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Influence of High-Dose Folic Acid on Methotrexate Efficacies and Safety in Japanese Rheumatoid Arthritis Patients

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ABSTRACT

Backgrounds Folic acid dose at ≦5 mg/week has been recommended for rheumatoid arthritis (RA) patients to decrease risk of methotrexate adverse effects. However, higher doses of folic acid is used in some cases. We examined the influence of high-dose folic acid on methotrexate efficacies and safety in Japanese RA patients.

Methods 502 RA patients of four hospitals prescribed methotrexate and folic acid were included. These patients were divided into two subgroups according to the threshold of folic acid dose by 5 mg/week. Basic patient characteristics, methotrexate doses, and the efficacies or adverse effects of methotrexate were retrospectively compared between the two patient subgroups.

Results The frequency of folic acid use at doses higher than 5 mg/week was significantly different between the four hospitals ($P < 0.001$). The prevalence of methotrexate adverse effects was not significantly different between the patients taking folic acid less and more than 5 mg/week. However, in the lower dose methotrexate subgroup (≦8 mg/week), the prevalence of patients exhibiting abnormal serum ALT concentrations in the patients using higher (> 5 mg/week) dose of folic acid was significantly higher than that in the lower (≦5 mg/week) folic acid-treated subgroup ($P = 0.029$). Folic acid dose between patients taking methotrexate less and more than 8 mg/week was not significantly different.

Major conclusion Folic acid dose was dependent on the hospitals, while efficacies and hepatotoxicity of methotrexate was not basically different between patients taking less and more than 5 mg/week of folic acid.

Introduction

The anti-folate methotrexate has been used as an anchor drug combined with disease modifying anti-rheumatic drugs (DMARDs) for treatment of rheumatoid arthritis (RA) patients. The primary function of folic acid is to act as a cofactor to various methyltransferases involved in serine, methionine, thymidine and purine biosynthesis [1]. Methotrexate inhibits dihydrofolate reductase (DHFR), an enzyme that participates in the reduction of folic acid into the active folate form, and then interferes thymidylate-purine synthesis to ameliorate immune cell proliferation [2]. Increase in extracellular adenosine concentration by the drug was also reported to be participated in inhibition of the function of neutrophils and lymphocytes [3].

The 2016 Guideline for the methotrexate treatment of RA recommended by the Japanese RA Society indicates that the drug dosage should be less than 16 mg/week, which are almost lower than those used in many western countries. However, adverse effects of methotrexate including myelosuppression, gastrointestinal and hepatic disfunction have been observed, and folic acid is prescribed to prevent and/or relief these undesirable effects of methotrexate [4, 5]. It has been recommended that RA patients under methotrexate treatment should be treated with 5 mg/week or less doses

of folic acid 24–48 h after the methotrexate administration [6]. However, the dose of folic acid may be independently determined among the hospitals or physicians, while folic acid at doses more than 5 mg/week may influence the efficacies of methotrexate. Therefore, it may be somewhat difficult for the pharmacists to make inquiries to the physicians, whether the dosage of folic acid should be changed or not in each case. It would be of benefits of the lower folic acid dosage in terms of reduced drug administrative burden and cost for the patients.

Then, we investigated, in the present study, the influence of folic acid doses on methotrexate efficacies and hepatotoxicity in Japanese RA outpatients came to our community pharmacies.

Materials and Methods

Patients

502 outpatients of four hospitals, who were diagnosed as RA and were prescribed methotrexate and folic acid from January to May in 2015 by Bohsei Pharmacy, Isehara, Japan, were included in this study. Diagnosis of RA were carried out according to the Japan College of Rheumatology 2014, which has been established on the basis of 2010 ACR/EULAR Classification Criteria for Rheumatoid Arthritis.

These patients were 121 males (24 %) and 381 females (76 %), and the number of the patients aged less than 65 were 272 (54.2 %) and those over than 65 were 230 (45.8 %), respectively. The median (range) age of these patients was 63 (18-84) years. These 502 patients include 70, 53, 304, and 75 outpatients treated in hospital A (located in Saitama prefecture with 395 beds), hospital B (located in Tokyo area with 520 beds), hospital C (located in Kanagawa prefecture with 804 beds) and hospital D (located in Kanagawa prefecture with 312 beds), respectively. The RA patients were divided into two groups according to the doses of folic acid prescribed by a threshold of 5 mg/week, and clinical characteristics were compared retrospectively between the two patient subgroups.

In 145 patients of three hospitals, from whom informed consent has been obtained to present their laboratory data, from June to August in 2015, efficacies and safety of methotrexate were examined, as described below. These 145 patients include 34, 19, and 92 outpatients treated in hospital A, B and C, respectively.

Methods

This study was approved by the Ethical Committee of Bohsei Pharmacy (authorization number 160503). The study was carried out according to the World Medical Association Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects. Doses of methotrexate and folic acid were investigated from the prescription history obtained from the four hospitals described above. The basic patient characteristics or doses, efficacies, and incidences of adverse effects of methotrexate were retrospectively compared between the folic acid higher-dose (> 5 mg/week) and the lower dose (≦5 mg/week) subgroups. The folic acid dose was basically adjusted in light of the changes of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations. None of the patients included in this study were treated with folinic acid.

Laboratory data in 145 patients from whom informed consent has been obtained from June to August in 2015, were examined for the efficacies and incidences of adverse effects of methotrexate, and these data were compared between the two patient subgroups. Methotrexate efficacies for treatment of the RA patients were estimated by the laboratory data including serum C-reactive protein (CRP) concentration, erythrocyte sedimentation rate (ESR), and serum matrix metalloproteinase (MMP) 3 concentration. Serum MMP3 amounts were measured using ELISA. MMP3 has been reported to be a predictor for structural remission in RA patients treated with methotrexate [7]. As one of the adverse effects of methotrexate frequently occur, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations were measured.

Statistical analyses

Statistical significance for the difference in the median of drug doses and laboratory data between any two patient subgroups was analyzed using the Mann Whitney's U-test. The paired t test was used to compare mean values between any two patient subgroups. The Kruskal-Wallis test was used for the multiple comparison of the data. The Chi-square test and the Fisher's exact provability test were performed for analyzing difference in the frequencies of values between any two patient subgroups. P values less than 0.05 were estimated to be statistically significant. All statistical analysis was done by GraphPad PRISM 4.0 (GraphPrism software Inc., USA).

Results

Profiles of RA patients treated with folic acid

Profiles of 502 patients examined in this study were shown in ▶ **Table 1**. The median (range) dose of methotrexate in these patients was 8 (2–16) mg/week, and the numbers of patients administered less and over than 8 mg/week methotrexate were 299 (59.6 %) and 203 (40.4 %), respectively.

All of the 502 patients were prescribed folic acid, and 421 of these patients (83.9 %) were administered 5 mg/week or less of folic acid. The percentages of patients administered with 7, 10, 15, 20, and 25 mg/week or more of folic acid were 0.6, 13.7, 0.8, 0.2, and 0.8 %, respectively, and thus the percentage of patients administered more than 5 mg/week of folic acid was 16.1 % (▶ **Table 2**).

The incidences of patients prescribed folic acid more than 5 mg/week were compared between four Hospitals included in this study. 38 out of 70 patients in hospital A, 17 out of 53 patients in hospital B, 25 out of 304 patients in hospital C, and 1 out of 75 patients in hospital D, respectively, were prescribed folic acid with doses more than 5 mg/week. Statistically significant difference in the incidence of the patients who were prescribed folic acid more than 5 mg/week was observed between four hospitals ($P < 0.001$).

Male patients prescribed folic acid with doses more than 5 mg/week were 13 out of 121, while such female patients were 68 out of 381, respectively, and the incidence was not statistically significant between male and female ($P = 0.064$). The patients aged less than 65 years, who were prescribed folic acid with doses more than

▶ **Table 1** Comparison for the basic profiles of the RA patients treated with less than (n = 421) and more than (n = 81) 5 mg/week of folic acid.

Profiles	Number of patients	Folic acid > 5 mg/week group	Folic acid ≦5 mg/week group	*P* value
Male/Female	121/381	13/68	108/313	0.064
Age (mean ± SD)	61.3 ± 13.4	60.3 ± 12.6	61.5 ± 13.6	0.448
Mean age less than 65/more than 65(years)	272/230	47/34	225/196	0.449
MTX dosage median (range) mg	8 (2–16)	8 (2–16)	8 (2–16)	0.725
Number of patients with MTX dose≦8 mg/ MTX dose > 8 mg	299/203	50/31	249/172	0.664

▶ **Table 2** Validation of folic acid doses for the treatment of methotrexate adverse effects in 502 RA patients.

Folic acid dose (mg/week)	Number of patients	% of patients
≦5	421	83.9
7	3	0.6
10	69	13.7
15	4	0.8
20	1	0.2
25 or more	4	0.8
Total	502	100

5 mg/week, were 47 out of 272, while those more than 65 years were 34 out of 230, respectively, and the incidence of the younger patients was not statistically significant as compared to that of the elder patients (P = 0.449) (▶ **Table 1**).

The median dose of methotrexate in all of the patients examined in this study was 8 mg/week, and then the incidence of the patients who were prescribed more than 5 mg/week of folic acid was compared between the two patient subgroups treated with the higher (> 8 mg/week) and the lower (≦8 mg/week) doses of methotrexate. In the patients treated with the lower (≦8 mg/week) doses of methotrexate, folic acid at doses more than 5 mg/week were prescribed in 50 out of 299 patients. Whereas, in the patients treated with the higher (> 8 mg/week) doses of methotrexate, folic acid at doses more than 5 mg/week were prescribed in 31 out of 203. The incidence of the higher folic acid use was not statistically significant between the two subgroups (P = 0.664) (▶ **Table 1**).

Relationship between dose of folic acid and patient outcome

Clinical efficacy and incidence of adverse effects of methotrexate were compared between the RA patients treated with the higher (> 5 mg/week) and the lower (≦5 mg/week) dose of folic acid in 145 patients (▶ **Table 3**). No significant differences in mean ages, male/female ratio, median periods treated with folic acid, and median doses of methotrexate were observed between the two patient subgroups (▶ **Table 3**).

CRP, ESR, and MMP3 values were examined in 128, 65, and 84 patients, respectively. The incidence of the patients showing abnormal value of CRP concentration (> 0.3 mg/dL) was not significantly different between the higher (> 5 mg/week) and the lower (≦5 mg/week) folic acid subgroups (P = 0.219). Similarly, the incidence of the patients exhibiting abnormal ESR values (> 7 mm/h for male and > 16 mm/h for female) was not significantly different between the higher (> 5 mg/week) and the lower (≦5 mg/week) dose of folic acid subgroups (P = 0.939). The prevalence of the patients exhibiting abnormal serum MMP3 concentrations (> 121 ng/mL for male and > 60 ng/mL for female) in the patients was not significantly different between the higher (> 5 mg/week) and the lower (≦5 mg/week) dose of folic acid subgroups (P = 0.832).

Serum AST and ALT concentrations were examined in 124 and 125 patients, respectively, for the monitoring of the adverse effects of methotrexate. The abnormal serum AST concentrations (> 34 IU/L) in the patients was not significantly different between the higher (> 5 mg/week) and the lower (≦5 mg/week) folic acid subgroups (P = 0.182). Similarly, the abnormal serum ALT concentrations (> 46 IU/L) were not significantly different between the higher (> 5 mg/week) and the lower (≦5 mg/week) folic acid subgroups (P = 0.455) (▶ **Table 3**).

Relationship between doses of folic acid and patient outcomes in patient subgroups treated with methotrexate at doses more or less than 8 mg/week

145 patients from three hospitals were then divided into two subgroups according to the median dose of methotrexate (8 mg/week). Clinical efficacy and incidence of adverse effects of methotrexate were compared between patients treated with the higher (> 5 mg/week) and the lower (≦5 mg/week) doses of folic acid, in both the high (> 8 mg/week; n = 66) and the low (≦8 mg/week; n = 79) dose-methotrexate subgroups, independently (▶ **Table 3**). No significant differences in the basic patient profiles including the mean ages, male/female ratio, median periods treated with folic acid, and the median doses of methotrexate were observed between the two patient subgroups to be compared (▶ **Table 3**).

High dose (> 8 mg/week) methotrexate group

In the high dose (> 8 mg/week) methotrexate group, the incidence of patients exhibiting abnormal serum concentration of each laboratory data did not significantly differ between the higher (> 5 mg/week) and the lower (≦5 mg/week) folic acid subgroups (▶ **Table 3**). CRP data were obtained from 58 out of 66 patients. The incidence of the patients showing abnormal CRP concentration (> 0.3 mg/dL) was not significantly different between the higher (> 5 mg/week) and the lower (≦5 mg/week) dose of folic acid subgroups (P = 0.256). ESR data were obtained from 28 patients. The incidence of the patients showing abnormal ESR values (> 7 mm/h for male and > 16 mm/h for female) was not significantly different between the higher (> 5 mg/week) and the lower (≦5 mg/week) folic acid subgroups (P = 0.264). MMP3 data were obtained from 35 patients. The incidence of the patients showing abnormal serum MMP3 con-

▶ **Table 3** Comparison for the basic profiles and clinical outcome between the RA patients under methotrexate therapy treated with the higher (> 5 mg/week) and the lower (≦5 mg/week) doses of folic acid.

Profiles	methotrexate > 8 mg/week patients			methotrexate≦8 mg/week patients			Total patients		
	Folic acid > 5 mg/week	Folic acid ≦5 mg/week	*P* value	Folic acid > 5 mg/week	Folic acid ≦5 mg/week	*P* value	Folic acid > 5 mg/week	Folic acid ≦5 mg/week	*P* value
Male/Female	2/16	10/38	0.300	2/ 18	9/50	0.434	5/33	19/88	0.353
Age (mean ± SD)	54.0 ± 13.1	57.0 ± 12.1	0.208	67.3 ± 10.0	63.9 ± 12.4	0.092	60.7 ± 13.2	60.4 ± 12.7	0.440
Duration of folic acid treatment median (range) days	714 (84–2522)	665 (65–2538)	0.526	717.5 (91–2520)	966 (21–2527)	0.928	717.5 (84–2527)	847 (21–2538)	0.645
MTX dosage median (range) mg	11 (10–16)	10 (10–17.5)	0.356	6 (4–8)	6.5 (4–8)	0.073	8 (4–16)	8 (4–17.5)	0.860
Patient ratio showing abnormal/normal CRP levels	2/13	12/31	0.256	4/11	19/36	0.565	6/24	31/67	0.219
Patient ratio showing abnormal/normal ESR values	4/3	7/14	0.264	5/6	16/10	0.367	9/9	23/24	0.939
Patient ratio showing abnormal/normal MMP3 levels	4/4	12/15	0.782	5/6	17/21	0.966	9/10	29/36	0.832
Patient ratio showing abnormal/normal AST levels	1/12	5/37	0.67	3/12	4/50	0.153	4/24	9/87	0.182
Patient ratio showing abnormal/normal ALT levels	0/13	2/40	0.324	3/12	2/53	0.029 *	3/25	4/93	0.455

The abnormal laboratory data means: CRP concentrations > 0.3 mg/dL, ESR values > 7 mm/h for male and > 16 mm/h for female, AST concentrations > 34 IU/L, ALT concentrations > 46 IU/L, MMP3 concentrations > 121 ng/mL for male and > 60 ng/mL for female.

centrations (> 121 ng/mL for male and > 60 ng/mL for female) was not significantly different between the higher (> 5 mg/week) and the lower (≦5 mg/week) folic acid subgroups (P = 0.782). Serum AST and ALT concentrations in 55 out 66 patients in this group were examined for the monitoring of the adverse effects of methotrexate. The ratios of the patients exhibiting abnormal/normal serum AST concentrations (> 34 IU/L) in the higher (> 5 mg/week) and the lower (≦5 mg/week) folic acid subgroups were 1/12 and 5/37, respectively, and there was no significant difference between the two subgroups (P = 0.67). Similarly, the ratios of the patients exhibiting abnormal/normal serum ALT concentrations (> 46 IU/L) in the higher (> 5 mg/week) and the lower (≦5 mg/week) folic acid subgroups were 0/13 and 2/40, respectively, and there was no significant difference between the two subgroups (P = 0.324) (▶ **Table 3**).

Low dose (< 8 mg/week) methotrexate group

In the low dose (≦8 mg/week) methotrexate group, CRP, ESR and MMP3 data were obtained from 70, 37, and 49 out of 79 patients, respectively.

The incidence of the patients showing abnormal CRP value was not significantly different between the higher (> 5 mg/week) and the lower (≦5 mg/week) folic acid subgroups (P = 0.565). The incidence of the patients showing abnormal ESR concentrations was not significantly different between the higher (> 5 mg/week) and the lower (≦5 mg/week) folic acid subgroups (P = 0.367). Similarly, the incidence of the patients showing abnormal serum MMP3 concentrations in the patients was not significantly different between the higher (> 5 mg/week) and the lower (≦5 mg/week) folic acid subgroups (P = 0.966).

Serum AST and ALT concentrations in 69 and 70 out 79 patients in this group, respectively, were examined for the monitoring of the adverse effects of methotrexate. The ratios of the patients showing abnormal/normal AST concentrations in the patients treated with the higher (> 5 mg/week) and the lower (≦5 mg/week) folic acid subgroups were 3/12 and 4/50, respectively, and the difference between the two subgroups was not statistically significant (P = 0.153). In contrast, the ratios of the patients exhibiting abnormal/normal ALT concentrations (> 46 IU/L) in the higher (> 5 mg/week) folic acid subgroup was 3/12, which was significantly higher than the ratio of 2/53 in the lower (≦5 mg/week) folic acid subgroup (P = 0.029) (▶ **Table 3**). The methotrexate doses at the day when the abnormal serum ALT concentration was observed were 4, 6, 6, 8, and 6 mg/week, respectively, in these five patients. The doses (periods) of folic acid administered in these patients were 10 (2), 10 (11), 10 (5), 3 (22), and 5 mg/week (46 months), respectively.

Discussion

Folic acid use at 5 mg or less per week has been recommended for RA patients having high risk of methotrexate adverse effects, while folic acid is used at doses more than 5 mg per week in some cases [5]. Then, we investigated, in the present study, the influence of folic acid dose on methotrexate efficacies and hepatotoxicity in Japanese RA patients. According to our present data of 502 RA patients from four hospitals located at Tokyo, Saitama and Kanagawa area, we concluded that incidence of folic acid use at higher than 5 mg/week was dependent on the hospitals, while efficacies and incidence of

adverse effects of methotrexate was not different basically between the RA patients taking less and more than 5 mg/week of folic acid.

The male/female ratio in the 502 RA patients from four hospitals examined in this study was 121/381, which was almost the same as that of the epidemiological data [8]. The study also included patients with wide range of ages from 18 to 84 years old. The Japanese therapeutic guide line for methotrexate treatment in RA patients in 2011 [9] recommended use of folic acid at doses less than 5 mg/week at 24–48 h after the last methotrexate administration. According to this indication, we divided the RA patients into two groups by the folic acid dose at 5 mg/week as a threshold, and then compared the efficacy and safety of methotrexate between these groups. The mean doses of folic acid were significantly different between the hospitals, and thus folic acid doses were seemed to be dependent on the physicians and/or hospitals.

Our present data basically showed that the efficacy of methotrexate as has been evaluated by serum CRP concentrations, ESR values, and MMP3 concentrations, as well as the adverse effects of the drug evaluated by serum AST and ALT concentrations, were not significantly different between the RA patients treated with > 5 mg/week and those treated with ≦5 mg/week of folic acid. Thus, the folic acid use at doses ranged from 2–35 mg/week may not largely influence the methotrexate efficacy, as well as its hepatotoxicity, in the RA patients examined in this study. However, in the lower methotrexate-dose (≦8 mg/week) group, the incidence of patients showing relatively high ALT concentrations was significantly high in the higher dose folic acid group. The results suggest that hepatotoxicity tended to occur in this group, and therefore the physicians have increased the dose of folic acid. These observations thus may also recommended that high dose of folic acid should be reconsidered immediately after recovery of the adverse-effect episodes of methotrexate. However, the number of the patients who showed ALT concentrations more than 46 IU/L was five, and thus further investigation should be carried out.

Almost all of the patients examined in this study showed normal range of serum AST and ALT concentrations, and thus folic acid use at doses less than 5 mg/week appeared to be enough to prevent hepatotoxicity of methotrexate. Our present data also suggested that folic acid at doses more than 5 mg/week had little efficacy over its doses less than 5 mg/week to decrease hepatotoxicity of methotrexate. However, this is a retrospective study, and therefore, the data for CRP, ESR, MMP3, AST and ALT were obtained at only one day-point for each patient at their visit to our pharmacy. RA patients under methotrexate therapy exhibiting severe drug adverse effects may occasionally require high amount of folic acid, and thus prescription of the high dose folic acid should be monitored carefully in such patients.

As mentioned above, the present study was carried out retrospectively, and thus we used transiently laboratory data as indirect indicators for the monitoring of methotrexate efficacy and safety in large number of RA patients. A prospective study for the influence of folic acid use in methotrexate-treated patients is also recommended, in which additional clinical indices including, for instance, SDAI/CDAI or DAS28 should be examined.

Dhir V et al. reported that additional folic acid use at doses more than 10 mg/week for the treatment of adverse effects caused by high dose methotrexate had little advantage over the therapy with usual dose of folic acid [6]. These observations are consistent with our present findings. It has also been reported that changes of folic acid dose from 5 to 1 mg/week was recommended to continue effective methotrexate therapy in RA patients [10, 11].

Conclusions

The present study showed that incidence of folic acid use at higher than 5 mg/week in RA patients treated with methotrexate was dependent on the hospitals, while efficacies and incidence of hepatotoxic side effect of methotrexate was not basically different between RA patients taking less and more than 5 mg/week of folic acid.

Conflict of interest

The authors declare that there is no conflict of interest in this study.

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