Prospective Study to Assess the Optimal Blood Concentration of Tacrolimus in Japanese Patients with Rheumatoid Arthritis

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(Received August 10, 2008; Accepted February 20, 2009; Published online February 23, 2009)

We performed a prospective study to assess the optimal blood concentration of tacrolimus in Japanese patients with rheumatoid arthritis (RA). The dose of tacrolimus (1-3 mg/day) was administered orally once daily after the evening meal, and the blood concentration of tacrolimus 12 hr after administration was quantified. A total of 23 patients were enrolled. Clinical efficacy was assessed using the Disease Activity Score in 28 joints and European League Against Rheumatism (EULAR) response criteria at each outpatient visit during months 1-6. The ratio of patients who showed a moderate or good response was 47.8% (11/23). The mean blood concentrations of tacrolimus in EULAR responders and EULAR non-responders were 5.5 ± 3.6 (mean \pm S.D.) and 3.1 ± 1.9 ng/ml, respectively (p = 0.069). Interestingly, although tacrolimus had lower mean blood concentrations (< 5 ng/ml) compared with transplant fields (10-20 ng/ml), six (35.3%) of 17 patients showed sufficient response to tacrolimus. In addition, in five patients, tacrolimus was co-administered with methotrexate (MTX). On the other hand, the mean blood concentrations of tacrolimus in patients who did or did not develop adverse events were 4.8 ± 4.4 (n = 8) and 4.0 ± 2.1 (n = 15) ng/ml, respectively (p = 0.624). We failed to clear the optimal blood concentration of tacrolimus in RA patients, but one of the most remarkable findings was the observation that patients in whom tacrolimus was combined with MTX reached a positive response at much lower tacrolimus concentrations compared to patients not co-treated with MTX.

Key words — tacrolimus, rheumatoid arthritis, blood concentration, therapeutic drug monitoring

INTRODUCTION

Rheumatoid arthritis (RA) is a collagen disease and an autoimmune disorder that requires early aggressive treatment to minimize the morbidity associated with its progression. Thus, the central treatment strategy is disease-modifying anti-rheumatic drugs (DMARDs) such as bucillamine, leflunomide, and especially methotrexate (MTX).^{1–3)} In recent years, tacrolimus has been applied in RA patients showing insufficient response to other DMARDs.^{4–13)}

Tacrolimus, a calcineurin inhibitor, is an immunomodulatory and anti-inflammatory agent. It diminishes the ability of calcineurin to dephosphorylate and translocate the nuclear factor of activated T-cells that initiates gene transcription for the synthesis of inflammatory cytokines such as tumor necrosis factor- α , interleukin-2, and interferon- γ .^{9,10)} Tacrolimus is routinely administered as prophylaxis for rejection in patients receiving allograft solid organ transplantation and for graft versus host disease in patients receiving allogeneic hematopoietic stem cell transplantation.^{14–17}) In these transplant fields, adverse event such as nephrotoxicity has remained as a significant problem in the clinic. It is well known that the relationship between the dosage and blood concentration of tacrolimus varies widely.¹³⁾ Therefore, a dose adjustment using the blood concentration measurement of tacrolimus is indispensable to achieve the target therapeutic range in which desired effect can be obtained without adverse events (10-20 ng/ml^{16, 18)} at trough level; 12 hr after administration). On the other hand, the dose of tacrolimus for RA [1.5-3 mg/day, per os

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(p.o.), once a day] is lower than that in the transplant fields (e.g., 0.06-0.3 mg/(kg·day), p.o., twice a day); that is, no strict management of the blood concentration of tacrolimus is needed in patients with RA. However, there is little information on the optimal blood concentration of tacrolimus for RA so far.

The purpose of this study was therefore to assess the relationship between the blood concentration of tacrolimus and clinical efficacy, or adverse events in patients with RA.

MATERIALS AND METHODS

Study Design — This study was prospectively performed following the Helsinki Declaration of 1975 as revised in 1996. The study design was approved by the Institutional Review Board of Kagawa University Hospital. All patients gave their written informed consent.

This study involved patients aged 16 years or older in whom RA had been diagnosed at least 6 months previously in this hospital, according to the American College of Rheumatology 1987 revised criteria. Patients had previously continued active RA despite receiving a therapeutic dosage of a specific DMARD for duration of time typically sufficient to elicit a therapeutic response. Subjects meeting any of the following criteria were excluded from this study: Serious hypersensitivity to tacrolimus; presence or history of malignancy; severe infection; use of contraindicated drugs to tacrolimus (*e.g.*, cyclosporin A, bosentan hydrate, potassium sparing diuretics).

Immunosuppressive **Therapy** — Tacrolimus (Prograf[®], Astellas Co. Ltd., Tokyo, Japan) was orally administered once daily after the evening meal. The dose range of tacrolimus was 1-3 mg/day. When DMARDs were switched to tacrolimus at study entry, a washout period of 1 week was needed. Patients were allowed to receive a stable dosage of the DMARDs including MTX, biologics, non-steroidal anti-inflammatory drugs (NSAIDs), and steroids ($\leq 10 \text{ mg/day}$ of prednisolone or its equivalent) during the entire period of the study. However, the dose of steroid could be reduced if the efficacy was seen. Tacrolimus was temporarily discontinued when a patient developed any infections and severe adverse events during the investigation period.

Clinical Assessment —— Clinical and safety assessments were conducted at baseline and at each outpatient visit during months 1–6. In addition, following our sample collection schedule, the blood concentration of tacrolimus 12 hr after administration was routinely quantified at each outpatient visit during months 1–6. If patients dropped out prior to month 6, their assessments were performed at the end of treatment. Clinical efficacy was assessed using the Disease Activity Score in 28 joints (DAS28) and European League Against Rheumatism (EU-LAR) response criteria.¹⁹⁾ To calculate the DAS28, information about disease variables is needed: tender and swollen joint counts as well as C-reactive protein (CRP). Using these data, the DAS28 can be calculated using the following formula:¹⁹⁾

DAS28-3 (CRP)

= $[0.56 \times \text{square (tender joint count 28)} + 0.28$

 \times square (swollen joint count 28) + 0.36

 $\times \ln(\text{CRP} (\text{mg/l}) + 1)] \times 1.10 + 1.15$

The primary endpoint of efficacy was based on EULAR response criteria using DAS28.¹⁹⁾ Two time-points were defined at baseline and at the end of treatment. The secondary endpoint of efficacy was based on changes of DAS28 scores from baseline to end of treatment. EULAR responder was defined as a patient who showed a moderate or good response in this report.

Safety was evaluated in terms of adverse events, including clinical symptoms, abnormal changes in laboratory tests, and development of infection occurring in the interim.

Determination of Tacrolimus Concentrations — The concentrations of tacrolimus in whole blood were quantified using the $IMx^{\mathbb{R}}$ Tacrolimus II assay (Abott Laboratories, Abbott Park, IL, U.S.A.) with microparticle enzyme immunoassay technology.

Statistical Analysis — Values are indicated as the mean \pm standard deviation (S.D.). Change of DAS28 scores was analyzed using the Wilcoxon signed-rank test. Comparison of the mean blood concentration of tacrolimus between EULAR responders and EULAR non-responders was analyzed using Welch's *t*-test. The correlation between mean blood concentration of tacrolimus and change of DAS28 scores, and the relationship between the initial blood concentration of tacrolimus and tacrolimus dose per body weight, were analyzed using simple linear regression. The mean blood concentrations of tacrolimus with or without adverse events were analyzed using Welch's t-test. Laboratory values of renal, liver, and glucose tolerance function parameters between baseline and end

RESULTS

A total of 23 Japanese patients were enrolled in this study between February 2006 and June 2007. Tacrolimus was started at 1 mg/day in eighteen patients, 2 mg/day in two patients, and 3 mg/day in three patients. Thereafter tacrolimus was continued at 1 mg/day in eight patients, 1.5 mg/day in seven patients, 2 mg/day in five patients, and 3 mg/day in three patients. Eighteen patients completed the 6-month treatment with tacrolimus, but the other five patients discontinued the study in the interim; the reasons for discontinuation were "a lack of efficacy" in two patients and "additional RA therapy in addition to tacrolimus" in three patients [concomitant MTX (n = 1) and leukocytapheresis treatment (n = 2)]. Their characteristics are shown in Table 1.

In the primary endpoint of efficacy, 11 (47.8%) of 23 patients showed a moderate or good response in EULAR response criteria. The secondary endpoint of efficacy, change of DAS28 scores is shown in Fig. 1. As a whole, DAS28 scores significantly decreased at the end of treatment compared with baseline (p < 0.01). The mean blood concentrations of tacrolimus in EU-LAR responders and EULAR non-responders were 5.5 ± 3.6 (n = 11) and 3.1 ± 1.9 (n = 12) ng/ml,

 Table 1. Patient Characteristics

No. of patients	23	
Age (years)	56.1 ± 13.0	
Body weight (kg)	53.1 ± 13.0	
Gender (male/female)	3/20	
Tender or painful joint count	6.3 ± 4.4	
Swollen joint count	7.3 ± 4.4	
CRP (mg/dl)	1.49 ± 1.36	
DAS28 score	4.30 ± 0.98	
Concomitant drug use		
Steroids	23	
NSAIDs	21	
Biologics	5	
MTX	16	
DMARDs (except for MTX)	2	
Steroid dose ^{<i>a</i>}) (mg/day)	6.1 ± 2.3	
MTX dose (mg/week)	8.5 ± 2.9	

a) Steroid dose was indicated as prednisolone or its equivalent. Unless otherwise stated, all values are expressed as mean \pm S.D.

respectively, which were not significantly different (p = 0.069, Fig. 2A). No significant correlation was also seen between the mean blood concentrations of tacrolimus and change of DAS28 scores ($r^2 = 0.092$, p = 0.158, Fig. 2B). Interestingly, six (35.3%) of 17 patients who had lower



Fig. 1. Change of DAS28 Scores from Baseline to End of Treatment



Fig. 2. Comparison of the Mean Blood Concentration of Tacrolimus between EULAR Responders and EULAR Non-responders (A) and Relationship between Mean Blood Concentration of Tacrolimus and Change of DAS28 Scores (B)

Closed and open circles show the blood concentration of tacrolimus with or without co-treatment of MTX, respectively. The number of patients who were co-administered with and without MTX was 16 and 7, respectively.

mean blood concentrations (< 5 ng/ml) showed sufficient response to tacrolimus (Fig. 2A). MTX was co-administered in five (8.3 ± 2.6 mg/week), and not in one of them. However, the other 10 patients cotreated with MTX (8.9 ± 3.0 mg/week) showed no response to tacrolimus. There was no significant difference in MTX dose between EULAR responders and EULAR non-responders (p = 0.710).

The blood concentration of tacrolimus after the initial dosing was significantly correlated with the dose per body weight ($r^2 = 0.307$, p = 0.006, Fig. 3). Significantly, it should be noticed that the blood concentration of tacrolimus exceeded 10 ng/ml in two patients receiving 1 or 2 mg/day. During the investigation period, one of the two patients initially took clarithromycin (CAM; 400 mg/day), interacting with tacrolimus at cytochrome P450 3A4 (CYP3A4).

As for adverse events, eight (34.8%) of 23 pa-



Fig. 3. Relationship between Initial Blood Concentration of Tacrolimus and Tacrolimus dose per Body Weight

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tients experienced seven clinical symptoms and one abnormal value in laboratory tests that were possibly related to tacrolimus during the investigation period. The subjective symptoms were alopecia (n = 3), abdominal pain (n = 2), diarrhea (n = 2), flu syndrome (n = 2), stomach pain (n = 1), chest pain (n = 1), and stomatitis (n = 1), but those were not serious. The abnormal value in laboratory tests was slight liver damage of function parameters (n = 1). The mean blood concentrations of tacrolimus in patients who did or did not develop adverse events were 4.8 ± 4.4 (n = 8) and 4.0 ± 2.1 (n = 15) ng/ml, respectively, which were not significantly different (p = 0.624). Table 2 shows creatinine clearance (CCr), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T.Bil), and hemoglobin A_{1c} (HbA_{1c}) values which are representatively substituted as renal, liver, and glucose tolerance function parameters for convenience, respectively. No significant difference between the baseline and the end of treatment in CCr, AST, ALT, and T.Bil was seen, whereas HbA_{1c} significantly increased at the end of treatment compared with the baseline (p = 0.022), but it is not serious. No abnormal changes were observed in other laboratory tests (data not shown).

DISCUSSION

There is little information on the optimal blood concentration of tacrolimus for RA. In this prospective study, we failed to demonstrate a clear relationship between the mean blood concentrations of tacrolimus and the clinical efficacy, or adverse events. However, it is suggested that tacrolimus in combination with MTX may be clinically effective treatment at much lower blood concentrations of tacrolimus compared to patients not co-treated with MTX.

Tacrolimus has been reported to be a clinically effective and well tolerated treatment for RA. How-

Table 2.	CCr, AST, ALT,	T.Bil, and HbA_{1c}	Values

	Baseline	End of treatment	<i>p</i> -value
CCr ^{a)} (ml/min)	101 (38 –211)	91 (35 –221)	0.120
AST (IU/l)	16 (11 - 66)	18 (9 - 39)	0.488
ALT (IU/l)	14 (7 - 70)	16 (4 – 74)	0.908
T.Bil (mg/dl)	0.4 (0.2- 0.8)	0.4 (0.2- 0.7)	0.711
HbA _{1c} (%)	5.4 (4.5- 6.7)	5.7 (4.6- 7.0)	0.022

a) CCr was calculated with the Cockcroft-Gault equation using the serum creatinine value. Values are expressed as median (range).

ever, the optimal blood concentration of tacrolimus for RA remains unclear because of the small sample size and/or lower blood concentration.^{11,12} In this study, the ratio of patients who showed sufficient response to tacrolimus was 47.8% (11/23), whereas the incidence of adverse events was 34.8% (8/23) and they were mild; that is, its efficacy and safety were comparable to these previous reports.^{11–13} Interestingly, in this study, regardless of lower mean blood concentrations of tacrolimus (< 5 ng/ml) compared with transplant fields (10-20 ng/ml), six (35.3%) of 17 patients showed sufficient response to tacrolimus (Fig. 2A). The difference between RA patients and transplant patients may be due to the fact that in transplantation tacrolimus is used to prevent allo-reactivity, while in RA tacrolimus is used to suppress already existing auto-immunity. On the other hand, five of six patients who showed sufficient response to tacrolimus were co-administered MTX (Fig. 2A). One of the most remarkable findings is the observation that patients in whom tacrolimus was combined with MTX reached a positive response at much lower tacrolimus concentrations compared to patients not co-treated with MTX. Concomitant treatment of tacrolimus with MTX may have a synergistic effect due to different mechanism of action between tacrolimus and MTX. Tacrolimus specifically suppresses the activation and proliferation of T-cells. Therefore, we consider that tacrolimus in combination with MTX may be effective for RA patients with insufficient response to DMARD therapy even when the blood concentration of tacrolimus is low (< 5 ng/ml). In fact, Kremer et al.⁶) has novel reported that tacrolimus in combination with MTX is safe and well-tolerated and provides clinical benefit. There are several clinical reports regarding tacrolimus monotherapy for RA in Japan.^{5,9,11-13} This is a novel report regarding the tacrolimus co-administrated with MTX for Japanese patients, which suggested the clinical effectiveness with much lower tacrolimus concentrations.

Significantly, there was two deviant patients exhibited a high blood concentration of tacrolimus $(\geq 10 \text{ ng/ml}, \text{ Fig. 3})$. These patients had no renal or liver dysfunction. One possible reason is the impact of the concomitant drugs interacting with tacrolimus at CYP3A4. Suzuki and Takeuchi mentioned the importance of paying attention to concomitant drugs [e.g., CAM and itraconazole (ITCZ)].¹³⁾ One of the two patients initially took CAM at 400 mg/day during the investigation pe-However, in other two patients who had taken CAM (400 mg/day) or ITCZ (50 mg/day) during the investigation period, no influence on blood concentration of tacrolimus was observed. Thus the intensity of impact of concomitant drugs is unknown. On the other hand, in the previous study of tacrolimus for RA,¹¹⁾ the high blood concentration of tacrolimus (> 10 ng/ml at 12 hr after administration) has been reported in ten of 103 patients (9.7%). Seven of 10 patients experienced adverse events, but those were not serious. It has been reported the good positive correlation between the blood concentrations of tacrolimus and adverse events in transplant recipients.¹⁷) Therefore, to avoid adverse events induced by higher blood concentration, we recommend measuring the blood concentration of tacrolimus even in RA patients treated with a low dose of tacrolimus when a drug that inhibits

riod.

CYP3A4 is co-administered. It goes without saying that a therapeutic drug monitoring of tacrolimus is necessary for patients with renal and/or liver dysfunction and after the dose of tacrolimus was increased. In adverse events, tacrolimus for RA was well tolerated because of a lower blood concentration compared with transplant fields. In the previous

study, the clinical adverse events were generally gastrointestinal symptoms, renal function abnormalities, and any infections.^{4, 6–9, 11, 12} In this study, HbA_{1c} significantly increased at the end of treatment compared with baseline (Table 2), but not seriously so. In this study (Table 1), steroids at the dose of 6.1 ± 2.3 mg/day was co-administered in all patients. Therefore, we consider that the abnormal glucose tolerance function may have been caused by tacrolimus in addition to steroids.

In the future, the optimal blood concentration of tacrolimus for RA should be investigated in a large number of patients, because we failed to demonstrate it. We should be careful when interpreting the result of this small prospective study in a single center; however, a novelty in this study is the observation that patients in whom tacrolimus was combined with MTX reached a positive response at much lower tacrolimus concentrations compared to patients not co-treated with MTX.

Acknowledgements The authors are sincerely grateful to the outpatients who kindly agreed to participate in this study. The authors have no conflicts of interest in relation to this paper.

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