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Combination of methotrexate and sulphasalazine in patients with rheumatoid arthritis: pharmacokinetic analysis and relationship to clinical response

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- 1 The influence of sulphasalazine (SASP) on the pharmacokinetics of low dose methotrexate (MTX) and the relation between pharmacokinetic variables and clinical response was studied in 15 patients with active rheumatoid arthritis despite >6 months of SASP treatment.
- 2 SASP was stopped for 2 weeks. Thereafter a single oral dose of 7.5 mg MTX was administered after a standard breakfast. Blood was sampled initially every 30 min, thereafter hourly during 8 h. Urine was sampled every hour. Then 2000 mg SASP daily + 7.5 mg MTX weekly was given. After 4 weeks the same procedure was repeated supplemented with concomitant administration of 1000 mg SASP. Clinical measurements included Ritchie articular index, number of swollen joints, ESR and the disease activity score. Pharmacokinetic analysis was performed using a two-compartment model with first order absorption and lag time. Results are given as mean (s.d.). Paired *t*-test or signed rank test were applied in the statistical analysis.
- 3 Pharmacokinetics of MTX without *vs* with SASP, means \pm s.d. were as follows: AUC: 673 ± 179 *vs* 628 ± 210 (95% confidence interval [CI] of the difference was -71 to 159) $\text{ng ml}^{-1} \text{ h}$, MRT: 5.2 ± 1.3 *vs* 5.2 ± 1.1 (95% CI -0.4 to 0.4) h, $t_{\frac{1}{2},z}$: 4.3 ± 1.1 *vs* 4.2 ± 1.1 (95% CI -0.3 to 0.5) h, V/F : 59.3 ± 29.3 *vs* 65.5 ± 25.3 (95% -23.8 to 11.4) l, CL/F : 12.3 ± 5.0 *vs* 13.5 ± 4.8 (95% CI -4.5 to 2.3) l h^{-1} . CL_R/F : 6.2 ± 1.3 *vs* 6.3 ± 2.1 (95% CI -1.3 to 1.1) l h^{-1} . All *P* values were ≥ 0.3 .
- 4 A weak correlation existed between the change of ESR and the MRT, the $t_{\frac{1}{2},z}$ and the V/F (Spearman correlation coefficients of 0.43, 0.50 and 0.50 respectively, $0.05 < P < 0.1$).
- 5 There is no significant influence of chronic SASP administration on the pharmacokinetics of MTX or *vice versa*. Of the clinical variables, only the ESR correlated consistently with some pharmacokinetic variables of MTX.

Keywords sulphasalazine methotrexate combination pharmacokinetics rheumatoid arthritis clinical study

Introduction

Rheumatoid arthritis (RA) is a disease which is often characterized by progressive and irreversible joint destruction, which occurs early in the course of the disease as is seen by radiographical analysis [1]. The

results of current drug therapy are not at all satisfactory, as is shown in various 'survival'-curves (i.e. analysis of the duration of use) of second-line antirheumatic drugs [2]. To tackle these problems proposals were brought forward to leave the current strategy of giving antirheumatic drugs in a sequential way, starting with the least

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toxic one [3, 4]. One of these proposals is to combine different disease modifying antirheumatic drugs (DMARDs).

Various review articles and editorials have addressed the issue of combining DMARDs [5, 6]. The general impression is that while definite conclusions cannot be drawn due to lack of randomized controlled studies, there are some clues that combination therapy is more effective but also more toxic. Which drugs to combine and how to use these combinations, e.g. to employ the so called step-down bridge approach [4] or to start with one and when a satisfactory response is lacking add another [5], is unclear.

The present study focuses on RA patients who are resistant to sulphasalazine (SASP) therapy. Methotrexate (MTX) was chosen to be added to SASP because it is likely to be superior to some other DMARDs with respect to efficacy and toxicity [7, 8]. Also, it has a relatively quick onset of action and the side effects are generally quite manageable. In the sparse literature describing case histories in which this combination was used, a favourable effect has been described [9].

SASP consists of two compounds, sulphapyridine and mesalazine, linked by an azo-bond, which is split by intestinal bacteria. SASP is variably absorbed, with estimates ranging from 10 to 30%, and undergoes extensive enterohepatic recycling. The metabolites of SASP have complex dispositional characteristics [10]. MTX is an antifolate drug. There is interindividual variation in its bioavailability. It is bound to plasma proteins (35–50%) and undergoes mainly renal elimination (tubular secretion) [11].

There are very few data concerning the toxicity and no reliable data addressing the pharmacokinetic interaction of this combination. Theoretically there is the possibility of a pharmacokinetic interaction at the renal level between MTX, SASP, sulphapyridine and other metabolites, since these are anionic drugs that could compete for the tubular organic anion excretory mechanism [12, 13]. The interaction of sulphamethoxazole-trimethoprim with MTX leading to increased toxicity is well known. This interaction could be due to interference with folate dependent metabolism, although for sulphonamides as sulphasalazine such an effect has only been described *in vitro* [14], not *in vivo*. The possibility of a pharmacokinetic explanation of this increased toxicity has been explored [15, 16], but not concerning other sulphonamides like SASP and its metabolites, with low-dose MTX.

Few data also exist relating pharmacokinetic parameters or blood levels to the clinical response of MTX or SASP, and there are no data on this relationship in patients using the combination of the two drugs. When there is a pharmacokinetic interaction, e.g. an impeded excretion of MTX due to competition for renal tubular excretory pathways, it could lead to prolonged exposure to the drug, thus explaining possible clinical results of the combination.

In the present study we tried to answer the primary question of whether there is a pharmacokinetic interaction between SASP and MTX, and the secondary

question of whether pharmacokinetic parameters can predict response and/or toxicity in RA patients treated with the combination of SASP and MTX.

Methods

Patients

As part of a larger open randomized clinical trial (40 patients) comparing MTX and the combination of MTX and SASP, the first consecutive 15 patients with RA (ACR criteria 1987) randomized to the combination were included in the study. All were receiving SASP before inclusion in the study for at least 6 months, but did not experience sufficient efficacy of the drug. All had to have an estimated creatinine clearance (Cockcroft's rule) $>75 \text{ ml min}^{-1}$, normal haematological and liver enzyme values. All patients had NSAIDs as co-medication, in a dose which was kept strictly the same throughout the study, as was all other co-medication. Ethics Committee approval was obtained from the Commissie Experimenteel Onderzoek op Mensen, Academic Hospital Nijmegen, the Netherlands. All patients gave their written informed consent.

Experimental design

After taking a serum sample for measurement of trough levels (15 h after the last dose of SASP) of SASP, sulphapyridine and acetylated sulphapyridine, the medication was discontinued for 2 weeks. NSAIDs were continued in a stable dose throughout the study, and other co-medication was kept the same throughout the period of the study. Two weeks later the pharmacokinetic investigation was performed: patients received three tablets of 2.5 mg MTX (Emtrexate[®], Pharmachemie, Haarlem, The Netherlands) given in a single dose, after a standardized breakfast. Blood was collected every 30 min for the first 2 h and hourly for a total of 8 h thereafter. Urine was collected hourly.

Subsequently patients were given 7.5 mg of MTX weekly in a single dose on the same day of the week combined with SASP tablets 500 mg (Salazopyrine EC[®], Pharmacia, Uppsala, Sweden) two tablets twice daily.

After 4 weeks the same pharmacokinetic investigation was repeated; additionally a sample for determination of SASP and metabolites was taken before ingestion of the tablets, and the patients received two tablets of 500 mg of Salazopyrine EC[®] together with the MTX in a single dose.

The concentration of MTX in plasma was determined using the TDX-immunoassay [17] (lower detection limit 4.5 ng ml^{-1} , interday CV 1.4%, intraday CV 0.4%). In urine the MTX concentration was determined using h.p.l.c. analysis (lower detection limit $0.25 \text{ } \mu\text{g ml}^{-1}$, interday CV 4.8%, intraday CV 1.4%) [18]. SASP and metabolite concentrations were measured by h.p.l.c. (lower detection limit $1 \text{ } \mu\text{g ml}^{-1}$, interday CV 1.3–3.4%, intraday CV 1.0–3.2%) [19].

The MTX drug levels were analysed using both model-dependent and model-independent methods. Curve fitting was done by using the nonlinear regression program NONLIN [20], in which plasma concentration data were weighted reciprocally ($1/C$) and cumulative amounts excreted weighted equally. Plasma concentration-time curves were analyzed according to a linear open two compartment model with first order absorption, using the following equation:

$$C(t) = A_1 \cdot e \exp(-(t-t_{lag})/\tau_1) + A_2 \cdot e \exp(-(t-t_{lag})/\tau_2) - (A_1 + A_2) \cdot e \exp(-(t-t_{lag})/\tau_{abs})$$

where:

- A_1 : intercept of the i -th exponential phase on the Y-axis (ng ml^{-1})
 τ_i : time constant of the i -th exponential phase (h)
 τ_{abs} : absorption time constant (h)
 t_{lag} : absorption lag time (h)

The cumulative amount of drug excreted in the urine unchanged *vs* time was calculated as follows:

$$A_e(t) = A_e^\infty \cdot [1 - e \exp(-(t-t_{lag})/\tau_e)]$$

where:

- A_e^∞ : cumulative amount of drug excreted unchanged in urine to time infinity (mg)
 τ_e : excretion time constant (h)
 t_{lag} : excretion lag time (h)

From these equations the following pharmacokinetic parameters can be derived:

$$\begin{aligned} \text{AUC} &= A_1 \cdot (\tau_1 - \tau_{abs}) + A_2 \cdot (\tau_2 - \tau_{abs}), \\ \text{AUMC} &= A_1 \cdot (\tau_1^2 - \tau_{abs}^2) + A_2 \cdot (\tau_2^2 - \tau_{abs}^2) \\ \text{MRT} &= \text{AUMC}/\text{AUC} \\ t_{1/2, \lambda_z} &= \ln 2 \cdot \tau_2 \\ \text{CL}/F &= D/\text{AUC} \\ V/F &= D \cdot \text{AUMC}/(\text{AUC})^2 - D \cdot \tau_{abs}/\text{AUC} \\ \text{CL}_R/F &= (A_e^\infty/D) \cdot \text{CL}/F \end{aligned}$$

where:

AUC: area under the curve from $0 \rightarrow \infty$, AUMC: area under the first moment curve from $0 \rightarrow \infty$, MRT: mean residence time, $t_{1/2, \lambda_z}$: elimination half life, CL: total body clearance, F : fraction absorbed (unknown), V : volume of distribution, CL_R : renal clearance.

AUC and AUMC were also calculated model-independently by application of the linear trapezoidal rule (from $t=0$ to 8 h and by extrapolation of the terminal phase of the plot to infinity).

Clinical evaluation

Patients were evaluated 2 weeks before entry, and at weeks 0, 4, 8, 12, 16, 20 and 24. All clinical evaluations were performed by one observer (CJH).

The following changes comparing week 0 and week 24 were evaluated: the DAS (disease activity score [21]), the number of painful joints (53 joints were evaluated), the Ritchie Articular Index, the number of swollen joints (maximum of 48 joints) and general wellbeing expressed by the patient on a visual analogue scale of 0–100 mm. Compliance was checked by interviewing the patient.

Laboratory evaluation consisted of ESR, haemoglobin

content (mmol l^{-1}) and haematocrit, mean red cell volume (fl), WBC count with differential count, platelet count, alanine and aspartate aminotransferase, gamma glutamyl transferase, alkaline phosphatase and creatinine in serum ($\mu\text{mol l}^{-1}$).

Toxicity was monitored every 4 weeks by interviewing the patient, physical examination and laboratory investigations. The following laboratory values were considered as an adverse drug reaction: a WBC count of less than $3.5 \cdot 10^9 \text{ l}^{-1}$, platelets less than $120 \cdot 10^9 \text{ l}^{-1}$, a decrease of haemoglobin content of $> 1.0 \text{ mmol l}^{-1}$, an increase of serum creatinine of $> 25\%$ and an increase of liver enzymes above the normal levels.

Statistical analysis

To compare the values of the derived pharmacokinetic parameters and blood levels during single drug use and during the use of the combination, paired t -tests were used in the case of normally distributed values and signed rank tests were employed when data had a skewed distribution. 95% confidence intervals were calculated using $2.145 \cdot \text{s.e.mean}$.

The goodness of fit to the plasma concentration and cumulative excretion data were evaluated through the deviations between observations and model-predicted values expressed as: $r^2 = 1 - \Sigma(\text{Dev})^2/\Sigma(\text{Obs})^2$.

To test the association between the derived pharmacokinetic parameters during the use of the combination and clinical variables (the change from week 0 to week 24 concerning the Ritchie articular index, the number of swollen joints, the ESR, the DAS score, and the occurrence of toxicity), Spearman rank correlation coefficients were tested.

Results

Fifteen RA patients were included in the study. The mean age was 59.5 years (s.d. 11.7), the female/male ratio was 12/3, the mean duration of the illness was 4.8 years (s.d. 4.1), the mean duration of SASP-use was 18.7 months (s.d. 12.2). Figure 1 shows the mean plasma levels of MTX (with and without SASP) and Figure 2 the mean cumulative urinary excretion of MTX. Table 1 shows the model-dependent values of the derived parameters of MTX pharmacokinetics. Model-independent analysis revealed virtually the same values (data not shown). The goodness of fit expressed as the mean coefficient of determination was 0.97 (s.d. 0.07), range 0.66–0.99, indicating very acceptable fits. The pharmacokinetics of MTX with or without MTX were very similar (Table 1). The steady state trough levels of SASP and metabolites were not influenced by co-administration of MTX either (Table 2). The clinical results of 24 weeks of treatment are shown in Table 3. Six patients experienced some form of toxicity. There were no consistent correlations between the derived pharmacokinetic parameters and the results of treatment or toxicity except for the decline in ESR which correlated

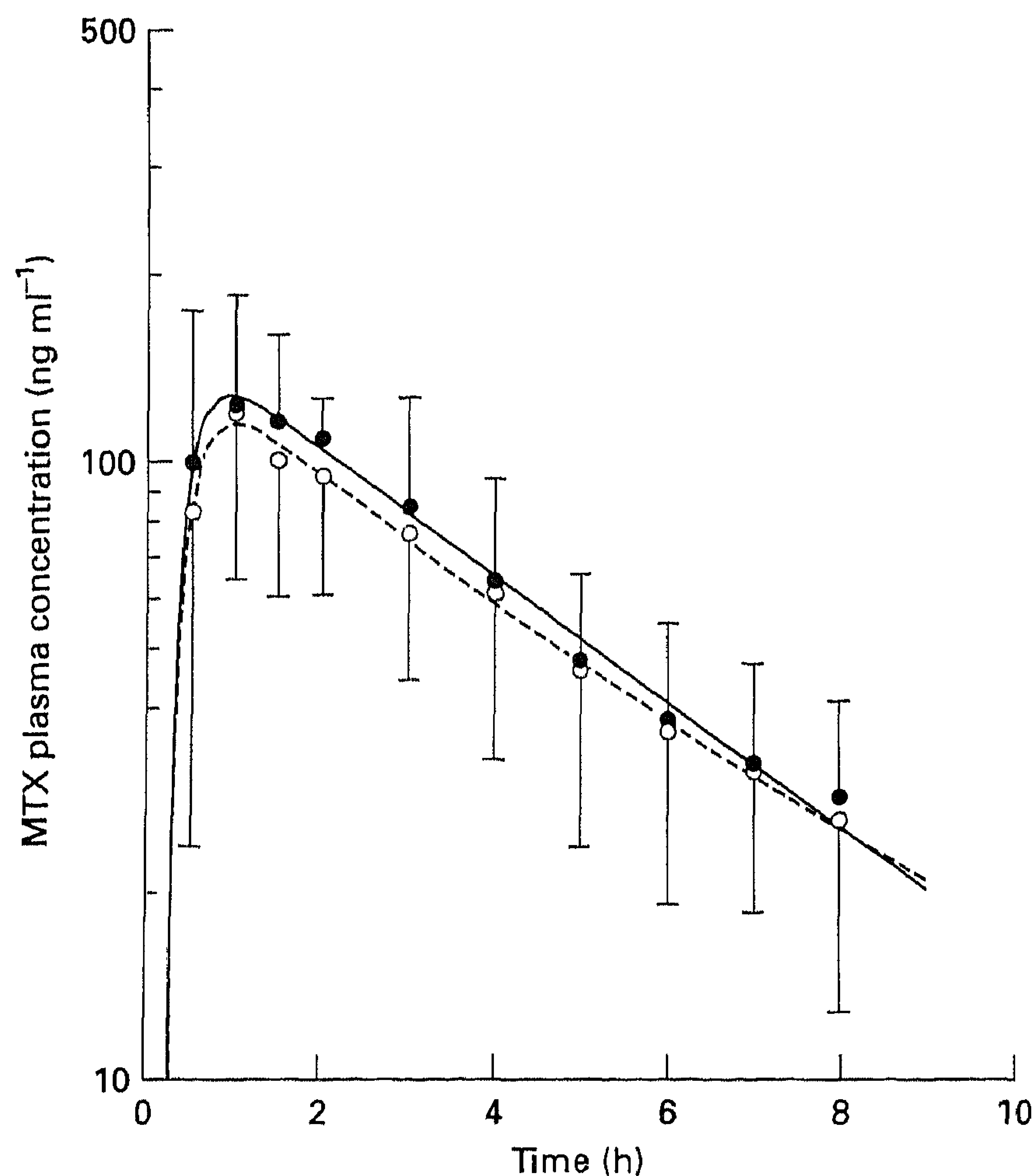


Figure 1 Plasma concentration-time curves of methotrexate without (●) and with sulphasalazine (○). Values are presented as mean and s.d.

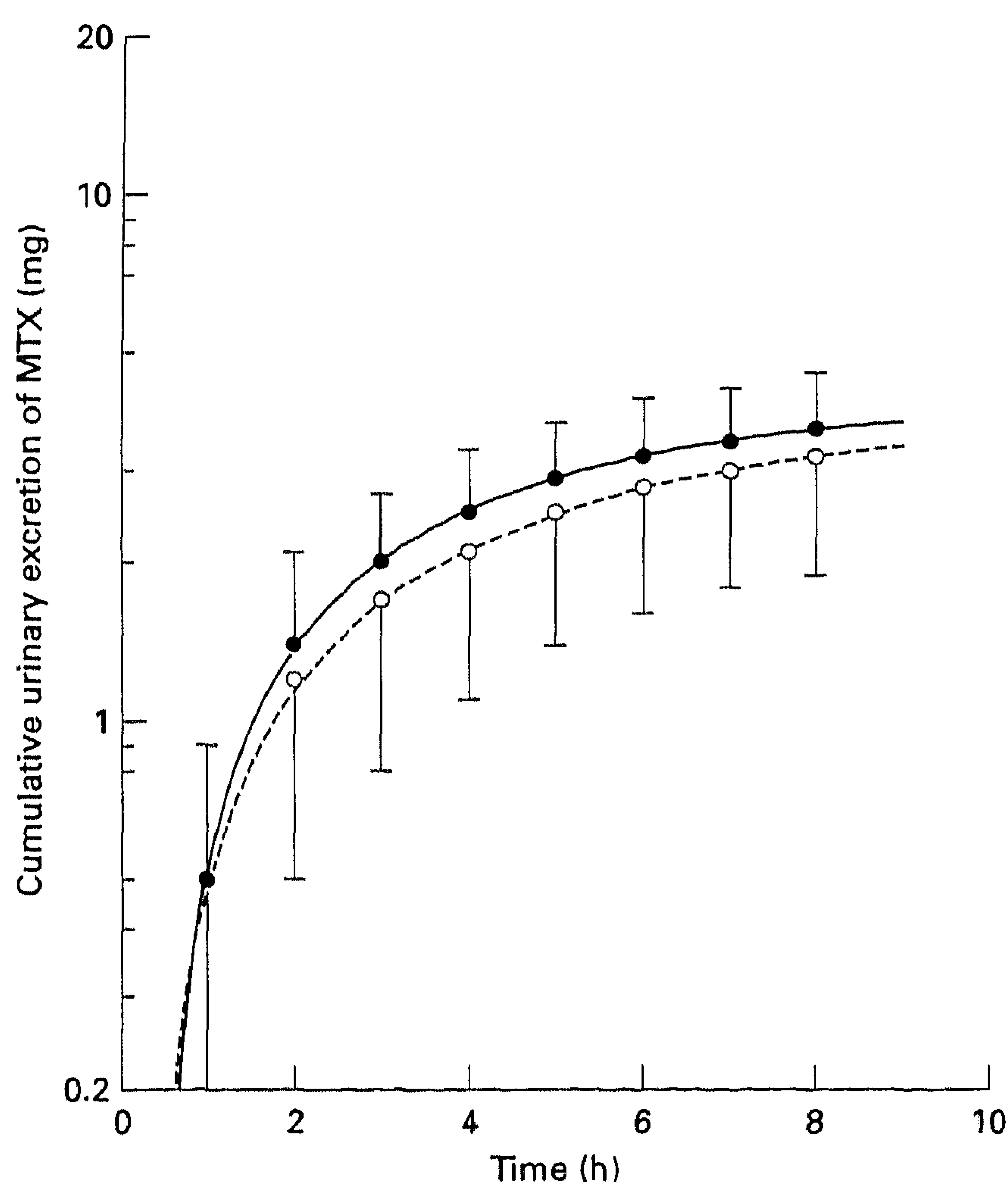


Figure 2 Cumulative urinary excretion of methotrexate without (●) and with sulphasalazine (○). Values are presented as mean and s.d.

with MRT, $t_{\frac{1}{2}}$ and volume of distribution (Spearman rank correlation coefficients 0.44, 0.51 and 0.50, P values 0.05, 0.05 and 0.1, respectively).

Discussion

This study shows that there is no pharmacokinetic interaction between SASP and MTX in doses used to

treat rheumatoid arthritis. Nor was there a consistent correlation between pharmacokinetic parameters of MTX and measures of efficacy and toxicity.

In the 15 RA patients studied, the mean values of the key parameters of single (low) dose MTX pharmacokinetics, e.g. the area under the curve (AUC), the mean residence time (MRT) and the total body clearance were statistically not different, without and with concomitant chronic administration of SASP. Also, the trough serum levels of SASP and major metabolites were comparable with or without MTX. A type II error seems unlikely given the relatively narrow 95% confidence intervals of the individual differences between the pharmacokinetic variables of the drugs administered singly and in combination. The disease activity of the patients variably decreased and there was considerable interindividual variation in the pharmacokinetics of the single dose MTX, but there was no consistent correlation between the two. A weak correlation existed between ESR and the pharmacokinetic parameters MRT, $t_{\frac{1}{2}}$ and V/F ($0.05 \leq P < 0.1$). We chose to study the kinetics of MTX in detail and of SASP only by trough levels for the following reasons. First, given the large difference between the dose of MTX and SASP, the latter is possibly more likely to influence the pharmacokinetics of the first than *vice versa*. Second, because we wanted to study the steady state of SASP, trough levels reflect well enough the levels of SASP and its major metabolites to which the body is exposed. The improved clinical response to the combination of MTX and SASP, previously reported in a clinical trial [22], cannot therefore be explained by altered pharmacokinetics of MTX or SASP and its major metabolites.

The values of the pharmacokinetic parameters of MTX found in our study are in line with those found by others [23, 24]. This is the first study specifically dealing with the possible pharmacokinetic interaction between SASP and MTX. Although one other study [25] mentioned this combination in children with juvenile rheumatoid arthritis, no reliable data concerning the pharmacokinetic interaction between the two compounds are extractable from that article.

We measured MTX levels in blood and urine for 8 h. There was an excellent fit of the model to the data. The comparability of model-dependent and model independent results was good. We believe it is unlikely that longer sampling times would have led to other conclusions.

The dose of NSAIDs and other concomittant medication was kept strictly the same, avoiding a possible bias in the results. Although some studies have mentioned an influence of NSAIDs on (renal) clearance of MTX [26, 27], others found no pharmacokinetic interaction between the two when using low dose MTX, as was concluded in a recent review [28]. An influence of the concomittant administration of NSAIDs on the results is therefore unlikely.

There are various abstracts describing the relationship between the pharmacokinetics of MTX and clinical results of the drug in rheumatoid arthritis. Furst [29] found that toxicity correlated with 1 h levels of MTX. In a study by Pons *et al.* [30] the elimination constant,

Table 1 The pharmacokinetics of MTX without and with concurrent administration of sulphasalazine

	AUC (ng ml ⁻¹ h)	MRT (h)	t _{1/2,z} (h)	V/F (l)	CL/F (l h ⁻¹)	CL _R /F (l h ⁻¹)
MTX	673 (179)	5.2 (1.3)	4.3 (1.1)	59.3 (29.3)	12.3 (5.0)	6.2 (1.3)
MTX+SASP	628 (210)	5.2 (1.1)	4.2 (1.1)	65.5 (25.3)	13.4 (4.8)	6.3 (2.1)
Difference (95% CI)	44 (-71,159)	0.0 (-0.4,0.4)	0.1 (-0.3,0.5)	-6.2(-23.8,11.4)	-1.1 (-4.5,2.3)	-0.1 (-1.3,1.1)
P value	0.4	0.5	0.6	0.3	0.4	0.8

AUC=area under curve, MRT=mean residence time, t_{1/2,z}=half life of elimination, V=volume of distribution, F=fraction absorbed (unknown), CL=total body clearance, CL_R=renal clearance. Values are presented as means (s.d.) unless otherwise stated.

Table 2 Trough-levels* of sulphasalazine (SASP) and major metabolites during SASP only and after 4 weeks of combination treatment with SASP and methotrexate (MTX)

	SASP (µg ml ⁻¹)	Sulphapyridine (µg ml ⁻¹)	ac-Sulphapyridine (µg ml ⁻¹)
SASP only	3.3 (2.1-8.2)	10.4 (6.3-21.4)	10.5 (7.0-16.8)
SASP + MTX	3.3 (2.8-9.0)	11.2 (7.0-18.9)	12.5 (7.5-18.3)
P value	0.2	0.4	0.7

*Medians (1st-3rd quartile).

Table 3 Clinical results of the 15 patients

Variable	Week 0	Week 24	P value*
Ritchie articular index	16 (12-26)	3 (2-7)	0.0001
Number of painful joints	26 (17-32)	4 (2-10)	0.0001
Number of swollen joints	19 (18-28)	9 (2-14)	0.0001
Morning stiffness (min)	120 (30-180)	5 (0-90)	0.01
ESR (mm)	40 (30-53)	18 (11-37)	0.007
VAS general health (mm)	64 (36-81)	24 (19-45)	0.02
Disease activity score	4.9 (4.7-6.2)	2.9 (1.8-3.8)	0.001

Results are expressed as medians (upper and lower quartiles). *The change between week 0 and week 24 was tested.

but not the AUC correlated with the number of tender joints. However, no consistent pattern of correlation between pharmacokinetics and efficacy or toxicity of MTX emerges. A study by Lafforgue *et al.* [31] failed to show any relationship between pharmacokinetics and efficacy. This is somewhat surprising because a dose-effect relationship is clinically obvious for all who work with this agent in rheumatoid arthritis and this was demonstrated in a study by Seideman [32]. Although our results have to be interpreted carefully because the pharmacokinetics of MTX were compared with the clinical results of the combination, in our study too no consistent correlation emerged. In most studies including ours, however, a small number of patients was investigated, and given the large interindividual variation a type II error (assuming no correlation when in fact there may be one) possibly conceals important relationships.

In conclusion, no pharmacokinetic interaction between low-dose MTX and SASP could be demonstrated in patients with rheumatoid arthritis. Although the sample size was relatively small for definite corre-

lational analysis, there was no consistent relationship between pharmacokinetic parameters of MTX and efficacy and/or toxicity.

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