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ORIGINAL ARTICLE

Hirofumi Shoda · Shigeko Inokuma · Noriyuki Yajima
Yoshiaki Tanaka · Taminori Oobayashi · Keigo Setoguchi

Higher maximal serum concentration of methotrexate predicts the incidence of adverse reactions in Japanese rheumatoid arthritis patients

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Abstract Weekly pulsed low-dose methotrexate (MTX) is a standard regimen for rheumatoid arthritis (RA). Severe adverse reactions to MTX, such as pneumonia and cytopenia, sometimes occur; however, it is difficult to predict the development of these adverse reactions. In this article, we examine the serum concentrations of orally administered MTX of 69 Japanese patients with RA in the clinical setting. The maximum serum concentration (C_{max}) after the first dose of the weekly administration and the time at which C_{max} was obtained (T_{max}) were analyzed. C_{max} correlated with the administered dose before measurement. The average T_{max} was 2.0 ± 0.8 h, and none of the patients showed a T_{max} of more than 4 h. In addition, we demonstrated that the weekly MTX dosage and the mean dosage of steroids were significantly higher in patients with adverse reactions than in those without them, and the C_{max} after the first dose of the weekly administration particularly correlated with the incidence of adverse reactions ($P < 0.001$). In fact, the cut-off point of C_{max} (0.16 μmol/l) was a sensitive predictor of the adverse reactions (sensitivity 81% and specificity 67%). We concluded that C_{max} after the first dose of weekly administration is a useful parameter for predicting the development of adverse reactions to MTX.

Keywords Adverse reactions · Methotrexate · Pharmacokinetics · Rheumatoid arthritis

H. Shoda (✉)
Department of Allergy and Rheumatology, Graduate School of Medicine, the University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
Tel. +81-3-3815-5411; Fax +81-3-3815-5954
e-mail: shoda-tky@umin.ac.jp

S. Inokuma · N. Yajima · Y. Tanaka · K. Setoguchi
Department of Allergy and Immunological Diseases, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan

T. Oobayashi
Department of Laboratory Medicine, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan

Introduction

Weekly pulsed oral low-dose methotrexate (MTX) therapy is to date the standard regimen for rheumatoid arthritis (RA). It is highly effective, and the American College of Rheumatology definition of a 20% improvement (ACR20) achievement is approximately 60%[1–4] in preventing bone erosion and improving mortality and daily activity.[5,6] However, adverse reactions to MTX have limited its usage. Although nausea, appetite loss, and diarrhea are usually not severe, cytopenia, pulmonary injury, and sometimes liver dysfunctions can be life threatening. To date, little is known about the predictive risk factors for these adverse reactions, and it is sometimes difficult to detect early signs of severe adverse reactions.

Recently, the pharmacodynamics and pharmacokinetics of the MTX of Japanese RA patients were reported. Shiozawa et al.[7] demonstrated that the mean maximal serum MTX concentration was achieved at 1–2 h after the intake of the first dose of the week, and the pharmacodynamics of the MTX of the Japanese patients was comparable with those overseas. Another group also showed the relationship between the serum MTX concentration[8]. Hiraga et al.reported that the improvement of C-reactive protein (CRP) levels by MTX therapy significantly correlated with the area under the concentration–time curve for MTX. However, the relationship between the serum MTX concentration and its adverse reactions in the case of low-dose therapy for RA patients is unclear. It is well known that the serum concentration of MTX is related to the cytotoxity of MTX in the field of high-dose therapy for malignancy. In this article, we measured the serum MTX concentration after the first dose of weekly administration in the clinical setting. The maximal serum MTX concentration after the first dose of the week could be obtained up to 3 h after the intake of MTX. We demonstrated that the maximal serum MTX concentration after the first dose of the week significantly correlated with the incidence of the adverse reactions, and was sensitive to predicting the development of the adverse reactions to MTX.

Patients and methods

Patients and MTX administration

Japanese RA patients who underwent weekly pulsed oral low-dosage MTX therapy in the Tokyo Metropolitan Komagome Hospital, and whose serum concentration of MTX was measured from January 1999 to December 2003, were included. RA was diagnosed on the basis of the ACR criteria.[9] The weekly dosage of MTX was started from 2 mg, and gradually increased by 2 mg to a maintenance dosage. The maintenance dosage was defined by the achievement of clinical remission of arthritis, which was determined by the rheumatologists, based on the decrease of CRP levels and the clinical improvement of joint inflammation. The highest dosage was limited at 16 mg. A weekly dosage of MTX was divided into one, two, or three portions at intervals of 12 h. We prescribed 2 mg MTX capsules in this study.

Measurement of serum MTX concentration

The serum MTX concentration was measured after a maintenance dosage of MTX was set. Serum samples were obtained 1 h, 2 h, and 3 h after intake of the first portion of weekly administration. If the concentration 3 h after the administration of the first dosage was the highest measured, then it was measured again 1 h later, that is, 4 h after the administration. For patients who were administered 2–6 mg/week, the serum MTX concentration was measured after the first portion of 2 mg; for those whose weekly dosages were 8–12 mg and 14–16 mg, the measurements were carried out after the first portion of 4 mg and 6 mg, respectively. The patients took MTX after meals. The serum MTX concentration was measured by a fluorescence polarization immunoassay (Abbott-TDx analyzer, Global Medical Instrumentation, Ramsey, MN, USA). The maximum concentration (C_{max}) in this study was defined as the highest MTX concentration among the data obtained at three or four time points after MTX administration. T_{max} in this study was defined as the time when C_{max} was obtained.

Background clinical data and adverse reactions to MTX

We examined the patients' records on age, sex, erythrocyte sedimentation rate (ESR), serum CRP, and the creatinine level immediately before the start of MTX therapy, and coadministered the drugs, ESR, CRP, and the creatinine level at the time of the serum MTX concentration measurement. Adverse reactions to MTX were also examined from the patients' records, including nausea or gastrointestinal distress, skin or mucosal lesions, liver dysfunction (defined as both the AST and ALT levels twofold that of the upper normal limits or higher), leukopenia (white blood cell count <3000/μl), anemia (hemoglobin level <8 mg/dl with a decrease by more than 2 mg/dl within 3 months), thrombocytopenia (platelet count <100 000/μl), interstitial pneumonia, and fever, the cause of which could only be attributed to MTX intake. Coadministered steroids were studied in terms of the dosage equivalent to the prednisolone dosage. We observed the occurrence of adverse reactions caused by MTX therapy after the measurement of the serum MTX concentrations until December 2003. The MTX dosages at the occurrence of the adverse reactions were the same as the dosages at the measurement of the serum concentrations.

Statistical analysis

The correlations among the administered dosage of MTX, C_{max}, and T_{max} were examined by multiple comparisons (Bonferroni's method). Clinical backgrounds and laboratory data were compared using the paired *t* test or the chi-square test between patients with and those without adverse reactions. The threshold C_{max}, which differentiates patients with adverse reactions from those without them, and its sensitivity and specificity were calculated from the receiver operating characteristic (ROC) curve.

Results

Patients' profile

The total number of patients included in this study was 69 (58 women and 11 men), who were followed up until January 2004. The average age at the start of MTX therapy was 61.0 ± 9.0 years (range 42–79). The mean follow-up duration was 39.7 ± 12.0 months (range 7–60 months). The average maintenance dosage of MTX was 7.2 ± 3.4 mg/week. Forty-seven patients took 4–6 mg/week as the maintenance dosage of MTX, 18 patients took 8–12 mg/week, and 4 patients took more than 14 mg/week. Fifty-four patients (78%) took nonsteroidal anti-inflammatory drugs (NSAIDs) and 37 patients (54%) took steroids, together with weekly MTX at a fixed maintenance dosage (Table 1). No patients took folate during the observed period of study.

Pharmacokinetics of orally administrated MTX

The relationship between C_{max} after the first portion of the weekly administration and the dosages of MTX are shown in Fig. 1. The serum MTX concentrations after the first por-

Table 1. Background features of rheumatoid arthritis (RA) patients undergoing weekly pulsed oral low-dosage methotrexate (MTX) therapy

Total number of patients (women:men)	69 (58:11)
Age (range)	60.8 ± 8.9 (42–79) years
Follow-up period (range)	39.7 ± 12.0 (7–60) months
Maintenance dosage of MTX (range)	7.2 ± 3.4 (4–16) mg/week
Coadministered drugs	
Steroid	37 (54%)
Nonsteroidal anti-inflammatory drug	54 (81%)
Other disease-modifying antirheumatic drug	18 (27%)

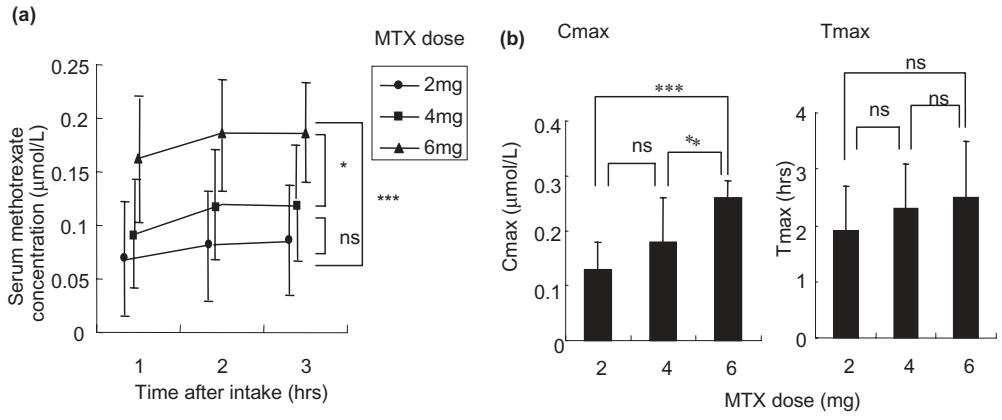

Fig. 1. Pharmacokinetics of methotrexate (MTX). **a** The average of the serum MTX concentrations after the first portion of weekly administration from 1 h to 3 h. **b** The relationship between maximum serum MTX concentration (C_{max}), time when C_{max} were obtained (T_{max}), and the MTX dosage before measurement. *ns* no significant difference, $^{*}P < 0.05$, $^{***}P < 0.001$

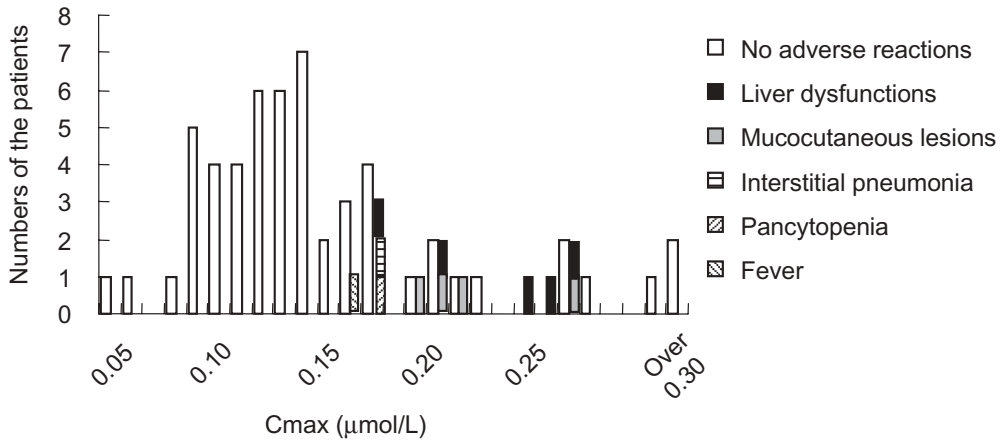

Fig. 2. The maximum serum MTX concentration (C_{max}) after the first dose of the weekly administration and the incidence of the adverse reactions caused by MTX; □ No adverse reactions; ■ Liver dysfunctions; ▨ Mucocutaneous lesions; ▭ Interstitial pneumonia; ▨ Pancytopenia; ▧ Fever

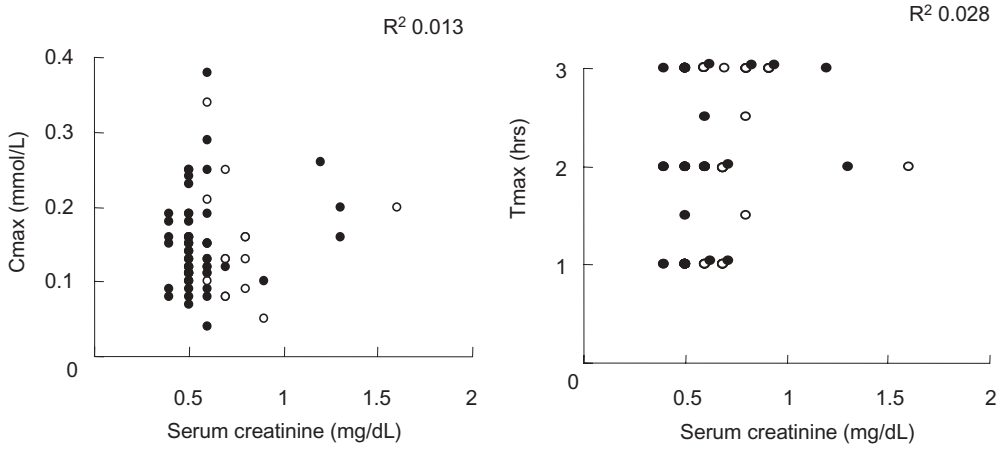

Fig. 3. The correlation between serum creatinine levels and pharmacokinetics of MTX; *filled circles*, female patients, *open circles*, male patients

tion of the weekly administration increased significantly in accordance with the dosages of MTX before measurement (Fig. 1). C_{max} after the first portion of the weekly administration and the numbers of patients for each range of C_{max} are shown in Fig. 2. The average C_{max} was 0.15 ± 0.07 μmol/l (range 0.04–0.38); 56 patients (81%) had a C_{max} of 0.08–0.20 μmol/l. Two patients showed a C_{max} higher than 0.30 μmol/l.

Twenty-six patients (38%) showed a T_{max} of 1 h, 22 patients (32%) 2 h, and 24 patients (35%) 3 h; none of the patients showed a T_{max} of 4 h. The average T_{max} was 2.0 ± 0.8 h. There was no significant correlation between the dosage of MTX and T_{max} (Fig. 1). Between patients with and those without coadministered steroids or NSAIDs, no significant differences were observed either in C_{max} or in T_{max}. In this study, we did not observe a significant correlation between the serum creatinine levels and pharmacokinetics of MTX (Fig. 3).

Comparison of background data between patients with and without adverse reactions

Adverse reactions to MTX developed in 12 patients (17.4%). The observed adverse reactions for each range of C_{max} are

listed in Table 2 and Fig. 2. In patients with adverse reactions to MTX, both the maintenance dosage of MTX and, especially, C_{max}, were significantly higher than those in patients without them (Table 2). Age, serum creatinine level, CRP level, or ESR either at the start of MTX therapy or at the measurement of the serum MTX concentration were not statistically different between the two groups, although the CRP level at the start of MTX therapy and the disease duration before MTX therapy induction tended to be higher and longer in patients with adverse reactions. Nine of 12 patients (75%) with adverse reactions took steroids in contrast to 28 of 57 patients (49%) without them. The steroid dosage for patients with adverse reactions was higher than that for patients without them (4.9 ± 3.4 mg vs. 2.4 ± 3.5 mg as prednisolone, $P < 0.05$). T_{max} did not significantly dif fer between patients with and those without adverse reactions.

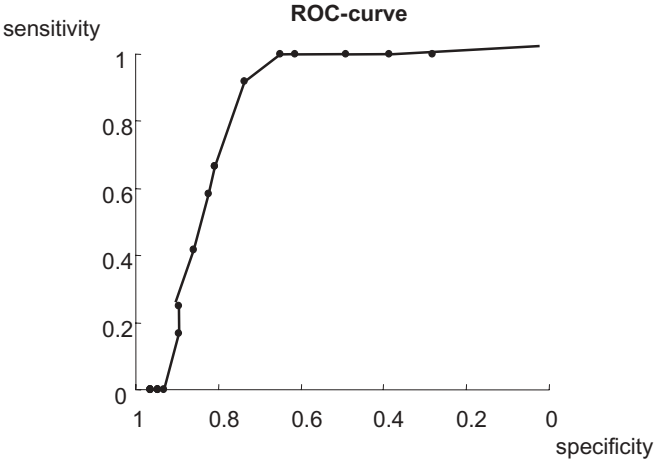

Fig. 4. Receiver operating characteristic curve of C_{max} after the first dosage of the weekly administration to differentiate patients with or without adverse reactions. The threshold C_{max} was calculated to be 0.16 μmol/l, which possessed a sensitivity of 81%, and a specificity of 67%

C_{max} and adverse reactions

The numbers of patients with adverse reactions, the organs involved and the corresponding C_{max} level are shown in Fig. 2. Liver dysfunction and mucocutaneous lesions were commonly observed. Although gastrointestinal disorders, such as nausea and diarrhea are known to be common adverse reactions caused by MTX, no included patient showed these adverse reactions during the observation periods of this study. In fact, three patients claimed nausea soon after the induction of MTX, but these reactions spontaneously disappeared until the maintenance dosages were set. By ROC curve analysis, the threshold C_{max} after the first portion of the weekly administration, which differentiates patients with adverse reactions from those without them, was calculated to be 0.16 μmol/l; its sensitivity for the occurrence of adverse reactions was 67%, and its specificity was 81% (Fig. 4).

Discussion

The pharmacokinetics of weekly pulsed oral low-dosage MTX for Japanese RA patients was analyzed in a clinical setting. Previous studies demonstrated that C_{max} after the administration of 7.5 mg and 10 mg of MTX was approximately 0.5 μmol/l.[10–13] This concentration was considerably higher than that in our study, in which the Japanese patients took low dosages of 2–6 mg of MTX before the measurement. In addition, the previous data and our data suggested that the administrated dosages of MTX would be linearly correlated with C_{max}. Notably, MTX was commonly effective for arthritis in our patients, despite a lower C_{max} than those values reported in Western countries.[11–13] Hiraga et al.[8] reported that the mean C_{max} of MTX after the first administration of the week was 0.241 μmol/l, and demons trated the efficacy of MTX. The relationship between the efficiency of MTX and C_{max} needs to be further studied in

Table 2. Comparison between patients with and those without adverse reactions to MTX

	Adverse reactions		*P*-value
	Present	Absent	
Number of patients	12	57	
Age at start of MTX (years)	62.7 ± 7.2	60.7 ± 9.3	NS
Disease duration until MTX starts (months)	126.3 ± 123.4	72.3 ± 96.7	NS
MTX			
Maintenance dosage (mg/week)	9.6 ± 4.2	6.7 ± 3.0	0.03*
C_{max} (μmol/l)	0.20 ± 0.04	0.14 ± 0.07	<0.001***
T_{max} (h)	2.2 ± 0.8	2.0 ± 0.9	NS
CRP at start of MTX (mg/dl)	5.1 ± 5.4	3.3 ± 2.9	NS
CRP at measurement (mg/dl)	0.8 ± 0.6	0.5 ± 0.4	NS
ESR at start of MTX (mm/h)	68.4 ± 47.6	62.7 ± 27.0	NS
ESR at measurement (mm/h)	45.7 ± 24.4	37.8 ± 19.0	NS
Cre at start of MTX (mg/dl)	0.68 ± 0.35	0.60 ± 0.19	NS
Cre at measurement (mg/dl)	0.65 ± 0.29	0.61 ± 0.16	NS
Patients administered with steroids	7 (75%)	24 (42%)	0.04*
Steroid equivalent to PSL dosage (mg)	4.9 ± 3.4	2.4 ± 3.5	0.04*

CRP, C-reactive protein; Cre, creatinine; PSL, prednisolone; ESR, erythrocyte sedimentation rate; NS, no significant difference
* $P < 0.05$, *** $P < 0.001$

Japanese RA patients. We observed that T_{max} varied from 1 h to 3 h after an administration of MTX dose. Previous studies reported that the T_{max} of orally administered MTX before or after meals was between 1 h and 2 h.[8,10–13] Robert et al.[12] reported no significant effect of food on MTX absorption or bioavailability, and there were no differences in C_{max} and T_{max} between fasting patients and those taking food. In contrast, Oguey et al.[13] reported that food delayed T_{max} from 1.3 h to 2.0 h, which was not statistically significant. Although the present study did not examine the exact effect of food, our RA patients usually took MTX after meals in a practical setting, and almost one-third of the patients showed T_{max} values of 1 h, another one-third 2 h, and the last one-third 3 h. Notably, no included patient showed a T_{max} of more than 4 h, and it is generally sufficient for obtaining T_{max} to measure the serum MTX concentration up to 3 h after the first portion of the weekly administration in the clinical setting.

There are some factors which could modify the pharmacokinetics of MTX. Because MTX is partially removed from the kidneys, renal dysfunction could modify the pharmacokinetics of MTX. In the present study, almost all the patients generally preserved intact renal function, and it is difficult to demonstrate a significant correlation between serum creatinine levels and the pharmacokinetics of MTX (Fig. 3). However, patients with higher serum creatinine levels tended to show higher C_{max} and T_{max} (Fig. 3). Moreover, one female patient who had a unilateral functional kidney showed a serum creatinine level of 1.6 mg/dl, and a C_{max} of 0.20 μmol/l 3 h after the intake of 2 mg of MTX, and these results suggested that the patients with renal dysfunction could modify the pharmacokinetics of MTX. Although we could not demonstrate it in this study, an impaired renal function could cause an increase of the serum MTX concentration, and could be an important risk factor for the development of the adverse reactions to MTX. Adjunctive drugs may modify MTX absorption and metabolism; previous studies showed that steroids and some types of NSAID modify the pharmacokinetics of intramuscularly administrated MTX.[14–16] In the present study, no modifications in MTX pharmacokinetics caused by coadministered steroids or NSAIDs were observed. However, as there are manifold combinations of factors, it is difficult to evaluate the effects of coadministered drugs to MTX pharmacokinetics in a clinical setting.

In the present study, patients with adverse reactions had a significantly higher C_{max} after the first dose of weekly administration than those without the adverse reactions. The provisional threshold C_{max} at which adverse reactions occurred was calculated to be 0.16 μmol/l. Eleven of 12 patients with adverse reactions (92%) showed a C_{max} higher than the provisional threshold C_{max}. In contrast, two patients (2.9%) who showed a C_{max} higher than 0.30 μmol/l had no adverse reactions for 52 months and 48 months. Notably, the provisional threshold of the maintenance dosage of MTX was less sensitive or specific than C_{max} (the provisional threshold of the MTX dosage: 7.0 mg/week, sensitivity 75%, specificity 61%). Because this study was designed as a retrospective study, and thus used the medical records, there were some limitations. The maintenance dosages of MTX were determined by the subjective decisions of the clinicians, and minor adverse reactions, such as slight nausea, might not have been mentioned in the medical records. Nevertheless, we thought that higher C_{max} after the first dose of the weekly administration could be a promising parameter for predicting the development of the adverse reactions to MTX.

In summary, two points need to be emphasized. First, we measured the serum MTX concentration of the Japanese RA patients after the first dose of the weekly administration. The C_{max} after the first dose of the weekly admini stration significantly correlated with the dosage of MTX administered immediately before the measurement. In addition, to obtain the C_{max} after the first dose of the week, it is sufficient to measure the serum MTX concentration up to 3 h after the administration in a general clinical setting. Second, a higher C_{max} after the first dose of the weekly administration, obtained by this sampling method, was a good indicator of the risks for adverse reactions. Our conclusion is that the serum MTX concentration is worth monitoring to predict whether the adverse reactions will occur.

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