Mod Rheumatol (2005) 15:405–409
DOI 10.1007/s10165-005-0434-6

ORIGINAL ARTICLE

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Serum levels and pharmacodynamics of methotrexate and its metabolite 7-hydroxy methotrexate in Japanese patients with rheumatoid arthritis treated with 2-mg capsule of methotrexate three times per week

Received: July 11, 2005 / Accepted: September 12, 2005

Abstract Methotrexate (MTX) is the first-choice drug for rheumatoid arthritis (RA); however, the pharmacodynamics of MTX in Japanese patients with RA treated legitimately according to the government recommended dosage, 6 mg per week, are unknown. Methotrexate and its metabolite, 7-hydroxy MTX (7-OH MTX), were measured in sera of 16 outpatients with active RA in the first week of MTX treatment and 4–12 weeks after the introduction at 0, 1, 2, 4, and 8 h after administration of the first and the third 2-mg capsule, followed by sampling at 48, 96, and 168 h. The mean maximal serum drug concentration (mean C_{max}) of MTX attained at 1–2 h after ingestion of the first capsule was 0.215 and 0.252 μM, respectively, in the first and the follow-up week. The mean C_{max} after ingestion of the third capsule was 0.223 μM and 0.357 μM. The mean C_{max} of 7-OH MTX was 0.0334 and 0.0289 μM for the first capsule, and 0.0495 and 0.0672 μM for the third capsule. The results indicate that MTX does not accumulate or deposit in the body of Japanese patients with RA when treated with 6 mg per week, and pharmacodynamics of MTX are comparable to those in overseas patients treated with 7.5 mg per week.

Key words Methotrexate (MTX) · Pharmacodynamics · Rheumatoid arthritis (RA) · Serum concentration

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Introduction

Methotrexate (MTX) is the first-choice drug for rheumatoid arthritis (RA) both worldwide[1] and within Japan.[2] Long-term use of MTX in clinics has established its clinical effectiveness and side effects.[1–4] However, the serum levels and pharmacokinetics of MTX prescribed to Japanese patients with RA legitimately in accord with the restriction of Ministry of Health, Labour and Welfare have not yet been investigated, while the pharmacokinetics of MTX in healthy individuals and rheumatoid patients in relation to absorption rate, biological availability, relationship between serum level and the amount of MTX administered, and the influence of meals and age have been reported from abroad.[5–10] The present study was designed firstly to clarify the pharmacodynamics of MTX in Japanese patients with active RA who were newly started on MTX treatment in accord with the government dosage guideline. Secondly, we aimed to clarify racial differences with regard to MTX treatment.

Patients and methods

Patients

Seventeen Japanese patients with RA fulfilling the diagnostic criteria of the American College of Rheumatology[11] were investigated for this study. Only active rheumatoid patients with age ≥20 years old, disease duration ≥6 months, tender joints ≥4, swollen joints ≥2, and erythrocyte sedimentation rate (ESR) ≥31 mmHg were included in the study. Patients were excluded from the study when they received any other disease-modifying antirheumatic drugs or ≥5 mg of prednisolone daily, or when nonsteroidal anti-inflammatory drugs were changed just before or during the study course. The study was performed from April 1996 to March 1997 at the National Kakogawa Hospital (now Konan Kakogawa Hospital) under written consent of patients based on approval of the institutional review board.

Table 1. Clinical profile of patients

	Initial assessment	Follow-up assessment	*P* value[c]
Grip strength (mmHg)	91.3 ± 47.0[a]	100.4 ± 31.1	NS
	80.0 (31.0–184.0)[b]	89.5 (60.0–146.0)	
ESR (mm/h)	83.3 ± 34.2	68.8 ± 37.7	NS
	80.0 (23.0–152.0)	72.0 (7.0–130.0)	
Tender joints	13.2 ± 6.1	4.9 ± 5.2	<0.001
	13.0 (5.0–26.0)	4.0 (0.0–19.0)	
Swollen joints	16.9 ± 8.3	12.9 ± 7.1	0.011
	17.5 (3.0–33.0)	14.0 (1.0–26.0)	
Joint score	111.4 ± 26.2	85.0 ± 28.5	0.008
	105.5 (58.0–145.0)	83.0 (22.0–121.0)	
Duration of morning stiffness (min)	438.0 ± 456.1	77.2 ± 186.6	0.094
	210.0 (0.0–960.0)	0.0 (0.0–570.0)	
ADL score	22.0 ± 2.3	16.0 ± 5.7	NS
	22.0 (20.0–24.0)	16.0 (12.0–20.0)	
Lansbury index	89.4 ± 32.1	56.4 ± 17.5	0.031
	91.0 (42.4–137.5)	51.1 (39.0–78.6)	
C-reactive protein (mg/dl)	6.3 ± 3.8	4.3 ± 4.1	0.026
	6.1 (1.6–14.8)	2.6 (0.2–13.2)	
Rheumatoid factor (IU/ml)	1434.9 ± 1687.8	1550.5 ± 3225.6	0.020
	789.0 (16.0–5066.0)	378.0 (16.0–12729.0)	

ESR, erythrocyte sedimentation rate; ADL, activity of daily living; NS, not significant
[a] Mean ± SD
[b] Median (range)
[c] Wilcoxon test

Study design

The patients started on a course of MTX treatment. A 2-mg capsule of MTX was administered at a time. A total of three capsules was administered orally per week to a patient: the first capsule was administered at 09:00 and the second at 21:00, followed by the third capsule administered at 09:00 on the following day. Blood samples were obtained at 0, 1, 2, 4, and 8 h after administration of the first MTX capsule on the first day, and at 0, 1, 2, 4, and 8 h after administration of the third MTX capsule on the second day which was followed by sampling at 48 h (3rd day), 96 h (5th day), and 168 h (8th day). This type of sequential measurement was performed and evaluated in the first and the follow-up weeks of the MTX treatment. Serum levels of MTX and 7-hydroxy (OH) MTX were quantified as described.[12] The patients were evaluated for the following points: grip strength (mmHg), ESR (mm/h), tender joints, swollen joints, joint score,[13] duration of morning stiffness (min), activity of daily living (ADL) score according to modified Health Assessment Questionnaire,[14] Lansbury index, C-reactive protein (mg/dl), rheumatoid factor (IU/ml), and Steinbrocker's functional class.[15]

Results

Clinical findings

The profiles of 17 Japanese patients with RA were: male/female, 4/13; mean age, 66.8 (median 69, range 39–81) years old; mean disease duration, 12.5 (median 10, range 1.2–41.0) years; mean C-reactive protein, 6.3 ± 3.8 mg/dl (median 6.1, range 1.6–14.8). Among 17 patients with RA enrolled in the study, the follow-up clinical assessment was made at 12 weeks in 8 patients, at 8 weeks in 6 patients, and 4 weeks in 3 patients (average 9.2 ± 3.1 weeks). Among the latter 3 patients, MTX was discontinued at 4 weeks, because MTX was subjectively very effective and discontinued in a 69-year-old woman, because of nausea, vomiting, and loss of appetite in a 69-year-old woman, and because of stomach ache in an 80-year-old woman. Clinical assessment of 17 patients showed that joint counts including the number of tender and swollen joints, Lansbury index, and C-reactive protein were significantly improved (Table 1). Steinbrocker's functional class was marginally improved after treatment with MTX (Table 2). No clinically significant changes were observed in the laboratory tests: White blood cell count decreased from 7323 ± 1668/mm^3 to 6406 ± 1858/mm^3 ($P = 0.034$); platelet count decreased from 36.4 ± 11.1 × 10^4/mm^3 to 32.3 ± 14.4 × 10^4/mm^3 ($P = 0.040$); serum albumin increased from 3.25 ± 0.46 g/dl to 3.44 ± 0.41 g/dl ($P = 0.047$); and serum glutamic pyruvic transaminase increased from 13.3 ± 7.9 IU/ml to 18.8 ± 14.8 IU/ml ($P = 0.016$).

Table 2. Change in Steinbrocker's functional class

	Class				
	I	II	III	IV	*P* value[a]
Initial assessment	0	7	8	2	
Follow-up assessment	1	11	2	2	0.031

[a] Wilcoxon test

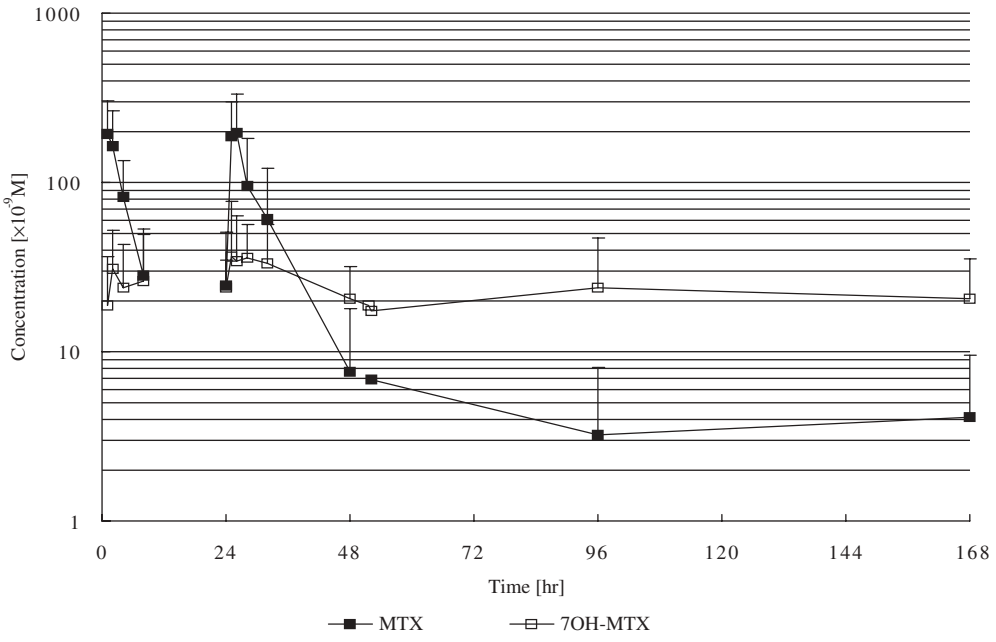

Fig. 1. Time course of serum levels of methotrexate (*MTX*) and 7-hydoroxy-MTX (*7-OH MTX*) measured in the first week of MTX treatment (n = 16). Mean ± S.D. is indicated

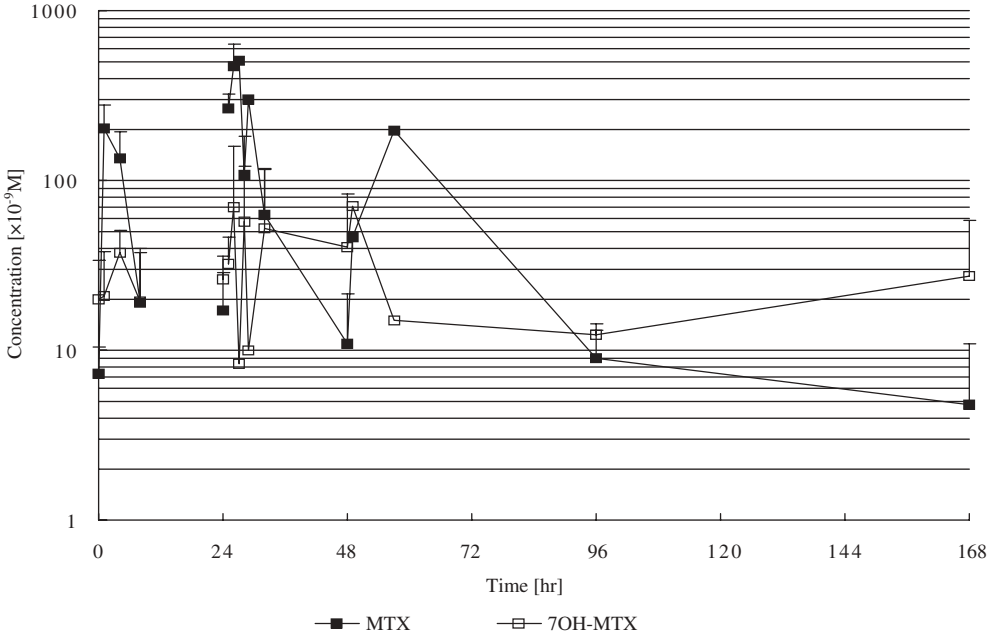

Fig. 2. Time course of serum levels of methotrexate (*MTX*) and 7-hydoroxy-MTX (*7-OH MTX*) measured in the follow-up week of MTX treatment (n = 16). Mean ± S.D. is indicated

Serum levels and pharmacodynamics of MTX

Except for a 69-year-old woman whose blood sample at 0 h was not available, serum levels of MTX and its metabolites were studied in all of the other 16 patients. The maximal serum drug concentration (C_{max}) of MTX was attained at 1–2 h after administration of the first 2-mg capsule of MTX both in the first week of MTX treatment (Fig. 1) and in the follow-up week (Fig. 2). The mean C_{max} values at this time point were similar (0.215 and 0.252 μM) as measured in the first week of MTX treatment (Fig. 1) and the follow-up week (Fig. 2). The half-lives of serum MTX ($t_{1/2}$) were also similar (2.4 and 2.3 h), as measured in the first and the follow-up week of MTX treatment, respectively (Tables 3 and 4).

Serum MTX as measured after ingestion of the third capsule at 09:00 on the following day peaked again at 1–2 h both in the first and the follow-up week of MTX treatment, respectively (Figs. 1 and 2). Meanwhile, though the C_{max} value and the $t_{1/2}$ values after ingestion of the third capsule in the first week were similar to those of the first capsule, the C_{max} value in the follow-up week was 60% higher than those measured in the first week (0.357 vs. 0.223 μM) and $t_{1/2}$ values were 30% shorter (2.2 vs. 3.2 h) (Figs. 1 and 2).

Further, serum levels of the MTX metabolite, 7-OH MTX, were below the assay limits in two patients, and thus

Table 3. Pharmacokinetic parameters for methotrexate (MTX)

	C_{max} (μM)				t_{max} (h)				$t_{1/2}^{0-8}$ (h)				AUC$^{0-8}$ (μM/h)			
	Initial assessment		Follow-up assessment		Initial assessment		Follow-up assessment		Initial assessment		Follow-up assessment		Initial assessment		Follow-up assessment	
	1st[a]	3rd[b]	1st	3rd	1st	3rd	1st	3rd	1st	3rd	1st	3rd	1st	3rd	1st	3rd
Mean	0.215	0.223	0.252	0.357	1.2	1.8	1.0	1.5	2.4	3.2	2.3	2.2	0.825	1.10	1.08	1.01
SD	0.107	0.112	0.060	0.156	0.4	1.7	0.0	0.5	0.6	2.2		0.5	0.524	0.88	0.33	0.36
Max.	0.437	0.483	0.294	0.747	2.0	8.0	1.0	2.0	3.5	10.6	2.3	3.3	2.04	3.62	1.31	1.53
Min.	0.103	0.118	0.210	0.184	1.0	1.0	1.0	1.0	1.5	1.7	2.3	1.6	0.281	0.307	0.843	0.54
N	11	16	2	11	11	16	2	11	11	15	1	10	11	16	2	10

[a] 1st: after the administration of 1st 2-mg capsule of MTX
[b] 3rd: after the administration of 3rd 2-mg capsule of MTX

Table 4. Pharmacokinetic parameters for 7-hydoroxy-MTX (7-OH MTX)

	C_{max} (μM)				t_{max} (h)				$t_{1/2}^{0-8}$ (h)				AUC$^{0-8}$ (μM/h)			
	Initial assessment		Follow-up assessment		Initial assessment		Follow-up assessment		Initial assessment		Follow-up assessment		Initial assessment		Follow-up assessment	
	1st[a]	3rd[b]	1st	3rd	1st	3rd	1st	3rd	1st	3rd	1st	3rd	1st	3rd	1st	3rd
Mean	0.0334	0.0495	0.0289	0.0672	3.8	3.0	4.5	17.5	10.0	22.3		27.6	0.174	0.625	0.140	0.326
SD	0.0197	0.0395	0.0174	0.0601	2.7	2.1	4.9	42.1	7.1	22.8		35.9	0.094	0.747	0.096	0.379
Max.	0.0659	0.172	0.0412	0.231	8.0	8.0	8.0	144.0	20.2	84.8		93.9	0.394	2.40	0.208	1.34
Min.	0.0125	0.00978	0.0166	0.0149	1.0	0.0	1.0	1.0	2.3	1.8		1.2	0.0706	0.0165	0.0720	0.0297
N	9	14	2	11	9	14	2	11	7	12	0	6	9	14	2	10

[a] 1st: after the administration of 1st 2-mg capsule of 7-OH MTX
[b] 3rd: after the administration of 3rd 2-mg capsule of 7-OH MTX

serum levels of 7-OH MTX were measured in 14 patients. The mean C_{max} values after ingestion of the first capsule as measured in the first and the follow-up week of MTX treatment were 0.0334 and 0.0289 μM, respectively. The mean C_{max} values after ingestion of the third capsule were 0.0495 and 0.0672 μM as measured in the first and the follow-up week (Figs. 1 and 2). There were no statistical differences among these values. The $t_{1/2}$ values for 7-OH MTX were variable at 1.2–94 h, as measured in either the first or the follow-up week (not shown). There was no significant correlation between the mean C_{max} values and body weight, renal function, and sex of patients (data not shown).

Discussion

Kashiwazaki et al. have shown in a double-blind controlled study performed in Japan that the dose of 6 mg per week was optimal for Japanese patients with RA;[16] this was adopted officially by the Ministry of Health, Labour and Welfare as the recommended dosage, and the maximum dosage is restricted to 8 mg per week for application of MTX to Japanese patients with RA. We have therefore legitimately followed this guideline and administered a total of 6 mg of MTX per week at 09:00 and 21:00 on the first day and at 09:00 on the following day. The present study was designed to measure serum levels of MTX and its metabolite 7-OH MTX, faithfully following this treatment schedule. Our clinical evaluation showed that the treatment with 6 mg MTX per week was somewhat effective in the follow-up weeks 4–12 but not fully satisfactory for complete remission of RA, although the effectiveness of MTX has already been proven by other investigators.[3,4,16]

In the present study, we found that the C_{max} and $t_{1/2}$ values were almost identical when measured in the first and the follow-up weeks of MTX treatment. While the C_{max} value measured after the third ingestion of MTX in the follow-up week was 60% higher than those measured in the first week, the area under the curve (AUC) was similar between them. Further, since the C_{max} value was basically highly variable in each individual as depicted in Figs. 1 and 2, we may conclude that MTX does not accumulate or deposit in the body of Japanese patients with RA treated with 6 mg MTX per week.

Serum levels of a pharmacologically inert MTX metabolite, 7-OH MTX, as measured in either the first week or the follow-up week of MTX treatment, were also similar. Further, since it was noted that the serum concentration of 7-OH MTX was less than 20% that of MTX, we may conclude that the metabolism of MTX was not changed during the period of this study.

The mean C_{max} values such as 0.215 or 0.252 μM obtained in the present study are almost compatible with the reports from abroad. Hamilton and Kremer showed that the mean C_{max} values before and after meals were 0.381 and 0.319 μM, respectively, when treated with 7.5 mg MTX per week.[9] It was noted that the mean C_{max} values are somewhat lower

than those of Hamilton and Kremer; this difference must be due to the dose of MTX administered each time, as shown by Halprin et al.[8] However, it was also noted that the mean C_{max} value of 0.357 μM was attained in the follow-up week of MTX treatment, indicating that serum levels of MTX comparable to those obtained abroad with 7.5 mg per week could be maintained during the treatment with 6 mg per week in Japanese patients. Overseas the weekly dosage of MTX is often increased to 15–20 mg per week when the effects of an initial dose of 7.5 mg per week are not satisfactory;[2] therefore, we may suggest that the dosage of MTX should be raised to 12–16 mg per week in Japanese patients, proportional to those abroad, when the legitimate dosage is not sufficient.

Acknowledgments We thank Mr. Tomokazu Yoshida, Mr. Kenji Udo, and Mr. Akira Yamamoto, and Weiss Co. for measurement of MTX and its metabolites and data gathering.

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. Suzuki Y. Methotrexate for the treatment of rheumatoid arthritis in Japan – much more still remains to be resolved (in Japanese). Nippon Rinsho 2002;60:2331–8.
3. Choi HK, Hernan MA, Seeger JD, Robins JM, Wolfe F. Methotrexate and mortality in patients with rheumatoid arthritis: A prospective study. Lancet 2002;359:1173–7.
4. Weinblatt ME, Maier AL, Fraser PA, Coblyn JS. Long-term prospective study of methotrexate in rheumatoid arthritis: Conclusion after 132 months of therapy. J Rheumatol 1998;25:238–42.
5. Kozloski GD, DE Vito JM, Kisicki JC, Johnson JB. The effect of food on the absorption of methotrexate sodium tablets in healthy volunteers. Arthritis Rheum 1992;35:761–4.
6. Ahern M, Booth J, Loxton A, McCarthy P, Meffin P, Kevat S. Methotrexate kinetics in rheumatoid arthritis: Is there an interaction with nonsteroidal antiinflammatory drugs? J Rheumatol 1988;15:1356–60.
7. Herman RA, Veng-Pedersen P, Hoffman J, Koehnke R, Furst DE. Pharmacokinetics of low-dose methotrexate in rheumatoid arthritis patients. J Pharm Sci 1989;78:165–71.
8. Halprin KM, Fukui K, Okawara A. Blood levels of methotrexate and the treatment of psoriasis. Arch Dermatol 1971;103:243–9.
9. Hamilton RA, Kremer JM. The effect of food on methotrexate absorption. J Rheumatol 1995;22:630–2.
10. Kevat S, Ahern M, Hall P. Pancytopenia induced by low-dose methotrexate for rheumatoid arthritis. Aust N Z Med J 1988; 18:697–700.
11. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The american rheumatism association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
12. Hirai T, Kitamura M, Inoue Y. Sensitive analytical procedure for methotrexate and its main metabolite 7-hydroxymethotrexate in serum by high-performance liquid chromatography (in Japanese). Yakugaku Zasshi 1994;114:602–10.
13. Lansbury J. Clinical appraisal of the activity index as a measure of rheumatoid activity. Arthritis Rheum 1968;11:599–604.
14. Pincus T, Summey JA, Soraci SA Jr, Wallston KA, Hummon NP. Assessment of patient satisfaction in activities of daily living using a modified Stanford Health Assessment Questionnaire. Arthritis Rheum 1983;26:1346–53.
15. Steinbrocker O, Traeger CH, Batterman RC. Therapeutic criteria in rheumatoid arthritis. JAMA 1949;140:659–62.
16. 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). Jpn J Inflamm 1996;16:483–58.