throughout. In the etanercept group, 60 patients were switched to either infliximab (27 patients), tocilizumab (25 patients), or adalimumab (8 patients), and 62 patients stopped administration of etanercept during the study observation period. The remaining patients continued etanercept treatment throughout. The overall survival rates of the first biologic agent at year 3 were 0.48 (95% confidence interval [95% CI] 0.41–0.55) for infliximab and 0.61 (95% CI 0.55–0.66) for etanercept. Our analysis was restricted to infliximab or etanercept because few patients receiving adalimumab or tocilizumab were registered in the REAL database and golimumab and certolizumab pegol were not approved in Japan at the time this study was conducted.

Unexposed group. Among 574 RA patients in the biologics unexposed group, 3 patients had received biologic DMARDs within 90 days before their enrollment in the REAL. These 3 patients were excluded from our analysis in consideration of the pharmacokinetic and pharmacodynamic property of biologic DMARDs and their possible effects on development of infection. Fifteen patients who had received biologic DMARDs and stopped them over 90 days before their enrollment in the REAL were included in this analysis. Therefore, 571 RA patients who initiated or were receiving nonbiologic DMARDs and not receiving biologic DMARDs at enrollment in the REAL were included in the unexposed group (1,104.1 PY). At enrollment, 347 patients (60.8%) of the patients in the unexposed group were being treated with MTX, 127 patients (22.4%) with sulfasalazine, 103 patients (18.0%) with tacrolimus, 95 patients (16.6%) with bucillamine, and 29 patients (5%) with other nonbiologic DMARDs.

Followup. For those patients who initiated nonbiologic DMARDs or biologic DMARDs at entry, the start of the observation period was the date these agents were first administered. For those patients enrolled in the unexposed group already receiving treatment with nonbiologic DMARDS at the time of study entry, the observation period started from the date of their enrollment in the REAL database.

Observation was stopped either 3 years after the start of the observation period, the day a patient died or met the exclusion criteria (14), or on November 30, 2009, whichever came first. For the unexposed group, stopping all nonbiologic DMARDs or starting any biologic DMARDs stopped followup. For the anti-TNF group, stopping therapy with either infliximab or etanercept ended observation. Patients were followed even after development of SAEs, as long as they did not meet the above criteria for stopping observation. The date of the last administration of infliximab or etanercept was retrieved from medical records and reported by the participating physicians. The mean ± SE followup was 2.04 ± 0.92 years for the anti-TNF group and 1.93 ± 0.99 years for the unexposed group. Figure 1 shows the number of patients for each year and the number who dropped out from each group during observation. Four hundred forty-two patients (34%) of all patients (n = 1,298) were followed up for 3 years.

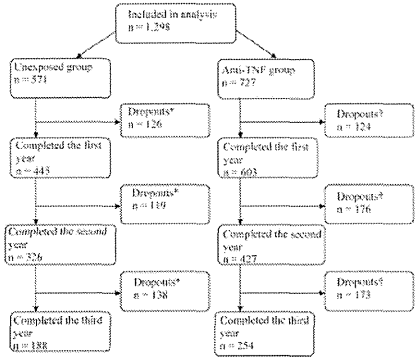

Figure 1. Distribution of numbers of patients with rheumatoid arthritis during the 3-year observation period. * = dropouts from the unexposed group include patients who started biologic disease-modifying antirheumatic drugs (DMARDs) or patients whose observation did not complete the next 1 year; † = dropouts from the anti–tumor necrosis factor (anti-TNF) group include patients who stopped infliximab or etanercept or switched to biologic DMARDs, except infliximab and etanercept, or patients whose observation did not complete the next 1 year.

Definition of SAEs. Our definition of an SAE, including an SI, was based on the report by the International Conference on Harmonisation (30). In addition, bacterial infections that required intravenous administration of antibiotics, as well as opportunistic infections, were also regarded as SAEs (14) (see Supplementary Table 1, available in the online version of this article at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)2151-4658).

Statistical analysis. Crude IRs per 100 PY and crude IR ratios (IRRs) with their 95% CIs were calculated. We conducted 2 analyses in this study. In the primary analysis (analysis 1), risk factors for SIs during continuous treatment with infliximab or etanercept for up to 3 years were identified. We also calculated the risk of TNF antagonists for SIs in the first year and in the second and third years combined to investigate time dependence of the risk. In the secondary analysis (analysis 2), the risks for SIs were compared between treatment with infliximab and etanercept.

Analysis 1. We included both patient groups and the entire observation period for each patient as described above for analysis 1 and added risk windows as follows. When a patient no longer received either infliximab or etanercept, the patient was excluded from the study on the day of the last administration of the agents and a 90-day postdiscontinuation risk window was applied (14). Any SAEs occurring within the risk window were attributed to the effects of the TNF antagonists. No risk window was needed for the unexposed group. For multivariate analysis, Poisson regression models were employed to estimate

Table 1. Comparison of RA patients treated with or without TNF antagonists infliximab or etanercept at the start of the observation period*

	Unexposed group (n = 571)	All anti-TNF groups (n = 727)	Infliximab group (n = 335)	Etanercept group (n = 392)	P†
Age, years	59.3 ± 13.1	56.3 ± 13.4‡	53.7 ± 13.9	58.5 ± 12.7	< 0.001
Women, %	83.2	82.0	79.3	85.1	0.045
Disease duration, years	8.9 ± 9.3	9.5 ± 8.6‡	8.1 ± 8.0	10.6 ± 9.0	< 0.001
Steinbrocker class 3 or 4, %§	10.7	30.7‡	28.4	32.7	0.211
Steinbrocker stage III or IV, %§	39.6	53.0‡	45.1	59.7	< 0.001
DAS28-CRP	3.4 ± 1.2	4.5 ± 1.2‡	4.6 ± 1.1	4.5 ± 1.3	0.197
N	567	723	335	388	
MTX use, %	60.8	68.8‡	99.1	42.9	< 0.001
MTX dosage, mg/week	6.4 ± 2.0	7.6 ± 2.2‡	7.9 ± 2.2	7.0 ± 2.1	< 0.001
MTX dosage >8 mg/week, %	4.4	10.6‡	18.2	4.1	< 0.001
Use of immunosuppressive drugs except for MTX, %¶	20.1	4.3‡	1.2	6.9	< 0.001
Oral corticosteroid use, %	58.3	71.5‡	69.0	73.7	0.16
Prednisolone or equivalent dosage of corticosteroids, mg/day	4.6 ± 2.1	5.7 ± 3.0‡	5.3 ± 2.7	6.0 ± 3.2	0.006
Prednisolone or equivalent dosage of corticosteroids ≥10 mg/day, %	1.9	9.1‡	5.7	12.0	0.003
No. of previous DMARDs	2.2 ± 1.2	2.5 ± 1.2‡	2.3 ± 1.1	2.7 ± 1.2	< 0.001
Chronic pulmonary disease, %#	18.7	21.6	11.9	29.8	< 0.001
Diabetes mellitus, %	5.8	12.0‡	8.7	14.8	0.011

* Values are the mean ± SD unless otherwise indicated. For univariate analysis, the chi-square test for categorical variables and the Student's *t*-test or Mann-Whitney test were used to compare continuous variables among groups. RA = rheumatoid arthritis; TNF = tumor necrosis factor; DAS28-CRP = 3-variable Disease Activity Score including 28-joint counts using the C-reactive protein level; MTX = methotrexate; DMARDs = disease-modifying antirheumatic drugs.
† Between the 2 anti-TNF antagonists.
‡ $P < 0.05$ versus the unexposed group.
§ Steinbrocker classification (28) was used to define RA disease stages and classes.
¶ Immunosuppressive drugs include tacrolimus, leflunomide, mizoribine, and cyclosporine.
# Chronic pulmonary diseases include interstitial pneumonia, chronic obstructive pulmonary disease, bronchial asthma, prior pulmonary tuberculosis, and bronchiectasis.

the risk for SIs with TNF antagonist treatment. To analyze the time-dependent risk for SIs, observation periods were divided into the first year and the second and third years combined.

Analysis 2. To compare the risk for SIs between the use of infliximab and etanercept in the anti-TNF group, the treatment period with the first TNF inhibitor for each patient was evaluated without setting a risk window because most of the patients who had stopped the first biologic agent started treatment with the second one immediately. We applied propensity score (PS) methodology to calculate the likelihood of being treated with TNF antagonists. First, we made a multivariate logistic regression model with the use of TNF antagonists as the dependent variable and the following as independent variables: age, sex, the 3-variable Disease Activity Score including 28-joint counts using the C-reactive protein level (DAS28-CRP), the presence of chronic pulmonary comorbidity, diabetes mellitus, calendar year of entry in the REAL, Steinbrocker stage (III or IV), MTX (≤8 or >8 mg/week), and oral corticosteroid (prednisolone or equivalent dosage <10 or ≥10 mg/day) at enrollment. We applied the Hosmer-Lemeshow goodness-of-fit test to assess how effectively the model described the outcome variable (i.e., the use of TNF antagonist: yes/no). We used the PS to select representative patients receiving TNF antagonist treatment: the patients with a PS >0.4 were included in analysis 2 and different cutoff values for PS were used for sensitivity analyses (31). To compare the risk for SIs between etanercept and infliximab, we employed Poisson regression models in the anti-TNF group patients with various combinations of adjusting factors, including the PS, to calculate the relative risks (RRs) of etanercept with 95% CIs, using infliximab as the reference.

These statistical analyses were conducted using SPSS, version 16.0, and R statistical language software, version 2.8.1. All *P* values were 2-tailed and *P* values less than 0.05 were considered statistically significant.

RESULTS

Baseline characteristics of patients. This study included a total of 1,298 patients: 727 in the anti-TNF group and 571 in the unexposed group. Baseline data for the patients are shown in Table 1. Compared to the unexposed group, the anti-TNF group was younger ($P < 0.001$), had more severe disease activity ($P < 0.001$), and was treated with higher doses of MTX ($P < 0.001$) and oral corticosteroids ($P < 0.001$). Significantly more patients with diabe-

Table 2. Number and IRs of SAEs in RA patients treated with and without the TNF antagonists infliximab or etanercept*

	Unexposed group (*n* = 571)	Anti-TNF group			Etanercept vs. infliximab, crude IRR (95% CI)	Anti-TNF vs. unexposed group, crude IRR (95% CI)
		All (*n* = 727)†	Infliximab (*n* = 335)‡	Etanercept (*n* = 392)§		
Patient-years (PY)	1,104.1	1,480.1	583.31	787.94		
All SAEs						
No. of events	95	213	61	123	1.49 (1.10–2.03)	1.67 (1.31–2.13)
IR/100 PY (95% CI)	8.60 (7.00–10.47)	14.39 (12.55–16.42)	10.46 (8.07–13.34)	15.61 (13.03–18.56)		
Serious infection						
No. of events	30	82	28	44	1.16 (0.72–1.87)	2.04 (1.34–3.10)
IR/100 PY (95% CI)	2.72 (1.87–3.83)	5.54 (4.44–6.84)	4.80 (3.26–6.84)	5.58 (4.11–7.42)		
Serious respiratory tract infection						
No. of events	17	42	16	26	1.20 (0.65–2.24)	1.96 (1.10–3.48)
IR/100 PY (95% CI)	1.45 (0.86–2.30)	2.84 (2.07–3.80)	2.74 (1.63–4.35)	3.30 (2.21–4.76)		
Serious infection leading to death					NA	0.75 (0.15–3.69)
No. of events	3	3	0	3		
IR/100 PY (95% CI)	0.27 (0.08–0.72)	0.20 (0.06–0.54)	0	0.38 (0.11–1.02)		

* Note that the number of severe adverse events (SAEs) in the All column is not the sum of the Infliximab and Etanercept columns. IRs = incidence rates; TNF = tumor necrosis factor; IRR = IR ratio; 95% CI = 95% confidence interval; NA = not applicable.
† The continuous treatment period with infliximab or etanercept for each patient was evaluated.
‡ Patients with rheumatoid arthritis (RA) given infliximab as the first TNF inhibitor in the Registry of Japanese RA Patients for Long-Term Safety (REAL) were included. The treatment period with infliximab for each patient was evaluated.
§ Patients with RA given etanercept as the first TNF inhibitor in the REAL were included. The treatment period with etanercept for each patient was evaluated.

tes mellitus ($P < 0.001$) were seen in the anti-TNF group compared to the unexposed group. In the anti-TNF group, the etanercept group compared to the infliximab group was older ($P < 0.001$), had a longer disease duration ($P < 0.001$), used MTX less frequently ($P < 0.001$), was treated with higher doses of oral corticosteroids ($P = 0.006$), and had higher percentages of chronic pulmonary comorbidity ($P < 0.001$) (see Table 1 for definition) and diabetes mellitus ($P = 0.011$) (Table 1).

Types and occurrence of SAEs. Among the 1,298 patients, 308 SAEs were reported during the observation period, 95 in the unexposed group and 213 in the anti-TNF group. The crude IRR comparing the anti-TNF group with the unexposed group for SAEs was 1.67 (95% CI 1.31–2.13) and for SIs was 2.04 (95% CI 1.34–3.10); both of these IRRs were significantly elevated. The IRs of SAEs, SIs, and serious respiratory tract infections in the infliximab group and the etanercept group are shown in Table 2. The crude IRR comparing the infliximab group with the etanercept group for SAEs was 1.49 (95% CI 1.10–2.03) and for SIs was 1.16 (95% CI 0.72–1.87). The IRs of SAEs, SIs, serious respiratory tract infections, and SIs leading to death are summarized in Table 2.

In the anti-TNF group, there were 82 SIs, including 21 opportunistic (14 cases of herpes zoster, 4 PCP, 3 pulmonary cryptococcosis, and 1 pulmonary nontuberculous mycobacterial infection) and 61 other infections. In the unexposed group, 30 SIs occurred, including 12 opportunistic (4 cases of herpes zoster, 3 PCP, 2 pulmonary tuberculosis, and 3 pulmonary nontuberculous mycobacterial infections) and 18 other infections. The names of the SIs in each site of infection are listed in Table 3. The respiratory system was the most frequent site of infection (n = 59), followed by skin and subcutaneous tissue (n = 24), gastrointestinal (n = 6), urinary tract (n = 5), and bone and joints (n = 5). Four of the latter 5 patients had histories of joint surgery. Three patients in each group died from SIs.

Continuous treatment with TNF antagonists and other risk factors contributing to the development of SIs (analysis 1). We initially performed univariate analyses to compare patients who did and did not develop SIs (data not shown) and selected the following variables for multivariate analysis: age, sex, chronic pulmonary comorbidity, diabetes mellitus, disease duration, calendar year, the number of previous DMARDs, Steinbrocker class, the use of immunosuppressive drugs, mean DAS28-CRP, and the mean dose of MTX and oral corticosteroids during the observation period. We used Poisson regression models and identified continuous use of TNF inhibitors as an independent risk factor for the development of SIs (RR 1.97, 95% CI 1.25–3.19; $P = 0.0045$) (Table 4). Among the confounding factors, we found that increasing age (RR 1.45 per 10-year increment, 95% CI 1.20–1.77; $P < 0.001$), chronic pulmonary comorbidity (RR 1.77, 95% CI 1.15–2.70; $P = 0.009$), mean DAS28-CRP score (RR 1.33, 95% CI 1.05–1.66; $P = 0.015$), mean dosage of MTX >8 mg/week (RR 2.14, 95% CI 1.15–3.87; $P = 0.013$), and mean dosage of oral prednisolone ≥10 mg/day (RR 2.49, 95% CI 1.08–5.50; $P = 0.027$) were significantly associated with SIs. The

Table 3. Classification of serious infections*

Site and name of infection	No. of infections		No. of deaths	
	Anti-TNF group	Unexposed group	Anti-TNF group	Unexposed group
Pulmonary				
Bacterial pneumonia	27	9	1	2
Fungal pneumonia†	7	3	0	1
Bronchitis	4	0	0	0
Nontuberculous mycobacterial infection	1	3	0	0
Empyema	1	0	0	0
Tuberculosis	0	2	0	0
Aspiration pneumonia	1	0	1	0
Infectious pneumatocele	1	0	0	0
Total	42	17	2	3
Skin				
Herpes zoster	14	4	0	0
Cellulitis	4	2	0	0
Total	18	6	0	0
Gastrointestinal				
Infectious gastroenteritis	3	0	0	0
Acute suppurative cholangitis	1	0	0	0
Appendicitis	1	0	0	0
Infection due to drain replacement‡	0	1	0	0
Total	5	1	0	0
Urinary				
Pyelonephritis	3	1	0	0
Urinary tract infection	1	0	0	0
Total	4	1	0	0
Bone and joints				
Infectious arthritis	3	1	0	0
Osteomyelitis	0	1	0	0
Total	3	2	0	0
Others				
Sepsis	4	1	0	0
Surgical wound infection	0	2	0	0
Bacteremia	1	0	0	0
Bacterial meningitis	1	0	1	0
Sinusitis	1	0	0	0
Viral meningitis	1	0	0	0
Unidentified	2	0	0	0
Total	10	3	1	0

* Anti-TNF = anti–tumor necrosis factor.
† Fungal pneumonia included *Pneumocystis jirovecci* pneumonia and cryptococcal pneumonia.
‡ For the treatment of cholangiocellular carcinoma.

Poisson regression analysis also revealed that the RR of TNF inhibitors in the first year was significantly elevated (RR 2.40, 95% CI 1.20–5.03), but not in the second and third years combined (RR 1.38, 95% CI 0.80–2.43).

Comparison of risk for SIs between infliximab and etanercept (analysis 2). We next investigated possible differences between the TNF inhibitors in their contribution to risk for development of SIs. The PS of each patient was calculated by logistic regression model as described in the Methods. The model fit well; the Hosmer-Lemeshow goodness-of-fit statistics did not show a significant difference between observed and predicted frequencies ($P = 0.164$). The patients with a PS of <0.4 (17.6% of the infliximab group and 20.9% of the etanercept group) were considered not representing those receiving TNF antagonists and we excluded them from the following analysis. We constructed 3 Poison regression models to calculate the RR from the use of etanercept for the development of SIs compared to infliximab. In the first model, we adjusted for age, sex, Steinbrocker class, chronic pulmonary comorbidity, diabetes mellitus, observation period, and the PS. The second model added the mean dosage of MTX (≤8 or >8 mg/week) and the mean dosage of oral corticosteroids (<10 or ≥10 mg prednisolone or equivalent/day) to the adjusting factors in the first model. The third model added the calendar year and the number of previous nonbiologic DMARDs to the adjusting factors in the second

Table 4. Multivariate analysis of independent risk factors for serious infections during continuous use of TNF antagonists in the Registry of Japanese Rheumatoid Arthritis Patients for Long-Term Safety database*

	RR (95% CI)†	P
TNF antagonist (infliximab or etanercept)	1.97 (1.25–3.19)	0.0045
Age by decade	1.45 (1.20–1.77)	< 0.001
Chronic pulmonary disease	1.77 (1.15–2.70)	0.009
Diabetes mellitus	1.20 (0.69–1.97)	0.49
Mean DAS28-CRP (per 1.0 increment)	1.33 (1.05–1.66)	0.015
Mean MTX dosage >8.0 mg/week‡	2.14 (1.15–3.87)	0.013
Mean prednisolone dosage ≥10 mg/day‡	2.49 (1.08–5.50)	0.027

* TNF = tumor necrosis factor; RR = relative risk; 95% CI = 95% confidence interval; DAS28-CRP = 3-variable Disease Activity Score including 28-joint counts using the C-reactive protein level; MTX = methotrexate.
† The RRs of biologic agents for development of serious infection for up to 3 years of the observation period were calculated using the Poisson regression model after adjusting for confounding factors of age, sex, disease duration, chronic pulmonary disease, diabetes mellitus, Steinbrocker class (28), calendar year, number of previous disease-modifying antirheumatic drugs, observation period, disease activity, immunosuppressive drugs, corticosteroid dose, and MTX dose.
‡ Mean dosage during the observation period.

model. The RR for using etanercept compared to infliximab in the first model was 1.28 (95% CI 0.73–2.30, P = 0.41), for the second model was 1.39 (95% CI 0.69–2.76, P = 0.35), and for the third model was 1.32 (95% CI 0.65–2.66, P = 0.44). We performed sensitivity analyses using different cutoffs for PS and observed essentially the same results.

DISCUSSION

This is the first epidemiologic study of patients with RA that uses a prospective cohort from an Asian country to investigate the association of SIs and use of TNF antagonists during 3 years and includes patients that changed to a second agent. In addition, we performed a head-to-head comparison of the risk for SIs between infliximab and etanercept. We demonstrated that the continuous use of TNF antagonists for up to 3 years was an independent risk factor for SIs (RR 1.97, 95% CI 1.25–3.19), but the risk was time dependent. We also revealed that the RR for SIs comparing the etanercept group with the infliximab group after adjusting for covariates was not significantly different.

Studies from European biologics registries analyzed the association of TNF antagonists with infections in patients with RA (32,33). There are some reports indicating that the risk for SIs was not increased by TNF antagonists (21–24), but other studies show significant associations between the use of these agents and development of SIs (14–20,34–36). Several of the latter studies revealed time dependence of the risk for SIs (15,16,18–20,34), which is compatible with our results where the risk for SIs was significantly elevated only in the first year and declined in the second and third years combined. The decrease in risk might be explained in part by the effect of dropout patients who developed SIs and stopped the TNF antagonist (34). Of 68 patients who developed SIs in the anti-TNF group, 22 discontinued the biologic agents. Patients who were not susceptible to SIs were more likely to remain in the cohort, which could contribute to reduced risk with increasing observation period.

Increasing age, presence of chronic pulmonary comorbidity, higher mean DAS28-CRP, mean dosage of MTX >8 mg/week, and mean dosage of oral prednisolone ≥10 mg/day were identified as independent risk factors for SIs in this study. Most previous studies have reported that increasing age, pulmonary comorbidity, and use of oral prednisolone were risk factors for infections (14,21–23,35,36) and for PCP (37) in RA patients treated with TNF antagonists. Conflicting results, however, have been reported regarding the association of disease activity and risk for SIs (23,36). Because disease activity is often improved rapidly and significantly by treatment with biologic agents, including TNF antagonists, it seems reasonable that baseline disease activity may not accurately predict infectious events. Mean disease activity during the observation period may serve as a better predictor, as our study indicates.

In Japan, the data from postmarketing surveillance programs conducted by pharmaceutical companies showed that the IRs of pneumonia, PCP, and tuberculosis occurring during the first 6 months of treatment with infliximab were numerically higher than those of etanercept (11–13). In the present study, however, we show that the risk for SIs of treatment with etanercept during the longer observation period was not significantly different from that of infliximab after adjusting for covariates. Some observational studies directly (23) or indirectly (17,20) compared the risk for SIs between treatment with infliximab and etanercept, and found no statistically significant difference. A recent meta-analysis including randomized controlled trials and their extension studies also supports the results of our study; the odds ratio of etanercept treatment for SIs indirectly compared with infliximab was 0.73 (95% CI 0.46–1.15), which was not statistically significant (38).

There are a number of limitations to our study. First, we have to consider possible selection bias in our study. All of the patients were enrolled from university hospitals or referral hospitals that are dedicated to the treatment of RA. The number of the unexposed group was smaller than that of the anti-TNF group in this study, which did not reflect the real world and may indicate unidentified selection bias. Although we estimated the risk of SIs after adjusting for variables that were clinically important, we had to interpret our data under these conditions. A second limitation is the effect of prevalent users on the analyses. In the exposed group, there were 273 prevalent nonbiologic DMARD users who had already been receiving the nonbiologic DMARDs at enrollment in the REAL database, and the rest were incident nonbiologic DMARD users. Inclusion of these prevalent nonbiologic DMARD users in our cohort might lead to the underestimation of the incidence of SIs. However, the majority of these patients started new nonbiologic DMARDs or underwent dose escalations of nonbiologic DMARDs during the observation period (data not shown), reducing the degree of underestimation. Third, the mean observation periods for both groups were approximately 2 years; it is possible that we underestimated the rate of SIs in the third year. Fourth, the mean dose of MTX of our database is lower than those of Western cohorts. In Japan, the maximum approved dosage of MTX for RA has been increased since February 2011 and Japanese rheumatologists can now officially prescribe MTX up to 16 mg/week for patients with RA. Therefore, in the future, we will be able to conduct further studies to examine the risk of TNF antagonists in patients receiving a higher dose of MTX.

In conclusion, we have shown that the continuous use of TNF therapy for up to 3 years in Japanese patients with RA, including cases where a clinical switch to a second TNF antagonist was employed, time dependently increased the risk for SIs compared to treatment with nonbiologic conventional DMARDs. A comparison of actual long-term safety among different classes of biologic DMARDs using registry data will be necessary for choosing the appropriate treatment of RA and needs to be performed.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Harigai had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Acquisition of data. Komano, Michi Tanaka, Nanki, Ryuji Koike, Nagasawa, Amano, Nakajima, Atsumi, Takao Koike, Ihata, Ishigatsubo, Saito, Yoshiya Tanaka, Ito, Sumida, Tohma, Tamura, Fujii, Sugihara, Kawakami, Hagino, Ueki, Hashiramoto, Nagasaka, Miyasaka, Harigai.

Analysis and interpretation of data. Sakai, Komano, Michi Tanaka, Nanki, Ryuji Koike, Miyasaka, Harigai.

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APPENDIX A: MEMBERS OF THE REAL STUDY GROUP AND THEIR AFFILIATES

Members of the Registry of Japanese Rheumatoid Arthritis Patients for Long-Term Safety (REAL) Study Group and their affiliates who contributed to this work were as follows: Hideto Kameda (Saitama Medical University), Shinsuke Yasuda (Hokkaido University), Mitsuhiro Takeno (Yokohama City University), Shintaro Hirata (University of Occupational and Environmental Health), Taichi Hayashi (University of Tsukuba), Yoshinari Takasaki (Juntendo University), Tsuneyo Mimori (Kyoto University), Hiroaki Ida, Katsumi Eguchi (Nagasaki University), Kazuhiko Yamamoto (University of Tokyo), Shunichi Shiozawa, Yasushi Miura (Kobe University), Tetsuji Sawada (Tokyo Medical University Hospital), Hiroaki Dobashi (Kagawa University Hospital), Sae Ochi (Tokyo Metropolitan Bokutoh Hospital), Ayako Nakajima, Hisashi Yamanaka (Tokyo Women's Medical University), Kiyoshi Migita (National Hospital Organization Nagasaki Medical Center), and Hayato Yamazaki, Kaori Watanabe (Tokyo Medical and Dental University).

The following university and hospitals are also members of the REAL Study Group, but were not involved in the present study: Keio University, Kurashiki Kohsai Hospital, Tokyo Kyosai Hospital, and Yokohama City Minato Red Cross Hospital.

Successful Treatment of Refractory Takayasu Arteritis with Tacrolimus

To the Editor:

Takayasu arteritis (TA) is a systemic vasculitis that affects large-size vessels such as the aorta and/or its main branches. Persistent inflammation of TA leads to segmental stenosis, occlusion, dilatation, and/or aneurysm formation. TA is also accompanied by somatic symptoms, including fever, fatigue, and weight loss, and elevation of acute-phase reactants such as C-reactive protein (CRP) that correlate with disease activity. Although high-dose corticosteroid (CS) therapy is effective in TA, CS alone does not provide sustained remission in about half of patients[1]. CS-resistant patients with TA have been treated with immunosuppressants, including methotrexate (MTX) and azathioprine[1,2]. Anti-tumor necrosis factor (anti-TNF) therapy[3,4] and anti-interleukin 6 receptor antibody[5,6] are also promising treatments for TA. However, the new therapies for CS-resistant TA have yet to be standardized. We describe a patient with TA who was successfully treated with tacrolimus, a calcineurin inhibitor, after failed trial of conventional CS, MTX, and infliximab (IFX). This case suggests that tacrolimus is another potential alternative treatment option for refractory TA.

A 22-year-old woman was admitted to our hospital in August 2007 for persistent fever and neck pain. Examination showed tenderness around both carotid arteries, different blood pressures in the left and right upper extremities (right 109/54, left 90/47 mm Hg), weak left radial pulse, and murmur on the common carotid arteries and abdominal aorta. Blood analysis showed elevated CRP (10.3 mg/dl). Neck and chest magnetic resonance imaging (MRI) showed wall thickening and stenosis of the left common carotid artery and bilateral brachiocephalic arteries (Figure 1A, 1B). Based on these findings, she was diagnosed with TA. Treatment with 30 mg/day (0.6 mg/kg/day) prednisolone (PSL) relieved the symptoms and reduced CRP to within the normal range (< 0.05 mg/dl). The PSL dose was subsequently reduced to 10 mg/day.

However, in April 2008, the patient developed fever and tenderness at the same location around carotid arteries, with high CRP (1.57 mg/dl), suggesting relapse of TA. Accordingly, the dose of PSL was escalated to 30 mg/day and combined with MTX. The combination treatment induced remission of TA again. Nevertheless, the disease activity became exacerbated again after reduction of PSL dose to 12.5 mg/day despite continuation of MTX at 12.5 mg/week. IFX was added to the treatment in March 2009 (3 mg/kg at weeks 0, 2, and 6 and then at 8-week intervals). Although the dose of IFX was subsequently increased to 8.5 mg/kg and the administration interval was shortened to every 6 weeks, neck tenderness did not disappear and CRP levels remained positive (0.40–0.97 mg/dl), suggesting the arteritis was still active. Consequently, 3 mg/day tacrolimus was added in February 2010 and the dosage was increased to 4 mg/day to maintain an adequate serum trough level (~5 ng/ml). The CRP level decreased to normal range 5 weeks after start of tacrolimus, and remained within the normal range even after discontinuation of IFX in June 2010. The dose of PSL was subsequently tapered to 6 mg/day. MRI in February 2011 showed disappearance of the vascular wall thickening and absence of new stenosis of arteries (Figure 1C, 1D).

There is only limited evidence for the efficacy of immunosuppressants or biologics for CS-resistant TA[1,2]. Molloy, *et al*[4] reported an uncontrolled series of 25 patients with TA who received anti-TNF therapy; remission with discontinuation of PSL was achieved in 15 patients (60%) and remission with PSL ≤ 10 mg/day in an additional 7 patients (28%). Three patients did not respond to anti-TNF therapy, similar to our patient.

A thorough search of the PubMed database revealed that this is the first case of TA treated successfully with tacrolimus in spite of failure of anti-TNF therapy. In addition, we identified 2 patients with TA who improved significantly with calcineurin inhibitors. In the first patient, tacrolimus was effective in a patient resistant to CS and intolerant to MTX[7]. Cyclosporine was effective in another patient with CS- and MTX-resistant TA[8]. Cyclosporine is another calcineurin inhibitor that shares similar immunosuppressive action with tacrolimus. Studies in the field of organ transplantation suggest that tacrolimus is more efficacious than cyclosporine[9]. Therefore, for treatment of TA, tacrolimus may also be more useful, although there is not enough evidence to date to compare the efficacy between tacrolimus and cyclosporine.

Our case suggests that tacrolimus is a potential alternative therapy for patients with CS-resistant or anti-TNF-resistant TA; further studies with large number of patients are needed to confirm the efficacy of tacrolimus for TA.

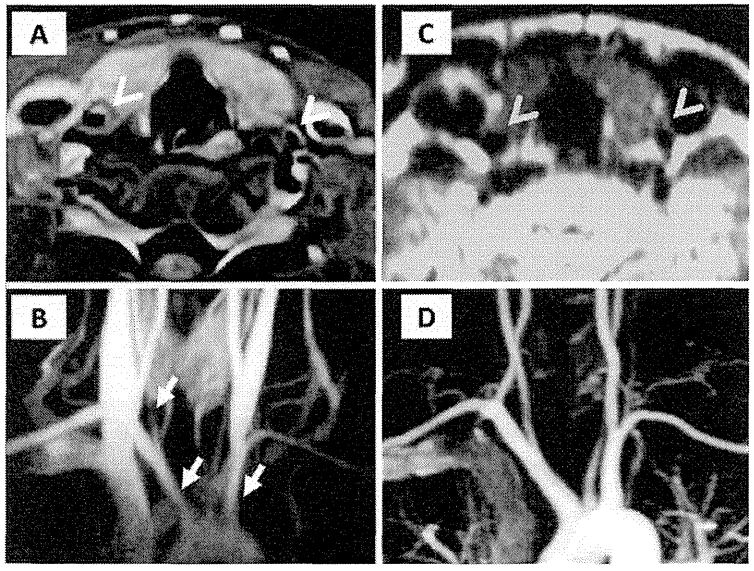

Figure 1. Neck and chest MRI findings. The left common carotid artery of this patient branches from the left brachiocephalic artery. A. T1-weighted image shows wall thickening of the left common carotid artery and right brachiocephalic artery (arrowheads). B. Magnetic resonance angiography shows stenosis of the right common carotid artery and bilateral brachiocephalic arteries (arrows) at diagnosis in August 2007. C. Magnetic resonance imaging in February 2011 during treatment with tacrolimus and subsequent sustained clinical remission shows normal vascular wall and (D) absence of new stenosis of arteries.

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Amelioration of Small Bowel Injury by Switching from Nonselective Nonsteroidal Anti-Inflammatory Drugs to Celecoxib in Rheumatoid Arthritis Patients: A Pilot Study

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Key Words
Nonsteroidal anti-inflammatory drugs · Cyclooxygenase-2 selective inhibitor · Small bowel injury · Video capsule endoscopy

Abstract
Background/Aims: Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used in patients with rheumatoid arthritis (RA) but have several side effects including mucosal damage in the small intestine. We aimed to evaluate whether the small bowel injury is ameliorated by switching from nonselective NSAIDs to celecoxib in patients with RA. ***Methods:*** Sixteen patients with RA who were treated with nonselective NSAIDs were enrolled in this study. Nonselective NSAIDs were converted to celecoxib for 12 weeks. Capsule endoscopy was performed before and after treatment with celecoxib. Videos were screened by gastroenterologists blinded to the patients' treatment. ***Results:*** Before the administration of celecoxib, reddened folds, denuded areas, petechiae/red spots and mucosal breaks were observed in 63, 63, 88 and 69% of the patients, respectively. In the 14 patients who completed this study, conversion to celecoxib significantly reduced the number of petechiae/red spots, the number of mucosal breaks, and Lewis scores. RA activity and cytokine levels in the peripheral blood were not significantly different before and after treatment with celecoxib. ***Conclusions:*** The incidence of small bowel injury by nonselective NSAIDs is high in patients with RA. Conversion from nonselective NSAIDs to celecoxib can be useful for protecting patients with RA from small bowel injury.

Introduction

Recent progress in the development of biologics, including disease-modifying antirheumatic drugs, has changed the treatment strategy for rheumatoid arthritis (RA) [1]. However, nonsteroidal anti-inflammatory drugs (NSAIDs) are still widely used because of their high efficacy for pain control and cost-effectiveness [2]. In spite of the usefulness of NSAIDs for reducing pain, patients who take NSAIDs are at a high risk for severe injury in the mucosa of the stomach and duodenum [3, 4]. Chronic NSAID users are estimated to suffer from ulcer complications (bleeding or perforation) at a rate of 1–4% each year, and NSAID use has been shown to be associated with up to 2,500 deaths per year in the UK population [5]. Previous randomized trials primarily focused on damage in the upper gastrointestinal (GI) tract, but recent reports have shown that lower GI events were also observed in patients with RA who use NSAIDs [6, 7].

NSAIDs act by inhibiting cyclooxygenase (COX), which converts arachidonic acid to prostaglandins [8, 9]. COX exists in two isoforms: COX-1, an essential enzyme to produce prostaglandins involved in the cytoprotective functions in the GI mucosa [10, 11]; COX-2, a predominantly cytokine-induced enzyme, produces prostaglandins to mediate pain and inflammation. Nonselective NSAIDs inhibit both COX-1 and COX-2, and gastroduodenal injury is suggested to mainly result from COX-1 inhibition. A selective COX-2 inhibitor has a characteristic of reducing mucosal damage compared to NSAIDs [12, 13], and celecoxib is one of the most commonly used selective COX-2 inhibitors. Several randomized controlled trials have compared the efficacy and side effects between celecoxib and nonselective NSAIDs and have shown that celecoxib reduced the relative risk of gastroduodenal ulcers compared to nonselective NSAIDs to 79% [12, 14, 15]. Over 24 weeks, the prevalence of endoscopically identified gastroduodenal ulcers in patients with RA taking celecoxib was nearly 4-fold lower than that of diclofenac. In addition, the incidence of mucosal injury to the small intestine was significantly lower in healthy subjects who received celecoxib than the subjects who received nonselective NSAIDs plus omeprazole [16, 17]. Available data, however, were limited to studies regarding the administration of nonselective NSAIDs or celecoxib in healthy volunteers.

Recently, video capsule endoscopy (VCE) enabled noninvasive visualization of the whole small intestine [18, 19]. VCE revealed approximately 90% of patients with RA suffer from small bowel injury regardless of NSAID use [20]. To date, clinical trials investigating the effect of COX-2 selective inhibitors on damage to the small intestine are lacking. In addition, the efficacy of switching from nonselective NSAIDs to celecoxib has not been investigated in patients taking NSAIDs. In this study, patients with RA with long-term use of nonselective NSAIDs were evaluated for mucosal damage of the small intestine by VCE. After the evaluation of mucosal injuries in the small intestine, nonselective NSAIDs were switched to celecoxib. We investigated whether the switching from nonselective NSAIDs to celecoxib ameliorates small bowel injury.

Patients and Methods

Study Subjects

Patients with RA who were older than 20 years and had been regularly treated with NSAIDs (loxoprofen, diclofenac, indomethacin, etc.) for more than 3 months (median 24 months; range 4–164 months) were consecutively recruited in the Department of Respiratory Medicine, Allergy and Rheumatic Disease, Osaka University Hospital, from January 2009 to April 2011. Exclusion criteria included: patients under treatment with biologics [anti-tumor necrosis factor (TNF)-α antibody, anti-interleukin (IL)-6 receptor antibody, etc.], high-dose corticosteroids (>10 mg/day of prednisolone), aspirin, or anti-ulcer drugs (misoprostol, teprenone, rebamipide, etc.); active GI ulcers; known or suspected complete or partial stenosis of the small intestine; inflammatory bowel disease; severe cardiovascular disease; malignancies; mental disorders; severe liver, renal and hematopoietic diseases, and patients who were pregnant or breastfeeding. Participants were not allowed to change their medications during the study, except for NSAID and celecoxib usage.

Study Design

This was a prospective, open-label, endoscopist-blinded, single-arm, and single-center study. Patients with RA treated with NSAIDs were assigned to receive celecoxib 200 mg twice daily for 12 weeks after the discontinuation of NSAIDs. VCE was performed before and after the treatment with celecoxib. Patients were evaluated for changes in the serologic markers of RA, serum cytokine concentrations, joint pain, and adverse events or side effects of celecoxib. The study protocols and informed consent forms were approved by the institutional review boards of Osaka University Hospital, and all patients signed a written consent form before being included in the study. The trial is registered at UMIN-CTR, No. UMIN000002554. The full trial protocol can be accessed at http://www.umin.ac.jp/ctr/.

Video Capsule Endoscopy

The VCE (Given video capsule system with the PillCam SB1 capsule; Given Imaging Ltd., Yoqneam, Israel) was performed as previously described [21]. Briefly, one day before the VCE examination, the subjects were required to fast after 9 p.m. After the subjects were fitted with sensor array and data recorder on the following morning at 9 a.m., they swallowed the VCE. Patients were not allowed to drink fluids until 2 h after swallowing the VCE, and only a light meal was allowed after a subsequent 2 h. The subjects avoided exposure to magnetic fields or radio transmitters, which may have interfered with image capture; otherwise, they were allowed to perform their daily activities. The sensor array and data recorder were removed 8 h after swallowing the capsule and were returned to the investigator to download the images onto a computer workstation for analysis. Videos were blinded before analysis by deletion of information including patients' name, date of birth, and examination date.

Methodology for Reviewing the VCE

The blinded videos were viewed by a physician with vast experience in VCE, and thumbnail pictures of potential abnormalities were created. These thumbnails were reviewed by three investigators. The damage scale from a previous classification for acute NSAID-induced small bowel damage by Maiden et al. [21] was used

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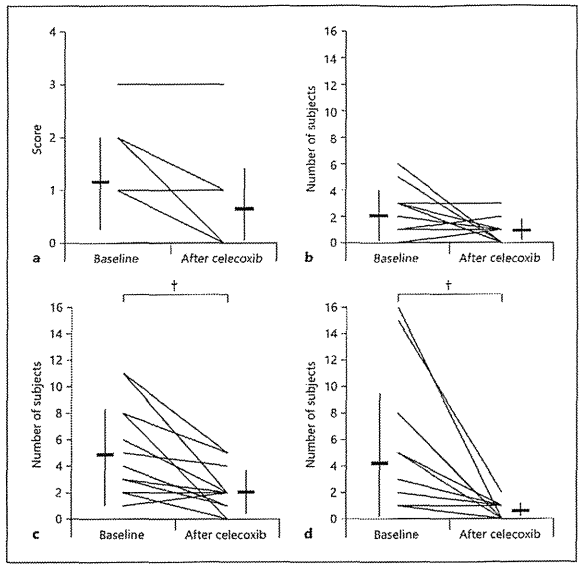

Fig. 1. Effect of the conversion from nonselective NSAIDs to celecoxib on the damage scale of small bowel lesions. The scores or number of lesions with each VCE finding [reddened folds (n = 14; **a**), denuded areas (n = 14; **b**), petechiae/red spots (n = 14; **c**) and mucosal breaks (n = 14; **d**)] were evaluated at baseline and after celecoxib treatment in the 14 patients who completed this study. † $p < 0.05$ after Bonferroni's correction. Bars indicate mean ± SD.

to evaluate mucosal injury as follows: category 1: reddened folds (≥1 valvulae conniventes showing discrete patchy or continuous erythema); category 2: denuded area (loss of villous architecture without a clear breach of the epithelium, may or may not be associated with surrounding erythema); category 3: petechiae/red spot (demarcated, usually circular, area of crimson mucosa with preservation of villi); category 4: mucosal break (mucosal erosions and/or ulcers, both represent discrete lesions with central pallor and surrounding hyperemia and loss of villi aphthae around the ulcer, and a punched out ulcer), graded by aphthae, circular ulcer, and punched out ulcer, and category 5: presence of blood. The severity of reddened folds was scored as 0 for none, 1 for mild, 2 for moderate, and 3 for severe. Additionally, the damage scale by Gralnek et al. [22], a capsule endoscopy scoring index for small bowel mucosal inflammatory change (Lewis score), was calculated for every patient by one endoscopist according to the judged thumbnails.

Assessing the Severity of RA

Changes in laboratory data including serum C-reactive protein (CRP), matrix metalloproteinase-3 (MMP-3) and blood hemoglobin (Hb) concentration were measured. Additionally, changes in serum cytokine concentrations were studied using the Bio-Plex human cytokine assay kit (Biorad, Hercules, Calif., USA) for platelet-derived growth factor-BB, IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, eotaxin, basic fibroblast growth factor, granulocyte colony-stimulating factor, granulocyte/macrophage colony-stimulating factor, interferon (IFN)-γ, IFN-inducible protein-10, monocyte chemoattractant protein-1, macrophage inflammatory protein (MIP)-1α, MIP-1β, regulated and normal T cell expressed and secreted, TNF-α, and vascular endothelial growth factor. Arthritis scores were evaluated before and after the treatment of celecoxib by Disease Activity Score in 28 joints calculated by using CRP (DAS28-CRP) as described previously [23]. The number of the joints with swelling (SJC28) and the number of the joints with tenderness (TJC28) among 28 joints were counted by the rheumatologist. The DAS28-CRP was calculated from SJC28, TJC28, pain visual analogue scale and serum CRP values.

Statistical Analysis

The mucosal injury scores and the blood test results were analyzed before and after the treatment with celecoxib among the 14 patients who had completed a previous study using Wilcoxon paired signed rank test and paired t test, respectively. The Bonferroni method was used to adjust for multiple comparisons; therefore, p values $<0.05/5 = 0.01$ for the mucosal injury parameters (fig. 1, 2) and $<0.05/4 = 0.0125$ for the blood test parameters (fig. 3) were considered statistically significant. JMP Pro version 10.0 (SAS Institute Inc., Cary, N.C., USA) was used for all the analyses.

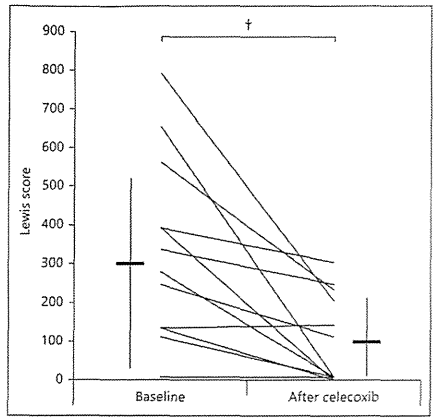

Fig. 2. Effect of conversion from nonselective NSAIDs to celecoxib on the scoring index for small bowel mucosal inflammatory change (Lewis score). The scoring index was evaluated at baseline and after celecoxib treatment in the 14 patients who completed this study. † $p < 0.05$ after Bonferroni's correction. Bars indicate mean ± SD.

Results

Patients

Sixteen patients underwent baseline VCE during the study period. Baseline characteristics for each subject are shown in table 1. Thirteen patients took loxoprofen; 3 patients took diclofenac, and 1 patient took indomethacin. One patient took both loxoprofen and diclofenac. Six patients took proton pump inhibitor (PPI) and 2 patients took histamine H2 receptor antagonist. Two patients dropped out because of general fatigue and disc herniation, which were not suggested as being related to celecoxib. Among the 14 patients who completed the study, no serious complications or side effects were observed.

Baseline VCE

Small bowel lesions were observed in 14 of 16 patients (88%) at baseline VCE. Reddened folds, denuded areas, petechiae/red spots and mucosal breaks were observed in 10 (63%), 10 (63%), 14 (88%) and 11 patients (69%), respectively. The presence of blood was not observed in any patient. The numbers of red spots and mucosal breaks were 5.5 ± 35.7 and 3.7 ± 5.1, respectively.

Efficacy of Switching from Nonselective NSAIDs to Celecoxib

Small bowel lesions were observed in 12 of 14 patients (86%) at posttreatment VCE. Similar to baseline VCE, the presence of blood was not observed in any patient. VCE findings were compared before and after treatment with celecoxib in the 14 subjects who completed this study. The proportion of patients having reddened folds, denuded areas, petechiae/red spots and mucosal breaks decreased from 71 to 50%, 64 to 50%, 93 to 79% and 71 to 36%, respectively. We performed quantitative analyses of small bowel injury in each patient before and after treatment with celecoxib. The numbers of petechiae/red spots and mucosal breaks were significantly decreased from 4.9 ± 3.5 to 1.9 ± 1.7 ($p = 0.002$; Bonferroni-adjusted $p = 0.010$) and 4.1 ± 5.4 to 0.4 ± 0.6 ($p = 0.004$; Bonferroni-adjusted $p = 0.0195$), respectively (fig. 1c, d). The scores of reddened folds and the number of denuded areas did not significantly differ before and after the treatment of celecoxib ($p = 0.031$, Bonferroni-adjusted $p = 0.157$; $p = 0.094$, Bonferroni-adjusted $p = 0.469$, respectively; fig. 1a, b). Lewis scores significantly decreased from 290 ± 248 to 92 ± 112 ($p = 0.002$; Bonferroni-adjusted $p = 0.010$; fig. 2).

Assessment of the Severity of RA

We next assessed serum and clinical markers for the disease activity of RA. There were no significant differences in Hb ($p = 0.029$; Bonferroni-adjusted $p = 0.114$), CRP ($p = 0.734$; Bonferroni-adjusted $p = 1$), MMP-3 ($p = 0.786$; Bonferroni-adjusted $p = 1$) and DAS28-CRP ($p = 0.167$; Bonferroni-adjusted $p = 0.667$) before and after treatment with celecoxib (fig. 3). We assessed serum cytokine concentrations by multiplex cytokine assay. There were no cytokines whose expressions were significantly different before and after treatment with celecoxib (table 2). Thus, we observed a significant reduction in small bowel injury by switching from nonselective NSAIDs to celecoxib after the 12 weeks of administration in patients with RA without a significant difference in the effectiveness of the medication on RA.

Discussion

Recently, VCE enabled to evaluate NSAID-induced small bowel injury, and we showed celecoxib use could reduce small bowel injury compared with nonselective NSAIDs. Goldstein et al. [16, 17] reported two randomized controlled trials using VCE in healthy volunteers and

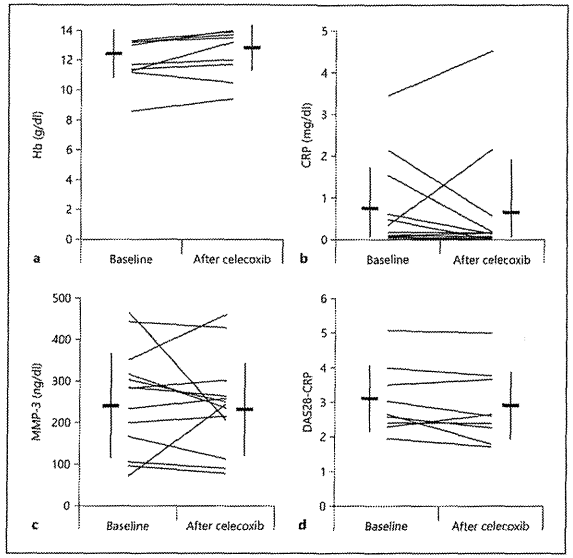

Fig. 3. Effect of conversion from nonselective NSAIDs to celecoxib on the activity of RA. Blood tests and disease activity scores were evaluated at baseline and after celecoxib treatment in 14 patients. **a** Blood Hb concentration (n = 14). **b** Serum CRP (n = 14). **c** *MMP-3* (n = 14). **d** *DAS28-CRP* (n = 9). Bars indicate mean ± SD.

Table 1. Details of patients and treatment for RA

Age, years	59.0±11.8[a]
Sex	
Male	6 (38%)
Female	10 (62%)
Body mass index	23.5±3.9[a]
Prior NSAID therapy	
Loxoprofen	13 (81%)[b]
Diclofenac	3 (19%)[b]
Indomethacin	1 (6%)
Treatments for RA other than NSAIDs	
Corticosteroids	12 (86%)
Methotrexate	11 (69%)
Salazosulfapyridine	6 (38%)
Bucillamine	2 (13%)
Laboratory tests	
CRP, mg/dl	0.65±0.96[a]
MMP-3, ng/dl	222±135[a]
DAS28-CRP	3.2±1.1[a]
Hb, g/dl	12.6±1.6[a]

[a] Mean ± SD.
[b] One patient took a combination of loxoprofen and diclofenac.

concluded that celecoxib was effective for decreasing small bowel injury in comparison to nonselective NSAIDs. Mizukami et al. [24] also reported that celecoxib reduced small bowel injury compared with loxoprofen. Although their reports clearly showed the efficacy of celecoxib in comparison to NSAIDs for reducing small bowel injury, these reports had limitations. The duration of the celecoxib/NSAID treatment was relatively short (2 weeks), and the subjects were not diseased patients who required NSAIDs. Chan et al. [25] reported that anemia caused by small bowel injury was lower in celecoxib than diclofenac and omeprazole in patients with RA taking either medication for 6 months. However, they did not directly evaluate this small bowel damage by radiologic or endoscopic methods such as VCE. Our results revealed that celecoxib reduced the number of petechiae/red spots and mucosal breaks. Lewis scores were significantly decreased after the conversion to celecoxib. Thus, we demonstrated for the first time that small bowel injury was significantly improved by switching from NSAIDs to celecoxib in RA patients.

Table 2. Multiplex immunobead assay at baseline (B) and after celecoxib treatment (P)

Cytokine	B, pg/ml	P, pg/ml	Fold change P/B[a]	p value[b]
PDGF-BB	105,441 (31,476–1,164,451)	97,588 (46,382–1,216,712)	0.89	0.58
IL-1β	37 (15–490)	30 (15–965)	0.96	0.5
IL-1ra	1,226 (526–1,911)	1,134 (500–2,721)	1.15	0.58
IL-2	3 (0–18.79)	18 (0–45.4)	5.26	0.84
IL-4	55 (34–94)	55 (33–86)	0.99	1
IL-5	25 (9–130)	27 (6–105)	1.02	0.47
IL-6	152 (28–1,430)	92 (47–5,369)	1.96	0.95
IL-7	158 (84–351)	170 (78–296)	1.05	0.67
IL-8	246 (108–39,611)	219 (126–90,527)	1.18	0.63
IL-9	568 (51–3,923)	656 (16–9,347)	1.25	1
IL-10	132 (12–14,014)	113 (21–14,527)	1.05	0.36
IL-12	331 (64–9,501)	324 (85–5,704)	0.74	0.76
IL-13	85 (23–311)	88 (17–297)	1.12	1
IL-15	1 (0–10)	19 (0–252)	30.93	0.63
IL-17	107 (0–307)	84 (0–1,117)	1.28	0.91
Eotaxin	730 (0–6,917)	726 (0–8,988)	1.44	0.5
Basic FGF	253 (110–447)	218 (100–6,136)	2.61	0.71
G-CSF	218 (123–350)	226 (120–363)	1.05	0.81
GM-CSF	46 (0–434)	59 (0–772)	1.30	0.97
IFN-γ	2,312 (1,052–4,574)	2,721 (1,059–5,314)	1.06	0.5
IP-10	21,271 (10,152–189,971)	27,085 (12,249–216,373)	1.28	0.27
MCP-1	252 (85–451)	324 (173–1,067)	1.41	0.1
MIP-1α	58 (14–5,554)	62 (24–4,666)	0.52	0.63
MIP-1β	1,143 (471–45,475)	957 (618–22,649)	0.47	0.86
RANTES	22,134 (13,799–39,568)	22,134 (17,876–40,407)	1.01	0.88
TNF-α	624 (220–1,543)	412 (282–1,665)	0.90	1
VEGF	2,650 (581–9,218)	2,537 (656–12,726)	1.23	0.39

Values for B and P are expressed as median (range). PDGF = Platelet-derived growth factor; FGF = fibroblast growth factor; G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte/macrophage colony-stimulating factor; IP-10 = IFN-inducible protein-10; MCP-1 = monocyte chemoattractant protein-1; RANTES = regulated and normal T cell expressed and secreted; VEGF = vascular endothelial growth factor.
[a] Fold change in mean cytokine level.
[b] Wilcoxon paired signed-rank test.

It has been reported that patients with RA have small bowel injury in high frequency regardless of their NSAIDs use [20]. In addition, Sugimori et al. [20] showed that patients with RA taking NSAIDs were at high risk of severe small bowel injury. Consistent with previous reports [16, 20, 21], we obtained the results that patients with RA taking NSAIDs had a high frequency of small intestinal injury. Although the proportion of RA patients who had mucosal injury was not significantly changed by switching NSAIDs to celecoxib, the severity of small bowel injury was significantly improved. In addition, the efficacy of pain control in patients with RA by celecoxib was not inferior to that of nonselective NSAIDs. These results indicate that COX-2 selective inhibition by celecoxib is beneficial for the protection of mucosal damage in the small intestine without sacrificing the efficacy of pain control in patients with RA. In spite of the absence of abdominal symptoms in most RA patients in this study, 71% of the patients with RA presented mucosal breaks by VCE and 50% presented abnormal Hb concentrations. Although it may not be necessary to switch from nonselective NSAIDs to celecoxib in all the patients, evaluation of the small intestine should be considered when the RA patients with chronic NSAID users develop persistent severe anemia but do not have diseases in the upper and lower GI tract. In such cases, switching from nonselective NSAIDs to celecoxib may be beneficial.

Maiden et al. [21] previously reported that long-term COX-2 selective agents caused small-bowel damage comparable to NSAIDs. The discrepancy in the effectiveness

of COX-2 selective agents between long- and short-term use might be caused by the fact that the majority of the subjects analyzed in their study were patients with osteoarthritis, and only 24–33% of RA patients were included. In addition, COX-2 selective agents other than celecoxib were used in 40% of the patients in their study (etoricoxib, 20%; rofecoxib, 15%; valdecoxib, 5%). Furthermore, they did not quantitatively evaluate the severity of small bowel injury. Even in their analysis, small bowel injury was observed in a lower percentage by COX-2 selective agents than by NSAIDs. Further long-term studies using celecoxib for the patients with RA are necessary to clarify this issue.

Consistent with the previous reports, we found that celecoxib did not worsen the severity of RA, including blood Hb concentration, CRP, MMP-3 and DAS28-CRP. No patients discontinued celecoxib because of a worsening of pain caused by RA. When we assessed serum cytokine concentrations by multiplex cytokine assay, which had been reported to correlate with disease activity of RA [26–28], there were no cytokines whose expressions were significantly different before and after treatment with celecoxib. These results indicate that the conversion from NSAIDs to celecoxib was tolerable without diminishing the efficacy of pain control for RA patients. We did not include patients on biologics, such as anti-TNF and anti-IL-6 receptor antibodies, because these medications might be protective for small bowel injury [29–31]. Infliximab has been reported to be protective for indomethacin-induced small bowel damage in rats [32]. Similarly, there are several reports showing the efficacy of antiulcer drugs on preventing NSAID-induced small bowel injury [33–36]. We excluded patients taking these drugs, although those were the majority of long-term NSAIDs users. On the other hand, we included patients taking gastric acid suppressants such as PPI and H2 receptor antagonist because it is still controversial whether the inhibition of gastric acid exacerbate small intestinal injury. Lansoprazole has been reported to ameliorate NSAID-induced small bowel injury in rats by inhibiting inducible nitric oxide synthase expression, while omeprazole has no effect [37, 38]. Conversely, there are some recent reports showing the opposite result that PPIs exacerbate or have no effect on NSAID-induced small intestinal injury [39–41]. Moreover, these reports concerned indomethacin-induced rat models and not humans. Additionally, because corticosteroids were reported to be either ulcerogenic [42, 43] or gastroprotective [44, 45], patients on high-dose corticosteroids were eliminated from our study. We tried to avoid medications which may affect the small intestinal injury as well as the severity of RA.

There are some limitations to this study; the sample size of the study is relatively small. This is neither a placebo-controlled study nor a crossover study. There was no control group continuing nonselective NSAIDs. Thus, we cannot completely deny the possibility that the lesions would have improved if the prior NSAIDs had been continued or switched to different nonselective NSAIDs. We did not set a washout period after the cessation of NSAIDs use because the joint pain of arthritis is not tolerable without medication with NSAIDs. The duration of medication by celecoxib in this study was relatively short. In spite of these limitations, this study clearly demonstrates that the COX-2 selective inhibitor celecoxib has a clinical benefit for the improvement of small bowel injury in RA without diminishing the efficacy of its pain control. Additional long-term and large-scale studies are necessary to clarify the role of celecoxib in small bowel injury.

In conclusion, the incidence of small bowel injury by nonselective NSAIDs is high in patients with RA. Conversion from nonselective NSAIDs to celecoxib can become a useful therapeutic option for the treatment of small bowel injury in patients with RA.

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Disclosure Statement

The authors declare that they have no conflicts of interest.

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ORIGINAL ARTICLE

Phase III Study of the Efficacy and Safety of Subcutaneous Versus Intravenous Tocilizumab Monotherapy in Patients With Rheumatoid Arthritis

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Objective. To evaluate the efficacious noninferiority of subcutaneous tocilizumab injection (TCZ-SC) monotherapy to intravenous TCZ infusion (TCZ-IV) monotherapy in Japanese patients with rheumatoid arthritis (RA) with an inadequate response to synthetic and/or biologic disease-modifying antirheumatic drugs (DMARDs).
Methods. This study had a double-blind, parallel-group, double-dummy, comparative phase III design. Patients were randomized to receive TCZ-SC 162 mg every 2 weeks or TCZ-IV 8 mg/kg every 4 weeks; no DMARDs were allowed during the study. The primary end point was to evaluate the noninferiority of TCZ-SC to TCZ-IV regarding the American College of Rheumatology criteria for 20% improvement in disease activity (ACR20) response rates at week 24 using an 18% noninferiority margin. Additional efficacy, safety, pharmacokinetic, and immunogenicity parameters were assessed.
Results. At week 24, ACR20 response was achieved in 79.2% (95% confidence interval [95% CI] 72.9, 85.5) of the TCZ-SC group and in 88.5% (95% CI 83.4, 93.5) of the TCZ-IV group; the weighted difference was −9.4% (95% CI −17.6, −1.2), confirming the noninferiority of TCZ-SC to TCZ-IV. Remission rates of the Disease Activity Score in 28 joints using the erythrocyte sedimentation rate and the Clinical Disease Activity Index at week 24 were 49.7% and 16.4% in the TCZ-SC group and 62.2% and 23.1% in the TCZ-IV group, respectively. Serum trough TCZ concentrations were similar between the groups over time. Incidences of all adverse events and serious adverse events were 89.0% and 7.5% in the TCZ-SC group and 90.8% and 5.8% in the TCZ-IV group, respectively. Anti-TCZ antibodies were detected in 3.5% of the TCZ-SC group; no serious hypersensitivity was reported in these patients.
Conclusion. TCZ-SC monotherapy demonstrated comparable efficacy and safety to TCZ-IV monotherapy. TCZ-SC could provide additional treatment options for patients with RA.

INTRODUCTION

Tocilizumab (TCZ) is a humanized monoclonal antibody directed against the interleukin-6 (IL-6) receptor that is approved for the treatment of patients with rheumatoid arthritis (RA), polyarticular-course and systemic juvenile idiopathic arthritis, and Castleman's disease by intravenous (IV) administration. Multiple phase III trials of TCZ, in combination with synthetic disease-modifying antirheumatic drugs (DMARDs) or as monotherapy, demonstrated an improvement of clinical symptoms and prevention of joint destruction (1–7).

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Significance & Innovations

- A subcutaneous formulation of tocilizumab (TCZ) would greatly contribute to improving the quality of life in patients with rheumatoid arthritis (RA) because it would allow for a shorter administration time compared with an intravenous formulation and for home administration.
- Subcutaneous TCZ monotherapy demonstrated comparable efficacy and safety to intravenous TCZ monotherapy in patients with RA who have had an inadequate response to synthetic and/or biologic disease-modifying antirheumatic drugs.

Previously, patients with RA who did not respond to treatment, such as the 19th century French impressionist painter Pierre-Auguste Renoir, had limited alternatives available (8). Many treatment choices are now available that have proven clinical efficacy, including anti–tumor necrosis factor (anti-TNF) agents and TCZ. Most anti-TNF agents require concomitant methotrexate (MTX) for maximum efficacy, whereas TCZ has similar efficacy with and *without* MTX (9).

To optimize a patient's treatment, the efficacy, safety, and route of administration for each therapy should be considered along with a patient's disease status in order to achieve clinical, functional, and structural remission or the lowest disease activity state possible (10,11). Some patients prefer therapies with a biologic agent that can be administered by subcutaneous (SC) injection rather than IV formulations, and prefer to receive treatments at home (12–14). An SC formulation of TCZ (TCZ-SC) would provide an additional treatment option for patients with RA.

The efficacy and pharmacokinetics of TCZ-SC monotherapy were evaluated in an open-label, phase I/II study conducted in Japan at 3 doses (81 mg every 2 weeks, 162 mg every 2 weeks, and 162 mg weekly) over 6 months (15). To further expand on these results, the noninferiority, multicenter phase II study MUSASHI (Multi-Center Double-Blind Study of Tocilizumab Subcutaneous Injection in Patients Having Rheumatoid Arthritis to Verify Noninferiority Against Intravenous Infusion) was conducted to compare the efficacy and safety of TCZ-SC monotherapy 162 mg every 2 weeks and TCZ-IV monotherapy 8 mg/kg every 4 weeks in Japanese patients with RA with an inadequate response to synthetic and/or biologic DMARDs.

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PATIENTS AND METHODS

Patient population. Eligible patients were ages 20–75 years and had RA for ≥6 months, as diagnosed using the 1987 criteria of the American College of Rheumatology (ACR) for the classification of RA (16). Additional inclusion criteria were as follows: an inadequate response of ≥12 weeks to any synthetic DMARD (MTX, sulfasalazine, bucillamine, and leflunomide), biologic DMARD (infliximab, etanercept, and adalimumab), or immunosuppressant (e.g., tacrolimus); ≥8 tender joints (of 68 joints); ≥6 swollen joints (of 66 joints); and an erythrocyte sedimentation rate (ESR) ≥30 mm/hour or a C-reactive protein level ≥1.0 mg/dl.

Exclusion criteria included active tuberculosis, a history of serious allergies, and active hepatitis B or C. All candidates underwent tuberculin reaction or QuantiFERON testing. Patients testing positive for latent tuberculosis were enrolled if treatment with isoniazid was initiated 3 weeks prior to initial administration of TCZ and continued for 9 months. Patients with class IV Steinbrocker functional activity were excluded. Patients were also excluded if they had received previous treatment with TCZ; had received plasmapheresis, surgical procedures (except with locally and low invasive operations), or dose changes or added-in DMARDs or immunosuppressants within 4 weeks of TCZ treatment; had received oral glucocorticoids at a dosage of >10 mg/day of prednisolone or equivalent; or had a dose increase, new administration, or IV or intramuscular injections of glucocorticoids within 2 weeks of TCZ treatment.

Study design. MUSASHI was a 24-week, phase III, randomized, double-blind, double-dummy study in Japanese patients with RA. The study protocol was approved by the Ministry of Health, Labour and Welfare of Japan and by the *local ethical committees*. All patients gave their written informed consent.

Patients were randomized 1:1 into 2 groups: 162 mg of TCZ-SC monotherapy every 2 weeks plus placebo TCZ-IV every 4 weeks or 8 mg/kg of TCZ-IV monotherapy every 4 weeks plus placebo TCZ-SC every 2 weeks. Throughout the study, DMARDs or immunosuppressants were not permitted. There was no washout period for synthetic DMARDs as long as treatment and dose were stable a minimum of 4 weeks prior to initial TCZ treatment. Concomitant use of low-dosage oral glucocorticoids (≤10 mg/day of prednisolone or equivalent without escalation from the baseline dosage) and 1 oral nonsteroidal antiinflammatory drug was permitted during the 24 weeks. Intraarticular injections of corticosteroids and hyaluronate preparations were avoided as much as possible.

Efficacy assessments. Efficacy assessments were conducted every 4 weeks. The primary end point was to demonstrate the noninferiority of TCZ-SC monotherapy to TCZ-IV monotherapy regarding the proportion of patients with 20% improvement in disease activity for ACR criteria (ACR20) responses at week 24 (17). Additional end points included ACR50 and ACR70 response rates, ACR/European League Against Rheumatism Boolean Index remission rates, Clinical Disease Activity Index (CDAI) remission rates, Disease Activity Score in 28 joints using the ESR (DAS28-ESR) remission rates, and a low disease activity rate at week 24. Mean changes in DAS28-ESR, CDAI score, and the proportion of patients who improved in the Japanese version of the Health Assessment Questionnaire (HAQ) by ≥0.3 units from baseline were assessed over time (18). For efficacy assessments, the per-protocol set (PPS) was used, excluding patients with protocol violations, early withdrawal, violations concerning concomitant medication use, or violations concerning the dose and administration. The last observation carried forward was used for any missing values. For patients receiving glucocorticoids or hyaluronic acid via intraarticular administration, any treated joints were treated as positive tender and swollen joints for that defined period.

Pharmacokinetics. Samples for pharmacokinetic analysis were collected at weeks 0, 2, 4, 8, 12, 16, 20, and 24. TCZ, which is not bound with the IL-6 receptor (free TCZ) in the serum, was determined by enzyme-linked immunosorbent assay (19). The lower limit of detection for free TCZ in serum was 0.1 μg/ml.

Safety and immunogenicity assessments. Safety and immunogenicity data were analyzed using the safety population, defined as all patients who received at least 1 dose of TCZ. Adverse events (AEs) and serious AEs were classified using the Medical Dictionary for Regulatory Activities, version 13.0. The number of patients with AEs and the total number of AEs were tabulated. Infusion and/or injection reactions were prespecified and classified as SC injection site reactions (ISRs; AEs at the site of SC injection), systemic reactions to SC injection (SIRs; AEs not at the site of SC injection within 24 hours of treatment), or IV infusion–related reactions (IRRs; AEs occurring within 24 hours of treatment). All AEs were graded as severe, moderate, or mild by physicians. Laboratory investigations were graded by Common Terminology Criteria for Adverse Events.

Blood samples for the anti-TCZ antibody screening assay were collected every 4 weeks. The anti-TCZ antibody screening assay was performed as previously described using a bridging enzyme-linked immunosorbent assay with an additional competitive displacement step as the confirmation assay (20).

Statistical analysis. The primary end point was analyzed using the PPS for the primary analysis and the modified intent-to-treat (ITT) population for the sensitivity analysis. The modified ITT population included all patients who received at least 1 dose of treatment. The noninferiority margin was set at 18%, as determined using the difference between the ACR20 results of SATORI (Study of Active Controlled Tocilizumab Monotherapy for Rheumatoid Arthritis Patients with an Inadequate Response to Methotrexate) (7); 18% was the more conservative criterion because it was less than one-third of the difference of the ACR20 response rate between the TCZ-IV monotherapy group and the control group in the SATORI study. Furthermore, it is less than half of the lower limit of the 95% confidence interval (95% CI) for the difference between the groups. The adjusted 95% CI for the difference between the ACR20 response rate in the TCZ-SC monotherapy and TCZ-IV monotherapy groups was calculated using the Mantel-Haenszel method, with patients stratified according to weight at enrollment (<60 or ≥60 kg) and previous use of anti-TNF agents. Noninferiority was demonstrated if the lower limit was not below the confidence limit for noninferiority (−18%). A sample size of 330 was calculated to provide 90% power to demonstrate the noninferiority of TCZ-SC monotherapy to TCZ-IV monotherapy. To determine the sample size, the ACR20 response rate was set to 70% because of the following assumptions: the ACR20 response rate at 24 weeks was 79.7% in the SATORI trial and the overall response rate potentially could be lower in the MUSASHI trial than in the SATORI trial because the patient population of inadequate anti-TNF responders was larger.

Simple logistic analysis was used to screen for potential predictive variables, including sex, age, weight (in kg, the fourth quartile versus the first to third quartiles), body mass index (BMI; in kg/m^2, the fourth quartile versus the first to third quartiles), disease duration, Steinbrocker class/stage, history of anti-TNF agents, rheumatoid factor, anti–cyclic citrullinated peptide antibody, glucocorticoid dose, number of previous DMARDs, DAS28-ESR, ACR core components, and IL-6 levels at baseline. Multiple logistic regression was used to identify the contributing baseline parameters to ACR20, ACR50, and ACR70 response rates in the TCZ-SC monotherapy group at week 24. The initial model contained the potential predictive variables and the predicting factor ($P \leq 0.05$) was identified in the final model by using a stepwise procedure.

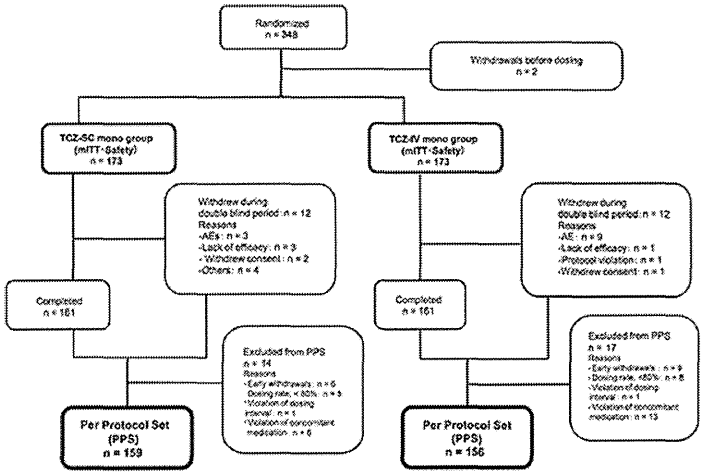


Figure 1. Patient disposition over 24 weeks (in the per-protocol set [PPS]). Two patients withdrew before treatment with tocilizumab (TCZ) was initiated. In the group receiving a subcutaneous injection of TCZ monotherapy (TCZ-SC mono), 3 patients withdrew because of adverse events (AEs), 3 patients withdrew because of a lack of efficacy, 2 patients withdrew consent, and 4 patients withdrew because of other reasons. In the group receiving an intravenous infusion of TCZ monotherapy (TCZ-IV mono), 9 patients withdrew because of AEs, 1 patient withdrew because of a lack of efficacy, 1 patient withdrew consent, and 1 patient withdrew because of a protocol violation. mITT = modified intent-to treat.

RESULTS

Patient disposition. A total of 348 patients were randomized (Figure 1). Two patients withdrew before treatment with TCZ and 346 patients were randomized into 2 groups; 173 patients in each group received the study drugs. Of these 173 patients, 161 (93.1%) completed the double-blind period in each group (Figure 1). In the PPS, 159 patients in the TCZ-SC monotherapy group and 156 patients in the TCZ-IV monotherapy group were eligible for analysis. The major reasons for patient exclusion from the PPS were receipt of <80% of the total dose, early withdrawal, and violations concerning concomitant medication use.

Baseline demographics and clinical characteristics. Patient demographics and clinical characteristics were similar between the TCZ-SC monotherapy and TCZ-IV monotherapy groups (Table 1). The patient population weighing ≥60 kg consisted of 23.3% in the TCZ-SC monotherapy group and 25.6% in the TCZ-IV monotherapy group. The percentages of patients who previously received anti-TNF agents were 18.9% in the TCZ-SC monotherapy group and 23.7% in the TCZ-IV monotherapy group (Table 1).

Clinical efficacy. The study met its primary end point of demonstrating the noninferiority of TCZ-SC monotherapy to TCZ-IV monotherapy. In the PPS, the ACR20 response rate at week 24 was achieved in 79.2% (95% CI 72.9, 85.5) of the TCZ-SC monotherapy patients and in 88.5% (95% CI 83.4, 93.5) of the TCZ-IV monotherapy patients (Figure 2A). The weighted difference between the groups was −9.4% (95% CI −17.6, −1.2), confirming the noninferiority of TCZ-SC monotherapy to TCZ-IV monotherapy. In the modified ITT population, the ACR20 response at week 24 was achieved in 79.2% (95% CI 73.1, 85.2) of the TCZ-SC monotherapy patients and in 86.0% (95% CI 80.9, 91.2) of the TCZ-IV monotherapy patients. The weighted difference between the groups was −7.0% (95% CI −15.0, 1.0), confirming the noninferiority of TCZ-SC monotherapy to TCZ-IV monotherapy in the sensitivity analysis. Another sensitivity analysis was conducted that was stratified according to disease duration and previous use of an anti-TNF agent. The weighted difference was −9.4% (95% CI −17.7, −1.1) and was consistent with the results of the PPS and modified ITT populations. ACR50 and ACR70 response rates at week 24 were also similar between the groups (Figure 2A).

The DAS28-ESR, CDAI, and Boolean Index remission rates at week 24 were 49.7%, 16.4%, and 15.7%, respectively, in the TCZ-SC monotherapy group. Conversely, the DAS28-ESR, CDAI, and Boolean Index remission rates at week 24 were 62.2%, 23.1%, and 16.0%, respectively, in the TCZ-IV monotherapy group (Figure 2B). A higher proportion of patients in the TCZ-IV monotherapy group (82.1% [95% CI 76.0, 88.1]) than in the TCZ-SC monotherapy group (65.4% [95% CI 58.0, 72.8]) achieved DAS28-ESR low disease activity at week 24. The mean change in DAS28-ESR and CDAI score decreased similarly over 24 weeks in both groups (Figures 2C and D). The proportions of patients who improved in physical function by ≥0.3 units (per the HAQ) from baseline between the TCZ-SC monotherapy and TCZ-IV monotherapy groups were 56.6% (95% CI 48.9, 64.3) and 67.9% (95% CI 60.6, 75.3), respectively, at week 24. The mean ± SD change in serum matrix metalloproteinase 3 (MMP-3) was similar in both groups (from 288.9 ± 204.7 ng/ml at baseline to 123.3 ± 89.9 ng/ml at week 24 in the TCZ-SC monotherapy group and from 290.0 ± 211.3 ng/ml at baseline to 101.7 ± 64.2 ng/ml at week 24 in the TCZ-IV monotherapy group).

To identify the background factors that influence efficacy, logistic regression analyses were applied to the ACR response rate. The result from stepwise regression, BMI in the fourth quartile (from 23.4 to 29.6 kg/m^2) at baseline, was detected as a significant variable for ACR20 response rate (63.4%; odds ratio [OR] 0.31 [95% CI 0.14, 0.70], P = 0.0048), ACR50 response rate (51.2%; OR 0.47 [95% CI 0.22, 0.98], P = 0.0444), and ACR70 response rate (24.4%; OR 0.39 [95% CI 0.17, 0.90], P = 0.0271).

Pharmacokinetics. The serum trough TCZ concentrations in the TCZ-SC monotherapy and TCZ-IV monotherapy groups were similar over time (Figure 3). More than 80% of patients maintained TCZ concentrations ≥1 μg/ml from week 4 onward in the TCZ-SC monotherapy group (Figure 3).

Table 1. Patient characteristics at baseline (per-protocol set)*

	TCZ-SC monotherapy (n = 159)	TCZ-IV monotherapy (n = 156)
Women, no. (%)	133 (83.6)	128 (82.1)
Age, years†	52.1 ± 12.6	51.8 ± 11.8
Body weight, median (min, max) kg†	53.0 (36.3, 83.3)	53.1 (37.5, 96.3)
Body weight, kg†	53.8 ± 8.7	54.4 ± 10.1
<60 kg, no. (%)	122 (76.7)	116 (74.4)
≥60 kg, no. (%)	37 (23.3)	40 (25.6)
Disease duration, years	7.3 ± 7.5	8.0 ± 7.3
Disease duration, median years	5.1	5.9
Steinbrocker functional class, no. (%)†		
I	25 (15.7)	20 (12.8)
II	112 (70.4)	118 (75.6)
III	22 (13.8)	18 (11.5)
Steinbrocker stage, no. (%)†		
I	20 (12.6)	8 (5.1)
II	53 (33.3)	60 (38.5)
III	47 (29.6)	42 (26.9)
IV	39 (24.5)	46 (29.5)
RF positive, no. (%)	126 (79.2)	131 (84.0)
ACPA antibodies, no. (%)	142 (89.3)	142 (91.0)
IL-6, pg/ml	39.1 ± 46.1	32.2 ± 42.8
SJC (in 66 joints)	14.3 ± 6.7	13.5 ± 6.8
TJC (in 68 joints)	18.1 ± 8.8	17.6 ± 9.4
Japanese HAQ score	1.18 ± 0.64	1.25 ± 0.65
Patient's pain assessment, mm	52.6 ± 23.1	58.4 ± 22.5
Patient's global assessment, mm	53.6 ± 24.9	59.7 ± 22.9
Physician's global assessment, mm	62.4 ± 20.0	61.3 ± 19.0
CRP, mg/dl	2.2 ± 2.3	2.1 ± 2.0
ESR, mm/hour	47.9 ± 24.4	48.8 ± 22.5
DAS28-ESR	6.1 ± 0.9	6.2 ± 0.9
CDAI score	34.2 ± 10.3	33.7 ± 10.8
Oral glucocorticoids administered, no. (%)	110 (69.2)	92 (59.0)
Dosage, mg/day‡	4.6 ± 2.3	4.7 ± 2.1
Previous MTX, no. (%)§	128 (80.5)	129 (82.7)
Dosage, mg/week§	8.2 ± 2.2	8.2 ± 2.3
Previous anti-TNF agents, no. (%)	30 (18.9)	37 (23.7)

* Values are the mean ± SD unless indicated otherwise. TCZ-SC = subcutaneous tocilizumab; TCZ-IV = intravenous tocilizumab; RF = rheumatoid factor; ACPA = anti–citrullinated protein antibody; IL-6 = interleukin-6; SJC = swollen joint count; TJC = tender joint count; HAQ = Health Assessment Questionnaire; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; DAS28-ESR = Disease Activity Score in 28 joints using the ESR; CDAI = Clinical Disease Activity Index; MTX = methotrexate; anti-TNF = anti–tumor necrosis factor.
† At randomization.
‡ Dosage is prednisolone or equivalent.
§ Patients who previously received MTX were analyzed within 4 weeks of initial TCZ treatment.

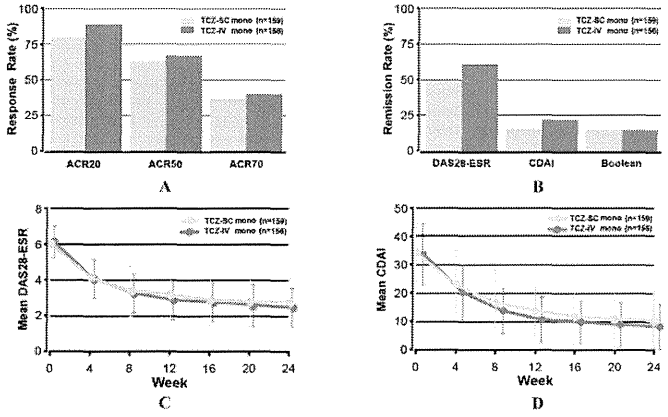


Figure 2. **A**, American College of Rheumatology (ACR) response rates of 20% (ACR20), 50% (ACR50), and 70% (ACR70) at week 24 (in the per-protocol set [PPS]) in patients receiving an intravenous infusion of tocilizumab monotherapy (TCZ-IV mono; n = 156) or a subcutaneous injection of tocilizumab monotherapy (TCZ-SC mono; n = 159). The ACR50 response rate in the TCZ-SC mono group was 63.5% (95% confidence interval [95% CI] 56.0, 71.0) and in the TCZ-IV mono group was 67.3% (95% CI 59.9, 74.7). The ACR70 response rate in the TCZ-SC mono group was 37.1% (95% CI 29.6, 44.6) and in the TCZ-IV mono group was 41.0% (95% CI 33.3, 48.7). The weighed differences of ACR50 and ACR70 response were −4.3% (95% CI −14.7, 6.0) and −3.8% (95% CI −14.5, 6.8), respectively. **B**, Disease Activity Score in 28 joints using the erythrocyte sedimentation rate (DAS28-ESR), Clinical Disease Activity Index (CDAI), and Boolean Index remission rates at week 24 (in the PPS). The rate of DAS28-ESR remission (<2.6) in the TCZ-SC mono group was 49.7% (95% CI 41.9, 57.5) and in the TCZ-IV mono group was 62.2% (95% CI 54.6, 69.8). The rate of CDAI remission (CDAI score ≤2.8) in the TCZ-SC mono group was 16.4% (95% CI 10.6, 22.1) and in the TCZ-IV mono group was 23.1% (95% CI 16.5, 29.7). The Boolean Index remission rate in the TCZ-SC mono group was 15.7% (95% CI 10.1, 21.4) and in the TCZ-IV mono group was 16.0% (95% CI 10.3, 21.8). **C**, DAS28-ESR over 24 weeks. The mean ± SD change in DAS28-ESR from baseline to week 24 in the TCZ-SC mono group was 6.1 ± 0.9 to 2.8 ± 1.4 and in the TCZ-IV mono group was 6.2 ± 0.9 to 2.5 ± 1.1. **D**, CDAI scores over 24 weeks. Error bars show the SD of the mean. The mean ± SD change in CDAI score from baseline to week 24 in the TCZ-SC mono group was 34.2 ± 10.3 to 10.3 ± 9.5 and in the TCZ-IV mono group was 33.7 ± 10.8 to 8.2 ± 7.8.

Safety. The safety profiles were comparable between the TCZ-SC monotherapy and TCZ-IV monotherapy groups, with the exception of ISRs, which occurred at a higher frequency in the TCZ-SC monotherapy group than in the TCZ-IV monotherapy group. Over 24 weeks, AEs occurred in 89.0% (154 of 173) and 90.8% (157 of 173) of patients, serious AEs occurred in 7.5% (13 of 173) and 5.8% (10 of 173) of patients, adverse drug reactions occurred in 83.2% (144 of 173) and 86.1% (149 of 173) of patients, and serious adverse drug reactions occurred in 3.5% (6 of 173) and 5.8% (10 of 173) of patients in the TCZ-SC monotherapy and TCZ-IV monotherapy groups, respectively. No deaths or malignancies were reported.

Infections were reported in 41.6% of the TCZ-SC monotherapy group and in 45.1% of the TCZ-IV monotherapy group. Nasopharyngitis was the most common event, occurring in 17.9% of the TCZ-SC monotherapy group and in 20.8% of the TCZ-IV monotherapy group. Serious infections (Table 2) occurred in 1.2% of patients in the TCZ-SC monotherapy group and in 2.9% of patients in the TCZ-IV monotherapy group.

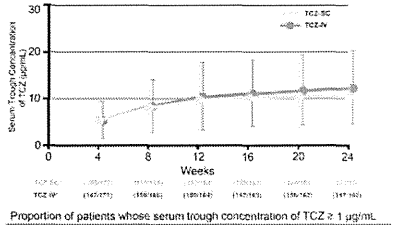
ISRs occurred in 12.1% of patients (21 of 173) in the TCZ-SC monotherapy group and in 5.2% of patients (9 of 173) in the TCZ-IV monotherapy group (placebo injection). The most common event was injection site erythema (16 patients [9.2%] in the TCZ-SC monotherapy group and 5 patients [2.9%] in the TCZ-IV monotherapy group). Other ISRs included injection site hemorrhage, pruritus, hematoma, swelling, pain, and urticaria (see Supplementary Table 1, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22110/abstract). All ISRs were mild, and no cases resulted in withdrawal from the study.

The incidence of SIRs from SC injection was 3.5% (6 of 173 patients) in the TCZ-SC monotherapy group, and the incidence of IV IRRs was 6.9% (12 of 173 patients) in the TCZ-IV monotherapy group. One patient (0.6%) in the TCZ-IV monotherapy group had an anaphylactic reaction after the second infusion (at week 4) and withdrew from the study; this patient tested negative for anti-TCZ antibodies and recovered without sequelae. No patients in the TCZ-SC monotherapy group experienced serious hypersensitivity, including anaphylactic reactions.

The proportion of patients who experienced elevations in lipid levels and liver function tests during the blinded period was similar between the TCZ-SC monotherapy and TCZ-IV monotherapy groups (Table 3). The proportion of patients who experienced a grade 3 decrease in neutrophils (<1,000 to 500 cells/mm^3) was 2.9% (5 of 173 patients) in each group; 1 patient in the TCZ-SC monotherapy group withdrew. No grade 4 neutropenia (<500 cells/mm^3) was reported.

The incidence of elevated serum levels of Krebs von den Lungen-6 (KL-6) that exceeded the upper limit of normal (500 units/ml) and reached ≥1.5 times the baseline value was 3.8% in the TCZ-SC monotherapy group and 1.9% in the TCZ-IV monotherapy group. The incidence of elevated serum levels of pulmonary surfactant protein D (SP-D) that exceeded the upper limit of normal (110 ng/ml) and reached ≥1.5 times the baseline value was 6.9% in the TCZ-SC monotherapy group and 6.2% in the TCZ-IV monotherapy group. Patients who experienced increased levels of KL-6 and SP-D did not have any events of interstitial lung disease.

The proportion of patients who tested positive for anti-TCZ antibodies in the screening and confirmation assays was 3.5% (6 of 173) in the TCZ-SC monotherapy group and 0% in the TCZ-IV monotherapy group. Five of the 6 patients tested positive for anti-TCZ antibodies before week 12. No patients who developed anti-TCZ antibodies experienced ISRs, SIRs, or lack of efficacy after developing anti-TCZ antibodies.



Proportion of patients whose serum trough concentration of TCZ ≥ 1 μg/mL

Week	4	8	12	16	20	24
TCZ-SC mono (%)	80.2	85.8	87.2	85.7	91.7	85.9
TCZ-IV mono (%)	73.1	83.3	86.6	84.7	90.1	89.4

*Numbers of patients whose serum TCZ concentrations were above the limit of detection (< 0.1 μg/ml) and were collected at each point

Figure 3. Mean serum trough tocilizumab (TCZ) concentrations over 24 weeks in patients receiving an intravenous infusion of TCZ monotherapy (TCZ-IV mono) or a subcutaneous injection of TCZ monotherapy (TCZ-SC mono). The table below the figure shows the proportion of patients in the TCZ-SC mono and TCZ-IV mono groups who had a serum trough TCZ concentration ≥1 μg/ml. At week 24, the mean ± SD serum trough TCZ concentration in the TCZ-SC mono group was 10.6 ± 7.8 μg/ml and in the TCZ-IV mono group was 12.4 ± 7.9 μg/ml.

Table 2. Summary of serious adverse events by patient*

SOC, preferred term	TCZ-SC monotherapy (n = 173)	TCZ-IV monotherapy (n = 173)
Infections and infestations		
Herpes zoster	–	2 (1.2)†
Pneumonia	–	2 (1.2)†
Cellulitis	1 (0.6)	1 (0.6)
Gastroenteritis	1 (0.6)	–
Gastrointestinal disorders		
Subileus	1 (0.6)†	–
Gastrointestinal hemorrhage	1 (0.6)	–
Ischemic colitis	–	1 (0.6)
Colonic polyp	1 (0.6)‡	–
Large intestine perforation	–	1 (0.6)
Vomiting	1 (0.6)†	–
Injury, poisoning, and procedural complications		
Spinal compression fracture	1 (0.6)‡	1 (0.6)†
Subdural hematoma	1 (0.6)†	–
Injury	1 (0.6)‡	–
Brain contusion	1 (0.6)†	–
Musculoskeletal and connective tissue disorders		
Synovitis	1 (0.6)‡	–
Spinal column stenosis	–	1 (0.6)†
Foot deformity	1 (0.6)‡	–
Respiratory, thoracic, and mediastinal disorders		
Pleurisy	–	1 (0.6)†
Chronic bronchitis	1 (0.6)‡	–
Asthma	1 (0.6)	–
Hepatobiliary disorders		
Hepatic function abnormal	–	1 (0.6)
Vascular disorders		
Hypertensive emergency	1 (0.6)†	–
Ear and labyrinth disorders		
Ménière disease	–	1 (0.6)
Nervous system disorders		
Intracranial hemorrhage	1 (0.6)†	
Metabolism and nutrition disorders		
Hyponatremia	1 (0.6)†	–
Immune system disorders		
Anaphylactic reaction	–	1 (0.6)
Benign, malignant, and unspecified neoplasms (including cysts and polyps)		
Neoplasm (benign)	1 (0.6)	–

* Values are the number (percentage). SOC = standard of care; TCZ-SC = subcutaneous tocilizumab; TCZ-IV = intravenous tocilizumab.
† Not related to the study drug. Occurred in the same patients, respectively.
‡ Not related to the study drug.

DISCUSSION

This noninferiority study was conducted to compare the efficacy of TCZ-SC monotherapy and TCZ-IV monotherapy in Japanese patients with RA who had inadequate responses to synthetic and/or biologic DMARDs. For the primary efficacy end point of ACR20 response rate at week

Table 3. Laboratory values*

	TCZ-SC monotherapy (n = 173)	TCZ-IV monotherapy (n = 173)
Shift in total cholesterol from baseline <200 mg/dl to worst value		
N	136	130
<200	39	37
200 to <240	65	58
≥240	32	35
Shift in HDL cholesterol from baseline <40 mg/dl to worst value		
N	29	14
<40	11	11
40 to <60	18	3
≥60	0	0
Shift in LDL cholesterol from baseline <100 mg/dl to worst value		
N	93	73
<100	17	17
100 to <130	51	44
130 to <160	24	8
160 to <190	1	4
≥190	0	0
Shift in ALT from normal at baseline to worst CTC grade		
N	164	165
Normal	124	124
Grade 1	35	32
Grade 2	4	7
Grade 3	1	2
Grade 4	0	0
Shift in AST from normal at baseline to worst CTC grade		
N	168	170
Normal	145	139
Grade 1	21	25
Grade 2	1	6
Grade 3	1	0
Grade 4	0	0
Shift in total bilirubin from normal at baseline to worst CTC grade		
N	173	172
Normal	149	154
Grade 1	21	13
Grade 2	3	5
Grade 3	0	0
Grade 4	0	0
Shift in neutrophils from normal at baseline to worst CTC grade		
N	170	172
Normal	130	125
Grade 1	19	20
Grade 2	16	22
Grade 3	5	5
Grade 4	0	0

* TCZ-SC = subcutaneous tocilizumab; TCZ-IV = intravenous tocilizumab; HDL = high-density lipoprotein; LDL = low-density lipoprotein; ALT = alanine aminotransferase; CTC = Common Terminology Criteria; AST = aspartate aminotransferase.

24, TCZ-SC monotherapy demonstrated noninferiority to TCZ-IV monotherapy in the PPS. The primary noninferiority analysis was made in the PPS, as recommended by the International Conference on Harmonisation E9 (21). To test the robustness of the noninferiority result, the results were validated by demonstrating the noninferiority of

TCZ-SC monotherapy to TCZ-IV monotherapy in the modified ITT population. From the results of secondary end points, the difference between TCZ-SC monotherapy and TCZ-IV monotherapy of ACR50 and ACR70 was smaller than ACR20. Furthermore, the mean change of the DAS28-ESR and CDAI score of TCZ-SC monotherapy was comparable to TCZ-IV monotherapy. These results support the noninferiority of TCZ-SC monotherapy to TCZ-IV monotherapy.

Two additional randomized, double-blind, phase III global studies (SUMMACTA and BREVACTA) evaluated TCZ-SC in combination with DMARDs in patients with RA from North America, Europe, South America, and Asia (other than Japan) (22,23). In the SUMMACTA study, TCZ-SC 162 mg every week was demonstrated to be noninferior to TCZ-IV 8 mg/kg every 4 weeks in combination with DMARDs using an ACR20 responder end point (noninferiority margin of 10%). The BREVACTA study demonstrated the superiority of TCZ-SC 162 mg every 2 weeks compared to placebo regarding the percentage of patients who achieved an ACR20 response at week 24. In both studies, the patients' mean body weight was 70–80 kg. In the MUSASHI study, TCZ-SC monotherapy dosing of every 2 weeks would be the most appropriate for Japanese patients with RA who have a lower body weight than patients in Western countries.

In Japan, the dose of TCZ-SC monotherapy of 162 mg every 2 weeks was selected from the previous phase I/II study with a mean body weight of 56 kg because it had a pharmacodynamic profile and TCZ trough concentration similar to those of the approved TCZ-IV dose of 8 mg/kg (15,24). In the current study, TCZ-SC monotherapy actually demonstrated TCZ trough concentrations comparable with those of TCZ-IV monotherapy despite a decrease in the given dose of TCZ in the TCZ-SC monotherapy group compared with the TCZ-IV monotherapy group if the weight is the same.

A previous TCZ-IV study reported that ≥1 μg/ml of serum TCZ was considered enough to suppress IL-6 signal transduction in the sera (19). In the current study, serum trough TCZ concentrations in the TCZ-SC monotherapy group were approximately equal to those in the TCZ-IV monotherapy group from week 4 onward, and most patients in both groups had TCZ concentrations ≥1 μg/ml. Prompt inhibition of IL-6 signaling by TCZ-SC monotherapy was also reflected in the time to improvement of disease activity, whereby the effectiveness of TCZ-SC monotherapy was approximately equal to that of TCZ-IV monotherapy from week 4 onward.

TCZ-SC monotherapy was administered as a fixed dose (162 mg), whereas the TCZ-IV monotherapy formulation was administered by body weight (8 mg/kg). In fact, trough TCZ concentrations tend to be lower in Japanese patients with a high body weight treated with TCZ-SC monotherapy (data not shown).

From the stepwise regression analyses, BMI in the fourth quartile at baseline was identified as a factor that contributed to low ACR response rates. However, more than half of patients in the fourth quartile of BMI achieved an ACR50 response. Therefore, it is unlikely that patients with high BMIs (23.4–29.6 kg/m^2) at baseline will have less response to therapy. With regard to the association between BMI and efficacy, further investigations are needed because the number of patients in the high BMI category was limited in this study. Previous use of anti-TNF agents was not identified as a factor that affected ACR response rates in the TCZ-SC monotherapy group. This suggests that the effect of TCZ-SC monotherapy on disease activity may be similar to that of TCZ-IV monotherapy in patients who have previously received anti-TNF agents.

Several studies have reported that TCZ as both monotherapy and in combination with DMARDs prevents joint destruction (4,6,9,23). The MMP-3 level in the TCZ-SC monotherapy group decreased at week 24 compared with baseline and was comparable with that in the TCZ-IV monotherapy group. Furthermore, the efficacy and serum TCZ trough concentrations were comparable between the TCZ-SC monotherapy and TCZ-IV monotherapy groups. These facts suggest that TCZ-SC monotherapy may also inhibit the progression of joint damage.

No new or unexpected safety issues were observed in this study. The safety profile of the TCZ-SC monotherapy group was similar to that of the TCZ-IV monotherapy group, except for ISRs. The incidence rate of ISRs was higher in the TCZ-SC monotherapy group than in the TCZ-IV monotherapy group. However, all events were mild and manageable. Although a direct comparison was difficult, the incidence of ISRs was not higher than that observed with other biologic agents that are administered by SC injection (10.4% with golimumab plus MTX and >30% with adalimumab monotherapy) (25,26). While the incidence rate of serious infection with TCZ-SC monotherapy was lower than with TCZ-IV monotherapy, there are not enough data to determine if this is a true difference. Additional data are being collected in the extension period. The serum levels of KL-6 and SP-D were reported to be elevated in patients with interstitial lung disease. The observed increase in serum KL-6 and SP-D levels was consistent with that in previous reports (27,28).

The number of patients who developed anti-TCZ antibodies was higher in the TCZ-SC monotherapy group than in the TCZ-IV monotherapy group. However, neither of these rates was numerically higher than the antidrug antibody rates reported for other biologic agents used to treat RA (29–32). None of the patients who tested positive for anti-TCZ antibodies experienced serious ISRs or hypersensitivity events, including anaphylaxis. The impact of anti-TCZ antibodies on efficacy was unclear because of the low number of patients who developed anti-TCZ antibodies. However, no patients who developed anti-TCZ antibodies experienced a lack of efficacy after developing anti-TCZ antibodies in this study.

The current study assessed the efficacy and safety of TCZ monotherapy without concomitant DMARDs. However, TCZ in combination with MTX was more commonly associated with elevated transaminases (9), and although the data on combination therapy with TCZ-SC are not yet available, the same effect is likely to be seen. Studies are currently ongoing to evaluate TCZ-SC in combination with DMARDs (22,23).

An SC formulation of TCZ would greatly shorten the administration time compared with the IV formulation

and would allow for home administration. Moreover, it would shorten the time and effort involved in the preparation of TCZ prior to administration and therefore would be more convenient for both patients with RA and health care professionals.

In summary, the noninferiority of TCZ-SC monotherapy to TCZ-IV monotherapy was confirmed. TCZ-SC monotherapy provided efficacy, safety, and serum trough concentrations of TCZ that were comparable with those of TCZ-IV monotherapy. The use of TCZ-SC monotherapy would provide an additional administration option for patients with RA.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Ogata had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Ogata.

Acquisition of data. Ogata, Tanimura, Sugimoto, Inoue, Urata, Matsubara, Kondo, Ueki, Iwahashi, Tohma, Ohta, Saeki, Tanaka.

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CASE REPORT

Expansion of range of joint motion following treatment of systemic sclerosis with tocilizumab

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Abstract *Systemic sclerosis (SSc) presents stiffness of* extremities due to sclerosis of the tissue especially at fingers, hands, and forearms. Here we report the case of a patient with diffuse cutaneous SSc who was administered anti-interleukin-6 receptor antibody tocilizumab (TCZ). Skin condition of SSc is evaluated by pinching the skin according to the Rodnan skin score, but sometimes tissue atrophy results in overestimation of the condition. To understand how the extremities softened after initiation of TCZ, we observed mobility of extremities. Range of motion (ROM) of joints was measured every four months after initiation of TCZ. The patient presented not only reduction of Rodnan score but also amelioration of mobility of extremities. The Rodnan skin score reduced from 35 to 7 within sixteen months, and ROM of most joints except ankle was expanded.

Keywords *Systemic sclerosis · Tocilizumab · Range of* joint motion

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Introduction

Systemic sclerosis (SSc) is a connective tissue disease that develops sclerotic changes in the skin and visceral organs. Patients present with stiffness of the limbs because of sclerosis in the skin and periarticular connective tissues. We present the case of a patient with SSc who showed improvement of joint motion after treatment with the anti-interleukin-6 (IL-6) receptor antibody tocilizumab (TCZ).

Although the etiology of SSc remains unclear, many factors have been proposed. IL-6 is a pleiotropic factor that plays a major role in inflammation; furthermore, it is a candidate factor that can reproduce the pathological conditions of SSc. Reportedly, culture supernatants of skin tissue or peripheral blood mononuclear cells from patients with SSc contain higher concentrations of IL-6 than those from normal controls [1, 2]. Elevation of serum IL-6 levels has also been reported, and these levels are reported to depend on skin score [3–5]. In addition, an anti-IL-6 antibody has been reported to suppress procollagen production by fibroblasts isolated from patients with SSc [6]. Given these facts, it is suggested that anti-IL-6 therapy may ameliorate the clinical symptoms of SSc. We have previously reported conventional therapy-resistant SSc cases that responded well to TCZ [7]. In our former study, two patients who were administered TCZ for six months showed a decrease in their modified Rodnan total skin (mRTS) scores, suggesting that their skin sclerosis could have been ameliorated by TCZ administration. However, the skin score in patients with SSc sometimes decreases spontaneously as a result of tissue atrophy. Therefore, it is necessary to examine not only the skin score but also the function of the extremities. In this case, we evaluated the range of motion (ROM) of joints before and after TCZ administration to investigate the effects of TCZ on mobility of extremities in a patient with SSc.

Case report

A 59-year-old woman noticed Raynaud's phenomenon and swellings in her fingers in 2004. This skin sclerosis developed from her fingers and expanded to her face and feet. She became aware of dyspnea on exertion, dysphagia, and stiffness of the hands, wrists, elbows, and shoulders. Although both anti-Scl-70 and anti-centromere antibodies were negative, she was diagnosed with SSc because skin biopsy revealed thick and tight collagen fiber bundles in the dermis. Antinuclear antibody was positive at a titer of 1:1280 with a speckled pattern. Closer examination revealed that anti-RNA polymerase III antibody was positive. Treatment was initiated with prednisolone at 10 mg/day, and cyclosporine was then added to this regimen, which was otherwise ineffective. In 2005, the patient developed subacute renal failure and hypertension; therefore, dialysis therapy was indicated. After temocapril at dosage of 4 mg/day and telmisartan at dosage of 80 mg/day were administered, her condition stabilized and hemodialysis was terminated. In 2007, the patient exhibited recurrent dyspnea on exertion and inadequate oral intake as a result of recurrent ileus with pneumatosis cystoides intestinalis. For a while, home parenteral nutrition was used; however, prolonged administration of antibiotics proved effective only to a limited extent. Though endoscopic examination showed normal esophageal mucosa, measurement of esophageal pressure indicated absence of peristaltic waves during swallowing. Chest computed tomography (CT) images detected no significant interstitial modification, however echocardiogram revealed pericardial effusion and elevated peak pressure gradient of tricuspid regurgitation (28 mmHg). Since right heart catheterization showed elevation of mean pulmonary artery pressure (25 mmHg), treatment with 125 mg/day bosentan was initiated. Her visceral organs became involved as described; furthermore, the skin sclerosis spread to her trunk. Her mRTS score was 35 in 2008. The patient had to use a wheelchair to move about, and she was unable to propel it by herself. Because her activities of daily living (ADL) were severely compromised because of skin sclerosis, we applied for a TCZ project which was supported by the National Institute of Biomedical Innovation (Ibaraki City, Osaka, Japan). After receiving informed consent by the patient and approval by the Ethics Committee of Osaka University Hospital, we initiated TCZ treatment. Laboratory data at TCZ initiation are presented in Table 1. The administration dosage and schedule of TCZ was 8 mg/kg every four weeks, which corresponds to the regimen used for rheumatoid arthritis. The following medications were administered concurrently: methylprednisolone (8 mg/day), telmisartan (40 mg/day), furosemide (80 mg/day), beraprost (120 μg/day), omeprazole (20 mg/day), cefdinir (300 mg/day), and bosentan (125 mg/day). ROM of the metacarpophalangeal joints of the hands as well as that of the wrist, elbow, shoulder, knee, and ankle joints was measured every 4 months using a goniometer.

ROM of the knee, wrist, and shoulder joints after TCZ initiation are shown in Fig. 1. ROM values, except for those in ankles, improved during the observation period. Skin sclerosis also improved over the course of treatment, and the patient's mRTS score decreased from 35 to 7. She could walk independently once again. In patients with SSc, problems concerning joint motion may result from sclerotic changes in the skin and subcutaneous tissue. In this case, the patient's knee, wrist, and shoulder joints, which were drastically affected, showed tendencies toward an inverse

Table 1 Laboratory data before TCZ therapy initiation

Blood cell count			Urine test			Biochemical data		
White blood cells	(3300–9400)	5660/μL	pH	(5.0–8.0)	5.0	Creatinine	(0.5–0.9)	2.09 mg/dL
Red blood cells	(390–510 × 10^4)	383 × 10^4/μL	Urine gravity	(1.005–1.030)	1.008	Aspartate aminotransferase	(<40)	19 IU/L
Hemoglobin	(12.0–15.0)	9.9 g/dL	Protein	(−)	−	Alanine aminotransferase	(<40)	11 IU/L
Hematocrit	(35.0–45.0)	30.6 %	Sugar	(−)	−	Gamma glutamyl transpeptidase	(8–51)	12 IU/L
Mean corpuscular volume	(84.0–98.0)	79.8 fL	Urobilinogen	(+/−)	+/−	Lactate dehydrogenase	(103–229)	241 IU/L
Mean corpuscular hemoglobin	(28.0–33.0)	25.8 pg	Bilirubin	(−)	−	Amylase	(44–153)	212 IU/L
Mean corpuscular hemoglobin concentration	(31.0–35.0)	32.3 %	Ketone	(−)	−	Creatinine kinase	(54–286)	11 IU/L
			Occult blood	(−)	−	Cholesterol	(150–220)	177 mg/dL
Platelet	(130–320 × 10^3)	187 × 10^3/μL				Albumin	(3.6–4.7)	3.5 g/dL
						C-reactive protein	(0.0–0.2)	0.13 mg/dL

Values in parentheses indicate normal limits at our hospital

relationship with the skin scores of the areas adjacent to the joints (Fig. 2a–c). In contrast, although the skin scores of the lower legs and dorsum of the feet improved, ROM of the ankle joint remained unchanged (Fig. 2d).

Discussion

This case report describes a patient with SSc who showed impaired mobility in addition to severe skin sclerosis. TCZ administration proved beneficial for the skin sclerosis, as described previously [7]. However, patients with SSc sometimes show improvements in their poor skin scores because of skin atrophy, considering that the Rodnan skin score is obtained by pinching the skin. In our patient, however, we observed an improvement in mobility of the limbs as well as the skin score. The patient regained the ability to walk independently, proving that the improved skin score not only represented the ease of pinching the skin but also functional improvement.

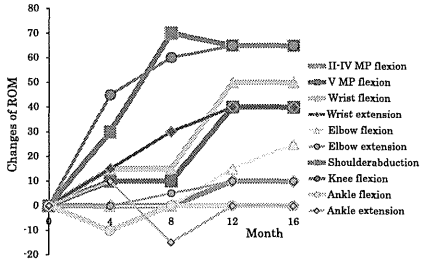

Fig. 1 Impact of tocilizumab (TCZ) on joint mobility in a patient with SSc. Joint range of motion (ROM) was initially set to zero, and data are expressed as the degree of improvement during the 16-month TCZ therapy. The *horizontal axis* indicates months after TCZ initiation. All ROM values, except for those in the ankles, improved considerably after 4 months and continued to improve until the end of study. The *unit* of angle is degrees

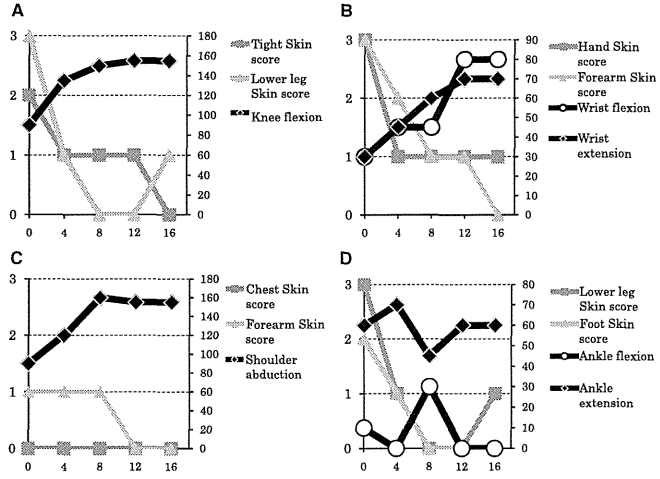

Fig. 2 Relationship between joint mobility and skin sclerosis in a SSc patient during TCZ treatment. The *left vertical axis* indicates the modified Rodnan total skin score, and the *right vertical axis* indicates ROM value. The *horizontal axis indicates months after* TCZ initiation. The skin scores for the knee (**a**), wrist (**b**), and shoulder (**c**) joints decreased as the ROM increased. In contrast, the ROM of the ankle joint did not improve, even though the skin scores of the lower leg and foot decreased to 0 after 8 months (**d**)

Several factors may contribute to the improvement in ROM during TCZ treatment in the present case. First, TCZ might act on arthritis. TCZ is a recognized medication for arthritis; therefore, it may improve joint movement through amelioration of joint inflammation. The patient, however, showed neither swelling nor tenderness of the joints before and during TCZ treatment. In addition, her C-reactive protein level was normal. Therefore, the observed improvement in ROM was probably not because of arthritis remission. Second, the ROM improvements might be the natural course of the disease or might indicate atrophic change. Four years passed between the onset of disease and initiation of TCZ treatment, and disability of limbs was worsening during this period. The fact that ROM improvement in the knee and shoulder joints was detected within the first 4 months of TCZ treatment gives an impression of the effect of this medicine. However, this possibility is remaining because there was no serial scoring data before TCZ treatment. Third, concomitant medicines might effect ROM improvement. Methylprednisolone and bosentan were used as concomitant medicines. Bosentan in particular might contribute to reduction of skin score, because bosentan has protective efficacy for skin ulcer in SSc [8]. The possibility that bosentan acts to improve ROM might remain, but there is no report which presents an effect of bosentan on ROM improvement. The reason why the ROM of the ankle joints remained unchanged is unclear. There may be a relationship with long-term wheelchair use. This patient also had kidney, heart, and bowel involvement, and it is unclear how they were affected by TCZ administration. Although she has remained free from dialysis or home parenteral nutrition to date, she continues to require angiotensin receptor blockers, proton pump inhibitors, diuretics, and antibiotics. There is a possibility that the internal organ symptoms are being affected by these medications. TCZ administration, however, clearly resulted in improvement in the skin score for this case as well as former reported cases [7], and in this case, it was clear that the skin score decrement after TCZ initiation was not because the skin became easy to pinch but because the tissue was becoming soft and easy to move.

There is currently no standard pharmacological guideline for treatment of SSc, despite numerous clinical trials on steroids, antirheumatic drugs, and immunosuppressive agents. While an effective low-dose corticosteroid therapy with prednisolone has been proposed for early-phase diffuse cutaneous SSc [9], patients are at risk of developing sclerodermal renal crisis [10]. The effectiveness of penicillamine in SSc treatment remains controversial [11]. On the other hand, the European League Against Rheumatism recommends methotrexate for treatment of skin sclerosis in patients with early diffuse SSc [12], but an opposing view was also presented [13]. Other immunosuppressive agents such as cyclophosphamide, cyclosporine A, tacrolimus, and mycophenolate mofetil have been evaluated for treatment of SSc. Though the beneficial effects of one-year oral administration of cyclophosphamide on skin thickening have been reported [14], the long-term safety of this medicine has not been verified. There are no data which present late-occurring toxicities of cyclophosphamide in patients with SSc, but there are several reports which present oncogenicity after withdrawal of this medicine in patients with systemic lupus erythematosus and rheumatoid arthritis [15]. The usefulness of cyclosporine is also controversial because of the associated risk of sclerodermal renal crisis [16, 17]. The effectiveness of mycophenolate mofetil as an immunosuppressive agent in SSc treatment also remains inconclusive [18, 19], though a recent study presented beneficial effects in patients with recent-onset SSc [20]. Finally, several biologic agents are currently being evaluated for treatment of skin involvement in SSc, of which only rituximab has shown efficacy [21, 22]. Therefore, effective treatment for this disease is an ongoing challenge.

The effect of TCZ on skin sclerosis, pneumonitis, or the other symptoms in patients with SSc remains unclear, and further studies are required to verify this. The efficacy of TCZ for patients with SSc is currently being evaluated in an open-label trial in Japan (UMIN0000055550) and a double-blind trial in Europe and North America (NCT01532869); the results of these trials should provide further information on unresolved issues.

In this report, we described time-course changes of ROM observed in a patient with SSc during treatment with TCZ. The relation between TCZ treatment and ROM changes observed in the patient is currently unclear.

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Conflict of interest T. Kishimoto holds a patent for TCZ, and receives royalties for ACTEMRA®. A. Ogata received a consulting fee from Chugai Pharmaceutical Co. Ltd. for providing medical advice. Other authors have no conflict of interest to declare.

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Tocilizumab Increases Serum Adiponectin and Reduces Serum Fatty Acid Binding Protein 4 in Patients With Rheumatoid Arthritis

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Abstract

Background: Recently tocilizumab, a humanized anti-interleukin-6 receptor antibody (IL-6R Ab), was clinically demonstrated to ameliorate metabolic syndrome. However, it is unknown whether blocking the IL-6R with tocilizumab directly impacts the adiponectin and fatty acid-binding protein 4 (FABP4) levels.

Methods: In this study, we measured the serum adiponectin and FABP4 levels in 18 patients with rheumatoid arthritis (RA) 3 months after treatment with tocilizumab.

Results: Our study revealed that treatment with tocilizumab decreased serum FABP4 levels and increased serum adiponectin levels in patients with RA. We also assessed the production of adipocytokines stimulated by IL-6R Ab using adipocyte precursors obtained from human fat tissue. Tocilizumab did not increase local adiponectin levels; however, the suppression of adiponectin secretion by IL-6 was completely abolished. Tocilizumab also directly suppressed FABP4 in human adipocytes.

Conclusion: This suggests that treatment with tocilizumab may be a novel approach to coordinately regulate adiponectin and FABP4 levels.

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Keywords: Rheumatoid arthritis; IL-6; Adiponectin; FABP4; Tocilizumab

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects approximately 1% of the general population and is associated with increased mortality, predominantly as a result of increased risk of cardiovascular disease (CVD) [1, 2]. Although the awareness of increased cardiovascular risk in patients with inflammatory diseases is increasing, the traditional risk factors for CVD in some patients remain suboptimally managed [3]. Recently, it was suggested that adipose tissue plays a role in chronic inflammatory diseases. It synthesizes and releases highly bioactive substances, including classical adipokines (such as leptin and adiponectin), and various pro-inflammatory cytokines (including tumor necrosis factor-α and interleukin-6 (IL-6)), which are collectively termed adipo(cyto)kines [4]. However, its ability to synthesize pro-inflammatory cytokines is not well understood.

Fatty acid-binding protein 4 (FABP4, also designated aP2 or adipocyte FABP) is expressed in adipocytes and other tissues and integrates inflammatory and metabolic responses [5, 6]. The expression of both FABP4 and adiponectin is regulated by peroxisome proliferator-activated receptor (PPAR)-γ [7]. However, the two proteins are differentially regulated because higher serum FABP4 levels [8, 9] and lower serum adiponectin levels [10, 11] have recently been found to be associated with metabolic syndrome (MetS) and CVD. Therefore, accumulating evidence suggests that the adipokine levels may act as biomarkers to dictate drug or dietary treatment strategies.

Tocilizumab, a humanized anti-IL-6 receptor antibody that blocks IL-6 signaling, is a novel therapeutic strategy for various autoimmune and inflammatory diseases, such as RA, Castleman's disease, and juvenile idiopathic arthritis [12]. Although tocilizumab increased plasma adiponectin levels in patients with RA [13], the relationship between IL-6 and FABP4 is still controversial [14, 15], and the effects of tocilizumab on FABP4 are unknown.

The aim of the present study was to evaluate whether treatment with tocilizumab leads to changes in serum adipokine levels in patients with RA.

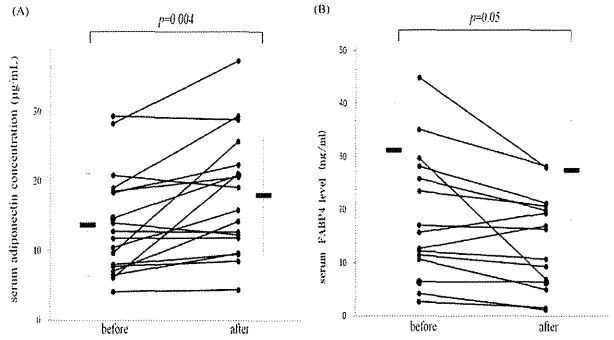

Figure 1. Treatment with tocilizumab increased serum adiponectin (A) and decreased fatty acid-binding protein 4 levels (B) in patients with RA. Bars represent mean ± SEM.

Materials and Methods

Patients

From July through December 2008, 16 patients (two males and 14 females) with active RA starting tocilizumab treatment were enrolled in this study between July and December 2008 at Osaka University Hospital. All patients had no history of medication use associated with PPAR-γ agonist (e.g., hypertension and diabetes) before enrolling in this study. They received a fixed dose of tocilizumab (8 mg/kg) in a single 1-h infusion every 4 weeks. Sixteen patients (89%) were also treated with oral prednisolone. Patient symptoms were assessed by disease activity score (DAS)-28, based on 28-joint counts for swelling and tenderness, tender joint count, and patient assessment. Serum FABP4 levels were measured using a human adipocyte FABP ELISA (BioVendor, Modrice, Czech Republic), and adiponectin levels were measured using an ELISA kit (Otsuka Pharmaceutical, Tokushima, Japan).

This study conformed to the Clinical Research Guideline of Osaka University Hospital and was approved by the institutional ethics committee. We obtained written informed consent to participate in this study from all patients.

Adipocyte culture and effects of tocilizumab on adipocytokine production in differentiated adipocytes

Preadipocytes were isolated from patients undergoing elective surgery who gave informed consent, as previously described [16]. Cells were differentiated into adipocytes by incubation in adipocyte differentiation medium (DM-2, Zen-bio®) at 37 °C with 5% CO_2. After 8 days, cells were plated in fresh adipocyte medium (AM-1, Zen-bio®) and treated with drug-containing medium every 48 h. The effects of tocilizumab and 10 μmol/L pioglitazone hydrochloride on adiponectin and FABP4 secretion were analyzed on day 12 after 48 - 96 h of treatment.

Immunoblotting of FABP4

Human FABP4 expression was assessed by western blotting. In brief, lysates from human adipocytes were collected and separated on 16% SDS-PAGE gels, and transferred to PVDF membranes. Membranes were blocked for 1 h at room temperature in blocking buffer (5% skim milk in 10 mmol/L Tris, 100 mmol/L NaCl, 0.1% Tween 20, pH 7.5), and then incubated with anti-FABP4 antibody (A-FABP C-15, Santa Cruz) at a dilution of 1:1,000 for 1 h at room temperature. After washing (3 × 5 min in 1 × TBS-0.05% Tween), membranes were incubated with secondary anti-mouse (Dako) or anti-goat (Wako) antibodies conjugated to horseradish peroxidase. The immune complexes were detected using ECL Advanced Western Blot Detection System (GE Healthcare, Buckinghamshire, UK).

Statistical analysis

All results are presented as mean ± SEM. The differences in CRP, DAS-28, and serum adipokine levels before and after tocilizumab treatment were analyzed using paired *t*-test. Student's *t*-test was used to compare the differences between the control and treatment groups. Values of $P < 0.05$ were considered to be statistically significant.

Results

The body weights of the patients were unchanged during the

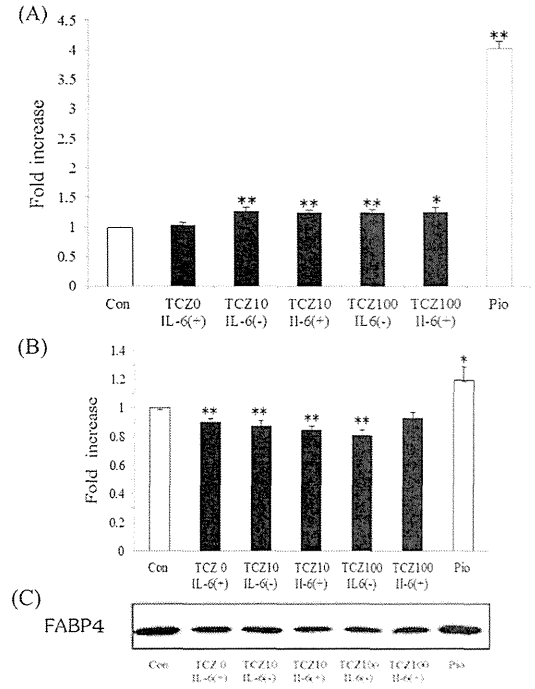

Figure 2. (A) Effects of interleukin-6 (IL-6) and tocilizumab on adiponectin production in human adipocytes. Cells were differentiated for 12 days, and then treated without (control) or with 10 μg/mL IL-6 antibody, and/or 100 μg/mL, or 10 mg/mL tocilizumab. Data are presented as means ± SEM of at least four independent experiments. *P < 0.05; **P < 0.01 vs. control. Pio: pioglitazone. (B) Effects of interleukin-6 (IL-6) and tocilizumab on fatty acid-binding protein 4 levels in human adipocytes. Cells were differentiated for 12 days and treated without (control) or with 10 μg/mL IL-6 antibody, and/or 100 μg/mL, 10 mg/ml or tocilizumab. Data are expressed as means ± SEM of at least four independent experiments.*P < 0.05; **P < 0.01 vs. control. Pio: pioglitazone. (C) A representative western blot of fatty acid-binding protein 4 expression in human adipocytes treated without (control) or with 10 μg/mL interleukin-6 antibody, and/or 100 μg/mL, or 10 mg/mL tocilizumab. Pio: pioglitazone.

study. Treatment with tocilizumab for 3 months significantly suppressed the inflammatory process, demonstrated by a decrease in mean serum CRP levels from 1.8 ± 0.4 to 0.2 ± 0.1 mg/dL ($P < 0.01$). Levels of DAS-28 also improved significantly, from 3.9 ± 0.3 to 1.8 ± 0.4 ($P < 0.01$). The baseline serum adiponectin concentrations were 13.7 ± 1.7 μg/mL, which increased significantly to 18.0 ± 2.1 μg/mL ($P < 0.01$, Fig. 1A) after treatment with tocilizumab for 3 months. In contrast, baseline FABP4 concentrations were 31.3 ± 9.0 ng/mL, which decreased to 27.4 ± 9.4 ng/mL after treatment ($P = 0.05$, Fig. 1B). Although previous reports suggested that treatment with steroids significantly decreased serum adiponectin levels [17], oral prednisolone did not affect serum FABP4 or adiponectin levels in our study (data not shown).

Next, the effect of IL-6 and tocilizumab on adiponectin and FABP4 was assessed in human adipocytes. After treatment of differentiated adipocytes with 100 μg/mL tocilizumab, adiponectin secretion was increased approximately 1.25 ± 0.04-fold ($P < 0.01$) compared with control. Tocilizumab treatment enhances adiponectin secretion by suppressing IL-6 (Fig. 2A). In contrast, FABP4 expression was decreased approximately 0.85 ± 0.03-fold ($P < 0.01$) in cells treated with tocilizumab compared with control. However, pioglitazone substantially increased FABP4 expression approximately 1.19 ± 0.10-fold

(Fig. 2B, C).

Discussion

In this study, we found for the first time that treatment with tocilizumab, an IL-6R Ab, for 3 months reduced serum FABP4 levels in patients with RA. Both adiponectin and FABP4 are downstream targets of PPAR-γ in adipocytes. PPAR agonists such as telmisartan and thiazolidines induce the secretion of adiponectin [16] and simultaneously increase serum FABP4 levels [18, 19]. A previous study demonstrated olmesartan, which has little PPAR-γ activity, decreased serum FABP4 in patients with hypertension [20], while little is known about the effect of medication on FABP4 levels.

FABP4 promotes atherosclerotic diseases by acting on macrophages [21]. FABP4-deficient macrophages display defects in cholesterol accumulation and decreased pro-inflammatory cytokines TNFα, IL-6, and MCP-1 levels by reducing IκB kinase and NF-κB activity [22]. Although FABP4 is a circulating protein, the mechanism by which it enters the circulation is unknown. In both cross-sectional and prospective studies, serum FABP4 levels were positively correlated with lipid profiles, hyperglycemia, and non-alcoholic fatty liver diseases [23]. In addition, a 12-year community-based cohort study in a Chinese population indicated that plasma FABP4 levels were a strong predictor of CVD [24]. Because elevated FABP4 levels are a risk factor for CVD in patients with end-stage renal disease [8] and are correlated with numerous metabolic syndrome symptoms [9], the up-regulation of FABP4 may induce unfavorable side effects in patients treated with PPAR agonists.

Tocilizumab, a monoclonal antibody that blocks both membrane-bound and circulating IL-6R has anti-inflammatory actions that extend beyond reducing the concentrations of C-reactive protein and fibrinogen [25]. Patients with chronic inflammatory diseases, such as RA, are at increased risk of developing CVD [3], which is in part caused by increased IL-6 levels. Although further studies are required to confirm that these effects are also mediated by factors including macrophages and IL-6 *in vivo*, the present results suggest that changes in adiponectin and FABP4 levels reflect metabolic defects in adipose tissue and thus may be a useful biomarker of CVD in patients with RA.

Tocilizumab was identified as an agent that may help prevent coronary heart disease [26]. Because the suppression of adiponectin gene expression by IL-6 is mediated in part by p44/42 MAP kinase [27], the inhibition of this signaling pathway by tocilizumab may induce adiponectin secretion. The concurrent decrease in FABP4 levels and increase in adiponectin levels induced by tocilizumab may help prevent CVD and metabolic syndrome. However, additional studies are required to define the mechanism behind the differential effects of tocilizumab on FABP4 and adiponectin expression. For example, tocilizumab may act as a selective PPAR gamma modulator (SPPARM) [28] to reduce oxidative stress [29] or improve hypoxia [30]. However, SPPARM exhibited limited effects on FABP4 gene expression in mature 3T3-L1 adipocytes [28] and inhibited the differentiation of human preadipocytes compared with PPAR-γ agonists. SPPARM also displayed a diminished ability to induce FABP4 mRNA expression compared with rosiglitazone [29], whereas in human trophoblasts cultured under hypoxic conditions, the expression of FABP4 was enhanced [30].

In conclusion, tocilizumab treatment decreases FABP4 levels in patients with RA and could provide a novel therapeutic approach to prevent CVD.

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