Mod Rheumatol (2004) 14:135–142
DOI 10.1007/s10165-004-0280-y

ORIGINAL ARTICLE

Yoshiko Hiraga · Yoshimitsu Yuhki · Katsumi Itoh
Kohji Tadano · Yasushi Takahashi · Masaya Mukai

Pharmacokinetics and efficacy of low-dose methotrexate in patients with rheumatoid arthritis

Received: June 25, 2003 / Accepted: December 9, 2003

Abstract This article evaluates the relationship between the pharmacokinetics of methotrexate (MTX), its efficacy in the treatment of rheumatoid arthritis (RA), and serum folic acid (FA) levels. The pharmacokinetics of MTX was studied in 29 patients with RA treated with low-dose MTX. The weekly dose of MTX was given orally at 2–4 mg every 12 h over a period of 24–36 h. Blood samples were taken 4 h after the first administration in any given week. A Bayesian method was used to estimate individual MTX pharmacokinetic variables. We then investigated the efficacy of MTX and the serum FA levels in these patients. We examined C-reactive protein levels (CRP) and the erythrocyte sedimentation rate (ESR), and analyzed the values obtained before and after MTX treatment in order to evaluate the efficacy of the MTX treatment. The degree of improvement in CRP and ESR was significantly correlated with the length of time the MTX concentration–time curve remained above 0.02 μM in one week. Furthermore, the degree of improvement in CRP was also significantly correlated with the area under the concentration–time curve (AUC) for MTX. These results suggest that serum MTX measurements could be useful in determining individual patient regimens.

Key words Methotrexate (MTX) · Pharmacokinetics · Rheumatoid arthritis (RA)

Y. Hiraga · Y. Yuhki · K. Itoh · K. Tadano · Y. Takahashi
Department of Pharmacy, Sapporo City General Hospital, Sapporo, Japan

M. Mukai (✉)
Division of Clinical Immunology and Hematology, Department of Medicine, Sapporo City General Hospital, Kita 11-jo, Nishi 13-chome, Chuo-ku, Sapporo 060-8604, Japan
Tel. +81-11-726-2211; Fax +81-11-726-9541
e-mail: masaya-mukai@hokkaido.med.or.jp

Introduction

Methotrexate (MTX) has been shown to be effective in the treatment of rheumatoid arthritis (RA), and many rheumatologists in the West select MTX as their initial disease-modifying antirheumatic drug (DMARD).[1] However, its efficacy is estimated at 60%–70%.[2–4] Indeed, there are quite a few patients for whom MTX has no effect.

Numerous authors have reported on the use and efficacy of MTX in patients with RA. The pharmacokinetics of low-dose MTX has also been studied repeatedly. To date, analyses of population pharmacokinetics, and investigations of pharmacokinetics using a Bayesian method[5] with limited sampling have been performed.[6–9] In these studies, large interindividual variability in the pharmacokinetics of low-dose MTX has been observed,[10,11] whereas Lebbe et al.[12] reported only a modest intraindividual variability. Therefore, if the efficacy of low-dose MTX correlates with its pharmacokinetics, it would be useful to determine a suitable regimen for each patient by investigating the pharmacokinetics of each individual. Several studies have demonstrated that MTX therapy is more efficient at a high dosage in RA and juvenile rheumatoid arthritis (JRA), although there is a higher risk of adverse reactions.[13–15] However, a clear relationship between the pharmacokinetics and the efficacy of low-dose MTX has not yet been established.[12,16] Although Wallace et al.[17] found that the improvement in JRA was significantly associated with MTX dosage and with the mean 1-h serum MTX level, Lafforgue et al.[12] suggested that "the MTX 1 h serum concentration is a somewhat partial and inaccurate approach to MTX pharmacokinetics."

The aim of this study was to estimate the pharmacokinetic parameters of low-dose MTX using a serum sample analysis at a single time point, and then investigate the relationship between the pharmacokinetics of MTX and its efficacy in the treatment of RA. We further investigated the effect of folic acid (FA), which is sometimes co-administrated to diminish the toxicity of MTX, on the pharmacokinetics of MTX and its efficacy. We examined

C-reactive protein levels (CRP) and erythrocyte sedimentation rate (ESR), and analyzed the values obtained before and after MTX treatment to evaluate the efficacy of MTX. Here, we report the potential correlation between the concentration-based pharmacokinetics of MTX and its efficacy.

Materials and methods

Patients

The pharmacokinetics of MTX was studied in 29 outpatients with RA who began low-dose MTX therapy at Sapporo City General Hospital. Six patients were male and 23 were female, and the group had the following parameters (mean ± SD): age 59.2 ± 10.0 years (range 41–75 years); body weight 57.5 ± 11.6 kg (range 37–83 kg). Serum creatinine concentration was 0.66 ± 0.15 mg/dl (range 0.4–1.0 mg/dl). Their disease stages were I (1 patient), II (15 patients), III (5 patients), and IV (8 patients). The classes were I (5 patients), II (14 patients), and III (10 patients). Twenty-two patients were taking nonsteroidal anti-inflammatory drugs, and 21 patients were being treated with low-dose prednisolone daily. Nineteen patients had received several DMARDs (salazosulfapyridine, bucillamine, and actarit). The duration of MTX treatment was 20.1 ± 22.1 months (range 1–99 months). The MTX treatment had no toxic reactions in any patient. No patient had evidence of ongoing renal or hepatic disease.

We investigated the efficacy of MTX and serum FA levels in these patients, and analyzed the following relationships: the pharmacokinetics and the efficacy of MTX, serum FA levels and the pharmacokinetics of MTX, and serum FA levels and the efficacy of MTX.

In the investigation of serum FA levels, one patient whose serum FA level was out of the detection limit was excluded. No patient in this study received FA supplementation.

We obtained informed consent from all patients.

MTX administration

The dosage of MTX was 6.55 ± 2.06 mg/week (range 4–10 mg/week). The weekly dose of MTX was given orally at 2–4 mg every 12 h over a period of 24–36 h.

Determination of MTX levels

Blood samples were taken approximately 4 h (3.61 ± 0.55 h) after the first administration of MTX in any given week. The blood samples were centrifuged for each patient separately, and serum MTX concentrations were determined using a fluorescence polarization immunoassay technique (FPIA; TDX, Abbott Laboratories, Abbott Park, IL, USA).

Determination of CRP, ESR, and serum FA levels

Samples were collected for the measurement of CRP, ESR, and serum FA levels on the same day that samples were collected for the MTX assay. We defined these values for CRP and ESR as the "values after MTX treatment (= after value)." In addition, we defined the values for CRP and ESR, which were recorded in clinical charts just before the initiation of MTX therapy, as the "values before MTX treatment (= before value)."

CRP was determined by latex immunoassay. ESR was found by the Westergren method. Serum FA levels were determined using a chemiluminescent immunoassay.

Pharmacokinetic analysis

A two-compartment model with first-order absorption and with lag time was employed as the pharmacokinetic model of MTX. This model was defined by the following parameters: absorption rate constant (k_a), volume of the central compartment (V_d), transfer rate constants between the two compartments (k_{12} and k_{21}), total body clearance (CL), bioavailability (F), and lag time (t_{lag}).

The pharmacokinetic parameters were estimated using a Bayesian method[5] and the population values of a previous report by Godfrey et al.[6] For the Bayesian estimation, MULTI2 (BAYES)[18] was used on a personal computer running Windows Me. In this study, we assumed that t_{lag} was 0.225 h in accordance with the Godfrey report.[6]

On the assumption that the intervals between each administration were 12 h, and that the values of these parameters were constant for each patient, the following additional parameters were subsequently derived: maximal concentration (C_{max}), area under the concentration–time curve (AUC), the length of time that the concentration–time curve was above 0.02 μM in one week (0.02 μM time), the volume of distribution at a steady state (V_{ss}), and the terminal half-life ($t_{1/2\beta}$). C_{max}, AUC, and 0.02 μM time are illustrated in Fig. 1.

Relationships

The relationships between the pharmacokinetic parameters (C_{max}, AUC, and 0.02 μM time) and the clinical effect of MTX were investigated. We examined CRP and ESR in order to evaluate the efficacy of MTX. To clarify the effect of MTX therapy, the "quantity of change," the "rate of change," and the "rate of improvement" in CRP and ESR were calculated. The rate of change was examined logarithmically. We defined each variable as follows:

$$\text{Quantity of change} = \text{After value} - \text{Before value}$$

$$\text{Rate of change}\,(\log) = \log(\text{After value}/\text{Before value})$$

$$\text{Rate of improvement}\,(\%) = 100 \times (\text{Before value} - \text{After value})/\text{Before value}$$

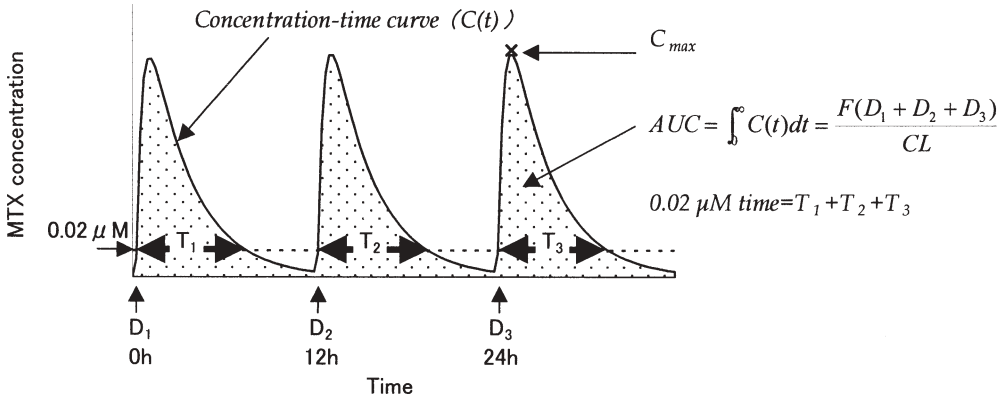

Fig. 1. Illustration of the maximal concentration (C_{max}), the area under the concentration–time curve (AUC), and the 0.02 μM time. D_1, D_2, and D_3 are the doses at each time. This figure shows an example of a concentration–time curve for a patient who received three doses of methotrexate (MTX) at 12-h intervals ($D_1 = D_2 = D_3$). *CL*, total body clearance

We investigated the correlations between the pharmacokinetic parameters of MTX and the "quantity of CRP change," the "rate of CRP change (log)," the "quantity of ESR change." and the "rate of ESR change (log)." In addition, we divided the patients into two groups using rate of improvement as the criterion in its median. We then compared the means of the pharmacokinetic parameters which were significantly correlated with the changes in CRP or ESR between a group of patients who showed greater improvement and a group of patients who showed less improvement.

Similarly, we investigated the correlations between serum FA levels and the pharmacokinetic parameters of MTX. These included not only C_{max}, AUC, and 0.02 μM time, but also CL/*F*, V_{ss}/F, and $t_{1/2\beta}$, in accordance with the report by Bressolle et al.[19]

In addition, we investigated the correlations between serum FA levels and quantity of CRP change, rate of CRP change (log), quantity of ESR change, and rate of ESR change (log).

Statistical evaluation

Correlations were examined using a simple linear regression analysis, and a statistical evaluation was performed using Pearson's correlation coefficient. Comparisons between two groups with equal variance were performed using Student's *t*-test, and those with unequal variance were performed using Student's *t*-test with Welch's correction. Statistical significance was defined as a P value <0.05. Data are reported as mean ± SD.

Results

MTX pharmacokinetic parameters

The pharmacokinetic parameters of MTX estimated using a Bayesian method are summarized in Table 1. All the pharmacokinetic parameters for MTX varied from patient to patient. In particular, C_{max}, AUC, and 0.02 μM time, which could be influenced by the use and the dosage of MTX administration, varied considerably; C_{max} was 0.113–0.465 μM (0.241 ± 0.103 μM), AUC was 1.10–6.46 μM·h (2.55 ± 1.20 μM·h), and 0.02 μM time was 11.9–44.3 h (28.8 ± 9.7 h). Furthermore, CL/*F*, which should have no connection with the use and dosage of MTX administration, also varied considerably, with a maximum value more than three times greater than its minimum value, i.e., 3.27 − 10.86 l/h (6.16 ± 1.79 l/h).

Table 1. Pharmacokinetic parameters of methotrexate (MTX) (n = 29). Data are mean ± SD, and minimum to maximum values

	Mean ± SD	(Min–Max)
V_d/F (l)	18.8 ± 4.0	(11.8–28.0)
CL/*F* (l/h)	6.16 ± 1.79	(3.27–10.86)
k_a (h^{-1})	2.30 ± 0.48	(1.46–3.12)
k_{12} (h^{-1})	0.181 ± 0.044	(0.110–0.287)
k_{21} (h^{-1})	0.181 ± 0.048	(0.095–0.264)
C_{max} (μM)	0.241 ± 0.103	(0.113–0.465)
AUC (μM·h)	2.55 ± 1.20	(1.10–6.46)
0.02 μM time (h)	28.8 ± 9.7	(11.9–44.3)
V_{ss}/F (l)	38.3 ± 9.2	(24.5–60.4)
$t_{1/2\beta}$ (h)	7.36 ± 1.29	(5.31–10.91)

Effect of MTX pharmacokinetics on CRP

The mean before and after values of CRP were 4.49 ± 3.02 mg/dl and 2.35 ± 2.48 mg/dl, respectively. Both quantity of CRP change and rate of CRP change (log) were inversely correlated with AUC and 0.02 μM time. These results show that CRP decreased as AUC increased and as the 0.02 μM time extended. They were especially strongly correlated with 0.02 μM time. In contrast, they were not correlated with C_{max} (Fig. 2), and they were not significantly correlated with either the weekly doses or the duration of MTX treatment.

A comparison between the group of patients whose CRP improved by 60% or more (n = 14, group A) and the group of patients whose CRP improved by less than 60% (n = 15, group B) showed a difference in AUC and 0.02 μM time. The mean AUC of group A was significantly larger than that of group B (3.05 ± 1.35 μM·h vs. 2.08 ± 0.82 μM·h; P = 0.031). Moreover, the mean 0.02 μM time of group A was significantly longer than that of group B (33.2 ± 7.1 h vs. 24.7 ± 10.1 h; P = 0.015) (Fig. 3).

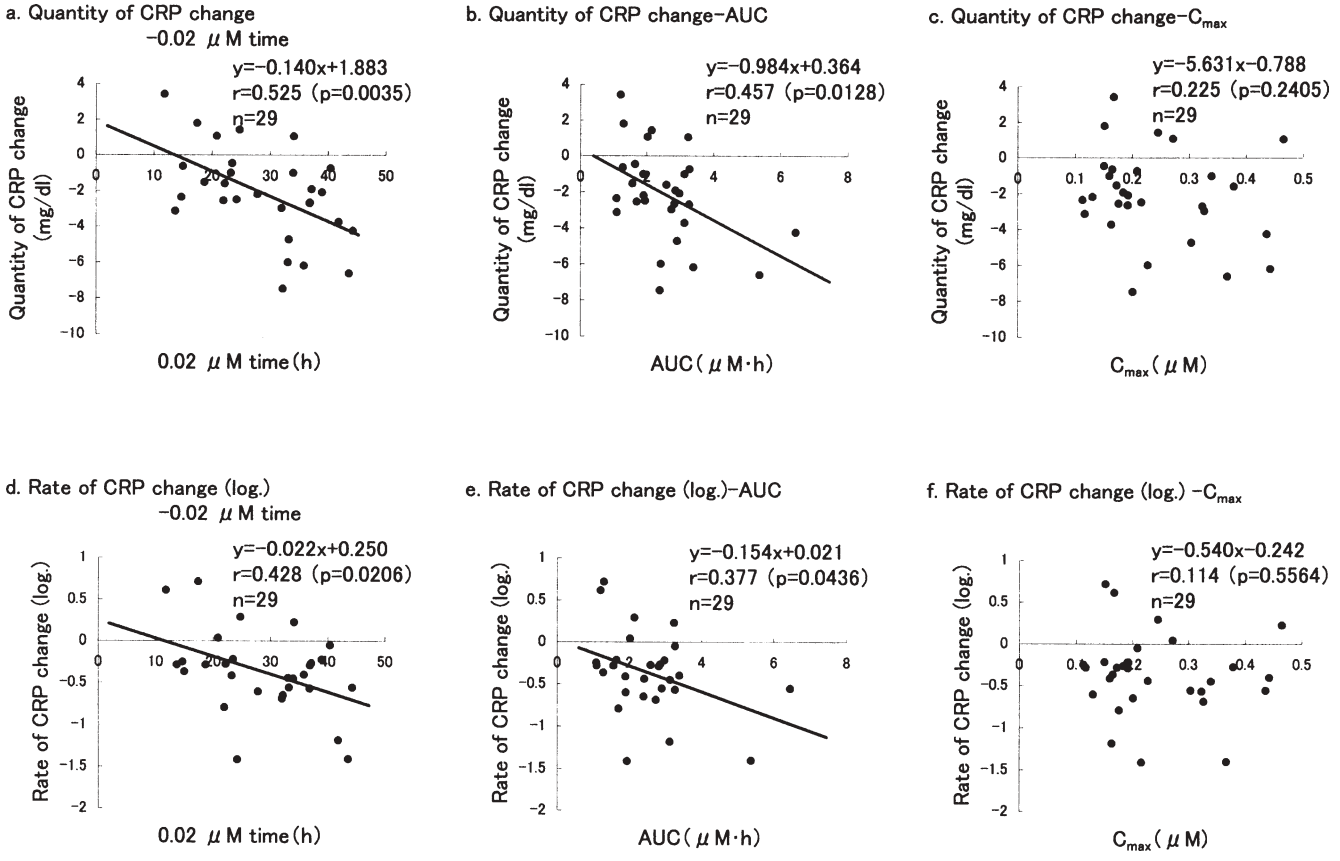

Fig. 2. Correlation between the change in C-reactive protein (*CRP*) and the pharmacokinetic parameters of MTX (0.02 μM time, AUC, and C_{max}) in patients with rheumatoid arthritis (*RA*)

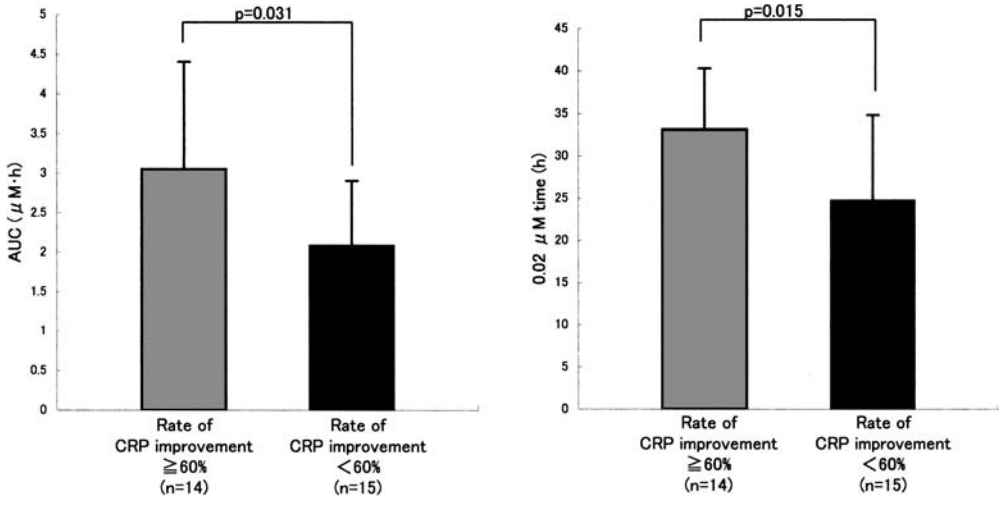

Fig. 3. Mean AUC and 0.02 μM time of MTX in RA patients with high (≥60%) and low (<60%) rates of CRP improvement. The patients were divided into two groups using the "rate of CRP improvement" as the criterion in its median

Effect of MTX pharmacokinetics on ESR

The mean before and after values for the ESR were 62.9 ± 29.8 mm/h and 43.4 ± 25.3 mm/h, respectively. Both quantity of ESR change and rate of ESR change (log) were inversely correlated with 0.02 μM time. These results show that ESR decreased as 0.02 μM time increased. Although they tended to be inversely correlated with AUC, the correlations did not reach statistical significance. Similarly, they were not significantly correlated with the C_{max} (Fig. 4), the weekly doses, or the duration of MTX treatment.

A comparison between the group of patients whose ESR improved by 20% or more (n = 14, group C) and the group of patients whose ESR improved by less than 20% (n = 15, group D) showed no significant difference in 0.02 μM time (30.2 ± 8.4 h vs. 27.3 ± 11.0 h; not significant), although the mean value for group C was slightly longer than that of group D (data not shown).

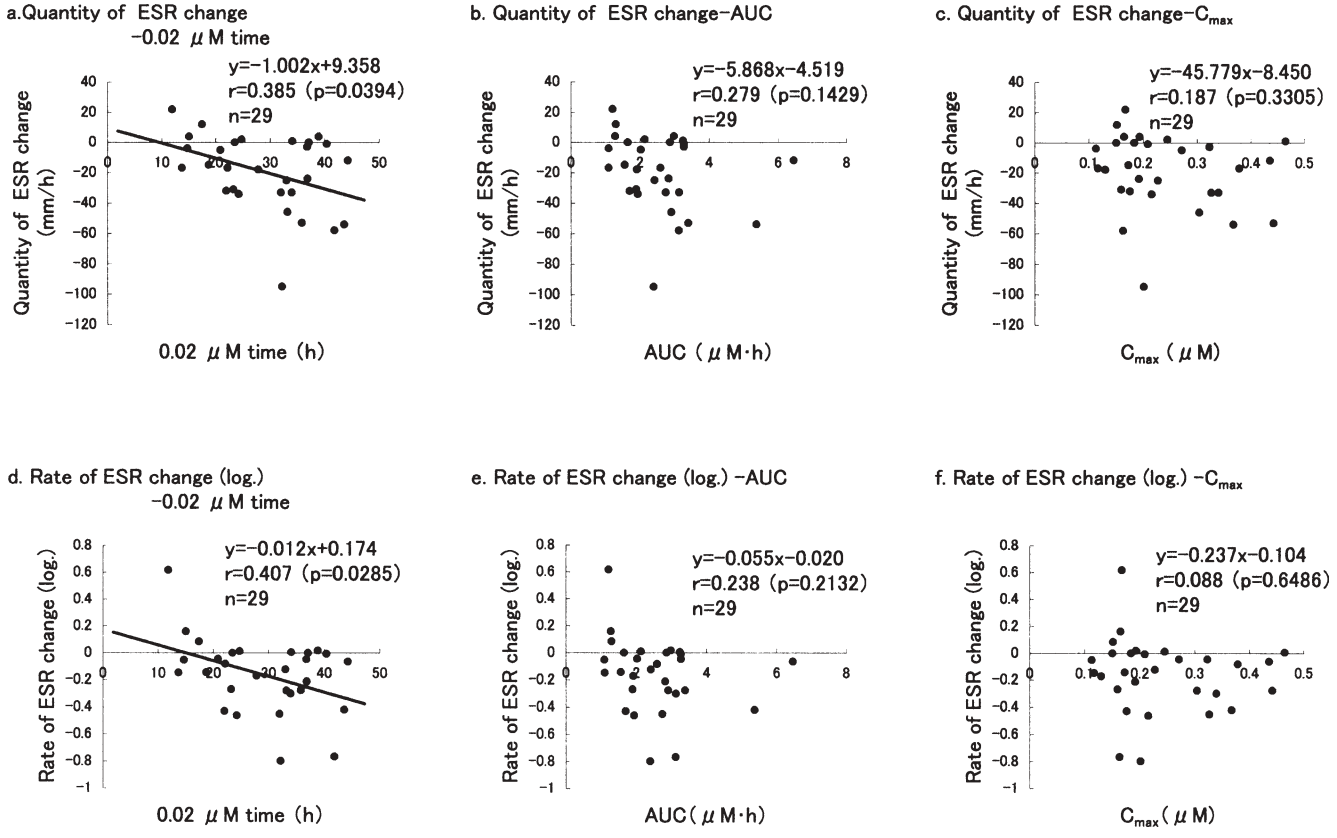

Fig. 4. Correlation between the change in erythrocyte sedimentation rate (*ESR*) and the pharmacokinetic parameters of MTX (0.02 μM time, AUC, and C_{max}) in patients with RA

Effect of serum FA level on the MTX pharmacokinetics/efficacy

The mean serum FA level was 6.78 ± 2.88 ng/ml. AUC and 0.02 μM time showed significantly positive correlations with serum FA levels. These results show that the increase in serum FA levels led to an increase in AUC and an extension of the 0.02 μM time. In addition, CL/*F* was inversely correlated with serum FA levels. The result shows that the increase in serum FA levels led to a decrease in CL/*F*. However, $C_{max,}$ V_{ss}/F, and $t_{1/2\beta}$ were not significantly correlated with serum FA levels (Fig. 5).

None of the values quantity of CRP change, rate of CRP change (log), quantity of ESR change, or rate of ESR change (log) was correlated with serum FA levels.

Discussion

In order to estimate pharmacokinetic parameters more precisely, multiple blood samples are preferred. However, this method is less convenient and entails higher costs. Bressolle et al.[7] reported a method to estimate individual pharmacokinetic variables for MTX using a Bayesian method with two plasma concentration measurements, taken 2 h and 8 h after drug administration.

Here we used the analysis of a single serum sample in order to minimize the outpatient burden. Although blood samples were taken only once, we believe that the precision of the pharmacokinetic estimation could be improved for the following reasons. Firstly, we took the serum samples about 4 h after MTX intake. At this time, Lebbe et al.[12] demonstrated a high correlation between AUC and blood concentrations, and therefore it seems reasonable to suppose that the phases of absorption and distribution, in which the concentrations could vary, should have been finished by that time. Secondly, we used population parameters that reflected the characteristics of each patient (age, sex, body weight, and creatinine clearance estimated by Yasuda's expression[20]) in accordance with the report by Godfrey et al.[6] Although the population model was obtained in Caucasian patients, there is no great difference between the Caucasian and Japanese population in the pharmacokinetics of MTX in high-dose treatments.[21,22] For this reason, we considered that we could apply these parameters to low-dose MTX for our Japanese patients.

However, we must also take into consideration the risk of obtaining a false result when we analyze pharmacokinetics using a Bayesian method with one blood sample. This is because fluctuations in the pharmacokinetics (e.g., a delay in absorption or elimination) resulting from any comedication[9] may change the outcome of the analyses.

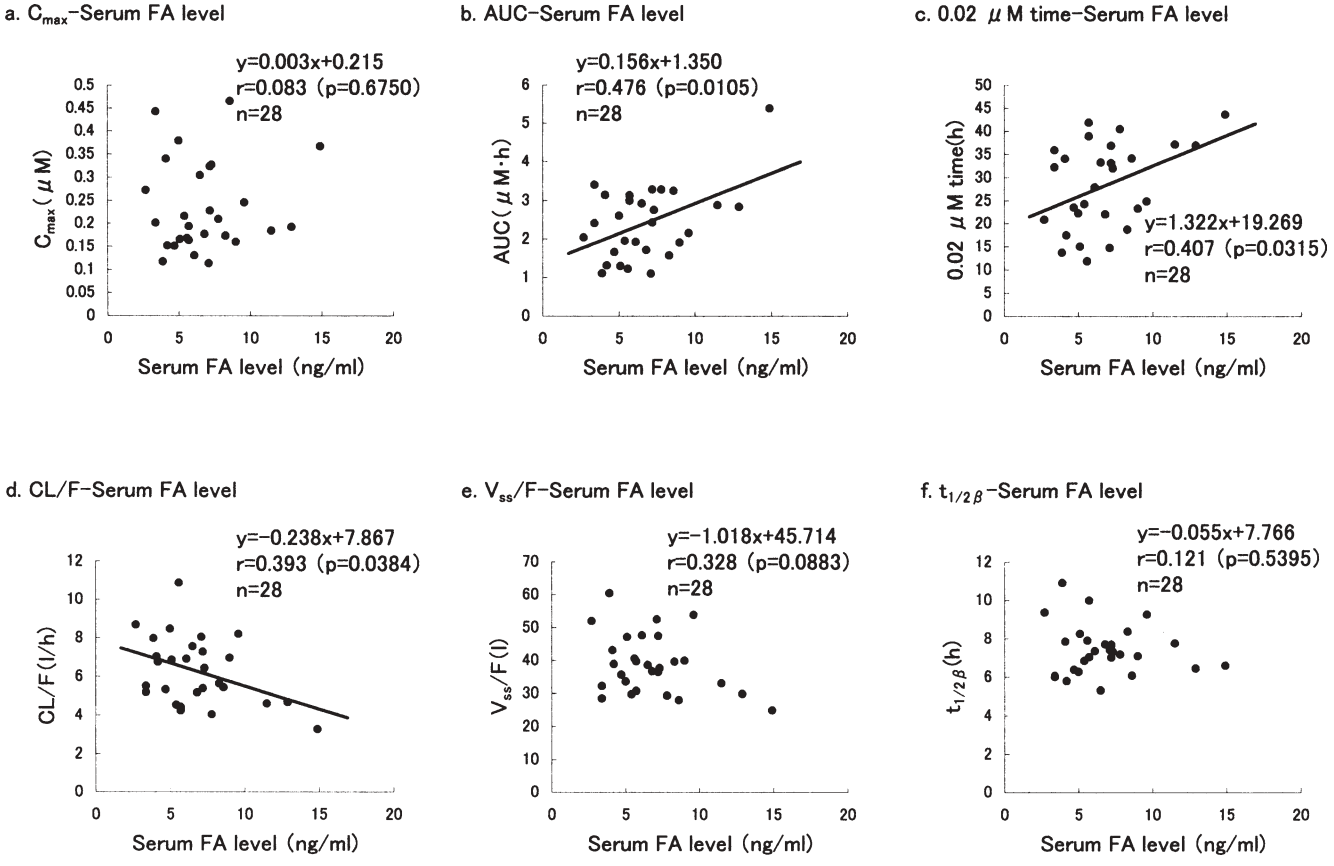

Fig. 5. Correlation between serum folic acid (*FA*) levels and the pharmacokinetic parameters of MTX (C_{max}, AUC, 0.02 μM time, total body clearance/bioavailability (*CL*/*F*), volume of distribution at a steady state/bioavailability (V_{ss}/*F*), and terminal half-life ($t_{1/2\beta}$))

In this investigation, although the mean volume of the central compartment (V_d) was smaller than that in other reports (range 25.2–34.81),[6–8] we consider that this result was derived from the difference in the average body weight of the subjects (range 64.2–75 kg).[6–8]

To date, no report has shown a definite correlation between the efficacy and the pharmacokinetics of MTX in the treatment of RA. Moreover, the precise mechanism of action of MTX in RA has not yet been elucidated.

In studies of high-dose MTX therapy, Bokkerink et al.[23,24] showed the time- and concentration-dependent effects of MTX on malignant human lymphoblasts. They also demonstrated that exposure to 0.02 μM MTX could inhibit DNA synthesis. We used this 0.02 μM concentration in this study,

Our results in this study indicate that the pharmacokinetics of MTX could be correlated with improvements in CRP and ESR. Although only a weak correlation between the change in ESR and the MTX pharmacokinetics was found, we may not have been able to evaluate the ESR change precisely owing to the low rate (mean 15%) of ESR improvement in the patients in this study. As a whole, the results show that a large AUC and a long 0.02 μM time led to improvements in CRP and ESR. Furthermore, the changes in CRP and ESR were strongly correlated with 0.02 μM time, and did not correlate with C_{max}. These results indicate that the efficacy of MTX on RA is time-dependent, i.e., MTX is effective when its concentration continues to be above a certain threshold level.

This is in accordance with the fact that the efficacy of MTX as an anticancer drug is time-dependent.[25] That is, the cytotoxicity of MTX is a function of both concentration and time.

Moreover, in their study on the effect of low-dose MTX therapy in RA, Totokawa et al.[15] reported that therapeutic effectiveness was observed in a group of elderly patients over 65 years old. They presumed that this result was related to an accumulation of MTX due to the decreased clearance in elderly patients. Indeed, a decrease in MTX clearance with age has been observed by other workers.[8,12]

From these reports and our results, it may be presumed that MTX is more effective in elderly patients because of its accumulation and the resulting maintenance of a certain MTX level .

However, patients with renal dysfunction or elderly patients who have a lower MTX clearance rate are liable to undergo adverse events. Thus, the continuance of a high MTX concentration could seriously influence not only its efficacy, but also any side-effects. Further studies will be needed to establish the relationship between the pharmacokinetics and the side-effects of MTX as well as its clinical effects.

FA is sometimes co-administrated to diminish the toxicity of MTX. However, only a few reports on the pharmaco-

kinetic interaction between FA and MTX have been published.

In this study, our results show that an increase in serum FA level relates to an increase in the AUC and a decrease in the CL/*F* of MTX. Although these results are at odds with the report by Bressolle et al.[19] in which they evaluated the effect of repeated doses of FA on the pharmacokinetics of MTX, our results could be interpreted as follows.

Recent studies have shown that the normal transport of MTX into cells is by a carrier-mediated and energy-dependent system. Therefore, cellular uptake of MTX is inhibited in a competitive manner by structural analogs, e.g., 5-methyl tetrahydrofolate, FA, etc.[26] Thus, concentrated FA could block the uptake of MTX, thereby maintaining an elevated MTX concentration in the serum, and this would lead to an increased AUC. With respect to the decrease in CL/*F*, it may be presumed that it was caused by the increase in AUC rather than by the delay in elimination, because $t_{1/2\beta}$ was not correlated with serum FA levels.

Indeed, Takeuchi et al.[27] using cells stably transfected with renal organic anion transporter (OAT-K1) cDNA, reported that OAT-K1-mediated MTX accumulation was inhibited in vitro in the presence of various FA derivatives. In addition, Horne and Reed[28] reported that the in vitro transport of MTX into human prostatic PC-3 cells was inhibited by FA and folate derivatives. On the other hand, recent studies have shown folate depletion in MTX-treated patients.[29,30] These results could also be accounted for by the hypothesis that MTX and FA might mutually inhibit their own transport into tissues in a competitive manner.

However, other mechanisms such as the inhibition of folate absorption and/or polyglutamylation have also been regarded as the causes of folate depletion by MTX.[29,30] A better understanding of the causal mechanism by which MTX depletes FA must be sought.

In this investigation, our results indicate that the pharmacokinetics of MTX could be correlated with its efficacy. In addition, we have shown that serum FA levels could affect the pharmacokinetics of MTX. Therefore, we investigated the relationships between serum FA levels and the changes in CRP and ESR in order to examine the effect of FA on the efficacy of MTX.

We found that the serum FA levels were not correlated with the change in either CRP or ESR, and thus it seems reasonable to suppose that the administration of FA does not directly affect the efficacy of MTX. However, because patients in this study received no FA supplementation, it cannot be denied that high doses of FA may change the MTX pharmacokinetics to a greater extent, and thus influence the efficacy of MTX.

Finally, we estimated the pharmacokinetics of MTX in each individual using a Bayesian method with population variables together with single concentration measurements, and found a relationship between the pharmacokinetics and the efficacy of MTX in the CRP and ESR of patients with RA. Our findings suggest that serum MTX measurements could be useful in determining an appropriate individual regimen.

This investigation was mainly a retrospective one with respect to laboratory data such as ESR and CRP. We could not carry out an adequate investigation into clinical conditions before the MTX treatment, and therefore we could find no correlations between the pharmacokinetic parameters of MTX and the improvements in the patients' evaluations of pain and function. We plan to examine other parameters in the future, including the American College of Rheumatology criteria, and thus advance the investigation of the relationship between the concentration/pharmacokinetics and the efficacy of MTX.

References

1. American College of Rheumatology Subcommittee on Rheumatoid Arthritis Guidelines. Guidelines for the management of rheumatoid arthritis: 2002 update. Arthritis Rheum 2002;46:328–46.
2. Weinblatt ME, Coblyn JS, Fox DA, Fraser PA, Holdsworth DE, Glass DN, et al. Efficacy of low-dose methotrexate in rheumatoid arthritis. N Engl J Med 1985;312:818–22.
3. Weinblatt ME, Kaplan H, Germain BF, Block S, Solomon SD, Merriman RC, et al. Methotrexate in rheumatoid arthritis. A five-year prospective multicenter study. Arthritis Rheum 1994;37:1492–8.
4. Kashiwazaki S, Ichikawa Y, Sugawara S, Nagaya I, Kawai S, Hakota M, et al. Determination of the clinical optimal dose of L-377 (methotrexate capsule) for the treatment of rheumatoid arthritis (in Japanese). Ensho 1996;16:437–58.
5. Sheiner LB, Rosenberg B, Melmon KL. Modelling of individual pharmacokinetics for computer-aided drug dosage. Comput Biomed Res 1972;5:411–59.
6. Godfrey C, Sweeney K, Miller K, Hamilton R, Kremer J. The population pharmacokinetics of long-term methotrexate in rheumatoid arthritis. Br J Clin Pharmacol 1998;46:369–76.
7. Bressolle F, Bologna C, Edno L, Bernard JC, Gomeni R, Sany J, et al. A limited sampling method to estimate methotrexate pharmacokinetics in patients with rheumatoid arthritis using a Bayesian approach and the population data modeling program P-PHARM. Eur J Clin Pharmacol 1996;49:285–92.
8. Debord J, Carpentier N, Sabot C, Bertin P, Marquet P, Treves R, et al. Influence of biological variables upon pharmacokinetic parameters of intramuscular methotrexate in rheumatoid arthritis. Int J Clin Pharmacol Ther 1998;36:227–30.
9. Suzuki T, Suzuki Y, Matsuyama T, Ohhashi H, Hashimoto H. Pharmacokinetics after an intermittent low-dose methotrexate treatment in patients with rheumatoid arthritis (in Japanese). Jpn J TDM 1998;4:340–5.
10. Sinnett MJ, Groff GD, Raddatz DA, Franck WA, Bertino JS Jr. Methotrexate pharmacokinetics in patients with rheumatoid arthritis. J Rheumatol 1989;16:745–8.
11. Lafforgue P, Monjanel-Mouterde S, Durand A, Catalin J, Acquaviva PC. Lack of correlation between pharmacokinetics and efficacy of low-dose methotrexate in patients with rheumatoid arthritis. J Rheumatol 1995;22:844–9.
12. Lebbe C, Beyeler C, Gerber NJ, Reichen J. Intraindividual variability of the bioavailability of low-dose methotrexate after oral administration in rheumatoid arthritis. Ann Rheum Dis 1994;53:475–7.
13. Furst DE, Koehnke R, Burmeister LF, Kohler J, Cargill I. Increasing methotrexate effect with increasing dose in the treatment of resistant rheumatoid arthritis. J Rheumatol 1989;16:313–20.
14. Seideman P. Methotrexate – the relationship between dose and clinical effect. Br J Rheumatol 1993;32:751–3.
15. Totokawa S, Furuya T, Nakajima A, Suzuki T, Kashiwazaki S. Effects of low-dose methotrexate therapy in rheumatoid arthritis: a comparison of three different dosage regimens (in Japanese). Ryumachi 1997;37:681–7.

16. Ravelli A, Di Fuccia G, Molinaro M, Ramenghi B, Zonta L, Regazzi MB, et al. Plasma levels after oral methotrexate in children with juvenile rheumatoid arthritis. J Rheumatol 1993;20: 1573–7.
17. Wallace CA, Bleyer WA, Sherry DD, Salmonson KL, Wedgwood RJ. Toxicity and serum levels of methotrexate in children with juvenile rheumatoid arthritis. Arthritis Rheum 1989;32:677–81.
18. Yamaoka K, Tanigawara Y. MULTI 2 (BAYES) (in Japanese). In: Hori R, editor. Population pharmacokinetics nyuumonn (Primer of population pharmacokinetics). 1st ed. Tokyo: Yakugyojihosya; 1988. p. 103–31.
19. Bressolle F, Kinowski JM, Morel J, Pouly B, Sany J, Combe B. Folic acid alters methotrexate availability in patients with rheumatoid arthritis. J Rheumatol 2000;27:2110–4.
20. Yasuda H. Age-related change in renal function. Differences between the Japanese and the Caucasians in a nomogram and a formula for evaluation of glomerular filtration rate (in Japanese). Med Biol 1980;101:83–6.
21. Sueyasu M, Mishima K, Shuto H, Hirakawa M, Kataoka Y, Oishi R, et al. Population pharmacokinetic parameters of high-dose methotrexate in osteogenic sarcoma (in Japanese). Jpn J TDM 1997;1:85–6.
22. Luyckx M, Cazin JL, Burnet C, Gosselin P, Demaille MC. Clinical pharmacokinetics of a 6-hour infusion of high-dose methotrexate. Preliminary trial of monitoring high infusion doses. Eur J Clin Pharmacol 1985;28:457–62.
23. Bokkerink JP, Bakker MA, Hulscher TW, De Abreu RR, Schretlen ED, van Laarhoven JP, et al. Sequence-, time- and dose-dependent synergism of methotrexate and 6-mercaptopurine in malignant human T-lymphoblasts. Biochem Pharmacol 1986;35: 3549–55.
24. Bokkerink JP, De Abreu RA, Bakker MA, Hulscher TW, van Baal JM, Schretlen ED, et al. Effects of methotrexate on purine and pyrimidine metabolism and cell-kinetic parameters in human malignant lymphoblasts of different lineages. Biochem Pharmacol 1988;37:2329–38.
25. Skipper HE, Schabel FM Jr, Mellett LB, Montgomery JA, Wilkoff LJ, Lloyd HH, et al. Implications of biochemical, cytokinetic, pharmacologic, and toxicologic relationships in the design of optimal therapeutic schedules. Cancer Chemother Rep 1970;54:431–50.
26. Fujimoto T. Methotrexate with leucovorin rescue (in Japanese). Igaku no Ayumi 1998;184:440–3.
27. Takeuchi A, Masuda S, Saito H, Hashimoto Y, Inui K. Transstimulation effects of folic acid derivatives on methotrexate transport by rat renal organic anion transporter, OAT-K1. J Pharmacol Exp Ther 2000;293:1034–9.
28. Horne DW, Reed KA. Transport of methotrexate into PC-3 human prostate cancer cells. Arch Biochem Biophys 2001;394:39–44.
29. Kremer JM, Galivan J, Streckfuss A, Kamen B. Methotrexate metabolism analysis in blood and liver of rheumatoid arthritis patients. Association with hepatic folate deficiency and formation of polyglutamates. Arthritis Rheum 1986;29:832–5.
30. Leeb BF, Witzmann G, Ogris E, Studnicka-Benke A, Andel I, Schweitzer H, et al. Folic acid and cyanocobalamin levels in serum and erythrocytes during low-dose methotrexate therapy of rheumatoid arthritis and psoriatic arthritis patients. Clin Exp Rheumatol 1995;13:459–63.