The population pharmacokinetics of long-term methotrexate in rheumatoid arthritis

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Aims Methotrexate is considered by many practitioners to be the agent of choice in the treatment of rheumatoid arthritis. The pharmacokinetics of methotrexate have been reported to exhibit significant intersubject variability. Therefore, this study was undertaken to evaluate the population pharmacokinetics of methotrexate during long-term administration in adults with rheumatoid arthritis.

Methods Methotrexate pharmacokinetics were evaluated in a 36 month study of 62 adults with rheumatoid arthritis. Patients received oral or intramuscular doses of methotrexate weekly with pharmacokinetic studies performed every 6 months. Data were analyzed with nonlinear mixed effects modeling.

Results Three thousand two hundred and sixty post oral or intramuscular dose serum methotrexate concentrations comprising 425 individual concentration *vs* time profiles were modeled using NONMEM. Covariates that significantly ($P < 0.005$) influenced the disposition of methotrexate were age (AGE, years), body weight (BW, kg), creatinine clearance (CL_{CR} , $l\ h^{-1}$), gender (GEN; 0 = male, 1 = female), dose (DOSE, μmol), and fed *vs* fasted state (FED; 0 = fasted, 1 = fed). The final model describing the biexponential disposition of methotrexate was $clearance(CL, l\ h^{-1}) = (0.0810 \cdot BW + 0.257 \cdot CL_{CR}) \cdot (1 - 0.167 \cdot GEN)$; central volume (V_c, l) = $0.311 \cdot BW$; peripheral volume (V_p, l) = $0.469 \cdot BW - 0.169 \cdot AGE$; intercompartmental clearance ($Q, l\ h^{-1}$) = $4.27 \cdot (1 - 0.355 \cdot GEN)$; oral absorption rate constant (k_{apo}, h^{-1}) = $4.70 - 0.0439 \cdot DOSE \cdot (1 - 0.507 \cdot FED)$; intramuscular absorption rate constant (k_{aim}, h^{-1}) = $0.122 \cdot DOSE$; relative bioavailability (F) = 93.4%; and oral absorption lag time (LAG_{po}, min) = 13.5. Pharmacokinetic parameters (%CV) for a typical fasted male subject in this study were CL , $7.34\ l\ h^{-1}$ (27%); V_c , $23.5\ l$ (28%); V_p , $25.3\ l$ (31%); Q , $4.25\ l\ h^{-1}$ (41%); k_{apo} , $3.67\ h^{-1}$ (77%); and k_{aim} , $3.09\ h^{-1}$ (44%).

Conclusions The population pharmacokinetics of methotrexate in adults with rheumatoid arthritis were well described by this investigation. Substantial interpatient variability was explained by incorporating patient specific data into regression equations predicting pharmacokinetic parameters.

Keywords: population pharmacokinetics, methotrexate, rheumatoid arthritis, chronic

Introduction

Methotrexate is widely recognized to be the most effective drug in current use for the treatment of rheumatoid arthritis. While most patients receive 7.5 mg weekly as a starting dose, long-term prospective studies have documented the need to increase the dose of methotrexate in order to achieve an optimal therapeutic effect [1–5]. Indeed, the clinical efficacy of the drug is dose related [6, 7] although a definitive dose–concentration–effect relationship has not been elucidated.

Many investigators have described the pharmacokinetic profile of methotrexate in patients with rheumatoid arthritis

[8–21] under a variety of circumstances, e.g., with and without non-steroidal anti-inflammatory drugs (NSAIDs) [12–17], with and without food. [10, 18] All of these investigations have been of a traditional pharmacokinetic nature in which the pharmacokinetics are investigated under standardized conditions [8, 9, 11], or once with and once without the variable under question [10, 12–18]. These rather strict analyses may miss intrinsic variability in the pharmacokinetics among and within patients [22]. Furthermore, the ability to interpret repeated observations under different dosing conditions might also be hindered with conventional analysis [22].

An alternative approach to the pharmacokinetic analysis of drug behaviour is the use of population models, in which variability is expected to exist not only in the experimental conditions under investigation, but also in

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other facets of the experiment. For example, variation is also anticipated in the assay methodology, day to day patient variability, patient gender, age, weight, and dose of medication. These multiple variables have been included in analytical techniques referred to as non-linear mixed effects modeling. Using one software implementation of this analytical method, NONMEM [23], the pharmacokinetics of methotrexate during long-term use have been analyzed in patients with rheumatoid arthritis. The results of this analysis are the substance of this report.

Methods

Subjects

Sixty-two patients with definite or classical rheumatoid arthritis [24] were recruited from the outpatient population of the Division of Rheumatology of Albany Medical College. Twelve patients had received methotrexate chronically (a mean duration of 78 months prior to the initiation of this study) and 50 patients had not received methotrexate prior to study entry. These 50 patients were recruited between November 1988 and June 1992 and represent all patients with rheumatoid arthritis who began methotrexate therapy during that period. The 12 patients who had received methotrexate chronically were part of a long-term cohort previously reported [21]. Demographic features of patients are seen in Table 1.

Methotrexate administration

This study was an investigation of the pharmacokinetics of methotrexate during long-term administration [20, 21]. Pharmacokinetic parameters were determined on multiple occasions at approximately 6 month intervals. As part of the long-term study of methotrexate disposition, patients were randomized to receive methotrexate orally or intramuscularly for part of this study. All 62 patients had initial pharmacokinetic studies performed after taking a 7.5 mg dose of methotrexate orally. The 12 patients who had received methotrexate prior to entry into the study continued to receive their usual maintenance dose of methotrexate with the difference between their usual maintenance dose and 7.5 mg being administered after the last blood sample (24 h after the 7.5 mg dose). Many of the

pharmacokinetic studies were also performed following 7.5 mg doses. However, all patients had at least one pharmacokinetic study completed following the administration of their usual maintenance dose of methotrexate. Most doses were administered orally, but every patient completed at least one pharmacokinetic study following an intramuscular injection of methotrexate. In general, patients received the dose of methotrexate under fasting conditions and patients received the usual dose of the NSAID they were administered at study inception throughout the study. On one occasion, 12 to 18 months after study entry, the NSAID therapy of 46 patients was withheld for five estimated half-lives prior to the pharmacokinetic determination. Thirteen patients completed one pharmacokinetic study under fed rather than fasted conditions. The methotrexate dose was administered as a single dose once weekly and the dose was adjusted to clinical response throughout the study as previously described [20, 21].

Pharmacokinetic studies

Pharmacokinetic studies were conducted approximately every 6 months. Patients received either 7.5 mg methotrexate or their usual maintenance dose at 08.00 h after an overnight fast. Blood samples were collected prior to the 08.00 h dose and at 0.5, 1, 2, 3, 4, 6, 8 and 24 h after the dose. The predosing blood samples confirmed that all patients had no detectable methotrexate in their blood. Patients were allowed to consume food at 10.30 h, 2.5 h after receiving methotrexate. Urine was collected for 24 h for methotrexate and creatinine analyses. Serum and urine creatinine concentrations were determined by a picric acid methodology. Creatinine clearance was calculated as the product of the urine creatinine concentration and the urine flow rate (urine volume in 24 h) ratioed to the midpoint serum creatinine concentration. This calculation was performed only in subjects with completed urine collections and appropriate serum creatinine determinations. Blood and urine were analyzed by fluorescence polarization immunoassay for methotrexate concentration. The methotrexate assay had a limit of detection of 0.01 μM and an interday coefficient of variation of 4.9%.

Data analysis

The population pharmacokinetics of methotrexate were estimated from 425 protocol specified administrations comprised of 3260 methotrexate concentrations obtained from 62 individuals. An extended least squares approach to nonlinear mixed effects modeling (NONMEM IV level 2.0, PREDDP III level 1.0) [23] was used to estimate pharmacokinetic parameters, intersubject and residual variability (random effects), and explain this variability in terms of patient specific information such as age or body weight (fixed effects).

Selection of base pharmacokinetic model

The choice of the structural (or pharmacokinetic) model was based on a comparison of two and three compartment models with first order input and first order elimination

Table 1 Demographic summary.

Feature	Mean (s.d.)	Range
Age (years)	58 (12)	26–81
Body weight (kg)	75 (18)	45–120
Creatinine clearance (ml min^{-1}) [*]	81 (24)	20–207
Female:Male (%)	66:34	
NSAID use (%)	95	
Duration of RA (weeks)	153 (101.2)	
Methotrexate dosage (mg) ^{**}	10.4 (3.7)	5–25

NSAID, non-steroidal anti-inflammatory drug; RA, rheumatoid arthritis.

^{*}Summary for subjects who had a creatinine clearance assessment.

^{**}Range for oral and intramuscular dose.

from the central compartment. Analysis of model diagnostics favored selection of the two compartment model. Therefore, the base model biexponential disposition of methotrexate was described in terms of clearance (CL), volumes of the central (V_c) and peripheral (V_p) compartments, intercompartmental clearance (Q), and oral (ka_{po}) and intramuscular (ka_{im}) first order absorption rate constants (PREDPP subroutine ADVAN4 TRANS4 in NONMEM) [22]. Parameters for relative bioavailability (F) and oral absorption lag time (LAG_{po}) were statistically justified for inclusion as well.

Statistical model

Incorporation of random effect parameters into the model allows for the modeling of unexplained variability. Intersubject variability for each pharmacokinetic parameter was modeled with an exponential error structure as follows, using clearance as an example:

$$CL_i = TVCL_i \cdot e^{\eta_{CL,i}} \quad (1)$$

where CL_i is the 'true' value of the pharmacokinetic parameter in the i th study subject, $TVCL_i$ is the model predicted value of the PK parameter, and $\eta_{CL,i}$ is the intersubject variability term. η_{CL} is normally distributed with a mean of zero and a variance ω_{CL}^2 . It should be noted that the first order method used in this analysis approximates the exponential error model as a proportional error model. The distribution of each pharmacokinetic parameter was evaluated with probability density plots and quantile-quantile plots for normal and log-normal distributions. Plots were constructed in S-PLUS [25, 26] with Bayesian estimates of individual pharmacokinetic parameters obtained with the POSTHOC option in NONMEM. Residual variability, comprised of intrasubject variability, interoccasional variability, measurement error, and model misspecification, was modeled with a combination (proportional and additive) error structure as follows:

$$C_{i,j} = F_{i,j} \cdot (1 + \varepsilon_{1,i,j}) + \varepsilon_{2,i,j} \quad (2)$$

where $C_{i,j}$ is the j th methotrexate serum concentration observation in the i th individual, $F_{i,j}$ is the j th model predicted concentration in the i th individual, and $\varepsilon_{i,j}$ are the residual variability error terms. $\varepsilon_{i,j}$ each have an expected value of zero and variance σ^2 .

Construction of the regression model The regression model describes the relationship between a pharmacokinetic parameter and a covariate. A graphical approach to exploratory data analysis can be a useful tool in elucidating the presence of these relationships [26]. In this study, scatter plots (produced in S-PLUS) of individual Bayesian pharmacokinetic estimates against covariates were used to identify possible correlation between the covariates and the pharmacokinetic parameters. Covariates available for analysis included subject body weight (BW), age (AGE), creatinine clearance (CL_{CR}), and dose (DOSE) recorded in μ mol. Categorical predictors included gender (GEN, 0 = male, 1 = female), fed *vs* fasted state (FED, 0 = fasted, 1 = fed), and non-steroidal anti-inflammatory drug use (NSAID, 0 = no NSAID, 1 = NSAID administered).

Development of a regression model is an attempt to maximize the predictive ability of the model without sacrificing parsimony. To achieve this objective, covariates were first screened by individually testing each covariate alone in the base pharmacostatistical model to determine which ones significantly influenced methotrexate pharmacokinetics. Inclusion of a fixed effect parameter into the base pharmacostatistical model quantifies the relationship between the pharmacokinetic parameter and the covariate. It also forms the basis of hypothesis testing as to whether the covariate significantly improves the ability of the model to predict the observed concentration-time profile. The difference in the objective function value ($-2 \cdot \log$ likelihood) between two hierarchical models, defined as the log likelihood difference (LLD), is asymptotically χ^2 distributed with degrees of freedom equal to the difference in the number of parameters between the two models. During the covariate screening step, addition of one parameter to the model had to effect a LLD of at least 3.84 to achieve the desired level of significance of $\alpha = 0.05$. Traditional methods of model building reported in the literature assemble all significant covariates into a intermediate full model that is then subject to a backwards elimination procedure to produce a parsimonious model [22, 27].

In the present investigation, the number of PK parameter-covariate relationships found to be significant during the screening step would exceed NONMEM program limitations on the number of fixed effects parameters allowed in a full model. Therefore, to maximize the likelihood of the data, a forward selection model building procedure was used to produce the intermediate full model. Covariates were added into the model sequentially with order of inclusion governed by the magnitude of reduction in the LLD observed in the screening steps. Criteria for retention of a covariate included statistical significance ($P < 0.05$), reduction of unexplained intersubject variability for the associated PK parameter, and an improvement in model diagnostics. Additionally, fixed effects parameters whose 95% confidence interval encompassed zero were considered for removal from the model. The COVARIANCE routine in NONMEM was executed to produce standard errors of the fixed effects parameters. Ninety-five percent confidence intervals were constructed according to standard formula using the parameter standard errors.

The full model thus produced was then subjected to backwards elimination where each model parameter was fixed to a value of zero (1 degree of freedom). The more stringent criterion of statistical significance at the 0.005 level was used to account for multiple statistical tests corresponding to a $LLD \leq 7.88$ for 1 degree of freedom [22, 28]. For cases where the full model and a reduced model could not be expressed as hierarchical models, i.e. when a pharmacokinetic parameter represented by only a slope term ($V_c = 0.4 \cdot WT$) was tested by replacement with an intercept ($V_c = 0.4$), the LLD serves as a measure of relative probability between two models. [29] Subjective interpretation of the magnitude of relative probability as well as an increase in unexplained intersubject variability upon removal of the parameter of interest served as criteria for retention.

Results

Pharmacokinetic model

Analysis of residual plots from the two and three compartment model fits were compared. The three compartment

Table 2 Summary of covariate screening.

Covariate	Pharmacokinetic parameter						F
	CL	V _c	V _p	Q	ka _{po}	ka _{im}	
WT	153	89	55	39	24	4	
AGE	14	5	34	21	13		
CL _{CR}	114						
DOSE	9				46	30	
NSAID	12						
FED	35				67		76
GEN	123	23	71	41		4	

CL, Clearance; V_c, volume of the central compartment; V_p, peripheral volume; Q, intercompartmental clearance; ka_{po}, oral 1st order absorption rate constant; ka_{im}, intramuscular 1st order absorption rate constant; F, relative bioavailability; WT, weight; CL_{CR}, creatinine clearance; NSAID, nonsteroidal anti inflammatory drug use; FED, fed *vs* fasted administration; GEN, gender.

Numbers are the decrease in objective function value when the covariate was tested on the respective pharmacokinetic parameter. Only significant relationships are presented ($P < 0.05$).

Table 3 Forward selection model building summary.

Number	Model*	LLD**	Significant?***
1	Base model		
2	1 + F	−139.6	Yes
3	2 + LAG _{po}	−32.5	Yes
4	3 + CL ~ WT	−142.1	Yes
5	4 + CL ~ GEN	−49.8	Yes
6	5 + CL ~ CL _{CR}	−88.2	Yes
7	6 + V _c ~ WT	−78.1	Yes
8	7 + V _p ~ GEN	−9.3	Yes****
9	8 + V _p ~ WT	−13.1	Yes
10	9 + F ~ FED	−6.9	Yes****
11	10 + ka _{po} ~ FED	−47.0	Yes
12	11 + ka _{po} ~ DOSE	−28.1	Yes
13	12 + Q ~ GEN	−17.0	Yes
14	13 + Q ~ WT	9.3	No
15	13 + CL ~ FED	−2.6	No
16	13 + V _p ~ AGE	−15.7	Yes
17	16 + ka _{im} ~ DOSE	−37.1	Yes
18	17 + ka _{po} WT	9.2	No
19	17 + V _c ~ GEN	0.0	No
20	17 + V _p ~ GEN	0.0	No
21	17 + CL ~ AGE	−8.6	Yes****
22	17 + CL ~ NSAID	−8.1	Yes****

*Model indicates sequential steps taken to achieve a full model. '~' symbolizes 'as a linear function of.' Intercept terms are implicit.

**LLD is the change in objective function value obtained for the modeling step.

***Significance is at the $P < 0.05$ level (LLD > 3.84)

****Covariate dropped in final model as the 95% confidence interval enclosed zero. Intercept terms for CL, V_c, V_p, and ka_{im} were also eliminated for the same reason.

model did not provide a superior fit to the data compared to the two compartment model fit (LLD increased by 0.032, not statistically significant).

Statistical model

Probability density plots for each of the pharmacokinetic parameters supported the use of the exponential intersubject error models under the first order approximation (data not shown). Each pharmacokinetic parameter appeared normally distributed with the exception of ka_{im}, which was better described by a log-normal distribution. Each intersubject error parameter was tested for statistical significance. The variability term associated with oral absorption lag time failed to achieve significance ($P > 0.05$) and was therefore dropped from the model.

Additive and proportional residual error models were examined as alternatives to the combination residual error model. The combination model was statistically advantageous ($P < 0.001$) to both alternatives and was thus retained.

Development of the full regression model

Scatterplots suggested that the association of the pharmacokinetic parameters with the covariates could be adequately described through linear relationships for the range of the data available. Therefore, each continuous covariate was screened in a linear fashion. Table 2 summarizes the results of the covariate screening steps. Only those relationships that were screened and found significant ($P < 0.05$) are presented. The magnitude of LLD governed order of sequential addition to the pharmacostatistical model during model build up. Results of model building are presented in Table 3. Fixed effects parameters that were significant but omitted from the full model because 95% confidence intervals enclosed zero were FED as a predictor of F, GEN as a predictor of V_p, and CL as a function of AGE and NSAID. Practical interpretation of this outcome is that there was insufficient information to precisely estimate the parameter and reject the hypothesis that the parameter value was zero even though the relationship was supported by trends in the data. Reduction of the full model through backwards elimination resulted only in the loss of the intersubject variability term associated with the lag time for oral absorption. Hypothesis testing with the full model is presented in Table 4.

Final regression model

The final regression model was determined as follows. Parameter estimates and associated standard errors are presented in Table 5.

Clearance

$$CL (1 \text{ h}^{-1}) = (01 + 03 \cdot \text{WT}) \cdot (1 + 04 \cdot \text{GEN}) \quad (3)$$

$$CL (1 \text{ h}^{-1}) = (02 \cdot \text{CL}_{\text{CR}} \cdot 0.06 + 03 \cdot \text{WT}) \cdot (1 + 04 \cdot \text{GEN}) \quad (4)$$

N.B. The factor 0.06 converts CL_{CR} in ml min^{−1} to l h^{−1}. Equation 3 was used when an assessment of creatinine

Table 4 Hypothesis testing with the full pharmacostatistical model.

Hypothesis	Parameter tested	LLD	Significance
<i>Is clearance a function of...</i>			
Creatinine clearance	Fix 01 to 0	67.6	<<0.001
Weight	Fix 02 to 0	162.3	<<0.001
Gender	Fix 03 to 0	27.5	<<0.001
<i>Does central volume change with...</i>			
Weight	04 in place of 04*WT	115.9	*
<i>Is peripheral volume determined by...</i>			
Weight	Fix 05 to 0	10.1	<0.005
Age	06 in place of 06*WT	24.2	*
<i>Is intercompartmental clearance a function of...</i>			
Gender	Fix 08 to 0	25.5	<<0.001
<i>Does the intramuscular absorption rate constant change with...</i>			
Dose	09 in place of 09*DOSE	27.6	*
<i>Is the oral absorption rate constant a function of...</i>			
Dose	Fix 010 to 0	28.5	<<0.001
Fed vs Fasted	Fix 011 to 0	45.5	<<0.001

*The full and reduced models are not hierarchical models (ie. no degrees of freedom) therefore a significance level cannot be ascertained. However, the LLD serves as a measure of relative probability between two models.

Table 5 Final model parameter estimates and standard errors.

Model parameter	Estimate	Standard error
01	0.257	0.0586
02	0.081	0.00862
03	-0.166	0.0719
04	0.312	0.0135
05	0.468	0.0625
06	-0.169	0.0598
07	4.25	0.389
08	-0.354	0.0836
09	0.121	0.0111
010	4.62	0.48
011	-0.0416	0.014
012	-0.496	0.0638
013	0.225	0.0173
014	0.934	0.0181

clearance was absent, equation 4, when the assessment was present. NONMEM estimated 01 as 1.27, a value approximately equal to the mean creatinine clearance value (in 1 h^{-1}) times 02.

Volume of distribution

$$V_c (l) = 05 * WT \quad (5)$$

$$V_p (l) = 06 * WT + 07 * AGE \quad (6)$$

Intercompartmental clearance

$$Q (1 \text{ h}^{-1}) = 08 * (1 + 09 * GEN) \quad (7)$$

Absorption rate constants

$$ka_{im} (h^{-1}) = 010 * DOSE \quad (8)$$

$$ka_{po} (h^{-1}) = (011 + 012 * DOSE) * (1 + 013 * FED) \quad (9)$$

N.B. DOSE is the dose administered recorded in μM

Oral absorption lag time and relative bioavailability

$$LAG_{po} (h) = 014 \quad (10)$$

$$F = 015 \quad (11)$$

Figure 1 depicts a plot of methotrexate concentrations predicted by the base model *vs* the observed methotrexate concentrations. This may be contrasted to Figure 2, which contains methotrexate concentrations predicted by the final

Figure 1 Base model predicted methotrexate concentration (•) *vs* observed methotrexate concentration. Open points (◊) are model predictions using individualized pharmacokinetic parameter estimates. The line is identity.

Figure 2 Final model predicted methotrexate concentration (•) *vs* observed methotrexate concentration. Open points (°) are model predictions using individualized pharmacokinetic parameter estimates. The line is identity.

model versus observations. Note an improvement of fit as evidenced by the tighter, more random scatter about the line of identity. Figure 3 displays the observed and model predicted methotrexate concentrations versus time for a representative subject (69 year old female weighing 60 kg with a creatinine clearance of 69 ml min⁻¹) following oral administration of 27.5 µM methotrexate in the fed state.

Table 6 displays the expected pharmacokinetic parameters in a hypothetical fasted male subject possessing mean values of the covariates as well as mean values of the individualized pharmacokinetic parameter estimates. The median value is reported for the log-normally distributed intramuscular absorption rate constant. Additionally, as estimated by NONMEM, intersubject coefficient of variation (CV) for each pharmacokinetic parameter for the base and final models is presented. After reparameterization of the model, elimination half-life for the hypothetical male subject was found to be 7.52 h. CVs for residual error at methotrexate serum concentrations of 2.0, 1.0, and 0.5 µM were 27.5%, 26.2%, and 25.6%, respectively.

Figure 3 Representative model predicted (---) and observed (●) methotrexate concentrations *vs* time profile. Solid line (—) is prediction using individualized pharmacokinetic parameter estimates.

Table 6 Typical values and mean values of pharmacokinetic parameters.

Pharmacokinetic parameter	Typical value*	Mean value**	Final model CV***	Base model CV***
CL (l h ⁻¹)	7.34	6.05	0.272	0.449
V _c (l)	23.5	25.2	0.279	0.335
V _p (l)	25.3	25.7	0.312	0.512
Q (l h ⁻¹)	4.25	3.22	0.405	0.581
k _a _{po} h ⁻¹	3.67	1.88	0.767	1.34
k _a _{im} h ⁻¹	3.09	2.75	0.440	0.688
F	0.934	0.889	0.130	0.125
LAG _{po} (min)	13.5	13.5	NE	NE

CV, coefficient of variation; NE, not estimated.

*Typical value is the value of the pharmacokinetic parameter calculated using the final regression model and assuming a hypothetical male subject with mean values of all of the covariates.

**Mean value is the average of the individualized (POSTHOC) estimates of each pharmacokinetic parameter. The median value is reported for the log-normally distributed k_a_{im}.

***CVs are those estimated by NONMEM.

Discussion

Previous reports in the literature suggest that the disposition of methotrexate could be characterized by a two or three compartment model with first order absorption and elimination [7, 9, 21, 30]. A three compartment model was fitted to the present data. Model diagnostics in the form of residual plots and the log likelihood difference suggested that the more complex model offered no improvement in the fit to the data than the two compartment model. Subsequent modeling was implemented with a two compartment model.

In this analysis, mean and typical values for clearance, elimination half-life, and volume of distribution at steady-state, were similar to those measured by other investigators [8–18, 31–33]. For example, in a recent review, Bannwarth *et al.* summarized values of clearance as 4.8–7.8 l h⁻¹ and elimination half-life as 5–8 h [34]. Clearance increased with increasing renal filtration as assessed by creatinine clearance and increased with increasing body weight, presumably representing non-filtration elimination. In two separate studies, Stewart *et al.* reported statistically significant correlations between creatinine clearance and total body clearance of methotrexate [15, 17]. However, these relations were for one variable only and of poor predictive value. Combe *et al.* also reported a statistically significant correlation between methotrexate clearance and creatinine clearance, as did Lafforgue *et al.* [33, 35].

More recently, Bressolle *et al.*, in a population pharmacokinetic analysis, reported a statistically significant positive correlation between creatinine clearance and methotrexate clearance [31]. In this same work, and another published by the same authors, methotrexate clearance was found to be statistically significantly inversely correlated to patient age, although the relationship was not as strong as with creatinine clearance [31, 32]. Lafforgue *et al.* report similar findings on the relationship between age, creatinine clearance and methotrexate clearance [35]. In the present investigation, patient age appeared to be a significant negative predictor of methotrexate clearance during both the screening step

($P < 0.001$) and the model building phase ($P < 0.005$). However, in the full model, the parameter relating methotrexate clearance to age was not precisely estimated as evidenced by a 95% confidence interval enclosing zero. This may be because creatinine clearance, already present in the model, was inversely correlated with patient age. The clinical interpretation of such a result might be that age related decreases in methotrexate clearance are adequately accounted for by incorporation of creatinine clearance assessments. This work supports the need for renal function monitoring in patients receiving methotrexate and adjusting the dosage as appropriate.

After correcting for the effects of creatinine clearance and body weight, a gender-specific difference in clearance was found, with females presenting values about 17% less than that for males. This observation may be extrapolated to suggest that females are at increased risk for methotrexate toxicity and lower doses may be appropriate in female patients. The relationship between clearance and body weight represented about 83% of total clearance in the hypothetical typical male subject, therefore, significant changes in body weight may necessitate dose changes. The interaction of NSAID administration with methotrexate disposition has been studied extensively, with varying conclusions [7, 12–17, 33, 36]. In this investigation, NSAID use was found to have a significant effect on methotrexate clearance. However, the data were insufficient to quantitate the magnitude of effect, therefore this effect was not included in the final model.

Volume of the central compartment was approximately 31% of body weight, averaging 25.2 l in this study population. This value is somewhat lower than that reported by Bressolle *et al.* (34.8 l) but similar to that published by Sabot *et al.* (22.2 l) [30, 32]. Volume of the peripheral compartment increased with increasing body size as measured by body weight and declined with advancing age, likely due to age related loss of body water and changing body composition. Mean volume of distribution at steady-state was calculated as 50.9 l (0.679 l kg^{-1}) in this investigation, a value consistent with that reported by Sinnett *et al.* ($0.33\text{--}1.0 \text{ l kg}^{-1}$) and Combe *et al.* (0.87 l kg^{-1}) [9, 33]. The body weight dependence of distribution volumes would also support the need for dose change in the presence of significant weight gain or loss.

Oral absorption was found to be highly variable as evidenced by the high CV (76.7%). k_{apo} declined with increasing dose suggesting the presence of a saturable absorption process. The observation that k_a for oral administration was dose dependent is consistent with some studies in the literature which report a value of 5.8 h^{-1} following a 7.5 mg dose [9] and 2.1 h^{-1} following a 15 mg dose [10]. Additionally, administration in the fed state led to a substantially slowed absorption. The relative bioavailability of oral administration to intramuscular administration averaged 93.4%. Bioavailability appeared to be reduced in the presence of food ($P < 0.01$) but the degree of reduction could not be precisely estimated. Given the substantial variability in the rate, and to a lesser degree, the extent of absorption, clinicians cannot assume uniformity of oral methotrexate administration and may want to consider switching patients to the parenteral route of administration when patients have not responded ideally to a higher weekly oral dose.

The first order absorption rate constant for intramuscular administration increased with increasing dose (or with increasing injection volume). The mean absorption rate constant for i.m. administration (2.75 h^{-1}) was also in good agreement with those reported by Brooks [36] and Edelman [37]. Bressolle and colleagues reported an average absorption rate constant of 4.31 h^{-1} [32] upon gluteal administration followed by massage to uniformly distribute the drug, possibly increasing the surface area for absorption. In this investigation intramuscular doses were administered in various intramuscular sites without deliberate massage, possibly accounting for the lower absorption rate constant.

The population pharmacokinetics of methotrexate in adults with rheumatoid arthritis were determined using an extended least squares approach to nonlinear mixed effects modeling. Estimates of mean pharmacokinetic parameters correspond well with values previously reported in the literature. However, the population approach has provided estimates of intersubject variability and a method by which to explain some of this variability in terms of subject specific covariates. This can be seen in Table 6, as unexplained variability in pharmacokinetic parameters was reduced by 15 to 47% in the final model relative to the base model, with the exception of relative bioavailability. The population pharmacokinetics of methotrexate in adults with rheumatoid arthritis were well described by this investigation. Substantial interpatient variability was explained by incorporating patient specific data into regression equations predicting pharmacokinetic parameters and the clinical relevance and implications of the relationships have been presented.

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Appendix

Equation relating concentration in the central (sampled) compartment to the model parameters

$$C = \frac{k_a F D}{V_c} \times \left[\frac{(k_{21} - \alpha) e^{-\alpha t^*}}{(\beta - \alpha)(k_a - \alpha)} + \frac{(k_{21} - \beta) e^{-\beta t^*}}{(k_a - \beta)(\alpha - \beta)} + \frac{(k_{21} - \alpha) e^{-k_a t^*}}{(\alpha - k_a)(\beta - k_a)} \right]$$

Where k_a equals $k_{a_{im}}$, $F = 1$, and $t^* =$ time for intramuscular administration and k_a equals $k_{a_{po}}$ and $t^* =$ time-lag for oral administration. $k_{21} = Q/V_p$.

$\alpha =$

$$\left(\frac{CL}{V_c} + \frac{Q}{V_c} + \frac{Q}{V_p} \right) + \sqrt{\left(\frac{CL}{V_c} + \frac{Q}{V_c} + \frac{Q}{V_p} \right)^2 - 4 \left(\frac{CL \times Q}{V_c \times V_p} \right)}$$

2

$\beta =$

$$\left(\frac{CL}{V_c} - \frac{Q}{V_c} + \frac{Q}{V_p} \right) + \sqrt{\left(\frac{CL}{V_c} + \frac{Q}{V_c} + \frac{Q}{V_p} \right)^2 - 4 \left(\frac{CL \times Q}{V_c \times V_p} \right)}$$

2

Where each term is a reparameterization of a micro rate constant. $k_{10} = CL/V_c$, $k_{12} = Q/V_c$, $k_{21} = Q/V_p$