Splitting High-Dose Oral Methotrexate Improves Bioavailability: A Pharmacokinetic Study in Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To study the bioavailability of a divided higher oral dose of methotrexate (MTX), in comparison to a single dose, in adult patients with rheumatoid arthritis (RA).

Methods. A pharmacokinetic analysis was performed in 10 patients with RA taking a stable dose (25–35 mg weekly) of MTX. Separated by one week, a pharmacokinetic analysis was performed in each patient after an oral single dose, and after an equal but split dose separated by 8 hours. MTX serum concentrations were measured by a fluorescence polarization immunoassay technique. Analysis was performed by calculation of the area under the curve (AUC) by the trapezoidal rule and by means of an iterative 2-stage Bayesian population procedure, obtaining population and individual pharmacokinetic parameters. For the population analysis, data from 15 patients in our previous study comparing oral and subcutaneous administration of MTX were also used.

Results. The median MTX dose was 30 mg weekly (range 25–35 mg). The bioavailability of the split dose was 28% higher compared to the single dose ($p = 0.007$). In the population pharmacokinetic modeling, a 2-compartment model best described the serum MTX concentration versus time curves. The mean bioavailability after single-dose and split-dose MTX was 0.76 and 0.90, respectively, compared to subcutaneous administration. There was a statistically significant difference in the bioavailability of the 2 oral administration regimens ($p = 0.008$).

Conclusion. The bioavailability of oral higher dose MTX in adult patients with RA can be improved by splitting the dose. (J Rheumatol 2006;33:481–5)

Key Indexing Terms:
METHOTREXATE PHARMACOKINETICS SPLIT-DOSE RHEUMATOID ARTHRITIS

In daily clinical practice higher doses of methotrexate (MTX; ≥ 25 mg) are being used in the treatment of rheumatoid arthritis (RA), although the efficacy of higher doses has not been proven in clinical trials. In the higher-dose range of MTX the bioavailability of oral MTX is variable and limited in comparison to subcutaneous and intramuscular administration[1-3]. The bioavailability of oral higher-dose MTX (25–40 mg weekly) is roughly two-thirds that of parenteral administration[4]. To ensure good bioavailability, a parenteral route is often chosen, especially in the higher-dose ranges. For patients the parenteral route obviously has disadvantages. The injections can be painful and can induce local skin reactions, and when patients cannot administer the injection themselves they have to rely on others. An oral route of administration has clear advantages in this respect. We investigated whether splitting the oral dose can improve bioavailability, by reducing the individual dose to a level at which bioavailability is known to be almost complete.

Our hypothesis was that the bioavailability would be improved by splitting the dose; we performed a crossover pharmacokinetic study in adult patients with RA, comparing the bioavailability of oral higher-dose MTX in a single weekly dose and in a split dose with an interval of 8 hours.

MATERIALS AND METHODS

Patients and MTX administration. Ten patients with RA, who were treated with MTX in a stable (≥ 3 months) dose of ≥ 25 mg weekly, oral or parenteral, were recruited into the study. The local ethics committee approved the study and written informed consent was obtained from each patient.

Baseline information on diagnosis, age, sex, disease duration, dose, serum creatinine, folic acid supplementation, disease modifying antirheumatic drugs (DMARD), nonsteroidal antiinflammatory drugs (NSAID), and prednisolone

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was gathered. Folic acid supplementation was allowed, but not on the day of MTX intake. Leukopenia, thrombocytopenia, and transaminase elevations were reasons for exclusion.

Pharmacokinetics was studied twice in each patient with a one-week interval: once with their regular MTX dose by a single route of administration, and once with the same total dose of MTX divided into 2 doses with an 8 hour interval. The dosing order was not randomized, because an effect of the previous dosing was not considered likely given that MTX serum concentrations are undetectable within 48 hours.

Patients were admitted to hospital in the early morning. They were allowed to have breakfast at home, at least 1.5 hours before MTX intake. Comedication was continued during both sampling episodes in the same way. Other DMARD and prednisone were allowed, with stable doses throughout the study. The concurrent medication was taken at least 1.5 hours before, and more than 2 hours after MTX intake. Oral MTX (2.5 mg tablets) was administered with water. Blood samples were drawn from an indwelling catheter at Time 0 (preadministration) and at 0.5, 1.0, 1.5, 2, 4, 8, 12, and 24 hours after administration of MTX in one dose. For the split dose, blood was drawn at Time 0 (preadministration) and at 0.5, 1.0, 1.5, 2, 4, 8, 8.5, 9, 9.5, 10, 12, 24, and 32 hours. Blood samples were centrifuged and the serum stored at –20°C until analysis.

MTX assay. MTX serum concentrations were determined using a fluorescence polarization immunoassay technique (TDX-Abbott Diagnostics; MTXII, list no. 7A12)[5]. The lower limit of detection was 0.02 µmol/l. The standard deviation (SD) of the assay is described by the formula

$$SD = 0.01 + 0.05*C$$

where C = concentration, in µmol/l.

Bioavailability calculation. Bioavailability was calculated in 2 ways, as follows.

1. From the raw data the area under the curve (AUC) was calculated using the trapezoidal rule. The AUC was calculated up until the last measured concentration, in the single dose 24 hours, and in the split dose 32 hours. The mean AUC of the single and of the split dose regimen was compared, and by means of paired t test the outcomes were compared. A p value < 0.05 was considered significant. We also calculated the AUC until infinity, by extrapolation of the data. We tested the data for a normal distribution.

2. Pharmacokinetic analysis. For the population pharmacokinetic analysis, data from our previous study[4] were included that compared the oral and subcutaneous routes of administration in 15 patients with RA. Seven patients that participated in that study[4] were included in the present study. The data were analyzed by an iterative 2-stage Bayesian process using the MW\Pharm program, version 3.57, modified to allow simultaneous analysis of the 5 extravascular methods of administration[6]. MTX concentration data from our previous study and from the current study with both administration methods from all patients (n = 18) were analyzed simultaneously. In our previous study MTX was also administered subcutaneously[4]. The bioavailability (F) of the oral-dose MTX was calculated as a relative F, assuming the bioavailability of subcutaneous administration as 100%. The pharmacokinetic model selected was a 2-compartment model (parameters k_e, V_1, k_{12}, k_{21}) with first-order absorption with a lag-time, with separate parameters F (bioavailability), k_a (absorption rate constant), and T_{lag} (lag-time) for each administration. For the 2 split doses (SP1 and SP2), F, k_a, and T_{lag} were calculated separately. Measurement data were weighted according to the reciprocal of their variance ($1/SD^2$). A log-normal distribution for the pharmacokinetic population parameters was assumed. Goodness-of-fit was evaluated from visual inspection of the measured and calculated data points. The choice between a one- and 2-compartment model was based on Aikake's information criterion[7].

RESULTS

Ten patients with RA were included in the study. Seven had participated in the first pharmacokinetic study, and 3 new patients were included (Figure 1). The median age was 59 years (range 34–72) and the median creatinine clearance was 75 ml/min (range 49–124). The creatinine clearance was not significantly different in comparison to the first study. The MTX dose varied between 25 and 35 mg weekly, with a median of 30 mg. All patients received folic acid supplementation in varying doses (5–30 mg weekly), but not on the day of MTX intake. Hydroxychloroquine was used in one patient, chloroquine in one, prednisolone in 3, sulfasalazine in one, aurothiomalate in one, and NSAID in 7 patients.

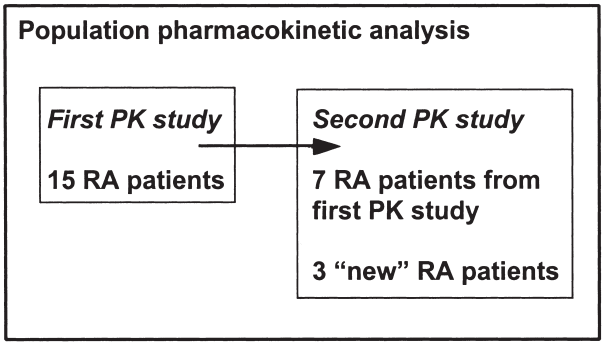

Figure 1. The 2-population pharmacokinetic (PK) analysis, including RA patients from the previous[4] and the current study. Total number of RA patients is 18.

The area under the curve, calculated from the raw data with the trapezoidal rule, of the split dose was significantly greater (mean 8.31 h·µmol/l, SD 1.80) than that of the single dose (mean 6.64 h·µmol/l, SD 2.15) (p = 0.007). The difference in AUC was 28% (mean AUC ratio 1.28, SD 0.24). The concentrations at 24 hours and 32 hours after single dose and split dose, respectively, were not detectable or were just above detection limit (Figure 2). When calculating the AUC to infinity by extrapolation of the data the results were as follows: mean AUC single-dose 6.76 h·µmol/l (SD 1.84) and mean AUC split-dose 8.42 h·µmol/l (SD 2.21) (p = 0.009).

Population pharmacokinetic analysis using MW-Pharm yielded the following results. A 2-compartment model fitted significantly better to the data than a one-compartment model (Aikake information criterion value –710 and –1616 for the one- and 2-compartment model, respectively). A total of 18 patients were analyzed: 15 from the previous study with an oral and a subcutaneous MTX dose; and 7 of these patients and 3 new patients were included in the current study. Mean bioavailability of the first split dose (F_SP1) was 0.89 (SD 0.13) and of the second split dose (F_SP2) 0.90 (SD 0.10), and the mean bioavailability of the split-dose regimen was 0.90 (SD 0.06) compared to subcutaneous MTX. The bioavailability of the single dose in the current study (PO2) was 0.76. The difference in bioavailability of the split dose compared to the single dose was statistically significant (p = 0.008; Table 1). The difference in bioavailability calculated by population analysis was smaller than that calculated by the trapezoidal rule. Complete population pharmacokinetic parameters are listed in Table 2.

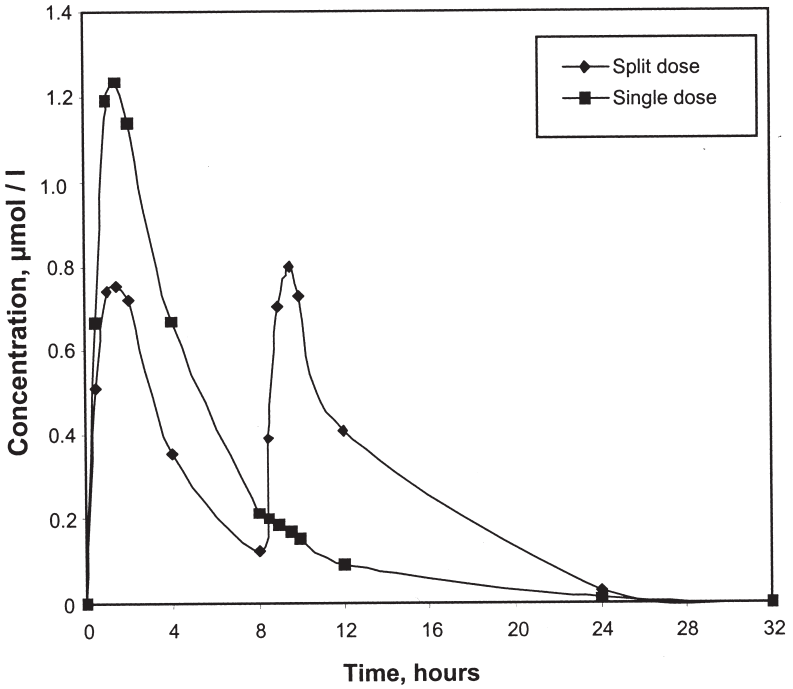

Figure 2. Plasma concentration-time curve of MTX administered in single dose and split dose. Values are means (n = 10).

Table 1. Population pharmacokinetic analysis calculating the bioavailability of MTX of the individual patients in the 2 pharmacokinetic studies.

Patient	Bioavailability ORAL/SC MTX (first study) F_PO1	Bioavailability ORAL/SC MTX, Single Dose (2nd study) F_PO2	Bioavailability of First Split Dose F_SP1	Bioavailability of 2nd Split Dose F_SP2	Bioavailability Comparing Split Dose and Single Dose of 2nd Study F_SP/PO2
1	0.63				
2	0.96				
3	0.21				
4	0.68				
5	0.55				
6	0.69				
7	0.77				
8	0.52				
9	0.27	0.58	0.93	0.98	1.65
10	0.79	0.91	0.94	0.87	1.00
11	0.73	0.71	0.65	0.81	1.03
12	0.73	0.72	1.02	0.80	1.25
13	0.65	0.87	0.88	0.99	1.08
14	0.56	0.77	0.81	1.00	1.18
15	0.85	0.91	1.06	0.85	1.05
16		0.69	0.91	0.93	1.34
17		0.67	0.92	0.91	1.37
18		0.84	0.85	0.87	1.02
Mean	0.60	0.67	0.89	0.90	1.18
SD	0.25	0.12	0.13	0.10	

MTX: methotrexate; SC: subcutaneous.

Table 2. Pharmacokinetic parameters of subcutaneous (SC), single-dose first study (PO1), single-dose second study (PO2), and split-dose of second study (SP1 and SP2) of MTX, calculated by means of population analysis (n = 18).

	Lag-Time	ka	F	V1**	k12**	k21**	ke**
SC	0.03	0.38	1*				
	0.04	0.11					
PO1	0.33	1.11	0.60				
	0.14	0.36	0.25				
PO2	0.35	0.86	0.76	0.140	0.69	0.55	0.77
	0.15	0.45	0.12	0.029	0.34	0.13	0.15
SP1	0.26	1.14	0.89				
	0.28	0.31	0.13				
SP2	0.33	0.77	0.90				
	0.21	0.38	0.10				

Values are mean (standard deviation). SC: subcutaneous; PO1: oral dose first study; PO2: single dose second study; SP1: first split dose; SP2: second split dose; lag time in hours; ka: absorption rate constant (h^{-1}); F: bioavailability; V1: volume of distribution of first compartment ($l.kg^{-1}$); K12 = rate constant of transport between compartment 1 and 2 (h^{-1}); K21 = rate constant of transport between compartment 2 and 1 (h^{-1}); ke: elimination rate constant (h^{-1}). * Fixed value (biovariability of SC administration assumed to be 100%). ** Same value for each administration.

DISCUSSION

Our data show that splitting the oral dose of MTX indeed improves bioavailability. In our previous study a limited bioavailability of oral higher-dose MTX was observed. Our current study was performed to examine whether splitting the oral dose would lead to better bioavailability, as an alternative for parenteral administration of MTX. Our results show that the split-dose regimen increases bioavailability with 28%. The bioavailability of the split dose is almost comparable to subcutaneous administration. When higher doses are used, splitting the oral dose is an option to improve bioavailability and is therefore a suitable alternative to subcutaneous administration.

Why do we consider bioavailability? The emphasis on enhanced bioavailability is clinically relevant when it is related to efficacy. Since a dose-effect relation was established for MTX doses up to 20 mg weekly[8,9], and the AUC of MTX concentration versus time is related to the dose, the AUC of oral MTX (and thus bioavailability) is of clinical importance. A retrospective study in children with juvenile idiopathic arthritis, in doses up to 20 mg weekly, showed improved efficacy when MTX oral administration was changed to parenteral[10]. The effect of higher-dose MTX (> 20 mg) in adult patients with RA, however, remains to be elucidated. In clinical practice higher doses are being used with good efficacy. Evidence in the literature for higher doses is scarce. A study by Furst, *et al* randomized for MTX 5 mg/m^2, 10 mg/m^2, and 20 mg/m^2. They included 6 patients with RA in the 20 mg/m^2 MTX group, and 2 patients experienced serious side effects. Folate supplementation was not given, however, and may have prevented some if not all of the adverse events. Efficacy of the higher doses was not evaluated, due to early withdrawal[8]. One randomized controlled trial of dose escalation of parenteral MTX in patients with RA showed no increased efficacy when the dose was escalated from 15 to 45 mg weekly, compared to MTX 15 mg weekly. The patients were using MTX for a mean period of 2.5 years, and still had active disease (defined as Disease Activity Score 28 > 3.2)[11]. Selection of patients with a long history of MTX use probably limits the ability to achieve further efficacy. It would be interesting to study this in patients with early RA.

A few remarks can be made concerning the methodology of our study. As in our previous study, all comedication was continued, and patients were allowed to have breakfast at home before coming to the hospital. Because of the time between comedication, breakfast, and MTX administration, an effect on MTX absorption is unlikely. The second dose in the split-dose regimen was at 5 PM, before the evening meal. The effect of food has been studied extensively and no effect on MTX absorption was found[12,13]. The 8 hour time separation of the split doses was chosen for practical reasons, for the pharmacokinetic analysis, and for patient convenience. Both doses can be taken before a meal (breakfast and dinner).

A limited absorption of the second dose of MTX can be expected when the time between doses is too short. However, although the difference in absorption rate of the 2 doses is significantly different (p = 0.04), the bioavailability of split doses as calculated in the population pharmacokinetic model is similar, suggesting that the gastrointestinal absorption of MTX is not limited after 8 hours. Diurnal variation in absorption could also play a role; however, since the F of the 2 split doses was the same, this argues against diurnal variation. One study of diurnal variation by Carpentier, *et al* compared the pharmacokinetics of intramuscular MTX administration at 10 AM or 6 PM, and they also found no differences[14].

The trapezoidal rule is the most robust way of calculating the AUC, but it has some drawbacks. The concentration pro-

file between 2 measurements is approximated by a straight line, resulting in inaccuracies in the estimated AUC. We calculated the AUC up until the last sampling time, i.e., 24 and 32 hours for the single dose and the split dose, respectively. When we calculated to infinity by data extrapolation, the results were the same. This can be understood easily, given the very low concentrations at 24 and 32 hours.

We used a population pharmacokinetic analysis, which included data from our previous study, to further determine the bioavailability. In this analysis bioavailability of the oral-dose MTX can be compared to subcutaneous administration, which presumably has 100% bioavailability. The pharmacokinetic model assumes first-order absorption kinetics starting abruptly after a lag-time, whereas the true absorption process may be expected to start more gradually after disintegration of the tablet and dissolution of the drug, possibly resulting in inaccuracies of the calculated bioavailability. Population pharmacokinetics examines drug absorption and disposition characteristics in the population. Variability between individuals, within individuals, and within the assay is taken into account, and can be corrected for in the model. Both individual and population pharmacokinetic parameters can be calculated. Particularly when only small numbers of patients can be studied in pharmacokinetic trials, combining the data can result in reliable estimates of the pharmacokinetic parameters. The use of data from our previous study also made it possible to compare the split-dose regimen to a subcutaneous route of administration without having to perform the analysis with subcutaneous administration again. A drawback of this method is that the patients in both studies with a one-year interval can differ in some aspects, for example in comedication or comorbid conditions, that can influence drug absorption and metabolism. We took account of this, and comedication was the same in patients that participated in both studies.

Calculations using the data from our previous study showed 18% improvement of bioavailability of the split-dose regimen. Surprisingly, the bioavailability of the single oral dose was higher in the current study in comparison to our previous study; in 3 out of 7 patients the difference was more than 30%. To reiterate, the studies were performed one year apart. Absorption can be influenced by several factors, but the circumstances of the oral MTX administration were largely the same in both studies. Comedication could also be a contributing factor, but the 3 patients in question had the same comedication. Apparently other factors must be responsible for the intraindividual variability in absorption.

However, both methods of calculating bioavailability yielded the same conclusion: the split-dose regimen showed improved bioavailability compared to a single dose. How do our results compare to other studies? No other studies are available in patients with RA. One study by Steele, *et al* in patients with various malignancies found an improved bioavailability of MTX 100 mg when divided into 4 doses of 25 mg. The bioavailability was 1.86 times that of the single dose[15].

The bioavailability of higher oral dose MTX is limited, and our data suggest that, in doses between 25 and 35 mg per week, the bioavailability of oral MTX is enhanced by a split-dose regimen. Intraindividual variation in absorption must be taken into account. In our study the bioavailability of the split-dose regimen is suggested to be comparable to the subcutaneous route of administration. When using higher doses of MTX, a split-dose regimen can thus be considered, as an alternative for parenteral administration. Further research is warranted to confirm our findings.

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