Patients with Rheumatoid Arthritis Treated with Methotrexate (MTX): Concentrations of Steady-state Erythrocyte MTX Correlate to Plasma Concentrations and Clinical Efficacy

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ABSTRACT. ***Objective.*** To investigate the accumulation of methotrexate (MTX) in circulating erythrocytes and the association with pharmacokinetic variables, weekly dose, and clinical efficacy in 2 cohorts of patients with chronic active rheumatoid arthritis (RA) undergoing MTX monotherapy.

Methods. Seventy-six patients with RA were included in this open prospective study: 40 were included before initiation of MTX therapy. Laboratory analyses, intracellular MTX concentrations in erythrocytes (Ery-MTX), and clinical examinations including toxicity data were performed prospectively for 52 weeks. Plasma concentrations of MTX were measured and area under the plasma concentration versus time curve (AUC) was estimated along with other pharmacokinetic variables in a population based software model.

Results. Ery-MTX rose after initiation of therapy and reached a steady state after 6–8 weeks. The correlation between steady-state Ery-MTX and dose was poor ($r^2 = 0.16$), whereas steady-state Ery-MTX levels correlated strongly with the estimated AUC ($r^2 = 0.51$, log-transformed variables). Both steady-state Ery-MTX levels and estimated AUC were significantly higher in patients responding to MTX therapy than in patients classified as nonresponders according to American College of Rheumatology core criteria and were similar to patients on longterm MTX therapy.

Conclusion. Our results indicate that clinical efficacy and Ery-MTX may have a causal relation and that measurement of Ery-MTX or estimation of AUC in a software model provides useful guidelines to the clinician when starting MTX therapy in patients with RA. The latter can be performed immediately after initiation of therapy. (First Release July 15 2008; J Rheumatol 2008;35:1709–15)

Key Indexing Terms:
RHEUMATOID ARTHRITIS METHOTREXATE ERYTHROCYTE
PHARMACOKINETICS TREATMENT EFFICACY

Low-dose orally administered methotrexate (MTX) has been used in the treatment of rheumatoid arthritis (RA) for many years. The beneficial effect is well established in large studies and MTX is still of great importance in the treatment of RA[1-7]. MTX is structurally related to folate and has antifolate properties. Administered in high doses for neoplastic diseases, MTX has antiproliferative effects inhibiting the enzymes dihydrofolate reductase and thymidylate synthetase involved in DNA synthesis. In low-dose regimens, MTX is retained intracellularly by the addition of glutamic acids, forming MTX polyglutamates[8,9]. The antiinflammatory effect is probably mediated by MTX polyglutamates inhibiting other enzyme systems, resulting in a release of the antiinflammatory agent adenosine. Although studies have addressed the issue, a direct inhibitory effect of MTX polyglutamates and the exact mode of action are still not completely clarified[4,10-15].

The initial dose of MTX treatment in RA is usually between 5 and 10 mg/week. It is administered orally and is rapidly absorbed from the gastrointestinal tract, although incompletely and with wide interindividual variability[16,17]. It is distributed to extravascular compartments and the majority of the drug is eliminated from the peripheral circulation within 24 hours. MTX accumulates in the erythrocytes as MTX polyglutamates and reaches a steady state after several weeks. The clinical effect or lack of efficacy is not concluded until after a minimum 2 months of therapy. At that time lack of efficacy or presence of side effects results

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Accepted for publication April 1, 2008.

in dosage escalation or discontinuation of MTX therapy. The clinical response is highly variable and unpredictable between patients and therefore there is a continuous search for metabolic and genetic biomarkers and the correlation to clinical response in low-dose regimes. The correlation between MTX polyglutamates and clinical efficacy of MTX therapy has been investigated, but with variable and inconclusive results[18-23]. However, 2 recent studies point to a value in monitoring low-dose MTX therapy by means of intracellular erythrocyte MTX concentrations[24,25].

The pharmacokinetics of low-dose MTX therapy have been described by many investigators in different clinical settings and study designs, but the usefulness of measuring plasma concentrations in low-dose therapy remains to be clarified[16,26-35].

The aim of our open prospective study was to investigate the use of MTX concentrations in erythrocytes (Ery-MTX) in the treatment of RA and the correlation between plasma MTX concentration and intracellular MTX concentrations. We measured the concentrations of MTX in erythrocytes and plasma in patients starting MTX therapy and in patients undergoing longterm treatment with MTX. Pharmacokinetic variables were estimated in a population based software model, and area under the plasma concentration versus time curve (AUC) was calculated by the trapezoidal method. Clinical efficacy and toxicity were evaluated clinically and biochemically and the results from MTX responders and nonresponders were compared. In addition, responders were compared to patients undergoing longterm MTX treatment.

MATERIALS AND METHODS

Eighty-one patients initially gave written consent and were included in the study. Of these, 5 patients were excluded because they proved not to fulfil the 1987 revised criteria for RA of the American College of Rheumatology (ACR)[36] or withdrew for personal reasons before any data were obtained. Of the remaining 76 patients, 6 withdrew after 10–24 weeks of followup, 1 died, and in 2 patients MTX therapy was discontinued because of side effects in the followup period.

All patients had chronic active RA and were treated with orally administered MTX. Forty patients were included before the initiation of MTX therapy after at least 4 weeks of washout if the patient had received other disease modifying antirheumatic drugs (DMARD). MTX dose was regulated by a rheumatologist independently from the study. The initial dose was 5 to 10 mg (5 mg in 4, 7.5 mg in 32, and 10 mg in 4 patients). The remaining 36 patients had been treated with MTX for 41 ± 25 months (mean ± SD) at the time of inclusion, with doses of 5 to 20 mg per week (5 mg in one patient, 7.5 mg in 9, 10 mg in 8, 12.5 mg in 6, 15 mg in 5, 17.5 mg in 6, and 20 mg in one patient). The dose had been stable for at least 3 months and no patient was receiving other DMARD. Change of dosage was independent of the study and followed a treatment strategy based on optimal balance between efficacy and side effects, increasing the weekly dose with 2.5 mg every 2 to 3 months until the desired clinical effect was achieved.

The study complied with the Declaration of Helsinki, and the research protocol was approved by the ethical committee, Aarhus, Denmark.

Clinical evaluation. Patients were evaluated clinically by the same physician at Weeks 0, 12–16, 28, and 52. Disease activity was assessed by swollen joint count (maximum 38), tender joint count (maximum 40), and global assessment of disease activity by the physician on a 5-point verbal rating scale. Questionnaires with global assessment of disease activity and pain on a numerical rating scale (0–10) and Stanford Health Assessment Questionnaire (HAQ score) for functional activity were completed by all patients at time intervals similar to blood sampling. Simultaneously, toxicity data were obtained by questionnaires and laboratory tests.

Response to MTX was evaluated according to preliminary ACR core criteria; response categories were none, 20%, or 50%[27]. Patients were classified as responders if they fulfilled the core criteria on at least one occasion during the followup period.

Laboratory assessments. Blood samples were drawn at Weeks 0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 28, and 52 and analyzed for C-reactive protein level, erythrocyte sedimentation rate (ESR), complete blood cell count, fraction of neutrophils, platelet count, and serum analyses of alanine aminotransferase (ALT), alkaline phosphatase and creatinine. Analyses were performed by routine methods.

Erythrocyte MTX analysis. MTX content in erythrocytes was measured in all blood samples by a radio chemical-ligand binding assay[18]. Briefly, erythrocyte concentrate was lyzed with phosphate buffer and the hemoglobin (mmol/l) determined photometrically (wavelength 540 nm). The samples were boiled for 10 min and centrifuged at 3000 rpm for 10 min, and the clear supernatants were stored at –80°C until tested for MTX content. This was performed with bovine dihydrofolate reductase (Sigma, St. Louis, MO, USA) as binder, [^{3}H]MTX (Moravek Biochemicals, La Brea, CA, USA) as tracer, and NADPH tetrasodium salt (Boehringer Mannheim, Indianapolis, IN, USA) as cofactor. Unlabeled MTX (Bie Berntsen, Denmark) diluted with phosphate buffer was prepared in concentrations from 2 to 12 nmol/l and used as standards. The standards were aliquoted in vials and kept frozen at –80°C. A new set of standard aliquots was thawed for each run. Controls were prepared at levels of 0.7, 2.0, and 5.0 nmol/l and were tested as unknown samples before being used as controls. The controls were kept frozen at –80°C and new preparation sets of standards and controls were never brought into use simultaneously. The reaction was performed in reaction buffer (phosphate buffer and NADPH tetrasodium salt) and terminated with ice-cold (4°C) dextran-coated charcoal (Sigma). This was followed by centrifugation at 2600 rpm for 15 min to pellet the charcoal, and radioactivity in supernatants was counted in a liquid scintillation beta counter. Standards, controls, and samples were run in duplicates. Quality criteria were linearity of the standard curve and accuracy of the controls. The sensitivity of the assay was < 1 nmol/l as determined by the activity of a blank plus 3 times the standard deviation of the lowest control. The coefficient of variation (CV%) of the assay was below 20% as determined by the largest CV% of the controls based on day to day results. Steady-state Ery-MTX was calculated as the mean level from Week 6, unless Ery-MTX increased even further, in which case steady-state Ery-MTX was calculated from Week 8.

Plasma MTX concentrations. For measurement of MTX plasma concentrations, heparinized venous blood samples (2 ml) were drawn before intake of MTX and at 1, 2, 4, 6, 12, and 24 hours after MTX intake. This was performed once for each patient in order to calculate the AUC for the current MTX dose.

Tubes were centrifuged at 1800 rpm for 10 min and plasma was collected and kept frozen at –80°C until analyzed for MTX content. MTX concentrations were measured by a fluorescence polarization immunoassay using a commercial kit for measuring low concentrations of MTX (Abbott Diagnostics, Abbott Park, IL, USA). Detection limit was 0.02 µmol/l and CV% of the assay was between 5% and 15% depending on the level.

Pharmacokinetic analysis. Data were analyzed in a 2-compartment model with first-order kinetics, which has been shown to characterize the disposition of MTX[28,33,34,37]. The following parameters were used: k_a: oral absorption rate constant (h^{-1}); Cl: systemic clearance (l/h); V_c: distribution volume of central compartment (l); Q: intercompartmental clearance (l/h); V_p: distribution volume of peripheral compartment (l). As usual in pharmacokinetic analyses of orally administered drugs, all clearance and volume terms are apparent parameters, i.e., Cl, V_c, Q, and V_p are scaled by the bioavailability (the fraction of the administered dose absorbed intact), F, of

MTX; i.e., Cl = Cl_u/F, etc., where Cl_u is the unscaled clearance. The unscaled parameters are what would have been measured in the hypothetical clinical situation with 100% bioavailability. To determine the unscaled parameters the scaled parameters should be multiplied by the bioavailability, which was not estimated in this study, however.

Data were analyzed in a nonlinear mixed-effects model where pharmacokinetic variables were assumed to follow normal distributions across individuals, and where the residual error was multiplicative and normally distributed. These assumptions were checked by graphic examination by QC plots of best linear unbiased predictors (BLUP). The model was fitted with the NLMIXED procedure in SAS rel. 8.02 (http://www.sas.com), with possibility for nonlinear mixed effects modeling using a first-order approximation and initial parameter values taken from Godfrey, *et al*[34]. For each patient the scaled BLUP for k_a, Cl, V_c, Q, and V_p were estimated and AUC (AUC_BLUP) was calculated as dose/Cl. AUC was also calculated by the trapezoidal rule (AUC_Trap).

Statistical analysis. Statistical analysis was performed by paired Student t-test (unpaired when appropriate), and Mann-Whitney test if data were not normally distributed. Comparison of frequencies was by Fisher's exact test. Correlation of data was estimated by weighted linear regression analysis. Differences were considered significant if p values were less than 0.05, and all statistical tests were performed using GraphPad Prism software and GraphPad Instat software or SAS rel. 8.02.

RESULTS

We obtained data for 93 series of MTX concentration measurements for a total of 73 patients. Fifty-four patients had only one series of measurements, 18 patients had 2, and one patient had 3. The reason for some patients having more than one series of measurements was change of the weekly dose for at least 8 weeks. The number of measurements within series was 3 for one series, 4 for one, 5 for 11, 6 for 18, and 7 measurements for 62 series. There were no plasma MTX results for 3 patients because of discontinuation of MTX therapy before the blood samples were drawn or for practical reasons.

Demographic data, clinical characteristics, and laboratory results at study entry are shown in Table 1. The demographic data were similar in the 2 groups, whereas disease activity was lower in the group established on MTX therapy. In Group 1, a total of 61% of the patients responded to MTX treatment, 46% had an ACR 50% response, and 15% obtained an ACR 20% response.

Toxicity. We noted 82.5% in Group 1 and 67.7% in Group 2 reported signs of side effects at at least one visit during the followup period. In Group 1, MTX therapy had to be discontinued in 2 patients for toxicity reasons. The most frequently reported side effects were gastrointestinal or mucocutaneous effects such as nausea, mouth ulcers, stomatitis, and diarrhea (55% of patients in Group 1 and 50% in Group 2). Other side effects reported were rashes, cough, flu-like symptoms, itch, and loss of hair (62.5% in Group 1; 38.9% in Group 2). The frequency of side effects was not significantly different in the 2 groups (p = 0.12), and there was no difference between responders and nonresponders in Group 1 (p = 0.86).

Intracellular concentration of MTX and correlation to MTX dose. The concentration of MTX measured in hemolyzed

Table 1. Demographic data and disease activity in patients starting MTX therapy (Group 1) and in patients well established on MTX therapy (Group 2). There was no difference between the 2 groups in demographic data, but disease activity was significantly higher in Group 1.

Characteristic	Group 1, n = 40	Group 2, n = 36	
Age, yrs	55.2 (12.4)	57.7 (10.7)	
Female, %	68	75	
Weight, kg	71.9 (15.4)	70 (11.3)	
Height, cm	169 (10)	168 (9)	
Disease duration, yrs	11.8 (10.2)	13.5 (9.4)	
MTX dose, mg/wk	7.5 (5–10)	11.9 (5–20)	
MTX therapy, yrs	0	3.4 (2.1)	
Patients (%) with concurrent use of			
Mild analgesic	83	72	
Opioid analgesic	25	25	
Corticosteroid	18	33	
Creatinine (μmol/l), females	67 (9)	67 (13)	
Creatinine (μmol/l), males	74 (8)	80 (8)	
Disease activity			
No. tender joints (max. 40)	10.8 (9.6)	4.5 (4.5)	p < 0.01
No. swollen joints (max. 38)	6.7 (6.5)	1.8 (2.4)	p < 0.001
Doctor's disease activity score (1–10)	4.7 (2.3)	3.3 (2.3)	p < 0.001
Doctor's global score (0–4)	1.9 (0.9)	1.2 (0.8)	p < 0.001
ESR, mm/h	31 (23)	27 (18)	NS
C-reactive protein, mmol/l	265 (217)	184 (141)	NS
HAQ	0.85 (0.59)	0.77 (0.56)	NS
Patient's disease activity score (1–10)	4.7 (2.3)	3.3 (2.3)	p < 0.01

HAQ: Health Assessment Questionnaire.

erythrocytes (Ery-MTX) increased during the first 6 to 8 weeks of therapy and reached a steady-state level in most patients at that time. Figure 1 shows mean ± 1 SD data of intracellular MTX concentrations measured in 32 patients starting MTX therapy of 7.5 mg weekly. Mean steady-state levels of Ery-MTX in 9 patients treated with 7.5 mg/week MTX for a longer period (Group 2) are shown in Figure 2. Weekly dose of MTX and steady-state Ery-MTX levels were only feebly correlated ($r^2 = 0.16$).

Pharmacokinetic results. The estimates of pharmacokinetic measures are shown in Table 2. The median AUC was 10 mg/(3.87 l/h) = 2.6 mg/l h. Steady-state Ery-MTX for a given series was estimated as the average of the Ery-MTX measurements, when the patient was considered to be in steady state. Figure 3 shows the relation between steady-state Ery-MTX and AUC_BLUP (untransformed values).

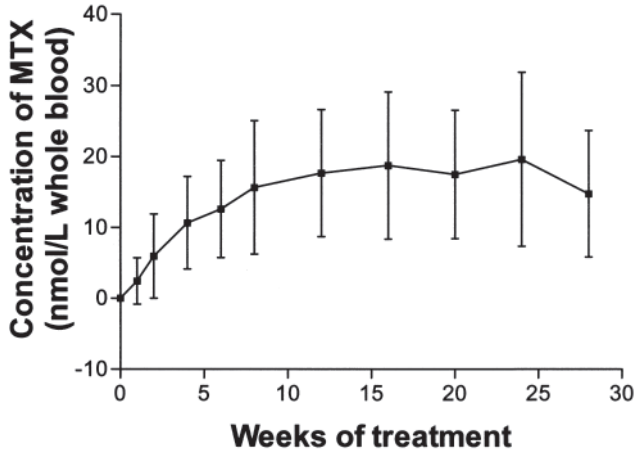

Figure 1. Ery-MTX levels (mean ± 1 SD) of 32 patients in Group 1 starting treatment with 7.5 mg MTX weekly. Results are from each visit during the first 28 weeks of followup.

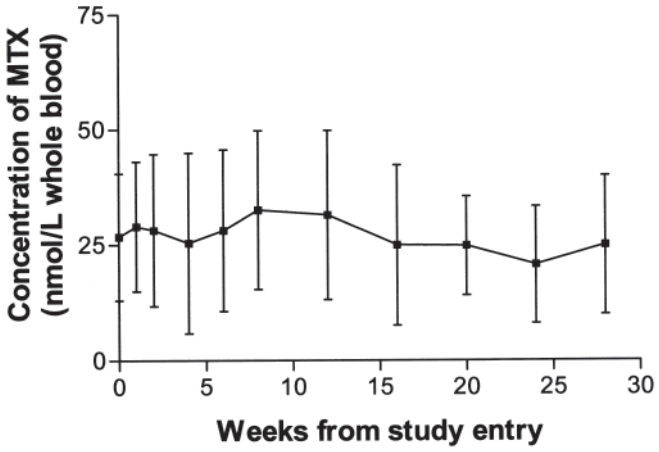

Figure 2. Ery-MTX levels (mean ± 1 SD) in 9 patients from Group 2 treated with 7.5 mg/week. Results are from each visit during the first 28 weeks of followup.

The correlation between plasma and intracellular MTX concentrations was estimated by weighted linear regression of the logarithm of steady-state Ery-MTX on the logarithm of AUC, with weights being the number of measurements on which the average concentration was based. Logarithmically transformed values were applied in order to obtain normal distribution of the residuals. AUC_BLUP was found to be significantly correlated with steady-state level of Ery-MTX ($p = 0.0001$, $r^2 = 0.51$, log values). Our results show that for a given value of AUC_BLUP, the mean Ery-MTX is approximately $9.97 \times \text{AUC\_BLUP}^{1.02}$. Moreover, the coefficient of variation (CV) of the random prediction error is approximately 99.9%.

AUC calculated by the trapezoidal method showed the same association to steady-state Ery-MTX level ($r^2 = 0.51$, log values), but here the residuals were not normally distributed and the correlation is therefore less interpretable.

MTX concentrations and clinical effect. Mean steady-state Ery-MTX levels and AUC_BLUP of responders in Group 1 were significantly higher than in nonresponders (Table 3). There was no significant difference between responders and nonresponders when AUC was calculated by the trapezoidal method. Patients who were classified as responders received significantly higher MTX doses than the nonresponding patients. There was no significant difference between Group 2 and responders in Group 1. The findings were similar if ACR 50% responders were evaluated [mean (SD) weekly dose 11.6 mg (3.7); Ery-MTX: 27.24 nmol/l (14.9); AUC_BLUP: 2.70 mg/l h (0.98); AUC_Trap: 2.30 mg/l h (0.78)], and were not statistically different from the "minimum ACR 20% response" criteria. The latter is expected since the majority were ACR 50% responders.

DISCUSSION

Low-dose MTX is widely used in the treatment of RA and is tolerated relatively well compared to other DMARD. Moreover, the average treatment period is significantly longer than with other drugs[38-40]. The main reasons for termination of MTX therapy are lack of efficacy and unacceptable side effects. Treatment with MTX usually requires dose adjustments until efficacy and side effects are balanced to a tolerable level. The clinical efficacy is evaluated according to several variables and it often lasts several weeks until a definite response/or nonresponse is concluded.

It is well known that MTX accumulates in erythrocytes during the first 6–8 weeks of treatment, as confirmed in our study. The steady-state Ery-MTX levels showed considerable interindividual variation, which may be explained by the well known variations in oral absorption and renal excretion of the drug. The correlation between dose and steady-state Ery-MTX was statistically significant, but weak, and was not applicable to predict the steady-state Ery-MTX level in a specific patient. Ery-MTX levels were significant-

Table 2. Estimates of pharmacokinetic parameters (scaled by bioavailability).

Parameter	Estimate	LCL (95%)	UCL (95%)	CV%
k_a Oral absorption rate constant (h^{-1})	2.06	1.68	2.53	800
CI Systemic clearance (l/h)	3.87	3.52	4.25	37
V_c Distribution volume of central compartment (l)	18.29	16.21	20.64	35
Q Intercompartmental clearance (l/h)	1.32	1.03	1.69	86
V_p Distribution volume of peripheral compartment (l)	13.01	9.05	18.70	152

LCL/UCL: lower and upper 95% confidence interval and coefficient of variation (CV%) of the estimated value.

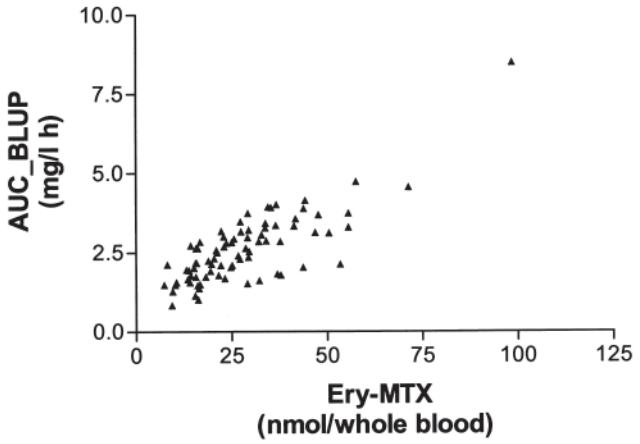

Figure 3. Relation of steady-state Ery-MTX and nontransformed AUC_BLUP values in all patients.

ly higher in responders than in nonresponders, which is in agreement with other studies demonstrating a correlation between intracellular MTX and efficacy or adverse effects, although conflicting results have been described[18-25]. It is interesting that nonresponders were treated with significantly lower doses than the responders in our study, and in this context it is worth emphasizing that the rheumatologist advising a specific dose regimen was independent of the study. Thus, nonresponders may have been found to be MTX responders if treated with a higher dose. A possible explanation may be that the nonresponders were maintained in a low-dose regimen because of signs of side effects, although there was no significant difference in the presence of side effects in the 2 groups. This underlines the need for markers to monitor MTX therapy, and that Ery-MTX may be a useful tool for the rheumatologist to increase dosages more aggressively in order to prevent undertreatment.

Steady-state Ery-MTX levels were significantly higher in responders than in nonresponders, and were similar in responders and in patients on longterm MTX therapy. It has been shown that the duration of MTX monotherapy without addition of other DMARD indicates effectiveness of the drug, and it is clear that patients well established on MTX therapy obviously tolerate MTX treatment and represent prolonged MTX responders[41]. Therefore it is tempting to speculate that clinical response is achieved when steady-state Ery-MTX reaches a certain level and that a certain steady-state Ery-MTX level is indicative of the therapeutic effect. This is in agreement with a recent study in which low levels of erythrocyte MTX polyglutamates were associated with high disease activity, although that study did not distinguish between patients well established on MTX therapy and patients starting MTX therapy[24]. Our study presents further evidence of the significance of intracellular levels of MTX and supports the hypothesis that the antiinflammatory effect is related to intracellular accumulation of MTX, although a direct causal relationship remains to be proven. There was no correlation between the presence of side effects and Ery-MTX concentrations in our study. This may probably be due to the relatively low dosing regimes and small variations in Ery-MTX levels. A larger study group with more pronounced variation in dose and Ery-MTX concentrations is needed to investigate this further.

Similar to the interindividual variation in steady-state Ery-MTX levels, we also found considerable interindividual

Table 3. Mean (SD) Ery-MTX, AUC, and dose for group 1 and Group 2. Group 1 is divided into responders and nonresponders. The first column of p values compares responders and nonresponders; second column of p values compares responders in Group q and Group 2.

	Group 1			Group 2	
	Responders	Nonresponders	p		p
Ery-MTX, nmol/l whole blood	25.74 (12.99)	17.44 (7.51)	0.013	32.52 (17.10)	0.138
AUC_BLUP, mg/l h	2.70 (0.86)	1.99 (0.75)	0.002	2.84 (1.27)	0.646
AUC_Trap, mg/l h	2.35 (0.79)	1.95 (1.40)	0.206	2.85 (1.35)	0.099
MTX dose, mg/wk	11.1 (4.2)	7.9 (1.9)	< 0.001*	11.4 (3.8)	0.648*

* Mann-Whitney test.

variation in MTX plasma concentrations. The findings are in accord with the variation in bioavailability, C_{max}, and AUC observed in other studies[16,17,27,34,37]. The pharmacokinetic variables of MTX may also be influenced by use of nonsteroidal antiinflammatory drugs (NSAID), which have been reported to decrease total clearance of MTX. This may have occurred in some patients in our study, although other investigators have not been able to show an influence of NSAID on MTX clearance[30,31,42-44].

In order to estimate pharmacokinetic parameters and predicted pharmacokinetic variables for each patient a software model was applied. The mean Cl 3.87 l/h found in our study is comparable to Cl values reported by others[27,34,45]. The correlation between AUC_BLUP and AUC_Trap was very poor for some patients, most likely because of the limited number of measurements in these patients. Since a credible AUC calculated with the trapezoidal method requires several blood samples the software model is useful in clinical situations where multiple blood sampling is inapplicable. AUC_BLUP and steady-state Ery-MTX levels were highly correlated, implying that MTX plasma concentrations predict steady-state Ery-MTX level, which may be indicative of the MTX response.

The pharmacokinetics of low-dose MTX have been described previously, but few studies have compared the pharmacokinetic variables with clinical outcome and intracellular MTX concentrations. In these studies there was either no correlation of the pharmacokinetic parameters with the clinical effect or there were correlations to one clinical parameter[16,35,45-47].

In summary we show that AUC estimated in a software model is highly correlated to steady-state Ery-MTX concentrations. We also show that MTX responders have significantly higher concentrations of intracellular MTX than nonresponders, which suggests an association between the level of intracellular MTX and clinical effect. Our findings are in agreement with the hypothesis that the effect is related to intracellular accumulation of MTX, and that the intracellular level is directly correlated to plasma concentrations and less closely correlated to the dose. It also indicates that either Ery-MTX or plasma MTX measurements, easily implemented in a standard laboratory, may be useful in optimizing MTX treatment and guiding the clinician in determining the appropriate dosage regimen for a given patient, thereby achieving the desired clinical efficacy more quickly.

Further studies are needed to validate and establish a routine method to predict Ery-MTX. It is necessary to investigate the prediction model as intended with a dosage strategy with simultaneous analysis of pharmacokinetic measures and individual Ery-MTX measurements and to combine this with a distinct clinical outcome. Finally, a limited sampling strategy is relevant to verify the prediction model with fewer plasma measurements, and such studies are in progress[48].

REFERENCES

1. Tugwell P, Bennet K, Gent M. Methotrexate in rheumatoid arthritis. Indications, contraindications, efficacy and safety. Ann Intern Med 1987;107:358-66.
2. Weinblatt ME, Maier AL, Fraser PA, et al. Longterm prospective study of methotrexate in rheumatoid arthritis: conclusion after 132 months of therapy. J Rheumatol 1998;25:238-42.
3. Case JP. Old and new drugs used in rheumatoid arthritis: a historical perspective. Part 1: the older drugs. Am J Ther 2001;8:123-43.
4. O'Dell JR. Methotrexate use in rheumatoid arthritis. Rheum Dis Clin North Am 1997;23:779-96.
5. Wluka A, Buchbinder R, Mylvaganam A, et al. Longterm methotrexate use in rheumatoid arthritis: 12 year followup of 460 patients treated in community practice. J Rheumatol 2000;27:1864-71.
6. Hoekstra M, van de Laar MA, Bernelot Moens HJ, et al. Longterm observational study of methotrexate use in a Dutch cohort of 1022 patients with rheumatoid arthritis. J Rheumatol 2003;30:2325-9.
7. Cronstein BN. Low-dose methotrexate: a mainstay in the treatment of rheumatoid arthritis. Pharmacol Rev 2005;57:163-72.
8. Chabner BA, Allegra CJ, Curt GA, et al. Polyglutamation of methotrexate. Is methotrexate a prodrug? J Clin Invest 1985;76:907-12.
9. Kremer JM. Toward a better understanding of methotrexate. Arthritis Rheum 2004;50:1370-82.
10. Kremer JM. The mechanism of action of methotrexate in rheumatoid arthritis: the search continues. J Rheumatol 1994;21:1-5.
11. Van Ede AE, Laan RFJM, Blom HJ, et al. Methotrexate in rheumatoid arthritis: an update with focus on mechanisms involved in toxicity. Semin Arthritis Rheum 1998;27:277-92.
12. Chan ES, Cronstein BN. Molecular action of methotrexate in inflammatory diseases. Arthritis Res 2002;4:266-73.
13. Cronstein BN. The mechanism of action of methotrexate. Rheum Dis Clin North Am 1997;23:739-55.
14. Dolezalova P, Krijt J, Chladek J, et al. Adenosine and methotrexate polyglutamate concentrations in patients with juvenile arthritis. Rheumatology Oxford 2005;44:74-9.
15. Morabito L, Montesinos MC, Schreibman DM, et al. Methotrexate and sulfasalazine promotes adenosine release by a mechanism that requires ecto'-5'-nucleotidase-mediated conversion of adenosine nucleotides. J Clin Invest 1998;101:295-300.
16. Bannwarth B, Pehourcq F, Schaeverbeke T, et al. Clinical pharmacokinetics of low-dose pulse methotrexate in rheumatoid arthritis. Clin Pharmacokinet 1996;30:194-210.
17. Seideman P. Methotrexate — the relationship between dose and clinical effect. Arthritis Rheum 1994;37:830-3.
18. Kamen BA, Nylen PA, Camitta BM, et al. Methotrexate accumulation and folate depletion in cells as a possible mechanism of chronic toxicity to the drug. Br J Haematol 1981;49:355-60.
19. Angelis-Stoforidis P, Vajda FJ, Christophidis N. Methotrexate polyglutamate levels in circulating erythrocytes and polymorphs correlate with clinical efficacy in rheumatoid arthritis. Clin Exp Rheumatol 1999;17:313-20.
20. Dervieux T, Furst D, Lein DO, et al. Polyglutamation of methotrexate with common polymorphisms in reduced folate carrier, aminoimidazole carboxamide ribonucleotide transformylase, and thymidylate synthase are associated with methotrexate effects in rheumatoid arthritis. Arthritis Rheum 2004;50:2766-74.
21. Fathi NH, Mitros F, Hoffman J, et al. Longitudinal measurement of methotrexate liver concentrations does not correlate with liver damage, clinical efficacy, or toxicity during a 3.5 year double blind study in rheumatoid arthritis. J Rheumatol 2002;29:2092-8.

22. Duby S, Karsh J, Prchal JF, et al. Measurements of red blood cell methotrexate concentrations and lymphocyte subsets during therapy of rheumatoid arthritis. Clin Exp Rheumatol 1985;3:5-9.
23. Kristensen K, Nielsen S, Karup Pedersen F, et al. Erythrocyte-methotrexate and disease activity in children treated with oral methotrexate for juvenile chronic arthritis. Scand J Rheumatol 2000;29:187-9.
24. Dervieux T, Furst D, Lein DO, et al. Pharmacogenetic and metabolite measurements are associated with clinical status in patients with rheumatoid arthritis treated with methotrexate: results of a multicentred cross sectional observational study. Ann Rheum Dis 2005;64:1180-5.
25. Dervieux T, Greenstein N, Kremer J. Pharmacogenomic and metabolic biomarkers in the folate pathway and their association with methotrexate effects during dosage escalation in rheumatoid arthritis. Arthritis Rheum 2006;54:3095-103.
26. Edelman J, Biggs DF, Jamali F, et al. Low-dose methotrexate kinetics in arthritis. Clin Pharmacol Ther 1984;35:382-6.
27. Herman RA, Vengpedersen P, Hoffman J, et al. Pharmacokinetics of low-dose methotrexate in rheumatoid arthritis patients. J Pharm Sci 1989;78:165-71.
28. Sinnet MJ, Groff GD, Raddatz DA, et al. Methotrexate pharamcokinetics in patients with rheumatoid arthritis. J Rheumatol 1989;16:745-8.
29. Brooks PJ, Spruill WJ, Parish RC, et al. Pharmacokinetics of methotrexate administered by intramuscular and subcutaneous injections in patients with rheumatoid arthritis. Arthritis Rheum 1990;33:91-4.
30. Tracy TS, Krohn K, Jones DR, et al. The effects of a salicylate, ibuprofen, and naproxen on the disposition of methotrexate in patients with rheumatoid arthritis. Eur J Clin Pharmacol 1992;42:121-5.
31. Tracy TS, Worster T, Bradley JD, et al. Methotrexate disposition following concomitant administration of ketoprofen, piroxicam and flurbiprofen in patients with rheumatoid arthritis. Br J Clin Pharmacol 1994;37:453-6.
32. Kremer JM, Petrillo GF, Hamilton RA. Pharmacokinetics and renal function in patients with rheumatoid arthritis receiving a standard dose of oral weekly methotrexate: association with significant decreases in creatinine clearance and renal clearance of the drug after 6 months of therapy. J Rheumatol 1995;22:38-40.
33. Kremer JM, Petrillo GF, Hamilton RA. Examination of pharmacokinetic variables in a cohort of patients with rheumatoid arthritis beginning therapy with methotrexate compared with a cohort receiving the drug for a mean of 81 months. J Rheumatol 1995;22:41-4.
34. Godfrey C, Sweeney K, Miller K, et al. The population pharmacokinetics of long-term methotrexate in rheumatoid arthritis. Br J Clin Pharmacol 1998;46:369-76.
35. Hiraga Y, Yuhki Y, Itoh K, Tadano K, Takahashi Y, Mukai M. Pharmacokinetics and efficacy of low-dose methotrexate in patients with rheumatoid arthritis. Mod Rheumatol 2004;14:135-42.
36. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
37. Seideman P, Beck O, Eksborg S, et al. The pharmacokinetics of methotrexate and its 7-hydroxy metabolite in patients with rheumatoid arthritis. Br J Clin Pharmacol 1993;35:409-12.
38. Scully CJ, Anderson CJ, Cannon GW. Long-term methotrexate therapy for rheumatoid arthritis. Semin Arthritis Rheum 1991;20:317-31.
39. Weinblatt ME. Efficacy of methotrexate in rheumatoid arthritis. Br J Rheumatol 1995;34 Suppl 2:43-8.
40. Wolfe F. Adverse drug reactions of DMARDs and DC-ARTs in rheumatoid arthritis. Clin Exp Rheumatol 1997;15 Suppl 17:S75-81.
41. Ortendahl M, Schettler JD, Fries JF. Factors influencing length of time taking methotrexate in rheumatoid arthritis. J Rheumatol 2000;27:1139-47.
42. Skeith KJ, Russell AS, Jamali F, et al. Lack of significant interaction between low dose methotrexate and ibuprofen or flurbiprofen in patients with arthritis. J Rheumatol 1990;17:1008-10.
43. Stewart CF, Fleming RA, Arkin CR, et al. Coadministration of naproxen and low-dose methotrexate in patients with rheumatoid arthritis. Clin Pharmacol Ther 1990;47:540-6.
44. Ahern M, Booth J, Loxton A, et al. Methotrexate kinetics in rheumatoid arthritis: is there an interaction with nonsteroidal antiinflammatory drugs? J Rheumatol 1988;15:1356-60.
45. Wiela-Hojenska A, Orzechowska-Juzwenko K, Swierkot J, et al. Monitoring methotrexate therapy in patients with rheumatoid arthritis. Int J Clin Pharmacol Ther 2004;42:434-41.
46. Lafforgue P, Monjanel-Mouterde S, Durand A, et al. Lack of correlation between pharmacokinetics and efficacy of low dose methotrexate in patients with rheumatoid arthritis. J Rheumatol 1995;22:844-9.
47. Bologna C, Anaya JM, Bressolle F, et al. Correlation between methotrexate pharmacokinetic parameters, and clinical and biological status in rheumatoid arthritis patients. Clin Exp Rheumatol 1995;13:465-70.
48. Eksborg S, Albertioni F, Beck O, Peterson C, Seideman P. Methotrexate in rheumatoid arthritis — a limited sampling strategy for estimation of the area under the plasma concentration versus time curve. Ther Drug Monit 1994;16:560-3.