was excreted as 7-OHMTX during 24 h after drug administration.

3 Plasma concentrations of MTX and 7-OHMTX were measurable at the end of the dose interval in most of the patients and may help to identify non-responders or patients with increased risk of side-effects.

Keywords methotrexate 7-hydroxymethotrexate plasma concentrations urine excretion rheumatoid arthritis

Introduction

Methotrexate (MTX) is a folic acid derivative that inhibits dihydrofolate reductase, thereby decreasing tetrahydrofolate formation and DNA synthesis. Its use is well established in the treatment of various forms of malignancy but it is now also being used increasingly for the treatment of rheumatological disorders and psoriasis (Owen & Cohen, 1979; Tugwell *et al.*, 1987; Wilkens *et al.*, 1984). In psoriasis MTX was initially given in low (2.5 mg) daily doses. In a retrospective evaluation of MTX it was found that the hepatotoxicity was less in psoriatic patients when MTX was given intermittently once weekly instead of by daily dosing (Dahl *et al.*, 1971, 1972). As a result the standard dosing schedule was changed to weekly dosing. MTX was later introduced in the treatment of psoriatic arthritis (PA) and rheumatoid arthritis (RA) using a weekly dosing schedule. No investigations of the effect of daily MTX dosing on hepatotoxicity and clinical effects in PA and RA have been reported.

Although the kinetics of MTX following high doses for the treatment of neoplastic disease have been studied extensively, studies of intermittent low-dose MTX treatment have been restricted to the first 24 h after dosing and plasma drug concentrations throughout the dosage interval have not been reported (Balis *et al.*, 1983; Christophidis *et al.*, 1979; Edelman *et al.*, 1984; Evans *et al.*, 1982; Furst *et al.*, 1986; Herman *et al.*, 1989; Pearson *et al.*, 1987; Shen & Azarnoff, 1978; Sinett *et al.*, 1989; Teresi *et al.*, 1989; Wang & Fujimoto, 1984). In most of the reported studies MTX was measured by immunological assay and consequently plasma concentrations of 7-OHMTX could not be determined. This metabolite may have both therapeutic and toxic effects (Schroder, 1990). A comparative pharmacokinetic study

Correspondence: Dr Peter Seideman, Department of Internal Medicine, Division of Rheumatology, Karolinska Institute, Danderyd Hospital, S-182 88 Danderyd, Sweden

of intramuscular and oral MTX is also indicated as i.m. administration is advocated to increase bioavailability in certain patients.

Methods

Patients

Nine patients (two males) with RA and current inflammatory activity about to start MTX treatment were included in the study after giving informed consent. Their mean age was 60 years (range 47–72 years) and their mean weight was 65 kg (range 53–83 kg). The study was approved by the Local Ethics Committee. No other drugs were allowed from 3 days prior to the study until its completion. The patients had no history of hepatic disease or renal insufficiency; the results of liver enzyme tests were normal and the patients had normal serum creatinine values. After an overnight fast each patient received 15 mg MTX as an i.v. bolus, an i.m. injection or orally (2.5 mg tablets) at about 08.00 h with at least 1 week intervals in a randomized cross-over design. The tablets were given with 100 ml water. No food was allowed until 2 h after dosage when a breakfast of tea or coffee, bread, butter and cheese was taken. Venous blood samples were collected from an indwelling catheter into heparinized Venoject® tubes before administration of MTX and at 1 h intervals for 8 h and thereafter at 24, 48, 72, 96, 144 and 168 h after dosage. For practical reasons sampling was not according to this protocol at the later time points in some patients. The i.v. injection was given through a separate catheter on the contralateral side. The plasma was separated within 1 h after blood sampling and frozen immediately at −20° C. The analyses were performed within 4 months. The patients were asked to empty their bladders before administration of MTX and urine was collected thereafter for 24 h in 4 portions (0–3 h, 3–6 h, 6–8 h and 8–24 h). Urine volumes were measured and samples were frozen at −20° C until analysis.

Assay of MTX and 7-OHMTX

The plasma and urine concentrations of MTX and 7-OHMTX were measured by h.p.l.c. with fluorometric detection as described by Beck *et al.* (1991). The lower limit of determination defined as a signal to noise ratio of 10 was 0.2 nmol l^{-1} for MTX and 1.0 nmol l^{-1} for 7-OHMTX. The intra-day assay variation was less than 8% in the ranges 1–50 nmol l^{-1} of MTX and 4.6–230 nmol l^{-1} of 7-OHMTX. In the present series of experiments the inter-day assay variation of plasma spiked with 100 nmol l^{-1} MTX and 460 nmol l^{-1} 7-OHMTX was 3.0% (n = 18) and 4.1% (n = 18), respectively.

Pharmacokinetic analysis

The plasma concentrations of MTX and 7-OHMTX were fitted using an extended least square procedure (Sheiner & Beal, 1985). The optimal number of exponential terms was estimated by the Schwartz criterion (Schwartz, 1978). The goodness of fit was established by visual inspection and comparison of estimated and observed concentrations.

Areas under plasma concentration-time curves were determined by integration of the fitted curves from 1 h (or from the lag time when evaluable) to 170 h.

Statistical evaluation

The Mann-Whitney U-test and the Wilcoxon matched-pairs signed-rank test were used for the comparison of non-paired and paired observations, respectively. Medians and approximate 95% confidence intervals were evaluated as outlined by Wilcoxon (Daniel, 1978).

Results

Representative plasma drug and metabolite data are shown in Figure 1a,b,c. The plasma concentrations of

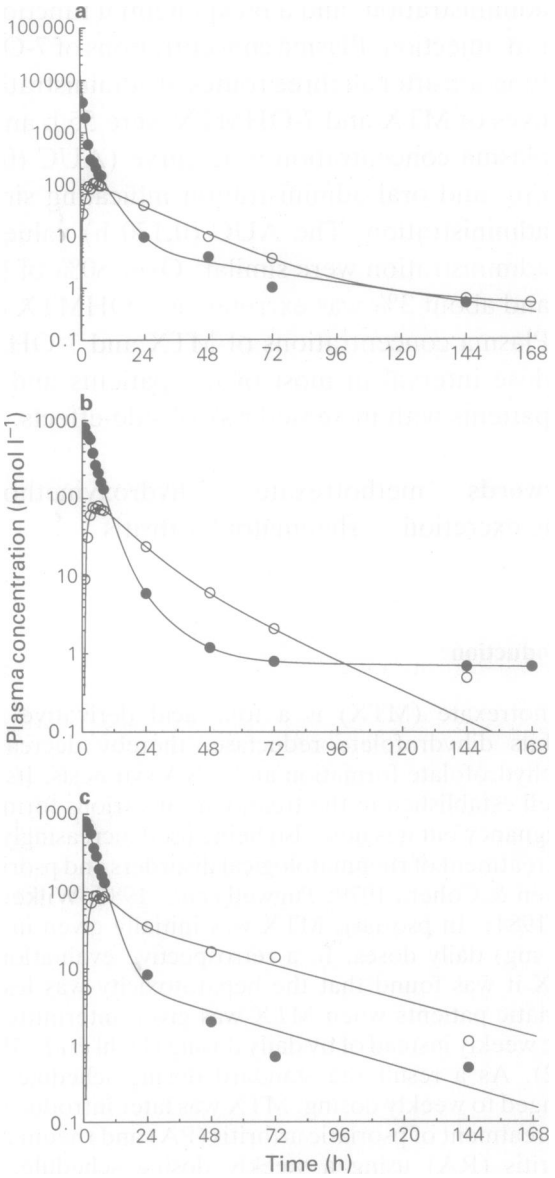

Figure 1 Plasma concentrations of MTX (●) and 7-OHMTX (○) in a representative patient (No 8) after 15 mg MTX given a) i.v., b) i.m. and c) p.o. Solid lines are fits of polyexponential functions.

Table 1 AUC values and 24 h urinary recoveries of MTX and 7-OHMTX in individual patients

	Plasma AUC (nmol l^{-1} h)					*24 h urinary excretion (% dose)*					
	MTX		*7-OHMTX*			*i.v.*		*Oral*		*i.m.*	
Patient	*Oral*	*i.m.*	*i.v.*	*Oral*	*i.m.*	*MTX*	*7-OHMTX*	*MTX*	*7-OHMTX*	*MTX*	*7-OHMTX*
1	4384	4100	3989	2793	2446	29.6	4.30	78.8	3.56	44.7	2.64
2	2529	2494	1438	1425	1130	107.5	4.49	84.1	3.43	98.0	2.54
3	4245	5395	5092	4950	4912	104.5	6.01	76.7	4.64	79.8	5.05
4	2579	4009	5912	5300	3805	104.4	10.7	84.5	8.87	105.1	12.7
5	4328	3676	2250	2105	—	104.4	4.10	111.0	2.84	101.3	2.60
6	2820	3688	814	737	742	69.0	2.06	42.1	2.12	59.4	1.67
7	3761	3956	2816	2157	2510	110.0	3.08	—	—	90.4	2.22
8	3394	3388	2295	2757	1589	85.8	4.28	72.3	2.90	96.5	3.33
9	3702	2896	1399	2189	1278	99.7	3.27	88.5	3.88	86.5	2.39
Median	3533	3699	2714	2473	2162	96.7	4.28	80.5	3.53	88.5	2.78
95% CI	2962–	3142–	1532–	1147–	1166–	67.0–	3.18–	63.1–	2.81–	69.6–	2.22–
	4073	4392	4104	3854	3679	106	6.99	94.9	6.15	98.9	7.57

MTX were best described by a triexponential function after intravenous and oral administration and by a biexponential function after intramuscular administration. The absorption phase after oral and intramuscular administration followed either zero- or first-order kinetics. The median terminal half-lives of MTX were 52 (95% CI 35–197 h), 49 (95% CI 36–86 h), and 59 (95% CI 40–108 h) after intravenous, oral and intramuscular administration, respectively.

Plasma concentrations of 7-OHMTX were described by a biexponential disposition function with either zero- or first-order input for all routes of administration. The median terminal half-lives of 7-OHMTX were 229 (95% CI 64–408 h), 49 (95% CI 20–309 h), and 141 (95% CI 38–305 h) h after intravenous, oral and intramuscular administration, respectively. On pooling data from all three routes of administration median half-lives of 55 h (95% CI 32–73 h) for MTX and 116 h (95% CI 44–387 h) for 7-OHMTX, were obtained. The trough plasma concentration (at 144–168 h) ranged between 0–1.3 nmol l^{-1} for MTX and between 0–3.7 nmol l^{-1} for 7-OHMTX.

The AUC (0, 170 h) of MTX did not differ between intramuscular and oral administrations (AUC ratio 0.98, 95% CI: 0.8–1.13) (Table 1) indicating a similar bioavailablity after these routes of administration. Also, AUC (0, 170 h) values of 7-OHMTX after intravenous, oral and intramuscular administrations were similar.

MTX was mainly excreted in urine as intact drug. The median 24 h recovery was 96.7%, 80.5% and 88.5% following intravenous, oral and intramuscular administration, respectively (Table 1). About 3% of the dose was excreted as the 7-OH metabolite over 24 h. The ratios of 24 h urinary recoveries of metabolite to drug were 0.0435, 0.0439 and 0.0404 after oral, i.v. and i.m. administration, respectively.

Discussion

The development of a sensitive h.p.l.c. assay enabled us to measure plasma MTX and 7-OHMTX concentrations over the entire dose interval of 1 week in rheumatic patients receiving MTX therapy. The observation of large variability in the data and a long terminal elimination half-life indicates the need for more extensive sampling to increase the accuracy and precision of the estimates of pharmacokinetic parameters. Individual values of the terminal half-lives of MTX and 7-OHMTX deviated substantially from median values, as well as from values obtained in the same individual for different routes of administration. MTX and 7-OHMTX concentrations at the end of the dosage interval were consistently near or below 1 nmol l^{-1}. The pharmacological and toxicological significance of these trace levels in RA patients remains to be determined. They may reflect release from intracellular pools. It has been established that both compounds are distributed intracellularly, partly in polyglutamated forms (Schröder, 1990). The measurement of MTX in erythrocytes showed a slow decline in concentration following termination of therapy (Schröder *et al.*, 1986; Schröder & Fogh, 1988). Therefore, the terminal elimination phase may be a useful measure of drug exposure in as much that plasma concentrations during this phase may reflect the intracellular pool of MTX.

The ability to measure MTX for a longer time explains why the data obtained in the present study were described by a triexponential function rather than the biexponential function reported by others (Edelman *et al.*, 1984; Furst *et al.*, 1986). The shorter elimination half-lives of MTX obtained in these studies are in accordance with our data during the first 24 h.

A study of RA patients by Herman *et al.* (1989) showed the oral bioavailability of MTX to be about 70%. In the present study plasma samples were not collected between 0 and 1 h and, therefore relative bioavailability after oral and i.m. administration could not be calculated accurately. However, the urine data show a slightly lower recovery (83%) after oral intake compared with i.m. administration (92%), supporting the results of Herman *et al.* (1989). In contrast, the median AUC ratio for oral and i.m. administration of 0.98, suggested a similar bioavailability for these routes of administration. This finding questions the clinical practise of changing from oral to i.m. administration to increase MTX bioavailabilty in a non-responder.

Similar metabolic ratios for the various routes of administration indicate that first pass metabolism is negligible with regard to the formation of 7-OHMTX. In a previous study of cancer patients given a dose of 30

mg/m^2 MTX with tritiated MTX as tracer, a greater recovery of metabolites was found in urine after oral intake compared with i.v. injection (Wang *et al.*, 1984). Metabolites other than 7-OHMTX may account for this observation. Although only a small fraction of MTX is converted to 7-OHMTX, the latter reaches significant concentrations in plasma often exceeding those of MTX.

MTX dosing in patients with rheumatic diseases and psoriasis remains arbitrary. It is mostly given as a single weekly dose or in three divided doses 12 h apart. Dermatologists use divided doses more often than rheumatologists. Despite its long and frequent use there are no studies comparing the clinical response to different dosing schedules. Our findings indicate that measurements of both peak and trough plasma MTX and 7-OHMTX concentrations may be useful in defining optimum dosing schedules and in identifying non-responders and patients at risk of developing side effects.

The skilful assistance of nurse Agneta Axelson is appreciated. The study was supported by grants from the Karolinska Institute Fund, the Swedish Association against Rheumatism Fund, the Swedish Association against Psoriasis Fund and the Smedby Fund.

References

Balis, F. M., Savitch, J. L. & Bleyer, W. A. (1983). Pharmacokinetics of oral methotrexate in children. *Cancer Res.*, **43**, 2342–2345.

Beck, O., Seideman, P., Wennberg, M. & Peterson, C. (1991). Trace analysis of methotrexate and 7-hydroxymethotrexate in human plasma and urine by a novel h.p.l.c. method. *Ther. Drug. Monit.*, **13**, 528–532.

Christophidis, N., Vajda, F. J. E., Lucas, I., Moon, W. J. & Louis, W. J. (1979). Comparison of intravenous and oral high-dose methotrexate in treatment of solid tumors. *Br. med. J.*, **1**, 298–300.

Dahl, M. G. G., Gregory, M. M. & Scheurer, P. J. (1971). Liver damage due to methotrexate in patients with psoriasis. *Br. med. J.*, **1**, 625–630.

Dahl, M. G. G., Gregory, M. M. & Scheuer, P. J. (1972). Methotrexate hepatotoxicity in psoriasis. Comparison of different dose regimens. *Br. med. J.*, **1**, 654–656.

Daniel, D. D. (1978). *Applied non-parametric statistics.* Boston: Houghyon Miflin Company.

Edelman, J., Biggs, D. F., Jamali, F. & Russel, A. S. (1984). Low-dose methotrexate kinetics in arthritis. *Clin. Pharmac. Ther.*, **35**, 382–386.

Evans, W. E., Stewart, C. F. & Huston, P. R. (1982). Disposition of intermediate dose methotrexate in children with acute lymphocytic leukemia. *Drug Intell. clin. Pharm.*, **16**, 839–842.

Furst, D. E., Haub, R., Faulkner, R. & Hoffman, J. (1986). Pharmacokinetics of low-dose methotrexate in rheumatoid arthritis (abstract). *Clin. Pharmac. Ther.*, **39**, 193.

Herman, R. A., Veng-Pedersen, P., Hoffman, J., Koehnke, Raachelle, & Furst, D. (1989). Pharmacokinetics of low-dose methotrexate in RA patients. *J. pharm. Sci.*, **78**, 165–171.

Owen, E. T. & Cohen, M. L. (1979). Methotrexate in Reiter's disease. *Ann. Rheum. Dis.*, **38**, 48–50.

Pearson, A. D., Mills, S., Amineddine, H. A., Long, D. R., Craft, A. W. & Chessels, J. M. (1987). Pharmacokinetics of oral and intramuscular methotrexate in children with acute lymphoblastic leukemia. *Cancer Chemother. Pharmac.*, **20**, 243–247.

Schröder, H. (1990). *In vivo* methotrexate kinetics and metabolism in human hematopoetic cells. *Danish Medical Bulletin*, **37**, 22–40.

Schröder, H., Clausen, N., Östergaard, E. & Pressler, T. (1986). Pharmacokinetics of erythrocyte methotrexate in children with acute lymphoblastic leukemia during maintenance treatment. *Cancer Chemother. Pharmac.*, **16**, 190–193.

Schröder, H. & Fogh, K. (1988). Methotrexate and its polyglutamate derivatives in erythrocytes during and after weekly low dose oral methotrexate therapy of children with acute lymphoblastic leukemia. *Cancer Chemother. Pharmac.*, **21**, 145–149.

Schwartz, G. (1978). Estimating the dimension of a model. *Ann. Stat.*, **6**, 461–464.

Sheiner, L. B. & Beal, S. L. (1985). Pharmacokinetic parameter estimates from several least squares procedures: Superiority of extended least squares. *Pharmacokin. Biopharm.* **13**, 185–201.

Shen, D. D. & Azarnoff, D. L. (1978). Clinical pharmacokinetics of methotrexate. *Clin. Pharmacokin.*, **3**, 1–13.

Sinett, M. J., Grof, G. D., Raddatz, D. A., Frank, W. A. & Bertino, J. J. (1989). Methotrexate pharmacokinetics in patients with rheumatoid arthritis. *J. Rheumatol.*, **16**, 745–748.

Teresi, M. E., Crom, W. R., Choi, K. E., Muire, J. & Evans, W. E. (1987). Methotrexate bioavailability after oral and intramuscular administration in children. *J. Pediatr.*, **110**, 788–792.

Tugwell, P., Bennet, K. & Gent, M. (1987). Methotrexate in rheumatoid arthritis. Indications, contraindications, efficacy and safety. *Ann. Int. Med.*, **107**, 358–366.

Wang, S. H. & Fujimoto, T. (1984). Clinical pharmacokinetics of methotrexate in children. *Clin. Pharmacokin.*, **9**, 335–348.

Wang, S. H., Huffman, D. H., Azarnoff, D. L., Stephens, R. & Hoogstraten, B. (1984). Effect of route of administration and effusions on methotrexate pharmacokinetics. *Cancer Res.*, **34**, 3487–3491.

Wilkens, R. F., Williams, J. & Ward, J. R. (1984). Randomized, double-blind, placebo controlled trial of low-dose pulse methotrexate in psoriatic arthritis. *Arthritis Rheum.*, **27**, 376–381.

(Received 17 December 1991, accepted 9 November 1992)