Mod Rheumatol (2008) 18:379–384
DOI 10.1007/s10165-008-0071-y

ORIGINAL ARTICLE

Efficacy of low-dose tacrolimus added to methotrexate in patients with rheumatoid arthritis in Japan: a retrospective study

Yoshitaka Morita · Yumi Sasae · Takeo Sakuta · Minoru Satoh · Tamaki Sasaki · Naoki Kashihara

Received: 19 September 2007 / Accepted: 11 March 2008 / Published online: 8 May 2008

Abstract The aim of this study was to assess if low-dose tacrolimus is efficacious for the treatment of rheumatoid arthritis (RA) when combined with methotrexate (MTX). The clinical courses of 32 RA patients who received tacrolimus plus MTX were analyzed retrospectively. Disease activity and clinical response were evaluated by DAS28 (disease activity score of 28 joints) and EULAR (European League Against Rheumatism) response criteria. Tacrolimus was started at 1 mg/day in five patients, 1.5 mg/day in 24 patients, and three mg/day in 3 patients. At six months, tacrolimus was continued at 1–2 mg/day in 27 of 32 patients, but was discontinued in five cases who showed no or inadequate response. Of the 32 patients, 47% were evaluated as having a moderate or good response at one month of tacrolimus therapy, and 72% at six months. No serious adverse events were observed. Our results suggest that the addition of tacrolimus at low dose to MTX for the treatment of patients with moderately active RA appears to be highly efficacious. Further studies are required for the appropriate use of this expensive immunosuppressant in the treatment of RA.

Keywords DAS · EULAR response · Methotrexate · Rheumatoid arthritis · Tacrolimus

Y. Morita (✉) · Y. Sasae · T. Sakuta · M. Satoh · T. Sasaki · N. Kashihara
Division of Nephrology and Rheumatology,
Department of Internal Medicine, Kawasaki Medical School,
577 Matsushima, Kurashiki, Okayama 701-0192, Japan
e-mail: morita@med.kawasaki-m.ac.jp

Introduction

Tacrolimus (Prograf, FK506) is an oral immunosuppressive agent. It exerts its immunosuppressive effects by inhibition of calcineurin, leading to interference with T cell activation. T cell is the most abundant infiltrating leukocyte in the inflamed synovium of patients with rheumatoid arthritis (RA), and plays an important role as effector cells in the perpetuation of the inflammatory process. Therefore, there has been interest in the use of tacrolimus for the treatment of RA [1]. Clinical studies of tacrolimus have been performed in Japan and the US to assess its efficacy in the treatment of RA [2–4]. In these studies, tacrolimus monotherapy was shown to be optimally effective against RA at a dosage of 3 mg/day. Oral tacrolimus was approved in Japan for the treatment of RA patients who otherwise do not respond to other disease-modifying anti-rheumatic drugs (DMARDs).

Several DMARDs are now available for the treatment of patients with RA, including weekly methotrexate (MTX), which is the current gold standard in RA therapy. However, in Japan, a lower dose of MTX is dispensed for the treatment of patients with RA than that used in the US and many European countries. The maximum MTX dosage officially approved in Japan is only 8 mg/week. Unfortunately, such a dose of MTX does not fully control RA in many patients. In those patients with a partial response to low-dose MTX, further therapeutic decisions are necessary. One of the strategies in such cases is to combine low-dose MTX with an additional agent ("step-up" combination therapy). Since the mechanisms of action of MTX and tacrolimus are different, tacrolimus can be a suitable candidate for use in combination with MTX.

A clinical trial of tacrolimus in the US has shown the advantages of combining tacrolimus with MTX [5].

The six-month, open-label study showed that 3 mg/day of tacrolimus plus 5–20 mg/week MTX provides clinical benefits. However, the high cost of tacrolimus has to be considered in any clinical use. The cost is almost comparable to that of biological agents in the Japanese market when it is prescribed at 3 mg/kg. We retrospectively evaluated the efficacy and safety of tacrolimus when added to MTX in Japanese patients with RA. The results indicated that the addition of tacrolimus to MTX is well-tolerated and highly efficacious, even at low doses ($\leq$1.5 mg/day).

Patients and methods

We collected clinical data of 32 RA patients who visited Kawasaki Medical School Hospital and Sayo Central Hospital (Sayo, Hyogo, Japan), and who started tacrolimus in addition to MTX before August 2007. All patients met the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) 1987 revised criteria for RA [7]. Of the 32, 28 patients had an inadequate response to MTX at $\geq$8 mg/week. In four patients, 8 mg/week of MTX was not tolerated because of the associated toxicity (bone marrow suppression or worsening of liver function tests). The clinical status was evaluated by the disease activity score of 28 joints (DAS28) using erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) [8]. We use the terms "DAS-ESR" for the original DAS28 using ESR and "DAS28-CRP" for DAS28 using CRP in this paper. The cut-off values of 2.6, 3.2 and 5.1 for DAS28-ESR were used to indicate remission, low disease activity and high disease activity, respectively [8]. The value of DAS28-CRP is reported to be less than that of the original DAS28-ESR [9, 10]. Based on the study by Inoue et al. [10], we used a value of DAS28-CRP of >4.1 as high activity, 2.7–4.1 as moderate activity, <2.7 as low activity, with <2.3 being defined as remission. We could not evaluate DAS28-ESR in two patients because of a lack of ESR data. The clinical response was analyzed using the European League Against Rheumatism (EULAR) improvement criteria [7]. To analyze the clinical response using DAS28-CRP, 4.1 and 2.7 were used as the thresholds for the high and low disease activities, respectively [11]. All adverse events that occurred during the combination therapy were also evaluated.

Statistical analysis

Data were expressed as mean ± SEM of the indicated number of samples studied. Wilcoxon's matched-pairs test was used to calculate levels of statistical significance. A *P* value of less than 0.05 denoted the presence of a statistically significant difference.

Results

Patients' characteristics

Table 1 shows the clinical features of the patients who received the combination of tacrolimus and MTX. All patients were treated with MTX for a minimum of six months at a stable weekly dose of 4–15 mg for at least four weeks before starting tacrolimus. None of the patients had serum creatinine levels greater than 1.0 mg/dl, HbA1c >6% or uncontrolled infection. Before commencement of tacrolimus therapy, 25 patients (78.1%) were taking 2–10 mg/day of prednisolone. The average DAS28-CRP and DAS28-ESR scores were 3.81 and 4.62, respectively. Based on the DAS28-CRP values, 12 of 32 patients (37.5%) were considered to have high disease activity, 19 patients (59.4%) moderate disease activity, and one patient (3.1%) to have a low disease activity. Likewise, evaluation by DAS28-ESR indicated 30.0% had high activity, 66.7% moderate activity, and 3.3% had low activity.

Table 1 Baseline characteristics of patients treated with tacrolimus and methotrexate

Male/female ratio	5/27
Age (years)	62.5 ± 1.9; 37–80
Duration of disease (years)	9.5 ± 1.3; 2–30
Stage	
I	0
II	10
III	9
IV	13
RF positive (%)	87.5%
Methotrexate user (%)	100%
Methotrexate dose (mg/week)	9.3 ± 0.4; 4–15
Steroid user (%)	78.1%
Prednisolone dose (mg/day)	4.7 ± 0.4; 2–10 (n = 25)
CRP (mg/dl)	2.72 ± 0.37 (n = 32)
ESR (mm/h)	66.9 ± 5.5 (n = 30)
Tender joint count (0–28 joints)	2.1 ± 0.4
Swollen joint count (0–28 joints)	2.5 ± 0.4
Patient's global assessement of disease activity (0–100 mm VAS)	47.7 ± 3.4
DAS28-CRP	3.81 ± 0.14 (n = 32)
DAS28-ESR	4.62 ± 0.14 (n = 30)

Data are mean ± SEM and range

RF Rheumatoid factor, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate, *VAS* visual analog scale, *DAS28* disease activity score of 28 joints

Doses of tacrolimus and discontinuation

Table 2 lists the doses of tacrolimus used in our patients. Tacrolimus was started at 1 mg/day in five patients, 1.5 mg/day in 24 patients, and 3 mg/day in three patients. At six months, tacrolimus was continued at 1–2 mg/day in 27 of the 32 patients. In the remaining five patients, tacrolimus was discontinued due to inadequate response. In four of these five patients, tacrolimus was started at 1.5 mg/day and then discontinued without evaluating the effect of a higher dose. In two of the three patients who started with 3 mg/day of tacrolimus, the treatment was considered effective. In these patients, the dose of tacrolimus was reduced to 1.5 mg/kg before the eighth month of treatment, and the efficacy was sustained up to 12 months.

Adverse events were observed in four patients. Serum creatinine levels increased slightly in two patients who were on 1.5 mg/kg of tacrolimus. When the dose of tacrolimus was further reduced to 1 mg/kg, serum creatinine levels returned to normal without a decrease in efficacy. HbA1c increased from 5.6 to 6.3% in one patient, but it improved without changing the dose of tacrolimus or the addition of a new drug. In one patient, blood pressure started to increase after the commencement of tacrolimus therapy. Although hypertension was controlled by antihypertensive therapy, tacrolimus was discontinued with insufficient efficacy.

Clinical response

We analyzed changes in CRP, ESR, tender joint count, swollen joint count, patient's global assessment of disease activity, DAS28-CRP, and DAS28-ESR from baseline values in 27 patients who continued tacrolimus for more than six months (Fig. 1). C-reactive protein, ESR, DAS28-CRP, and DAS28-ESR improved significantly at one month after the start of tacrolimus therapy. The mean DAS28-CRP value decreased from 3.92 at baseline to 2.40 at six months. The mean DAS28-ESR value also decreased from 4.72 to 3.28. Figure 2 depicts the categorized disease activity. Before starting tacrolimus therapy, about one-third of the patients were categorized as having high disease activity (44.4% in DAS28-CRP, 36.0% in DAS28-ESR). At six months, none of the patients exhibited high disease activity. A total of 51.9% of the patients exhibited clinical remission in DAS28-CRP. The remainder (48.1%) were categorized as having moderate or low activity. For DAS28-ESR, the proportion of patients exhibiting moderate activity, low activity, or remission at six months were 52.0, 36.0, or 12.0%, respectively.

We also analyzed the clinical response using the EULAR improvement criteria (Fig. 3). Patients who discontinued tacrolimus were included in the category of no response, since the reason for their discontinuation was no or inadequate response. Based on the criteria using DAS28-CRP, a moderate or good response was achieved in 46.9 and 71.9% of patients at one and six months, respectively. A similar finding was obtained from the original criteria using DAS28-ESR. A total of 46.7% and 70.0 patients were evaluated as having a moderate or good response at one and six months, respectively.

In 11 of 25 patients who were taking corticosteroids, the mean dose of prednisolone was reduced from 4.9 mg/day at baseline to 3.6 mg/day at six months. This suggests that low-dose tacrolimus therapy had a clinically significant steroid-sparing effect.

Table 2 Doses of tacrolimus at the start and after six months of therapy

	At 0 months	At 6 months
1 mg	5	6
1.5 mg	24	20
2 mg	0	1
3 mg	3	0
Discontinuation		5

Data are number of patients

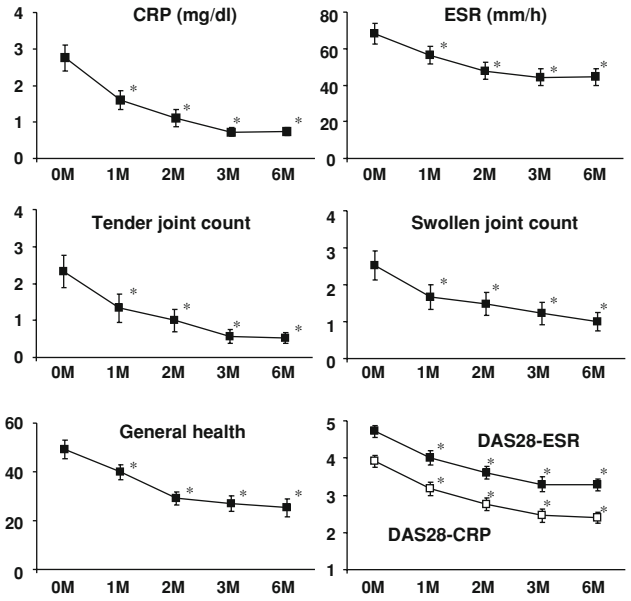

Fig. 1 Serial changes in C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), tender joint count (0–28 joints), swollen joint count (0–28 joints), patient's global assessment of disease activity (0–100 mm visual analog scale), disease activity score of 28 joints DAS28-CRP, and DAS28-ESR in patients who received tacrolimus in addition to methotrexate (MTX). Data are mean ± SEM. CRP, tender joint count, swollen joint count, general health, and DAS28-CRP, $n = 27$; ESR and DAS28-ESR, $n = 25$. *$P < 0.01$, compared with 0 months

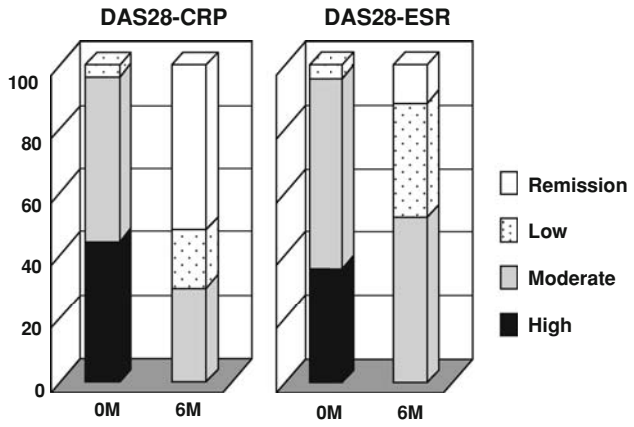

Fig. 2 Disease activity state before and six months after starting tacrolimus treatment, evaluated with DAS28-CRP ($n = 27$) and DAS28-ESR ($n = 25$)

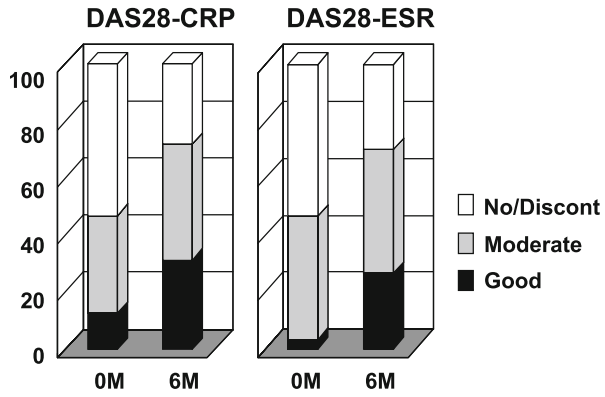

Fig. 3 Clinical response to the combination therapy of tacrolimus and MTX based on EULAR criteria using DAS28-CRP ($n = 32$) and DAS28-ESR ($n = 30$). Patients who discontinued tacrolimus were included in the category of no response, since the reason for their discontinuation was no or inadequate response

Discussion

It was demonstrated previously that tacrolimus at 3 mg/day plus MTX is safe and effective for the treatment of patients with RA [6]. The present study shows that the combination of tacrolimus and MTX is highly efficacious in patients with RA, even when administered at low doses (≤1.5 mg/day). Despite its retrospective nature, our study provides the basis for selecting a better approach among several therapeutic options for patients in whom MTX monotherapy had failed, which may allow more patients to achieve a therapeutic response.

The use of anti-cytokine therapy using biological agents constituted a major advance in RA therapy. There is convincing evidence that treatment with anti-TNF agents results in a significant improvement in the radiographic progression of disease. On the other hand, it is well-recognized that the disadvantages of anti-TNF agents are significant increase of infections, the high cost of medications, and the need for parenteral administration. To avoid potentially serious adverse events, treatment guidelines for the use of anti-TNF agents have been issued and utilized in a post-marketing survey in Japan [12, 13]. Thus, biologics should be considered in patients with highly active disease. The use of more than one DMARD such as MTX plus tacrolimus would be primarily indicated for patients with moderate disease activity.

The cost issues must be considered in the choice of treatment for RA patients. The high cost of tacrolimus is a matter of concern with regard to clinical use. Indeed, the cost is almost comparable to that of biological agents in the Japanese market when it is prescribed at 3 mg/kg. Our results show that tacrolimus is efficacious at a lower dose (≤1.5 mg/day) when it is used with MTX. Thus, compared with biological agents, this combination therapy is a lower cost option. However, to discuss the cost-effectiveness issue more precisely, more data on the impact of treatment on long-term outcome such as radiographic progression and work capacity are needed. Regarding this issue, a multicenter, double-blind clinical trial has started to assess the radiographic benefit of tacrolimus therapy in Japan.

The safety of tacrolimus for elderly patients with RA (≥65 years) was demonstrated previously in an open-label, noncontrolled study [14]. In that study, the initial dose of tacrolimus was set at 1.5 mg/day for all patients and then increased to 3 mg/day after six weeks if no changes were noted [14]. In our study, the initial dose of tacrolimus was ≤1.5 mg/day in the majority of patients (29 of 32 patients). Our patients were younger than those in the above study (mean 62.5 years). The adverse events observed included increased creatinine, hyperglycemia and hypertension. Nocturnal myoclonus in elderly patients with RA treated with tacrolimus was recently reported [15], but we did not observe any neurotoxic adverse effects. Thus, while the combination of tacrolimus and MTX appears to be relatively safe and well-tolerated, we advocate a close monitoring of renal function, blood sugar, blood pressure, and neurotoxicity.

C-reactive protein, ESR and DAS28 improved significantly after one month of the combination treatment of tacrolimus and MTX. The early appearance of the therapeutic effect of tacrolimus is consistent with the finding of a previous study using tacrolimus monotherapy [2]. Our findings suggest that the effects of tacrolimus plus MTX combination therapy should be evident at three months after starting the treatment. Based on this observation, we recommend reconsideration of the therapeutic regimen when no response is observed after six months of the combination therapy.

We used both DAS28-CRP and DAS28-ESR values to evaluate disease activity. While the value of DAS28-CRP is less than that of DAS28-ESR, the respective changes in DAS28 show a significant correlation [9, 10]. Based on the database of Japanese RA patients, Inoue et al. [10] proposed the use of DAS28-CRP threshold values as criteria for remission, low disease activity and high disease activity. We used their recommended cutoff values for DAS28-CRP in this study. As shown in Fig. 2, the high and moderate disease activities evaluated by using DAS28-CRP were almost consistent with those of DAS28-ESR. However, the proportions of patients with low disease activity and those in remission were different due to the scoring system. The inconsistency of the above criteria for low disease activity and remission have been discussed previously [10], and this issue would require further work. On the other hand, the EULAR improvement criteria evaluated by DAS28-CRP correlated well with the original criteria using DAS28-ESR (Fig. 3). These results suggest that the DAS28-CRP system can be used as an alternative to the DAS28-ESR to evaluate the clinical response to therapy of RA.

We should emphasize that this investigation is a pilot retrospective study on the effects of adding tacrolimus to MTX treatment in Japanese patients with RA. Patient selection bias might affect the results of this observational study, although we studied all patients for whom tacrolimus was added to MTX before August 2007. The selection of this combination therapy was determined by each attending physician. It could be pointed out that the disease activity of the studied patients is relatively mild. However, about one-third of the patients were evaluated as having high disease activity, while most of the remainder had moderate disease activity. Evaluation of physical function such as measuring changes in the disability index of the Health Assessment Questionnaire was not performed in this study, because of its retrospective nature. Thus, a randomized control trial is also needed to determine if the combination of tacrolimus and MTX is a better approach than switching to tacrolimus monotherapy.

Treatment with tacrolimus may overcome multidrug resistance that can occur in patients with RA. Tsujimura et al. [16] have recently demonstrated that P-glycoprotein, which is a product of the multidrug resistance-1 gene, is overexpressed on activated lymphocytes in patients with refractory RA. They proposed an interesting hypothesis that high levels of P-glycoprotein on lymphocytes may reduce the intracellular concentration of corticosteroids and certain DMARDs that are substrates of P-glycoprotein, resulting in drug resistance in patients with highly active RA [16]. It was reported previously that tacrolimus is an effective P-glycoprotein modulator that competitively inhibits P-glycoprotein from binding to other substrates and overcomes multidrug resistance [17]. Treatment with tacrolimus is demonstrated to increase the levels of intracellular dexamethasone in lymphocytes of patients with active RA [16]. Although further studies are necessary, this proposed mechanism supports the beneficial effects of tacrolimus in the treatment of some patients with RA.

In summary, our results suggest that the addition of low-dose tacrolimus to MTX in the treatment of moderately active RA appears to be well-tolerated and highly efficacious. However, due to the limited data, further studies are required for the assessment of this expensive immunosuppressant in the treatment of RA. Uncontrolled RA is associated with increased long-term disability and reduced life expectancy. Rheumatologists should thoroughly consider all possible benefits, theoretical risks and costs of each treatment regimen in each individual to provide the best care for their patients.

Acknowledgments We thank Dr. Takashi Hayashi (Sayo Central Hospital) for providing the data. The authors have no conflicts of interest to disclose.

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