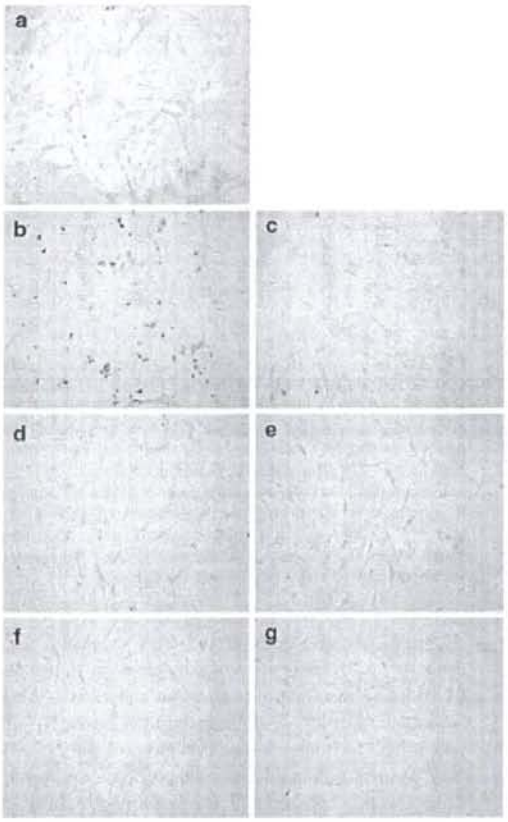


Fig. 4 Detection of apoptosis in RA synovial fibroblasts, by the TUNEL assay. Cells were incubated for 24 h. **a** Without any COX-2 inhibitors or with **b** celecoxib (40 μM), **c** etodolac (100 μM), **d** meloxicam (100 μM), **e** nimesulide (100 μM), **f** NS-398 (100 μM), or **g** rofecoxib (100 μM). Apoptotic cells exhibiting TUNEL staining are brown; normal cells counterstained with methyl green are blue (×200). Reprinted from Kusunoki et al. [23], with kind permission from John Wiley & Sons, Inc.

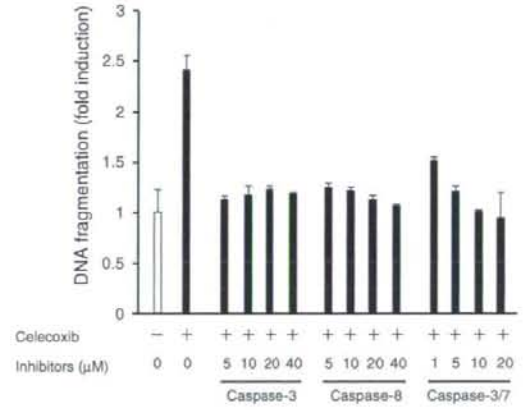


Fig. 5 Effect of caspase inhibitors on celecoxib-induced DNA fragmentation in RA synovial fibroblasts. Cells were incubated with celecoxib (40 μM) and with caspase inhibitors [Z-DEVD-FMK (a caspase-3 inhibitor), Z-IETD-FMK (a caspase-8 inhibitor), and Z-VAD-FMK (a caspase-3/7 inhibitor)] for 24 h at the indicated concentrations, after which DNA fragments in the cytoplasm were measured by enzyme immunoassay. The fold-induction of DNA fragmentation is shown relative to the control value (untreated cells). Representative results from two independent experiments are shown; values are the mean and SD from triplicate cultures. Reprinted from Kusunoki et al. [23], with kind permission from John Wiley & Sons, Inc.

that the pro-apoptotic effect of celecoxib on RA synovial fibroblasts was independent of COX-2 inhibition. This pro-apoptotic effect was suppressed by caspase inhibitors (Fig. 5). In addition, celecoxib did not cause transcriptional activation of PPARγ in RA synovial fibroblasts.

Epidemiological studies have shown that chronic intake of aspirin is associated with a reduction in the incidence of colorectal cancer [24]. NSAIDs have also been shown to exert a pro-apoptotic effect on various cell lines, particularly colon cancer cells [25]. We previously investigated the pro-apoptotic effect of six selective COX-2 inhibitors indicated above on human colorectal cancer cells, and found that only celecoxib induced apoptosis again, which was induced via a mechanism that was unrelated to COX inhibition [26]. We found that celecoxib reduced the phosphorylated Akt, an anti-apoptotic molecule, in colon cancer cell lines [26]. Several NSAIDs such as indomethacin [27], dicrofenac [28], salicylic acid [29], etodolac [30], nimesulide [31], and NS-398 [32] inhibited Akt activation in vitro experiments using cancer cell lines. Celecoxib alters intracellular calcium by inhibiting Ca^{2+} ATPases in the endoplasmic reticulum [33], and blocks TNF-induced activation of NF-κB [34]. However, these intracellular changes induced by NSAIDs were not consistent among several different cell types. The mechanisms of pro-apoptotic effects of NSAIDs on cancer cells as well as synovial fibroblasts are still remained to be studied.

TT101, a new derivative of celecoxib

Although celecoxib suppressed the proliferation of RA synovial fibroblasts and induced apoptosis at the optimal concentrations were higher (10–40 μM) compared with those for COX-2 inhibition (0.01–10 μM) [23]. The mean maximum plasma concentration of celecoxib in healthy volunteers was reported to be 1.4, 2.5, and 7.7 μM after single doses of 100, 400, and 800 mg, respectively [35], showing that insufficient concentrations for pro-apoptotic effect on RA synovial tissue. Therefore, we tried to develop potent inducer of apoptosis by modification of the

Table 1 Properties of bioactivities of several NSAIDs

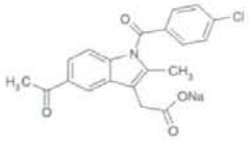
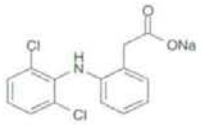
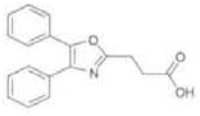
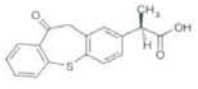
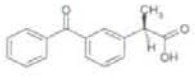
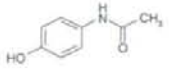
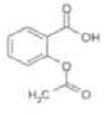
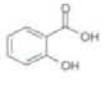
NSAIDs	Structure	Pro-apoptotic effect on RSF	Activation of PPARγ	Inhibitory effect of Akt activation	COX-1 IC_{50} (μM)	COX-2 IC_{50} (μM)	COX-1/ COX-2 ratio
Indomethacin		++ [15]	+ [15]	+ [27]	0.013 [15]	0.044 [15]	0.30
Diclofenac		++ [15]	+ [15]	+ [28]	0.076 [44]	0.026 [44]	2.92
Oxaprozin		++ [15]	+ [15]	NT	2.2 [15]	36 [15]	0.061
Zaltoprofen		++ [15]	+ [15]	NT	1.3 [15]	0.34 [15]	3.82
Ketoprofen		− [15]	− [15]	− [38]	0.11 [15]	0.88 [15]	0.13
Acetaminofen		− [15]	− [15]	− [39]	42 [15]	11 [15]	3.81
Aspirin		++ [17]	− [17]	− [38]	1.7 [45]	7.5 [45]	0.23
Salicilic acid		++ [17]	− [17]	+ [29]	4,956 [45]	34,440 [45]	0.14

Table 1 continued

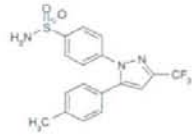
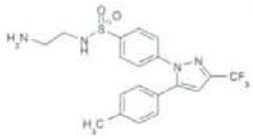
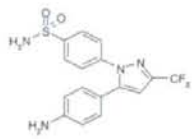
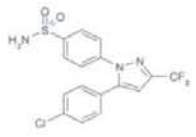
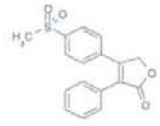
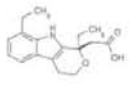
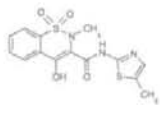
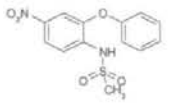
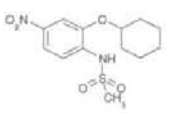
NSAIDs	Structure	Pro-apoptotic effect on RSF	Activation of PPARγ	Inhibitory effect of Akt activation	COX-1 IC_{50} (μM)	COX-2 IC_{50} (μM)	COX-1/ COX-2 ratio
Celecoxib		++ [23, 36]	− [23]	+ [26]	82 [44]	0.0032 [36]	25,625
TT101		+++ [36]	NT	− [36]	NT	0.31 [36]	NT
TT201		+ [36]	NT	− [36]	NT	0.13 [36]	NT
SC236		++ [36]	+ [40]	+ [41]	NT	0.0071 [36]	NT
Rofecoxib		− [23]	NT	NT	>100 [44]	0.048 [36]	>2,083
Etodolac		− [23]	NT	+ [30]	>100 [44]	53 [44]	>1.89
Meloxicam		− [23]	− [42]	NT	37 [44]	6.1 [44]	6.07

Table 1 continued

NSAIDs	Structure	Pro-apoptotic effect on RSF	Activation of PPARγ	Inhibitory effect of Akt activation	COX-1 IC_{50} (μM)	COX-2 IC_{50} (μM)	COX-1/ COX-2 ratio
Nimesulide		− [23]	− [43]	+ [31]	10 [45]	1.9 [45]	5.26
NS-398		− [15, 23, 36]	− [15, 23]	+ [32]	125 [44]	0.012 [36]	10,416

NT not tested

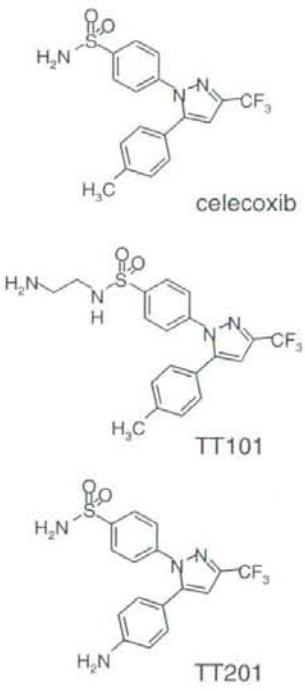


Fig. 6 Chemical structure of celecoxib and its derivatives

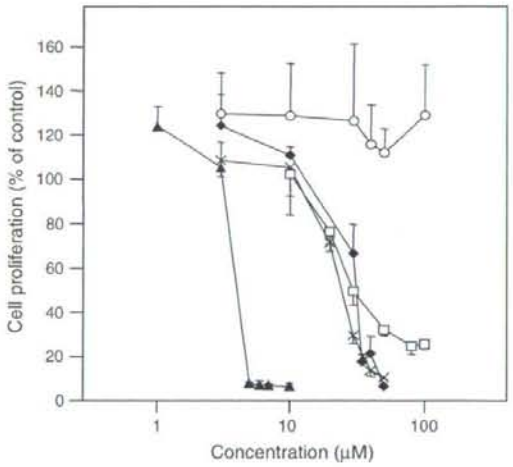


Fig. 7 Effect of the each drug on the proliferation of synovial fibroblasts obtained from patients with RA. Cells were incubated with celecoxib (*closed diamonds*), TT101 (*closed triangles*), TT201 (*open squares*), SC-236 (*crosses*), or rofecoxib (*open circles*) for 24 h. Then proliferative activity was estimated from the nuclear incorporation of BrdU and was expressed as a percentage of the control value (untreated cells). Data are the mean ± SD for triplicate cultures, and representative results from three independent experiments are shown. Reprinted from Kusunoki et al. [36], with kind permission from American Society for Pharmacology and Experimental Therapeutics

structure of celecoxib. We synthesized two celecoxib derivatives (TT101 and TT201) and analyzed their pro-apoptotic effect on RA synovial fibroblasts [36].

We summarized properties of several NSAIDs from the view point of bioactivities and structures (Table 1). The sulfonamide group of celecoxib was changed to an N-(2-aminoethyl)-sulfonamide group when developing TT101, whereas the tolyl group in the terminal aromatic ring of celecoxib was changed to an aminophenyl group to create TT201 (Fig. 6). Interestingly, TT101 was more potent with respect to suppression of hyperplasia (Fig. 7) and induction of apoptosis (Fig. 8) in RA synovial fibroblasts when compared to celecoxib. NSAIDs without sulfonamide group,

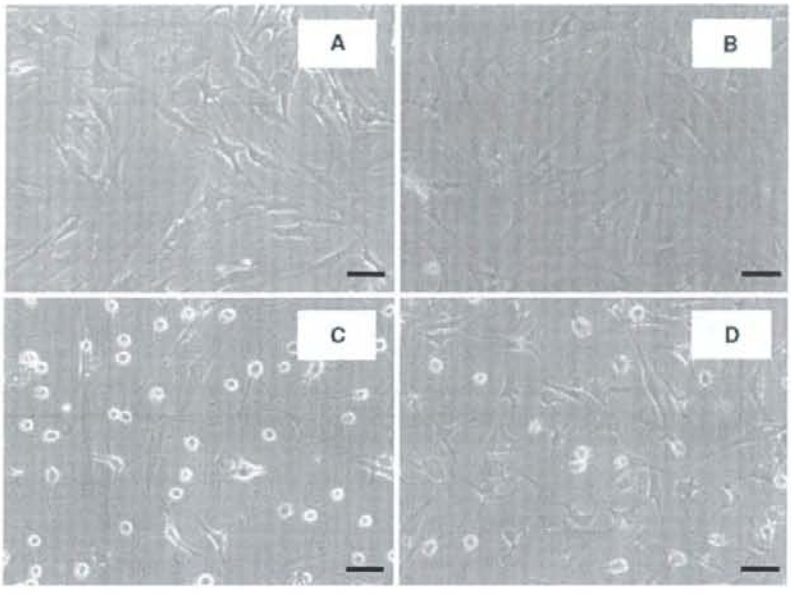


Fig. 8 Morphological changes of the synovial fibroblasts from RA patients (**a** and **c**) or osteoarthritis patients (**b** and **d**) as observed by light microscopy. Cells were incubated for 24 h without (**a** and **b**) or with (**c** and **d**) TT101 at a concentration of 7 μM. *Bar* 60 μm. Reprinted from Kusunoki et al. [36], with kind permission from American Society for Pharmacology and Experimental Therapeutics

such as indomethacin, diclofenac, oxaprozin, zaltoprofen, also induced apoptosis in RA synovial fibroblasts. However, they activated PPARγ in the cells, while celecoxib did not. A pro-apoptotic effect of TT201 was weaker than that of celecoxib. Therefore, conformations of TT101 and celecoxib except sulfonamide group are possibly important to maintain pro-apoptotic effect. We also measured the COX-2 inhibitory effect of these compounds in RA synovial fibroblasts and found that the order of potency for the COX-2 inhibition by these drugs was celecoxib > TT201 > TT101 [36]. The potent pro-apoptotic effect of TT101 was also observed in colon cancer cell lines [37]. Although the mechanism of action of TT101 remains unclear, it may have potential as a novel anti-proliferation drug for rheumatoid synovial fibroblasts and colon cancer cells.

Conclusion

Pro-apoptotic effects of NSAIDs including conventional NSAIDs, celecoxib and a derivative of celecoxib were reviewed. Although additional studies are needed, these results suggest that the induction of apoptosis caused by some NSAIDs may help to prevent the degradation of articular cartilage in RA after the inhibition of synovial hyperplasia and pannus formation.

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Competing interests S.K. has served as consultants to and/or received honoraria from Pfizer Japan (Tokyo), the manufacture relatives of celecoxib, and Astellas Pharma (Tokyo), the selling company of celecoxib. N.K. and S.K. hold a patent for TT101 and TT201. R.Y. has no competing interests.

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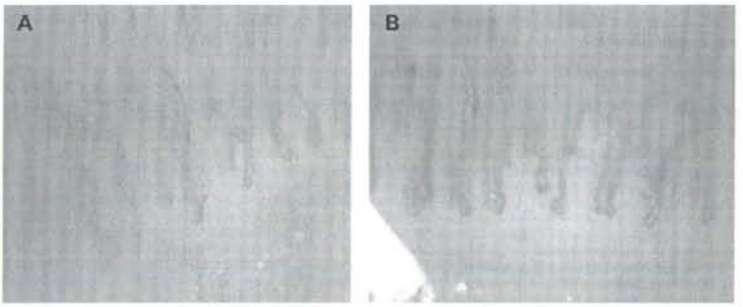

Figure 2 Higher magnification of selected areas of finger four in patient 2 (also shown in fig 1, B3) demonstrating the non-homogeneous distribution of capillary morphology. A. Segment with normalised pattern. B. Elongated, tortuous, partly enlarged capillaries with some thrombosis. Informed consent was obtained for publication of this figure.

Our findings support the reversibility of the microvascular changes in patients with SSc and MCTD on a short time interval. Intense immunosuppression seems to have an impact on vascular remodelling in patients with SSc and MCTD.

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Clinical value of second- and third-generation assays of anti-cyclic citrullinated peptide antibodies in rheumatoid arthritis

Anti-cyclic citrullinated peptide (CCP) antibodies are useful for the diagnosis of rheumatoid arthritis (RA) because of their higher specificity.[1] First-generation anti-CCP (CCP1) ELISAs were based on synthetic peptides derived from human filaggrin.[2] The second-generation anti-CCP (CCP2) test, which contains epitopes selected from libraries of citrullinated peptides, performs better than anti-CCP1.[3 4] Recently, a third-generation anti-CCP (CCP3) test was introduced. We compare the performance of the anti-CCP3 test with that of two anti-CCP2 tests, and assess their value in diagnosing RA.

A total of 502 participants were studied: 227 patients fulfilling the American College of Rheumatology criteria for RA,[5] 173 patients with non-RA autoimmune diseases (details are indicated in the footnote of table 1) and 102 healthy controls. The mean (SD) age of the patients was 60.2 (13.1) years and 53.8 (15.8) years, female patients comprised 78.4% and 89.0% and the mean (SD) disease duration was 10.3 (8.8) years and 9.1 (9.0) years in the RA group and non-RA group, respectively. One hundred and two healthy controls were also studied. Mean (SD) age was 39.4 (7.9) years and 40.2% were female.

Patients underwent serological tests for anti-CCP antibodies (Inova Diagnostics, CA, USA: anti-CCP3; Axis-Shield Diagnostics, Scotland, UK: anti-CCP2a and Inova Diagnostics: anti-CCP2i), rheumatoid factor (RF) by nephelometry (Mitsubishi Kagaku Iatron, Tokyo, Japan), RF-IgM by ELISA (Inova Diagnostics), anti-agalactosyl IgG antibody (CARF) (Sanko Junyaku, Tokyo, Japan) and RF-IgG (Sanko Junyaku). The cut-off values used for all tests were those specified by the manufacturer. Research ethics committee approval was obtained and all participants gave informed consent.

Table 1 shows the sensitivity and specificity of the serological tests. Figure 1 shows the relationship between the sensitivity and specificity of these tests at different cut-off values in receiver operating characteristic (ROC) curves. The ROC curves show three groups of tests; the anti-CCP tests, the RF/RF-IgM/CARF tests and the RF-IgG test. The area under the curve (AUC) for the anti-CCP tests is almost identical. We compared the anti-CCP antibody titres measured by these three anti-CCP tests, and noted a significant correlation between every test pair

Letters

Table 1 Sensitivity and specificity of serological tests in patients with and without* rheumatoid arthritis (RA)

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
CCP2a	76.5	86.1	87.8	73.8
CCP2i	70.0	91.3	91.4	69.9
CCP3	77.1	86.1	87.9	74.1
RF	73.2	69.8	75.6	67.0
RF-IgM	80.6	56.1	70.7	68.8
CARF	80.0	65.9	75.3	71.7
RF-IgG	11.7	93.2	69.4	44.2

*Non-RA includes systemic lupus erythematosus (n = 66), systemic sclerosis (n = 28), Sjögren's syndrome (n = 33), mixed connective tissue disease (n = 15), polymyalgia rheumatica (n = 6), polymyositis/dermatomyositis (n = 8), Behçet's syndrome (n = 4), adult-onset Still's disease (n = 3), antiphospholipid antibody syndrome (n = 1), psoriatic arthritis (n = 1), Churg–Strauss syndrome (n = 1), polyarthritis nodosa (n = 1), Wegener's granulomatosis (n = 1), pustulosis palmoplantaris (n = 1), Hashimoto's disease (n = 3) and autoimmune haemolytic anaemia (n = 1).
NPV, negative predictive value; PPV, positive predictive value; RF, rheumatoid factor.

(CCP3 vs CCP2a, $r_s = 0.860$, $p<0.01$; CCP3 vs CCP2i, $r_s = 0.860$, $p<0.01$; CCP2a vs CCP2i, $r_s = 0.935$, $p<0.01$ by Spearman's rank test). In healthy controls, anti-CCP2i, anti-CCP3 and RF were positive in 1.0%, 6.9% and 5.9%, respectively. The positive rate of anti-CCP3 test was slightly higher than that of the anti-CCP2i.

Recent studies reported in Belgium[6 7] and in Italy[8] demonstrated that the third-generation anti-CCP test had no better diagnostic performances than the second-generation assays in patients with RA. Our results also suggest that the performance of the newly introduced anti-CCP3 test does not exceed that of the anti-CCP2 tests in a Japanese population. However, the second- and third-generation anti-CCP tests are powerful aids in the diagnosis of RA in comparison with traditional serological tests such as rheumatoid factors.

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Competing interests: None.

Ethics approval: Research ethics committee approval was obtained.

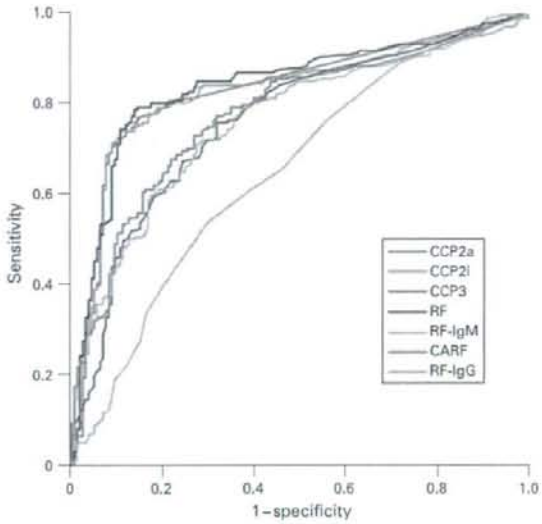

Figure 1 Receiver operating characteristic curves of anti-cyclic citrullinated peptide (anti-CCP) tests, rheumatoid factor (RF), RF-IgM, CARF and RF-IgG. Mean (SE) area under the curve: anti-CCP2a test, 0.83 (0.02); anti-CCP2i test, 0.83 (0.02); anti-CCP3 test, 0.84 (0.02); RF, 0.76 (0.03); RF-IgM, 0.76 (0.02); CARF, 0.78 (0.02); RF-IgG, 0.65 (0.03).

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ORIGINAL ARTICLE

Safety profile of tacrolimus in patients with rheumatoid arthritis

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Abstract We assessed the safety of tacrolimus therapy for rheumatoid arthritis. Forty-two patients who started tacrolimus therapy between April 2005 and July 2006 were investigated retrospectively using data from their medical records up to June 2007. The cumulative treatment continuation rate was assessed by the Kaplan–Meier method. Fisher's exact test was used to compare gastrointestinal symptoms between different tacrolimus doses and between the presence and absence of each concomitant medication. The mean (±SD) observation period was 288± 238 days. The cumulative treatment continuation rate was, respectively, 59.5% and 38.1% at 6 months and 1 year after the patients started treatment. Tacrolimus was discontinued in 28 patients, and was discontinued because of adverse reactions in 21 patients. Gastrointestinal symptoms were the most common adverse reactions (45.2%=19/42 patients), followed by infections and hyperglycemia. Tacrolimus was discontinued in 9/19 patients with gastrointestinal symptoms, and was discontinued within 60 days of starting treatment in seven of them. Nausea and vomiting led to discontinuation in seven patients (within 60 days of starting treatment in six of them). The incidence of gastrointestinal symptoms was higher in patients receiving a daily dose ≥2 mg than in those receiving <2 mg/day. During treatment of rheumatoid arthritis by oral tacrolimus therapy, gastrointestinal symptoms were common, early, and dose-dependent. However, these symptoms were not severe and did not cause any serious safety problems.

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Keywords Adverse reaction · Disease-modifying antirheumatic drug · Gastrointestinal symptoms · Immunosuppressant · Rheumatoid arthritis · Tacrolimus

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that features chronic synovitis as the main pathology and can be accompanied by various extraarticular symptoms; its etiology has not yet been clarified [1]. According to the ACR Guidelines, treatment with disease-modifying antirheumatic drugs (DMARDs) should be started within 3 months of diagnosing RA [2]. Methotrexate is a very widely used DMARD, and it is combined with or switched to other agents in methotrexate-resistant patients.

Tacrolimus is a substance derived from *Streptomyces tsukubaensis* that was discovered by Kino et al. in 1984 [3]. It is an immunosuppressant with a calcineurin inhibitory effect [4] and has been used to control allogenic immune reactions after organ transplantation [5, 6]. In 1999, the efficacy of tacrolimus for RA was demonstrated in an open-label study [7]. In Japan, a placebo-controlled clinical trial [8] and a trial comparing tacrolimus with mizoribin [9] have been performed, confirming its efficacy and safety for RA. Based on the results of these studies, administration of tacrolimus at an oral dose of 3 mg/day was approved in 2005 for the treatment of RA in Japan.

Both clinical studies and investigations in the organ transplantation field have shown that tacrolimus may cause characteristic adverse reactions, such as nephropathy, gastrointestinal disorders, glucose intolerance, and hypertension, which are not induced by cytotoxic immunosuppressants. However, few reports have been published

focussing on the safety of this drug when used for the treatment of RA. Therefore, we performed the present study to clarify the safety profile of tacrolimus by a retrospective investigation of RA patients treated with this drug at our department.

Materials and methods

The present study was performed by extracting tacrolimus-treated patients from those registered in our database. This study was approved by the Ethical Committee of Toho University School of Medicine. Before performing the study, written informed consent was obtained from all patients regarding the use of information in their medical records for research purposes.

Data obtained until June 2007 were retrospectively compiled from the medical records of 42 RA patients who started tacrolimus therapy between April 2005 and July 2006. Information about concomitant medications was collected in addition to information about tacrolimus therapy, including the duration of administration (days), dosage, and adverse reactions. The subjects were 9 men and 33 women with a mean (±SD) age of 60.0±13.4 years. According to Steinbrocker's stage classification [10], 1, 12, 5, and 24 patients were in stages I, II, III, and IV, respectively. According to Steinbroker's class classification [10], 33 and 9 patients were in classes II and III, respectively (there were no class I or IV patients).

Tacrolimus therapy

The initial dose of tacrolimus (Prograf®, $C_{44}H_{69}NO_{12}$–H_2O; Astellas Pharma, Tokyo) was 1–3 mg/day orally, which was administered once daily (in the morning or evening), twice daily (morning and evening), or three times daily (morning, afternoon, and evening). Subsequently, the dose was adjusted within the range of 1–3 mg/day at the discretion of the investigator. Other DMARDs, glucocorticoids, and nonsteroidal antiinflammatory drugs (NSAIDs) could be used at the discretion of the investigator.

Statistical analysis

The cumulative treatment continuation rate was investigated by the Kaplan–Meier method, and the significance of differences was determined by the log-rank test. Fisher's exact test was used to compare the incidence of gastrointestinal symptoms between different tacrolimus doses and between the presence and absence of each concomitant medication. For these analyses, $p<0.05$ was considered to indicate statistical significance.

Results

Cumulative treatment continuation rate

When the cumulative treatment continuation rate was investigated by the Kaplan–Meier method over a mean (±SD) observation period of 288±238 days, the rate was 59.5% at 6 months and 38.1% at 1 year after starting treatment with tacrolimus (Fig. 1). Treatment was discontinued in 28 of the 42 (66.7%) patients because of adverse reactions in 21 (50.0%) patients and for other reasons in 7 (16.7%) patients. When these 21 patients were classified according to the reason for discontinuation and compared by the Kaplan–Meier method (Fig. 1), they fitted into two groups: patients who discontinued tacrolimus within 60 days of starting therapy and patients who discontinued it after this period. Early discontinuation within 60 days of starting treatment was due to adverse reactions in all but one patient.

Adverse reactions

Table 1 shows all of the adverse reactions detected during tacrolimus treatment. For the patients who discontinued this drug because of adverse reactions, the symptoms that led to discontinuation are shown in relation to the time of onset. Gastrointestinal symptoms, such as nausea and vomiting, were the most common adverse reactions (19 patients, 45.2%), followed by infections and hyperglycemia. One patient died at 330 days after starting treatment. The cause of death was considered to be progression of chronic renal failure due to diabetes mellitus, and the relationship to tacrolimus therapy was classified as "possible."

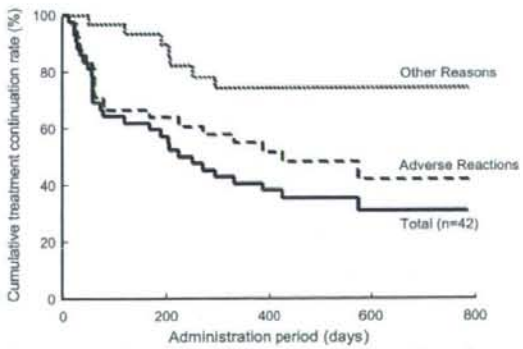

Fig. 1 Cumulative treatment continuation rate of tacrolimus therapy. Cumulative continuation rates are shown for all patients treated with tacrolimus and for patients who discontinued treatment due to adverse drug reactions and for other reasons. Among the 42 patients, 28 discontinued tacrolimus treatment (21 due to adverse reactions and 7 for other reasons). The cumulative treatment continuation rate was 59.5% at 6 months and 38.1% at 1 year after initiation

Table 1 Adverse reactions to tacrolimus in patients with rheumatoid arthritis

Adverse reactions	Number of patients (n=42)[a]	Patients who discontinued therapy (n=21)[a]			
		Day of onset			
		1–60	61–120	121–180	181
Clinical adverse reactions					
Gastrointestinal symptoms	19 (45.2)	7	1	0	1
Nausea, vomiting	13 (31.0)	6	0	0	1
Abdominal pain	6 (14.3)	1	1	0	0
Diarrhea	5 (11.9)	1	0	0	0
Appetite loss	3 (7.1)	1	0	0	0
Abdominal fullness	2 (4.8)	0	0	0	0
Infections	10 (23.8)	1	2	0	0
Respiratory infection	8 (19.0)	0	2	0	0
Any infection	2[b] (4.8)	1	0	0	0
Cardiovascular	4 (19.0)	0	0	0	0
Elevation of blood pressure	3 (7.1)	0	0	0	0
Tachycardia	1 (2.4)	0	0	0	0
Skin and appendages	3 (7.1)	1	0	0	0
Eruptions	2 (4.8)	1	0	0	0
Itch	1 (2.4)	0	0	0	0
Dizziness	3 (7.1)	1	0	0	0
Headache	2 (4.8)	2	0	0	0
Chest pain	2 (4.8)	1	0	0	0
Others	8[c](19.0)	0	0	0	0
Abnormal laboratory values					
Glycemia increased	6 (14.3)	0	0	0	3
Creatinine increased	3 (7.1)	1	0	0	2
Transaminase increased	3 (7.1)	0	0	0	0
HbA_{1c} increased	2 (4.8)	0	0	0	1
ALP increased	1 (2.4)	0	0	0	0
BUN increased	2 (4.8)	0	0	0	2
LDH increased	2 (4.8)	0	0	0	2
Uric acid increased	1 (2.4)	0	0	0	1
Triglyceride increased	1 (2.4)	0	0	0	1

ALP: alkaline phosphatase, *LDH*: lactate dehydrogenase

[a] More than one adverse event was reported for some patients. Percentages are rounded to the nearest decimal place. Values in parentheses are percentages.

[b] Urinary tract infection, herpes zoster.

[c] Insomnia, pleural effusion, metrorrhagia, hyposphagma, rib fracture, back pain, compression fracture of the lumbar spine, fatigue.

Gastrointestinal symptoms

Among the 19 patients with gastrointestinal symptoms, 13 (31.0%), 6 (14.3%), 5 (11.9%), 3 (7.1%), and 2 (4.8%) had nausea/vomiting, abdominal pain, diarrhea, anorexia, and bloating, respectively. For the patients who discontinued tacrolimus therapy because of adverse reactions, the reasons for discontinuation are listed according to the time of onset of their symptoms in Table 1. Of the 19 patients with gastrointestinal symptoms, 9 (47.4%) patients discontinued tacrolimus therapy and 7 (77.8%) of them discontinued it within 60 days of starting treatment. Seven patients discontinued this drug because of nausea or vomiting, and 6 (85.7%) of them discontinued it within 60 days of starting treatment.

The incidence of gastrointestinal symptoms stratified according to the dose of tacrolimus was compared by Fisher's exact test (Table 2). The incidence of such symptoms was 35.5% among the 11 patients receiving an initial dose of less than 2 mg/day, while it was 72.7% among the eight patients receiving an initial dose of at least 2 mg/day, and symptoms were significantly more common ($p<0.05$) at the higher initial dose. No significant relationship was noted between patient background factors (such as sex, age, RA stage, or administration times of tacrolimus) and the incidence of gastrointestinal symptoms (data not shown). The influence of concomitant treatment with other DMARDs, glucocorticoids, NSAIDs, H_2 receptor antagonists, or proton pump inhibitors on the incidence of gastrointestinal symptoms was assessed, but none of these drugs had a significant effect on the occurrence of gastrointestinal symptoms (data not shown).

Discussion

In the present study, tacrolimus therapy was discontinued by 21/42 patients because of adverse reactions. Previous clinical studies of tacrolimus performed in Japan have shown that treatment with this drug could be continued in 85.2% (1.5 mg/day) and 77.1% (3.0 mg/day) of subjects at 16 weeks after starting treatment [8] and in 62.1% (3.0 mg/day) [9] and

Table 2 Dose of tacrolimus and gastrointestinal symptoms

Tacrolimus dose	Number of patients	Patients with gastrointestinal symptoms, n (%)
<2 mg/day	31 (73.8%)	11 (35.5[a])*
≥2 mg/day	11 (26.2%)	8 (72.7[a])*
Total	42	19 (45.2)

[a] Percentage of patients from the respective groups.

*p=0.03724, Fisher's exact probability test

77.2% (1.5–3.0 mg/day) [11] of subjects at 28 weeks after starting treatment. A 6-month clinical study performed overseas showed that tacrolimus therapy could be continued in 42% and 52% of the 2 and 3 mg/day groups, respectively [12]. In the present study, the cumulative treatment continuation rate calculated by the Kaplan–Meier method was 59.5% at 6 months after starting treatment. This was similar to the results obtained in the above-mentioned clinical studies when the duration of administration is considered.

The main reason for discontinuation of tacrolimus therapy in the present study was gastrointestinal symptoms, especially nausea and vomiting, and these symptoms developed soon after the start of treatment in most cases. The mechanism(s) underlying the occurrence of gastrointestinal symptoms in patients taking this drug have not been elucidated yet. Tacrolimus has the same macrolide structure as erythromycin. When administered intravenously to dogs, erythromycin stimulates gastrointestinal motility and induces various symptoms [13], and its stimulatory effect on gastrointestinal motility was reported to be similar to that of motilin in dogs [14, 15]. Tacrolimus was also reported to stimulate small bowel motility in pigs [16]. In kidney transplant patients, the gastric emptying rate was higher after the administration of tacrolimus than after the administration of cyclosporine, which is also a calcineurin inhibitor [17]. Other reports from the transplantation field indicate that tacrolimus induces gastrointestinal symptoms more frequently than cyclosporine [18, 19, 20]. These findings suggest that the macrolide structure of tacrolimus may be involved in the development of gastrointestinal symptoms by mimicking the effect of motilin.

The present study showed that the incidence of gastrointestinal symptoms was significantly higher in patients receiving tacrolimus at doses ≥2 mg/day than in those receiving <2 mg/day, suggesting that these symptoms were dose-dependent. However, the dose range of tacrolimus used to treat our RA patients was narrower than that employed in the field of organ transplantation. According to Kondo et al. [8], gastrointestinal symptoms were noted in 3/62 patients (4.8%) receiving tacrolimus at 1.5 mg/day and in 7/63 patients (11.1%) receiving it at 3 mg/day. These findings indicate the same dose dependency as that observed in the present study. Accordingly, it is considered that tacrolimus therapy for RA patients should be started from a lower dose, such as 1.5 mg/day, and then should be increased while paying close attention to symptoms.

The incidences of gastrointestinal symptoms in the two randomized controlled trials performed in Japanese RA patients receiving 3 mg/day of tacrolimus were 11.1% [8] and 20.4% [9], respectively. Although the incidence of gastrointestinal symptoms in the present study (45.2%) seems higher than those in these studies, we could not find any background factors except administration doses, correlated with the symptoms. On the other hand, the incidences of tacrolimus-induced gastrointestinal symptoms were 5%, 21%, and 10% for patients undergoing hepatic, bone marrow, and kidney transplantation, respectively, in Japanese studies of transplant patients [21]. Gastrointestinal symptoms in the other study [22] developed in 7 out of 122 (5.7%) patients undergoing liver transplantation. These values in the transplantation fields appeared almost the same as those in the randomized controlled trials for RA. In contrast, the incidences of gastrointestinal symptoms were reported as 41.1% [23] and 29.3–43.9% (vomiting, constipation, nausea, and diarrhea) [24], respectively, in the renal transplantation studies, which are similar as that of the present RA study. We then suggest that the differences in the gastrointestinal symptoms among transplantation studies, randomized RA trials, and our present retrospective study were mainly attributable to the different cohorts, not to the essential differences in the disease properties, and other clinical background factors.

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Conflict of Interest Statement Dr. Shinichi Kawai has served as a consultant to and/or received an honorarium from Astellas Pharma Inc. (Tokyo, Japan), the manufacturer of tacrolimus.
No conflict of interest has been declared by Kimiko Akimoto, Yoshie Kusunoki, Shinichiro Nishio, and Kenji Takagi.

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ORIGINAL ARTICLE

Safety of long-term tacrolimus therapy for rheumatoid arthritis: an open-label, uncontrolled study in non-elderly patients

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Abstract In this study we focused on the safety of long-term tacrolimus therapy in non-elderly patients with rheumatoid arthritis who were treated with tacrolimus or mizoribine in a previous double-blind study. The patients received oral tacrolimus at a dose ≤3 mg once daily for 76 weeks. The safety analysis population included 115 patients aged 20–64 years. Adverse drug reactions presented as symptomatic events in 39 patients (33.9%), laboratory abnormalities in 38 patients (33.0%), and infections in 19 patients (16.5%). The major reactions were gastrointestinal disorders and hypertension as symptomatic events, increases of creatinine, urinary *N*-acetyl-β-D-glucosamidase and hemoglobin A_{1C} as laboratory abnormalities, and the common cold syndrome as infections. After 76 weeks of tacrolimus treatment, the ACR20 response rates of patients who had also received tacrolimus during the preceding double-blind study was 61.5% (compared with the status at baseline in the preceding study). The corresponding response rate for patients who had previously received mizoribine was 66.0%. The mean blood concentration of tacrolimus was 3.8–4.8 ng/mL. In conclusion, safety profiles of tacrolimus treatment for long-term seems to be similar to those of previous studies in patients with rheumatoid arthritis.

Keywords Long-term open study · Rheumatoid arthritis · Safety · Tacrolimus

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Introduction

Tacrolimus is an immunosuppressive agent with a macrolide skeleton that was discovered as a metabolite of *Streptomyces tsukubaensis*. In Japan, oral and parenteral formulations have been approved for use in patients with transplantation or autoimmune diseases, while tacrolimus ointment has been approved for the treatment of atopic dermatitis. The pathogenesis of rheumatoid arthritis (RA) has been reported to be related to immunocompetent T cells [1], and the T cell-selective immunosuppressant cyclosporine has shown efficacy for this disease [2].

The mechanism of action of tacrolimus differs from those of conventional disease-modifying antirheumatic drugs (DMARDs). Tacrolimus inhibits T cell activation and thereby suppresses the production of tumor necrosis factor-α and other inflammatory cytokines involved in the development of RA [3]. This drug has shown efficacy in animal models of RA, such as rats with collagen-induced arthritis and adjuvant-induced arthritis [4–6]. An early phase II study of tacrolimus in patients with RA demonstrated its usefulness as a new DMARD at doses of 1.5 and 3 mg/day [7]. A late phase II study [8] and phase III studies [9, 10] conducted in non-elderly

patients aged 20–64 years and elderly patients aged 65 years or older demonstrated the efficacy and safety of tacrolimus therapy for RA in both age groups. Based on these findings, tacrolimus was approved in 2005 for the treatment of RA with an insufficient response to existing therapy in Japan.

However, the maximum duration of treatment in these clinical studies was 28 weeks. The safety of long-term tacrolimus therapy has been investigated by a 12-month open study in the United States [11], but there have been no long-term studies in Japanese patients. Accordingly, we performed an open-label, uncontrolled study to focus on the safety of long-term tacrolimus treatment in non-elderly Japanese patients who had previously been enrolled in a phase III double-blind controlled trial [10].

Patients and methods

Patients

This study was conducted from May 2001 to December 2003 at 25 institutions. The subjects comprised RA patients who had participated in a previous double-blind controlled study of tacrolimus (the preceding study) [10] and had completed 28 weeks of treatment with tacrolimus or the control drug (mizoribine), or else had discontinued therapy due to lack of symptomatic improvement despite at least 12 weeks of treatment. All of the patients wished to receive long-term treatment with tacrolimus and were judged to be eligible for this study by their investigators.

The patients entering the present study had all met the following inclusion criteria at the time of their enrollment in the preceding study: (1) a diagnosis of RA according to the American College of Rheumatology (ACR) criteria (revised in 1987) [12]; (2) a disease duration of at least 6 months; (3) an age between 20 and 64 years at the time of giving informed consent; (4) an insufficient response to treatment with at least one DMARD other than tacrolimus or mizoribine before entering the study; (5) active disease (which was defined by a C-reactive protein level $\geq$1.0 mg/dL or an erythrocyte sedimentation rate $\geq$30 mm/h, at least six tender joints, and at least three swollen joints).

Patients were excluded from the present study for the following reasons: (1) study drug-related adverse events had occurred during the preceding study and the potential risks of long-term tacrolimus therapy were expected to outweigh the potential benefits, (2) surgery had been performed and the effects of surgical invasion were persistent, or (3) they had renal dysfunction, pancreatitis/glucose intolerance, hyperkalemia, severe liver dysfunction, heart disease (e.g., ischemic heart disease, arrhythmias requiring treatment, and heart failure), malignancy, severe infections, or severe drug hypersensitivity.

Methods

Prior to the present study, the Institutional Review Board at each participating institution approved the study protocol. All of the patients involved gave written informed consent. A multicenter open-label design was employed without a control drug.

There was an off-treatment interval in all patients between the end of the preceding study and the start of tacrolimus therapy in the present study. During the interval, blinding for the preceding study was maintained and the treatment of RA was not specified.

Tacrolimus was initiated at a dose of 3 mg orally once a day after the evening meal, which was the dosage for tacrolimus group in the preceding study. Dose reduction was possible (i.e., 3 mg/day was the maximum dose) depending on the patient's symptoms or adverse drug reactions at the discretion of the investigator. The duration of treatment in the present study was scheduled to be 76 weeks. The nonsteroidal anti-inflammatory drugs (NSAIDs) and steroids that were used prior to the study could be continued, but commencement of these drugs was prohibited. Use of two or more NSAIDs was avoided whenever possible, and dose reduction was allowed for NSAIDs and steroids during the study period. Concomitant use of other DMARDs (including gold salts, D-penicillamine, bucillamine, salazosulfapyridine, lobenzarit, actarit, cyclosporine, methotrexate, and mizoribine) was prohibited. Administration of any drug (e.g., astemizole or terfenadine) that could influence the blood level of tacrolimus was also prohibited.

Safety evaluation

Blood pressure and body weight were measured at the start of treatment (baseline), after 4 weeks of treatment, at 12-week intervals thereafter, and at the completion or discontinuation of treatment (the end of treatment). Laboratory investigations included hematology tests (red cell count, hemoglobin, hematocrit, platelet count, leukocyte count, and differential leukocyte count), biochemistry tests (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ-glutamyl transpeptidase (γ-GTP), total bilirubin, cholesterol, triglycerides, amylase, glucose, hemoglobin A_{1c} (HbA_{1c}), β_2-microglobulin, blood urea nitrogen (BUN), serum creatinine, uric acid, and serum electrolytes [Na, K, Cl, Ca, and Mg]), urinalysis (tests for protein, glucose, urobilinogen, and *N*-acetyl-β-D-glucosamidase (NAG)), and electrocardiography at baseline, after 2 and 4 weeks of treatment, at 4-week intervals thereafter (12-week intervals for electrocardiography), and at the end of treatment.

The blood level of tacrolimus was measured after 2 and 4 weeks of treatment, at 4-week intervals thereafter, and at the end of treatment by high performance liquid chromatography-tandem mass spectrometry using whole blood samples collected at 12 ± 4 h after drug administration. All adverse events were examined and those for which a causal relationship to tacrolimus could not be ruled out by the investigator were classified as adverse drug reactions. Adverse events other than laboratory abnormalities and infections were classified as symptomatic events to distinguish these from the other events.

Efficacy evaluation

Disease activity was assessed by determining the tender joint count (based on evaluation of 48 joints, including temporomandibular ($n = 2$), sternoclavicular ($n = 2$), shoulder ($n = 2$), elbow ($n = 2$), wrist ($n = 2$), metacarpophalangeal ($n = 10$), interphalangeal of thumb ($n = 2$), proximal interphalangeal ($n = 8$), hip ($n = 2$), knee ($n = 2$), ankle mortise ($n = 2$), ankle tarsal ($n = 2$), and ten complete sets of toe joints (each set was counted as one joint)) and the swollen joint count (based on evaluation of 46 joints, excluding the hips from those assessed for tenderness), with modification of the number of joints recommended by ACR [13], the C-reactive protein level, and the 1-h erythrocyte sedimentation rate (Westergren method) at baseline, at 4-week intervals thereafter, and at the end of treatment. At the same times, global assessment of disease activity by the investigator, global assessment of disease activity by the patient, and assessment of joint tenderness by the patient were also performed. At the end of treatment, clinical improvement was assessed according to both ACR response criteria (ACR20%/50%/70% responses) [14].

Statistical analysis

For the ACR20, ACR50, and ACR70 responses, last observation carried forward (LOCF) analysis was performed using the last observation recorded while patients were receiving the study drug. LOCF analysis was also used for DAS28. Wilcoxon signed rank test was used for comparison between baseline values and those at each time point. Statistical tests were two sided and $p < 0.05$ was taken as statistically significant unless otherwise specified.

Results

Patient characteristics

Among 204 patients who were treated during the preceding study, 103 were assigned to receive tacrolimus and 101 were given mizoribine. Tacrolimus was discontinued by 39 patients and mizoribine was discontinued by 68 patients. The main reasons for discontinuation of treatment were adverse events (12 patients in the tacrolimus group and 10 patients in the mizoribine group) and no response or worsening of RA (19 patients in the tacrolimus group and 52 patients in the mizoribine group) [10].

A total of 115 patients were enrolled in the present study. Among them, 65 patients had already been treated with tacrolimus in the preceding study and 50 patients had been treated with mizoribine. All of the patients received tacrolimus therapy in this study. There was an off-treatment interval for each patient ranging from 13 to 307 days. During this interval, the patients received other therapy for RA that did not include tacrolimus.

Treatment with tacrolimus for 76 weeks was completed by 67.8% of the subjects (78/115 patients), while tacrolimus was discontinued in 32.2% of them (37/115 patients) (Fig. 1). All 115 patients were included in the safety and efficacy analyses. The main reasons for discontinuation of treatment were adverse events in 15.7% (18/115 patients) and lack of improvement/worsening of RA in 13.0% (15/115 patients). Discontinuation due to an increase of creatinine occurred in 3.5% (4/115) of all patients. The mean age (±standard deviation) of the patients was 49.8±8.7 years at enrollment in the preceding study, and 66.1% (76/115 patients) had stage III or IV RA at that time (Table 1).

Treatment period and dose of tacrolimus

The median duration of treatment in the present study (including any period of suspension during the study) was 532.0 days. A dose of 3 mg/day was received by 91.0% (91/100) of the patients remaining on treatment after 6 months (28 weeks), 83.0% (73/88) of the remaining patients after 1 year (52 weeks), and 80.8% (63/78) of the remaining patients at completion (76 weeks), showing a gradual decrease. Thus, most of the on-study patients were receiving 3 mg/day during the study period, and most of the other patients were receiving 2.0 or 2.5 mg/day.

Safety

The incidence of adverse events was 88.7% (102/115 patients) and that of adverse events with a possible relationship to tacrolimus, defined as adverse drug reactions, was 58.3% (67/115 patients). During the study period, no deaths occurred. Serious adverse events occurred in ten patients, including 12 serious adverse drug reactions in six patients (diverticulitis of the colon, cerebral infarction, bronchopneumonia, multiple gastric ulcers, perforated duodenal ulcer, acute renal failure, pyelonephritis,

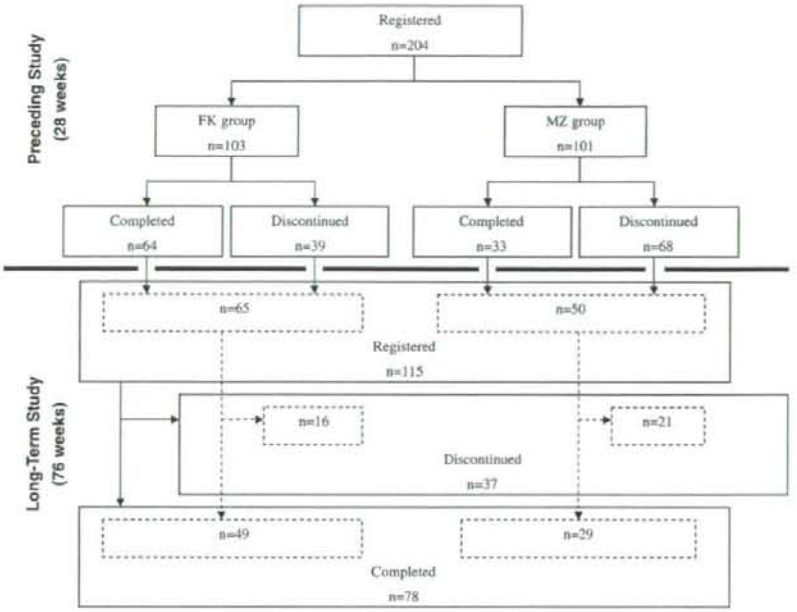

Fig. 1 Disposition of the patients. Patients enrolled in the preceding study were randomized to receive tacrolimus (*FK*) or mizoribine (*MZ*). Patients who completed 28 weeks of treatment with *FK* or *MZ*, or else discontinued therapy due to lack of symptomatic improvement despite at least 12 weeks of treatment, and who were judged to be eligible for long-term treatment with tacrolimus were enrolled in the present study. *n* total number of patients in each group, *FK* tacrolimus; *MZ* mizoribine

Table 1 Characteristics of the patients at enrollment in the preceding study

No. of patients analyzed	FK→FK, $n = 65$	MZ→FK, $n = 50$	Total, $n = 115$
Sex (no., female (%))	58 (89.2)	46 (92.0)	104 (90.4)
Age (years, mean ± SD)	49.6 ± 8.9	50.1 ± 8.5	49.8 ± 8.7
Body weight (kg, mean ± SD)	53.1 ± 7.2	53.9 ± 8.1	53.4 ± 7.6
Steinbrocker stage (no. (%))			
I	7 (10.8)	3 (6.0)	10 (8.7)
II	19 (29.2)	10 (20.0)	29 (25.2)
III	19 (29.2)	15 (30.0)	34 (29.6)
IV	20 (30.8)	22 (44.0)	42 (36.5)
Steinbrocker class (no. (%))			
1	10 (15.4)	5 (10.0)	15 (13.0)
2	48 (73.8)	35 (70.0)	83 (72.2)
3	7 (10.8)	10 (20.0)	17 (14.8)
4	0 (0.0)	0 (0.0)	0 (0.0)
Duration of RA (months, mean ± SD)	108.6 ± 98.3	131.4 ± 103.9	118.5 ± 100.9
Tender joint count (mean ± SD)	13.6 ± 7.3	13.6 ± 7.1	13.6 ± 7.2
Swollen joint count (mean ± SD)	10.5 ± 6.8	9.5 ± 4.3	10.1 ± 5.8
Erythrocyte sedimentation rate (mm/h, mean ± SD)	63.0 ± 28.5	57.0 ± 25.1	60.4 ± 27.1
C-reactive protein level (mg/dL, mean ± SD)	3.46 ± 2.85	3.75 ± 2.44	3.58 ± 2.67

proteinuria, increased creatinine, increased BUN, increased uric acid, and increased β_2-microglobulin). Cerebral infarction resulted in mildly remaining neurological deficits, but the other events resolved.

Symptomatic events occurred in 85 patients (73.9%), and were defined as adverse drug reactions in 39 patients (33.9%). The major reactions were hypertension, stomach ache, gastric ulcer, diarrhea, nausea, stomach discomfort, gastritis, pollakiuria, and alopecia, so there was a predominance of gastrointestinal disorders (Table 2). Most of these adverse reactions resolved or were alleviated. Treatment with tacrolimus was continued (including dose

Table 2 Symptomatic events

No. of patients analyzed	115
Adverse events: no. of patients (%); no. of events	85 patients (73.9%); 211 events
Adverse drug reactions: no. of patients (%); no. of events	39 patients (33.9%); 60 events
Withdrawals due to adverse drug reactions (%)	6 patients (5.2%)
Adverse drug reactions stratified by body system	**No. of patients (%)**
Central and peripheral nervous system disorders	
Tremor	1 (0.9)
Numbness of lips	1 (0.9)
Migraine	1 (0.9)
Dizziness	1[a] (0.9)
Respiratory system disorders	
Rhinitis (allergic)	1 (0.9)
Cardiovascular disorders (general)	
ST segment depression	1[a] (0.9)
Cardiomegaly	1 (0.9)
Increased blood pressure	1 (0.9)
Hypertension	4[a] (3.5)
Aggravated hypertension	1 (0.9)
Heart rate and rhythm disorders	
Extrasystoles	1 (0.9)
Vascular (extracardiac) disorders	
Cerebral infarction	1[a] (0.9)
Gastrointestinal system disorders	
Gingivitis	1 (0.9)
Stomatitis	1 (0.9)
Oesophagitis	1 (0.9)
Anorexia	1 (0.9)
Heartburn	1 (0.9)
Retching	1 (0.9)
Nausea	2 (1.7)
Vomiting	1 (0.9)
Stomach heaviness	1 (0.9)
Stomach discomfort	2 (1.7)
Stomach ache	3 (2.6)
Stomach pain	1 (0.9)
Gastritis	2 (1.7)
Gastric ulcer	3 (2.6)
Duodenal ulcer	1 (0.9)
Duodenal ulcer (perforated)	1[a] (0.9)
Diarrhea	3 (2.6)
Irritable bowel syndrome	1 (0.9)
Metabolic and nutritional disorders	
Abnormal glucose tolerance	1 (0.9)
Urinary system disorders	
Pollakiuria	2 (1.7)
Renal failure (acute)	1[a] (0.9)
Reproductive disorders (female)	
Menstrual irregularity	1 (0.9)
Visual disorders	
Conjunctivitis (allergic)	1 (0.9)
Disorders of other special senses	
Dysosmia	1 (0.9)
Skin and appendageal disorders	
Pruritus	1 (0.9)
Pruritus cutaneous	1 (0.9)
Urticaria	1 (0.9)
Erythema	1 (0.9)
Alopecia	2 (1.7)
Body as a whole-general disorders	
Hot flushes	1 (0.9)
Fever	1 (0.9)
Oedema of lower extremities	1 (0.9)
Fatigability	1 (0.9)
Weakness	1 (0.9)

[a] Tacrolimus treatment was discontinued in these patients

reduction or retreatment after withdrawal) in seven patients with persistent adverse reactions (migraine, aggravated hypertension, gastric ulcer, abnormal glucose tolerance, menstrual irregularity, dysosmia, and alopecia). Treatment was discontinued due to adverse drug reactions in six patients (dizziness, ST segment depression, hypertension, cerebral infarction, perforated duodenal ulcer, and acute renal failure), and these abnormalities resolved or were alleviated after discontinuation.

Abnormal changes in laboratory values were observed in 47 patients (40.9%), and were classified as adverse drug reactions in 38 patients (33.0%). The major reactions were increases of creatinine, urinary NAG, HbA_{1c}, BUN, and ALP, as well as a decrease of magnesium, showing a predominance of abnormal renal function and abnormal glucose tolerance (Table 3). Most of these adverse reactions resolved. Tacrolimus treatment was continued (including dose reduction) in most of eight patients with 18 persistent events [increased ALP (4), increased γGTP (2), increased uric acid (2), increased triglycerides, increased HbA_{1c}, increased total bilirubin, increased creatinine, increased BUN, increased urinary NAG, increased red cell count, increased hemoglobin, increased hematocrit, and decreased magnesium]. Treatment was discontinued in seven patients with ten events [increased creatinine (4), increased glucose (2), increased HbA_{1c} (2), increased

Table 3 Abnormal laboratory findings

No. of patients analyzed	115
Adverse events: no. of patients (%); no. of events	47 patients (40.9%); 103 events
Adverse drug reactions: no. of patients (%); no. of events	38 patients (33.0%); 78 events
Withdrawals due to adverse drug reactions (%)	7 patients (6.1%)

Adverse drug reactions	No. of patients (%)
Red cell count increased	1 (0.9)
Hemoglobin increased	1 (0.9)
Hemoglobin decreased	1 (0.9)
Hematocrit increased	1 (0.9)
Leukocytosis	1 (0.9)
Neutrophilia	1 (0.9)
Lymphopenia	1 (0.9)
Alkaline phosphatase increased	6 (5.2)
γ-glutamyl transpeptidase increased	4 (3.5)
Total bilirubin increased	1 (0.9)
Creatinine increased	14[1–4] (12.2)
Blood urea nitrogen increased	7[5] (6.1)
Uric acid increased	3 (2.6)
β_2-microglobulin increased	2 (1.7)
Amylase increased	1 (0.9)
Blood sugar increased	3[6,7] (2.6)
Hemoglobin A_{1C} increased	8[6,7] (7.0)
Triglycerides increased	2 (1.7)
Potassium increased	1 (0.9)
Magnesium decreased	6 (5.2)
Urinary sugar	2[6] (1.7)
Urinary protein	2 (1.7)
Urinary *N*-acetyl-β-D-glucosamidase increased	9 (7.8)

[1–7] Tacrolimus treatment was discontinued in these patients (reactions bearing the same number occurred in the same individual)

Table 4 Infections

No. of patients analyzed	115
Adverse events: no. of patients (%); no. of events	70 patients (60.9%); 105 events
Adverse drug reactions: no. of patients (%); no. of events	19 patients (16.5%); 23 events
Withdrawals due to adverse drug reactions (%)	5 patients (4.3%)

Adverse drug reactions	No. of patients (%)
Sinusitis	1 (0.9)
Sore throat	1 (0.9)
Upper respiratory tract infection	1 (0.9)
Common cold syndrome	6 (5.2)
Coughing	1 (0.9)
Bronchitis	1[a] (0.9)
Bronchopneumonia	1[a] (0.9)
Pneumonia	2[a] (1.7)
Candida esophagitis	2 (1.7)
Diverticulitis	1[a] (0.9)
Pyelonephritis	1[a] (0.9)
Tinea	1 (0.9)
Rash (acneiform)	1 (0.9)
Dermatitis	1 (0.9)
Herpes zoster	1 (0.9)
Purulence	1 (0.9)

[a] Tacrolimus treatment was discontinued in these patients

BUN, or urinary sugar], and these laboratory abnormalities all resolved or improved after discontinuation.

Infections occurred in 70 patients (60.9%), and were classified as adverse drug reactions in 19 patients (16.5%). The major infections considered to be adverse drug reactions were common cold syndrome, pneumonia, and Candida esophagitis (Table 4). All of these adverse infections resolved or were alleviated, but treatment was discontinued due to infections (bronchitis, bronchopneumonia, pneumonia, diverticulitis, and pyelonephritis) in five patients.

Blood level of tacrolimus

The mean whole blood concentration of tacrolimus was 3.8–4.8 ng/mL, with a median value of 3.6–4.5 ng/mL. One patient had a blood level greater than 20 ng/mL (21.19 ng/mL at 14 h and 40 min after taking 3 mg of tacrolimus on study day 449), but this patient's blood levels ranged between 4.67 and 7.12 ng/mL at the other times of assessment (8–16 h after administration) and no adverse drug reactions occurred throughout the study.

Efficacy

Compared with their status at baseline of the preceding study, the patients who received tacrolimus during both studies (FK→FK group) had an ACR20 response rate (number of responders/number of patients evaluated) of 64.6% (42/65) and 61.5% (40/65), respectively, at the end of the preceding and present studies, and the two response rates were similar. The corresponding ACR20 response rates of the patients who received mizoribine during the preceding study (MZ→FK group) were 12.0% (6/50) and 66.0% (33/50), respectively, with a better response at the end of the present study. Changes of the ACR50 and 70 response rates in the two groups were similar to those of the ACR20 (Fig. 2).

The median DAS28 value showed a significant decrease ($p < 0.001$) throughout the study from 8 weeks onward in the FK→FK group and from 4 weeks onward in the