14. Obach RS, Reed-Hagen AE, Krueger SS, et al. Metabolism and disposition of varenicline, a selective alpha4beta2 acetylcholine receptor partial agonist, in vivo and in vitro. *Drug Metab Dispos.* 2005;34:121-130.

15. Feng B, Obach RS, Burstein AH, Clark DJ, de Morais SM, Faessel HM. Effect of human renal cationic transporter inhibition on the pharmacokinetics of varenicline, a new therapy for smoking cessation: an in vitro-in vivo study. *Clin Pharmacol Ther.* 2008;83:567-576.

16. Burstein AH, Fullerton T, Clark DJ, Faessel HM. Pharmacokinetics, safety and tolerability following single and multiple oral doses of varenicline in elderly smokers. *J Clin Pharmacol.* 2006;46:1234-1240.

17. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron.* 1976;16:31-41.

18. Gough K, Hutchison M, Keene O, et al. Assessment of dose proportionality: report from the statisticians in the Pharmaceutical Industry/Pharmacokinetics UK Joint Working Party. *Drug Inf J.* 1995;29:1039-1048.

19. Ravva P, Gastonguay MR, Tensfeldt TG, Faessel HM. Population pharmacokinetic analysis of varenicline in adult smokers. *Br J Clin Pharmacol.* 2009;68:669-81.

INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Elevated Serum Levels of Resistin, Leptin, and Adiponectin are Associated with C-reactive Protein and also Other Clinical Conditions in Rheumatoid Arthritis

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Abstract

Objective Body fat is an important source of hormones and cytokines (adipokines) that not only regulate the energy balance, but also regulate the inflammatory and immune responses. This study investigated the association of clinical conditions with serum levels of adipokines in patients with rheumatoid arthritis.

Methods Serum levels of resistin, leptin, and adiponectin were measured by enzyme-linked immunosorbent assay in 141 patients (110 women) who fulfilled the 1987 revised criteria of the American Rheumatism Association for the diagnosis of rheumatoid arthritis and in 146 normal controls (124 women). Then the correlations between adipokine levels and clinical parameters were evaluated.

Results The serum resistin level did not differ between the patients and controls. However, serum leptin levels were significantly higher in male and female rheumatoid arthritis patients than in the corresponding controls, while the serum adiponectin level was significantly higher in female patients than in female controls. Multivariate analysis revealed that predictors of an elevated resistin level were female sex and C-reactive protein (CRP), while the leptin level was related to the body mass index and CRP. Predictors of an elevated adiponectin level were the use of prednisolone and CRP, however, CRP was negatively associated with adiponectin in patients with rheumatoid arthritis.

Conclusion The serum levels of resistin and leptin were positively associated with CRP level in patients with rheumatoid arthritis, suggesting that these adipokines may act as pro-inflammatory cytokines in this disease. The serum adiponectin level was elevated in the patients, however, it was negatively associated with CRP level. In addition, the serum levels of resistin, leptin, and adiponectin were also associated with female sex, BMI and the use of prednisolone, respectively.

Key words: rheumatoid arthritis, resistin, leptin, adiponectin, C-reactive protein

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Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease that is characterized by symmetrical synovitis, progressive joint damage, pain, fatigue, and disability. Although the exact cause of this disease is still unknown, investigation of its pathogenesis has confirmed a role for various pro-inflammatory cytokines, including tumor necrosis factor-α (TNFα), interleukin-1 (IL-1), and interleukin-6 (IL-6) (1-3). Accordingly, inhibition of these cytokines has become the new therapeutic strategy for RA.

Recent studies have demonstrated that cytokines secreted by adipocytes (adipokines) have an important physiological role. Adipokines, including resistin, leptin, and adiponectin, have been demonstrated to influence eating behavior and the energy balance, and have also been noted as new mediators of the inflammatory process (4, 5). Recently, we reported

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Table 1. Demographic Profile of the Patients with Rheumatoid Arthritis and Control Subjects

	Sex	RA patients (n=141)	Control subjects (n=146)	p value
Male : Female		31:110	22:124	
Age (years)	M	61.0 ± 12.7	45.6 ± 13.8	<0.001
	F	59.0 ± 14.0	57.5 ± 16.6	0.456
Height (cm)	M	166.8 ± 6.1	170.0 ± 6.2	0.069
	F	154.4 ± 6.6	156.0 ± 6.0	0.091
Weight (kg)	M	64.7 ± 11.1	64.5 ± 9.9	0.952
	F	52.9 ± 9.3	52.8 ± 7.0	0.956
BMI (kg/m^2)	M	23.2 ± 3.2	22.3 ± 2.8	0.29
	F	22.2 ± 3.8	22.2 ± 3.0	0.932
Rheumatoid factor positive, %	M	80.6	–	–
	F	89.1	–	–
Duration of RA (years)	M	7.8 ± 8.6	–	–
	F	11.4 ± 8.9	–	–
DAS28-ESR	M	3.4 ± 1.9	–	–
	F	3.8 ± 1.4	–	–
Stage of RA (I:II:III:IV)	M	12:5:6:8	–	–
	F	11:25:16:58	–	–
CRP (mg/L)	M	10.4 ± 10.5	–	–
	F	8.2 ± 14.4	–	–
ESR (mm/h)	M	23.7 ± 22.2	–	–
	F	33.6 ± 24.9		

Data are shown as the mean±SD; M, Male; F, Female; RA, rheumatoid arthritis; BMI, body mass index; DAS, disease activity score; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

Table 2. Current Medications in Patients with Rheumatoid Arthritis

Current medications	Male (n=31)			Female (n=110)		
	n	%	dosage	n	%	dosage
Prednisolone (mg/day)	10	32.3	4.4±2.6	52	47.2	5.2±2.1
Methotrexate (mg/week)	17	54.8	8.0±2.6	67	60.9	8.0±2.3
other DMARDs						
Salazosulfapyridine (g/day)	17	54.8	1.0±0.3	41	37.3	1.0±0.3
Bucillamine (mg/day)	11	35.5	132±64	19	17.3	192±42
Biological agents						
Infliximab (mg/kg/2 months)	1	3.2	3	8	7.3	3.7 ± 0.8
Etanercept (mg/week)	2	6.5	50	13	11.8	44 ± 11

Data are shown as the mean±SD; n, number of samples; DMARDs, disease modifying anti-rheumatic drugs

that adiponectin stimulates the production of IL-8 (6) and prostaglandin E_2 (7) by rheumatoid synovial fibroblasts. These findings suggest that adipokines may contribute to synovial inflammation in RA.

In the present study, we measured the serum concentrations of 3 adipokines (resistin, leptin, and adiponectin) in Japanese patients with RA and in normal controls to further investigate the role of these molecules in the pathogenesis of this disease.

Methods

Subjects

One hundred and forty-one patients with RA diagnosed according to 1987 revised criteria of the American Rheumatism Association (8) were enrolled in this study, and 146 healthy persons were also enrolled as controls. The demographic characteristics of the RA patients and the controls are shown separately for males and females in Table 1. Clinical features of the male and female RA patients are also shown in Table 1. The body mass index (BMI) was calculated as [body weight/height^2] (kg/m^2). Demographic characteristics did not differ between the RA group and the control group, except for the mean age of the males. Medications in the RA patients are shown in Table 2.

Disease activity score 28 (DAS28) was calculated with the following equation (9): DAS28 = 0.56 × $\sqrt{}$28TJC + 0.28 × $\sqrt{}$28SJC + 0.7 × ln ESR + 0.014 × GH, where 28TJC and 28SJC are the tender joint count and swollen

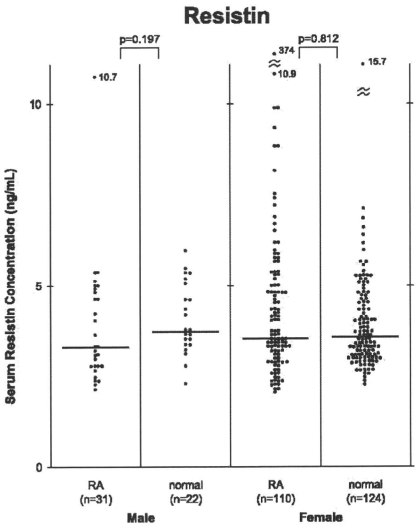

Figure 1. Serum resistin levels in RA patients and control subjects. Horizontal bars indicate median values. Statistical significance was determined by Mann-Whitney test.

joint count from 28 joints and general health (GH) is the patient's global assessment on a 100-mm visual analog scale (VAS).

This study was approved by the Ethical Committees of Toho University and Kitasato University. The RA patients and normal controls were recruited at Toho University Omori Hospital and the Research Center for Clinical Pharmacology of Kitasato University, respectively. Informed consent was obtained from both the patients and the normal controls. In all subjects, a blood sample was collected in the morning after an overnight fast. We did not provide any special dietary management information to the patients or normal controls.

Measurement of adipokines and other laboratory parameters

The serum concentrations of resistin, leptin, and adiponectin were measured by enzyme-linked immunosorbent assay (ELISA). Resistin and leptin ELISA kits were purchased from B-Bridge International, Inc. (Sunnyvale, CA, USA), while the kit for adiponectin was obtained from R&D Systems, Inc. (Minneapolis, MN, USA). Samples were prepared at the appropriate dilutions and paired samples were assayed together according to the instructions of the manufacturers. The intra- and inter-assay coefficients of variation for resistin, leptin, and adiponectin were: <4% and <7%, <8% and <10%, <5% and <7%, respectively. Rheumatoid factor was measured by nephelometry (Mitsubishi Kagaku Iatron, Tokyo, Japan). C-reactive protein (CRP) was also measured by nephelometry according to the manufacturer's specifications (Dade-Behring Inc., Deerfield, IL, USA). The erythrocyte sedimentation rate (ESR) was measured by the Westergren method.

Statistical analysis

Results are expressed as the mean and/or median. Statistical analysis was performed with StatFlex software (ver. 6; ARTEC Co., Ltd., Osaka, Japan). The significance of between-group differences in serum adipokine concentrations was determined by the Mann-Whitney non-parametric test, while differences of background data were evaluated by Student's *t*-test. Simple linear regression analysis was used to assess correlations between serum adipokine levels and patient characteristics, and stepwise forward multiple regression analysis was also performed. Logarithmic transformation was done for highly skewed variables (resistin, leptin, adiponectin, and CRP) when needed in order to satisfy the requirements of multivariate models. In all analyses, $p<0.05$ was considered to indicate statistical significance.

Results

Serum adipokine concentrations

There were no statistically significant differences in serum resistin levels between the RA patients [males: 3.3 (2.8-4.9) ng/mL, females: 3.5 (2.5-5.0) ng/mL] and normal controls [males: 3.7 (3.4-5.0) ng/mL, females: 3.6 (3.1-4.5) ng/mL] (Fig. 1). However, the resistin levels of female RA patients were broadly distributed. Therefore, we compared CRP levels between patients with resistin levels above the 75th percentile (>4.95 ng/mL) and those with resistin levels below the 75th percentile. We found that the CRP level of the former subgroup was significantly higher than that of the latter subgroup (19.1±21.8 mg/L vs. 4.3±7.7 mg/L, $p<0.001$).

The serum concentration of leptin was significantly ($p<0.001$) higher in male RA patients [median 11.2 (interquartile range, 5.1-20.3) ng/mL] than in normal male control subjects [2.7 (1.8-4.3) ng/mL], and serum leptin level was also significantly ($p<0.001$) higher in female RA patients [15.3 (7.3-26.7) ng/mL] than in normal female control subjects [7.4 (3.9-12.0) ng/mL] (Fig. 2). Serum leptin levels were significantly correlated with BMI in all subjects ($p<0.001$), except male RA patients ($p=0.955$), according to linear regression analysis. Since BMI is closely associated with the serum leptin concentration (10, 11), leptin levels were adjusted by BMI. As a result, the leptin/BMI ratios of RA patients [males: 0.51 (0.21-0.95), females: 0.69 (0.35-1.15)] were significantly ($p<0.001$) higher than those of normal control subjects [males: 0.12 (0.10-0.17), females: 0.33 (0.20-0.55)].

Female RA patients had significantly ($p<0.001$) higher serum adiponectin concentrations [10.1 (4.5-26.8) μg/mL] than

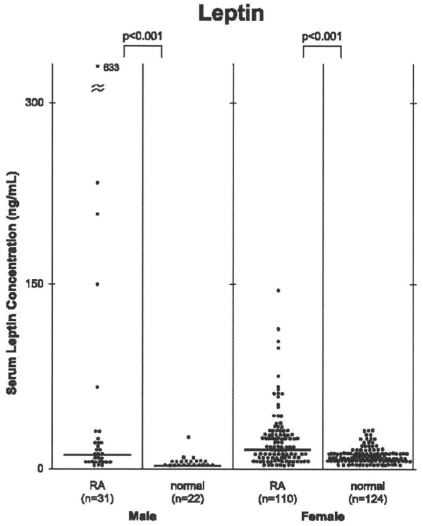

Figure 2. **Serum leptin levels in RA patients and control subjects. Horizontal bars indicate median values. Statistical significance was determined by Mann-Whitney test.**

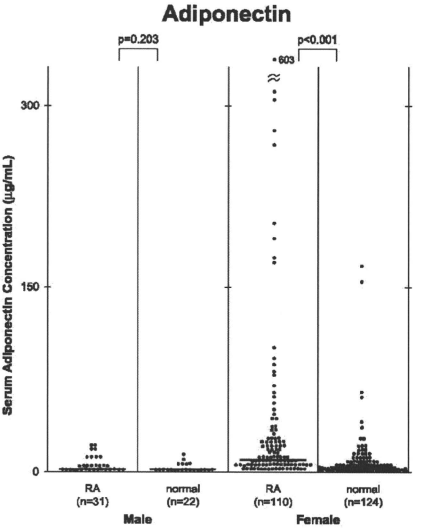

Figure 3. **Serum adiponectin levels in RA patients and control subjects. Horizontal bars indicate median values. Statistical significance was determined by Mann-Whitney test.**

normal female control subjects [3.6 (2.4-7.4) μg/mL], but no significant difference of adiponectin levels was observed in males (RA males: median 2.6 μg/mL; control males: median 2.3 μg/mL, p=0.203) (Fig. 3).

Correlations between adipokines and patient characteristics

We included various patient characteristics [sex, age, BMI, duration of RA, stage, CRP, ESR, DAS28-ESR, prednisolone, methotrexate, other disease modifying antirheumatic drugs (DMARDs), and biological agents] in a model predicting the serum levels of adipokines (resistin, leptin, and adiponectin) (Table 3-5, respectively).

As shown in Table 3, significant univariate predictors of the serum level of resistin included age, BMI, CRP, ESR, and DAS28-ESR. Inclusion of these univariate predictors in a multivariate model resulted in the final selection of female sex and CRP as significant predictors (Table 3, multivariate model).

Significant univariate predictors of the leptin level included BMI, CRP, and DAS28-ESR (Table 4, univariate model), while multivariate analysis resulted in the final selection of BMI and CRP (Table 4, multivariate model).

For adiponectin, significant univariate predictors included female sex, BMI, RA stage, CRP, and current prednisolone use (Table 5, univariate model). On multivariate analysis, the significant predictors were reduced to CRP and current prednisolone use (Table 5, multivariate model). In addition, a significant positive correlation was found between the serum adiponectin level and the dose of prednisolone in female RA patients by linear regression analysis (r=0.306, p<0.05). However, we did not find any significant correlation between serum adiponectin levels and the use of methotrexate and/or biological agents.

Discussion

We measured the serum levels of 3 adipokines (resistin, leptin, and adiponectin) in 141 RA patients and 146 normal controls. Most of the previous studies showed the serum levels of several adipokines in only around 50 patients (12-24). They indicated that the serum resistin (12, 13, 25), leptin (14-16, 23, 25) and adiponectin (14, 22-25) levels are higher in RA patients than in healthy controls, while negative results (14, 17-20, 24) were also reported. The present results showed significantly elevated serum levels of leptin and adiponectin, and a trend for an elevated serum resistin level in RA patients. In addition, we found that the serum levels of resistin, leptin and adiponectin in the same samples were all associated with CRP, and they were individually associated with the different clinical conditions of female sex, BMI, and prednisolone use, respectively.

Some previous reports described that the serum levels of these adipokines were associated with dietary supple-

Table 3. Crude and Adjusted Associations of the Serum Resistin Concentration and Patient Characteristics

Characteristic	Resistin*						
	Univariate			Multivariate (complex)		Multivariate (simplified)	
	β	p	R^2	β	p	β	p
Female	0.031	0.352	0.006	0.068	0.061	0.070	0.027
Age	0.004	0.047	0.028	0.002	0.259		
BMI	0.015	0.041	0.030	0.008	0.287		
RA duration	-0.005	0.082	0.022	-0.006	0.077		
Stage	0.029	0.227	0.010	0.022	0.441		
CRP*	0.083	<0.001	0.150	0.075	0.001	0.086	<0.001
ESR	0.004	0.001	0.080	0.001	0.556		
DAS28-ESR	0.043	0.025	0.037	-0.016	0.474		
Prednisolone	0.049	0.080	0.022	0.026	0.369		
Methotrexate	-0.002	0.938	0.000	-0.011	0.699		
Other DMARDs	0.017	0.563	0.002	0.023	0.428		
Biological agents	0.031	0.355	0.006	-0.004	0.911		
R^2				0.163		0.173	

β: regression coefficient; DMARDs: disease modifying anti-rheumatic drugs; Other DMARDs: one or more of sulfasalazine, bucillamine, injectable gold, and/or auranofin; DAS: disease activity score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; BMI: body mass index; R^2: coefficient of determination.

*Logarithmic transformation was done for highly skewed variables as needed to satisfy the requirements of multivariate models. Significant correlations ($p<0.05$) are underlined.

Table 4. Crude and Adjusted Associations of the Serum Leptin Concentration and Patient Characteristics

Characteristic	Leptin*						
	Univariate			Multivariate (complex)		Multivariate (simplified)	
	β	p	R^2	β	p	β	p
Female	0.037	0.746	0.001	0.150	0.223		
Age	0.008	0.258	0.009	0.004	0.516		
BMI	0.113	<0.001	0.138	0.103	<0.001	0.104	<0.001
RA duration	-0.005	0.621	0.002	-0.003	0.836		
Stage	0.130	0.111	0.018	0.079	0.411		
CRP*	0.216	<0.001	0.087	0.185	0.019	0.187	0.001
ESR	0.004	0.287	0.008	-0.008	0.111		
DAS28-ESR	0.149	0.022	0.038	0.111	0.153		
Prednisolone	0.176	0.064	0.025	0.005	0.959		
Methotrexate	0.067	0.502	0.003	-0.045	0.642		
Other DMARDs	-0.008	0.933	<0.001	0.040	0.678		
Biological agents	0.149	0.186	0.013	0.090	0.500		
R^2				0.162		0.187	

β: regression coefficient; DMARDs: disease modifying anti-rheumatic drugs; Other DMARDs: one or more of sulfasalazine, bucillamine, injectable gold, and/or auranofin; DAS: disease activity score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; BMI: body mass index; R^2: coefficient of determination.

*Logarithmic transformation was done for highly skewed variables as needed to satisfy the requirements of multivariate models. Significant correlations ($p<0.05$) are underlined.

ments (26-28). The present study did not investigate the relationship between the serum adipokine levels and dietary supplements; no special dietary management was provided for the patients or normal controls.

Tarkowski et al (29) demonstrated that resistin competes with lipopolysaccharide for binding to Toll-like receptor-4 and may act as a pro-inflammatory cytokine in human monocytes. In the present study, we found that the CRP level was higher in the subgroup of high serum resistin levels than in the subgroup of low serum resistin levels. In addition, the CRP level was a significant predictor of the higher serum resistin level according to multivariate analysis. These data suggest that an increased serum level of resistin may contribute to inflammation in RA patients. However, the reason for the gender difference, in which the female sex was associated with high serum resistin levels, is unknown.

Simons et al (30) described that TNFα and IL-1β stimulate leptin production by human preadipocytes. Some reports have described a significant positive correlation between the serum leptin level and the disease activity of RA (14, 15, 21). We also found a significant correlation between the serum leptin level and CRP by multivariate analysis in this study.

Table 5. Crude and Adjusted Associations of the Serum Adiponectin Concentration and Patient Characteristics

Characteristic	Adiponectin*						
	Univariate			Multivariate (complex)		Multivariate (simplified)	
	β	p	R^2	β	p	β	p
Female	0.654	<0.001	0.152	7.676	0.370		
Age	0.011	0.191	0.012	0.891	0.065		
BMI	-0.068	0.034	0.032	-2.626	0.139		
RA duration	0.025	0.061	0.025	-0.538	0.515		
Stage	0.294	0.003	0.060	3.774	0.572		
CRP*	-0.175	0.024	0.037	-11.144	0.042	-10.453	0.010
ESR	-0.106	0.678	0.001	-0.120	0.728		
DAS28-ESR	0.025	0.755	0.001	4.106	0.448		
Prednisolone	0.283	0.016	0.041	15.485	0.023	17.594	0.005
Methotrexate	0.070	0.572	0.002	-0.428	0.949		
Other DMARDs	-0.061	0.629	0.000	-1.853	0.786		
Biological agents	0.095	0.499	0.003	0.427	0.963		
R^2				0.055		0.090	

β: regression coefficient; DMARDs: disease modifying anti-rheumatic drugs; Other DMARDs: one or more of sulfasalazine, bucillamine, injectable gold, and/or auranofin; DAS: disease activity score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; BMI: body mass index; R^2: coefficient of determination.

*Logarithmic transformation was done for highly skewed variables as needed to satisfy the requirements of multivariate models. Significant correlations ($p<0.05$) are underlined.

Previous reports have shown that the serum leptin level is positively correlated with BMI, (10, 11) as observed in this study, except for male RA patients. We also found that leptin/BMI ratio of RA patients was significantly higher than that of normal control subjects. Based on these results, the absence of correlation between the serum leptin level and BMI in male RA patients might be explained by the influence of inflammation. Moreover, it was suggested that leptin may act as a pro-inflammatory cytokine in this disease.

Rho et al (25) suggested that leptin was associated with reduced radiographic joint damage as estimated by the Larsen score (31). In the present study, leptin as well as other adipokines were not associated with the Steinbrocker stage of RA. In general, high disease activity in RA patient is correlated with joint damage. The relationship between the serum leptin level and radiographic joint damage should be studied in the future.

The serum adiponectin level was significantly higher in female RA patients than in normal female controls. We also found the same trend in male RA patients, although the difference was not statistically significant. However, the serum CRP level was negatively associated with the adiponectin level in RA patients. Schäffler et al (32) reported that adiponectin was increased in the synovial fluid of RA patients compared with osteoarthritis patients, but they found no statistically significant correlations between adiponectin and ESR or CRP in RA patients. Our previous *in vitro* studies (6, 7) have suggested that adiponectin might be a pro-inflammatory cytokine for rheumatoid synovial fibroblasts. The discrepancies in the adiponectin studies between *in vitro* pro-inflammatory effects and various facets in clinical inflammatory conditions in RA patients remain to be studied.

In the present study, the serum adiponectin level was significantly correlated with current prednisolone use by multiple regression analysis, and was also significantly correlated with the dose of prednisolone by linear regression analysis. Maeda et al (33) reported the reciprocal suppression of adiponectin and TNFα production in adipose tissue. Corticosteroids inhibit the production of pro-inflammatory cytokines such as TNFα (34). Thus, the reduction of TNFα by prednisolone might be the cause of the increased serum adiponectin level in the present RA patients.

Laurberg et al (35) found that the plasma adiponectin level was increased by 13% in RA patients who received methotrexate treatment. Nishida et al (36) reported that serum adiponectin levels showed an increase during infliximab (TNFα inhibitor) therapy in RA patients. However, we did not find significant correlations between serum adiponectin levels and the use of methotrexate and/or biological agents in the present study. The reason for the absence of correlation between serum adiponectin levels and TNFα inhibitor therapy might be explained by the small number of patients receiving TNFα inhibitors, comparing with those receiving prednisolone.

In summary, the serum levels of resistin and leptin were positively associated with CRP level in patients with rheumatoid arthritis, suggesting that these adipokines may act as pro-inflammatory cytokines in this disease. The serum adiponectin level was elevated in the patients, however, it was negatively associated with CRP level. In addition, the serum levels of resistin, leptin, and adiponectin were also associated with female sex, BMI and the use of prednisolone, respectively.

The authors state that they have no Conflict of Interest (COI).

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References

1. Smolen JS, Aletaha D, Koeller M, Weisman MH, Emery P. New therapies for treatment of rheumatoid arthritis. Lancet **370**: 1861-1874, 2007.
2. Firestein GS. Evolving concepts of rheumatoid arthritis. Nature **423**: 356-361, 2003.
3. Arend WP. Physiology of cytokine pathways in rheumatoid arthritis. Arthritis Rheum **45**: 101-106, 2001.
4. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol **6**: 772-783, 2006.
5. Koerner A, Kratzsch J, Kiess W. Adipocytokines: leptin--the classical, resistin--the controversial, adiponectin--the promising, and more to come. Best Pract Res Clin Endocrinol Metab **19**: 525-546, 2005.
6. Kitahara K, Kusunoki N, Kakiuchi T, Suguro T, Kawai S. Adiponectin stimulates IL-8 production by rheumatoid synovial fibroblasts. Biochem Biophys Res Commun **378**: 218-223, 2009.
7. Kusunoki N, Kitahara K, Kojima F, et al. Adiponectin stimulates prostaglandin E_2 production in rheumatoid synovial fibroblasts. Arthritis Rheum **62**: 1641-1649, 2010.
8. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum **31**: 315-324, 1988.
9. Fransen J, van Riel PL. The Disease Activity Score and the EULAR response criteria. Clin Exp Rheumatol **23**: S93-S99, 2005.
10. Maffei M, Halaas J, Ravussin E, et al. Leptin levels in human and rodent: measurement of plasma leptin and *ob* RNA in obese and weight-reduced subjects. Nat Med **1**: 1155-1161, 1995.
11. Considine RV, Sinha MK, Heiman ML, et al. Serum immunoreactive leptin concentrations in normal-weight and obese humans. N Engl J Med **334**: 292-295, 1996.
12. Migita K, Maeda Y, Miyashita T, et al. The serum levels of resistin in rheumatoid arthritis patients. Clin Exp Rheumatol **24**: 698-701, 2006.
13. Šenolt L, Housa D, Vernerová Z, et al. Resistin in rheumatoid arthritis synovial tissue, synovial fluid and serum. Ann Rheum Dis **66**: 458-463, 2007.
14. Otero M, Lago R, Gomez R, et al. Changes in plasma levels of fat-derived hormones adiponectin, leptin, resistin and visfatin in patients with rheumatoid arthritis. Ann Rheum Dis **65**: 1198-1201, 2006.
15. Targońska-Stepniak B, Majdan M, Dryglewska M. Leptin serum levels in rheumatoid arthritis patients: relation to disease duration and activity. Rheumatol Int **28**: 585-591, 2008.
16. Gunaydin R, Kaya T, Atay A, Olmez N, Hur A, Koseoglu M. Serum leptin levels in rheumatoid arthritis and relationship with disease activity. South Med J **99**: 1078-1083, 2006.
17. Hizmetli S, Kisa M, Gokalp N, Bakici MZ. Are plasma and synovial fluid leptin levels correlated with disease activity in rheumatoid arthritis? Rheumatol Int **27**: 335-338, 2007.
18. Anders HJ, Rihl M, Heufelder A, Loch O, Schattenkirchner M. Leptin serum levels are not correlated with disease activity in patients with rheumatoid arthritis. Metabolism **48**: 745-748, 1999.
19. Wisłowska M, Rok M, Jaszczyk B, Stepień K, Cicha M. Serum leptin in rheumatoid arthritis. Rheumatol Int **27**: 947-954, 2007.
20. Popa C, Netea MG, Radstake TR, van Riel PL, Barrera P, van der Meer JW. Markers of inflammation are negatively correlated with serum leptin in rheumatoid arthritis. Ann Rheum Dis **64**: 1195-1198, 2005.
21. Lee SW, Park MC, Park YB, Lee SK. Measurement of the serum leptin level could assist disease activity monitoring in rheumatoid arthritis. Rheumatol Int **27**: 537-540, 2007.
22. Šenolt L, Pavelka K, Housa D, Haluzík M. Increased adiponectin is negatively linked to the local inflammatory process in patients with rheumatoid arthritis. Cytokine **35**: 247-252, 2006.
23. Härle P, Sarzi-Puttini P, Cutolo M, Straub RH. No change of serum levels of leptin and adiponectin during anti-tumour necrosis factor antibody treatment with adalimumab in patients with rheumatoid arthritis. Ann Rheum Dis **65**: 970-971, 2006.
24. Popa C, Netea MG, de Graaf J, et al. Circulating leptin and adiponectin concentrations during tumor necrosis factor blockade in patients with active rheumatoid arthritis. J Rheumatol **36**: 724-730, 2009.
25. Rho YH, Solus J, Sokka T, et al. Adipocytokines are associated with radiographic joint damage in rheumatoid arthritis. Arthritis Rheum **60**: 1906-1914, 2009.
26. Aggarwal BB. Targeting inflammation-induced obesity and metabolic diseases by curcumin and other nutraceuticals. Annu Rev Nutr **30**: 173-199, 2010.
27. Zhao WS, Zhai JJ, Wang YH, et al. Conjugated linoleic acid supplementation enhances antihypertensive effect of ramipril in Chinese patients with obesity-related hypertension. Am J Hypertens **22**: 680-686, 2009.
28. Nelson TL, Stevens JR, Hickey MS. Adiponectin levels are reduced, independent of polymorphisms in the adiponectin gene, after supplementation with alpha-linolenic acid among healthy adults. Metabolism **56**: 1209-1215, 2007.
29. Tarkowski A, Bjersing J, Shestakov A, Bokarewa MI. Resistin competes with lipopolysaccharide for binding to Toll-like receptor 4. J Cell Mol Med **14**: 1419-1431, 2010.
30. Simons PJ, van den Pangaart PS, van Roomen CP, Aerts JM, Boon L. Cytokine-mediated modulation of leptin and adiponectin secretion during in vitro adipogenesis: evidence that tumor necrosis factor-alpha- and interleukin-1beta-treated human preadipocytes are potent leptin producers. Cytokine **32**: 94-103, 2005.
31. Larsen A, Dale K, Eek M. Radiographic evaluation of rheumatoid arthritis and related conditions by standard reference films. Acta Radiol Diagn (Stockh) **18**: 481-491, 1977.
32. Schäffler A, Ehling A, Neumann E, et al. Adipocytokines in synovial fluid. JAMA **290**: 1709-1710, 2003.
33. Maeda N, Shimomura I, Kishida K, et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med **8**: 731-737, 2002.
34. Smoak K, Cidlowski JA. Glucocorticoids regulate tristetraproline synthesis and posttranscriptionally regulate tumor necrosis factor alpha inflammatory signaling. Mol Cell Biol **26**: 9126-9135, 2006.
35. Laurberg TB, Frystyk J, Ellingsen T, et al. Plasma adiponectin in patients with active, early, and chronic rheumatoid arthritis who are steroid- and disease-modifying antirheumatic drug-naïve compared with patients with osteoarthritis and controls. J Rheumatol **36**: 1885-1891, 2009.
36. Nishida K, Okada Y, Nawata M, Saito K, Tanaka Y. Induction of hyperadiponectinemia following long-term treatment of patients with rheumatoid arthritis with infliximab (IFX), an anti-TNF-alpha antibody. Endocr J **55**: 213-216, 2008.

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CASE REPORT

Exacerbation of adult-onset Still's disease, possibly related to elevation of serum tumor necrosis factor-alpha after etanercept administration

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Abstract

Adult-onset Still's disease (AOSD) is a systemic inflammatory disease of unknown etiology. A 44-year-old male patient presented with AOSD complicated by macrophage activation syndrome after etanercept therapy. His serum tumor necrosis factor-α (TNF-α) level was increased dramatically after etanercept therapy. The clinical course of this case suggests that the increased TNF-α level by etanercept administration might cause macrophage activation syndrome in this case.

Key words: adult onset Still's disease, etanercept, macrophage activation syndrome, TNF-α.

INTRODUCTION

Adult onset Still's disease (AOSD) is a systemic inflammatory disease of unknown etiology. It has been reported that serum levels of various proinflammatory cytokines, including interferon-gamma (IFN-γ), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), IL-1 and IL-18, are elevated in patients with active AOSD.[1–3] In particular, IL-18 has been shown to have a central role in the etiology of AOSD.[2,3] In an effort to control the excessive elevation of various cytokines in AOSD, attention has recently been paid to biological preparations, including TNF-α inhibitors, IL-6 inhibitors and IL-1 receptor antagonists.[4,5] We encountered a patient whose AOSD showed exacerbation after treatment with etanercept and analyzed the serum cytokine profile in detail.

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CASE REPORT

A 44-year-old man complained of arthralgia in February 2006. A high fever of >39°C was observed and arthralgia of various joints persisted. The patient was admitted to our University Hospital on August 15. Physical examination showed a salmon-pink rash on both upper extremities, both thighs and the back. There was tenderness of both wrists, both elbows and both ankles. Laboratory tests on admission gave the following results: white blood cell count, 9400/μL; erythrocyte sedimentation rate, 85 mm/h; and serum C-reactive protein, 33 mg/L. There was a significant increase in the serum ferritin level to 4917 ng/mL. Antinuclear antibody, rheumatoid factor and culture for various microorganisms were all negative. His clinical course is shown in Figure 1. Corticosteroid therapy (prednisolone 30 mg/day) was started after the patient was diagnosed as having AOSD. Corticosteroid therapy was continued in various doses as described in Figure 1. Since the patient did not respond well to corticosteroid therapy, treatment with etanercept (25 mg subcutaneously) was started. After the patient

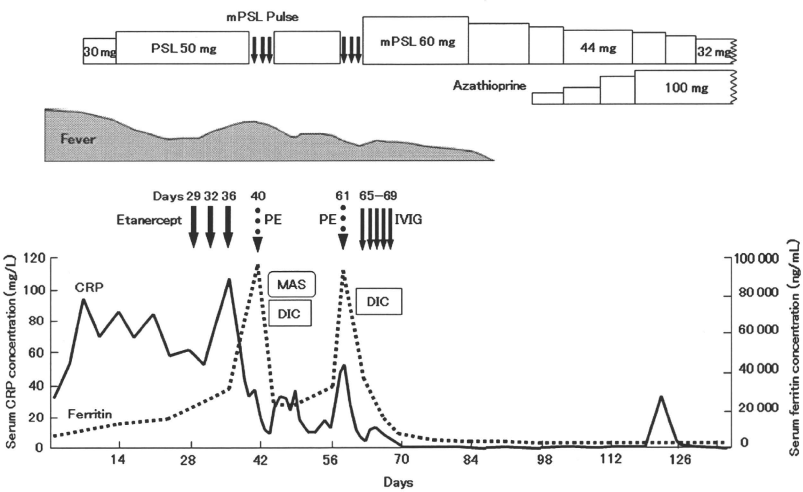

Figure 1 Clinical course of the reported case. PSL, prednisolone; mPSL, methylprednisolone; IVIG, intravenous immunoglobulin 0.5 mg/kg per day for 5 days; mPSL Pulse, mPSL 1000 mg/day for 3 days; DIC, disseminated intravascular coagulation; MAS, macrophage activation syndrome; PE, plasma exchange; CRP, C-reactive protein; IL-18, interleukin-18; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; IFN-γ, interferon gamma.

Table 1 Changes in serum proinflammatory cytokine levels in the reported case

Days after admission	29	32	36	43	61	70	85
Etanercept (mg)	25	25	25	–	–	–	–
Cytokines†							
TNF-α (pg/mL, normal < 5.0)	<5.0	874	642	101	<5.0	<5.0	<5.0
IL-18 (pg/mL, normal < 180)	39.9	126	295	195	203	469	45.2
IL-6 (pg/mL, normal < 4.0)	24.8	4.4	33	5.7	1.9	1.1	0.9
IFN-γ (IU/mL, normal < 0.1)	<0.1	7.2	27	<0.1	<0.1	<0.1	<0.1

†Cytokine concentrations were measured in the serum samples obtained in the morning before etanercept injection at 29, 32 and 36 days after admission.
TNF-α, tumour necrosis factor-alpha; IL-18, interleukin-18; IL-6, interleukin-6; IFN-γ, interferon gamma.

had received etanercept three times, he developed a high fever and disseminated intravascular coagulation. Splenomegaly was not detected by ultrasonographic examination. His laboratory tests were as follows: white blood cell count, 2300/μL; hemoglobin, 7.3 g/dL; platelet count, 44 000/μL; serum ferritin level, 96 335 ng/mL; fibrinogen, 160 mg/dL; and the serum triglyceride value, 321 mg/dL. Moreover, his bone marrow aspirate showed pathological features of hemophagocytosis. Therefore, we diagnosed his condition as macrophage activation syndrome (MAS), since these included elevated serum ferritin (>10 000 mg/mL) and triglyceride (>160 mg/dL), decreased fibrinogen (<250 mg/dL), low platelet count and bone marrow aspirate showing hemophagocytosis.[6] These symptoms were subsequently relieved by two cycles

of methylprednisolone pulse therapy, 2 × plasma exchange and a cycle of intravenous immunoglobulin 0.5 mg/kg per day for 5 days.

DISCUSSION

Immunosuppressants like methotrexate and cyclosporine and/or TNF-α inhibitors are used to treat corticosteroid-refractory AOSD.[4,5] However, in our patient, etanercept aggravated the symptoms of AOSD and the patient developed MAS as a complication of this drug. Stern *et al.*[7] reported a patient in whom etanercept treatment was associated with development of MAS due to reactivation of Epstein-Barr virus, although antibody titers for viruses including Epstein-Barr virus were within the normal range when our patient's condition deteriorated. The exacerbation in this case might be explained by the symptoms of AOSD itself; however, the patient's physical condition and laboratory measures changed just after commencement of etanercept therapy. In addition, serum IL-18 levels in patients with AOSD complicated by MAS have been reported to be extra-high,[3] at approximately 1000 times more than those of our case. Thus, we assessed that MAS in this patient was triggered by etanercept therapy.

Zou *et al.*[8] reported that INF-γ and TNF-α productions by T cells increased after treatment with etanercept in patients who had ankylosing spondylitis. They also reported that infliximab (a chimeric monoclonal anti-TNF-α antibody) down-regulated the TNF-α production by T cells, while etanercept (a soluble TNF receptor) up-regulated the TNF-α. Therefore, in our patient an increase of serum TNF-α induced by etanercept may have led to exacerbation of the patient's condition. Etanercept is a fusion protein of p75 TNF-α receptor and Fc component of human IgG. The increased concentrations of cytokines including TNF-α could be trigged by the binding of etanercept with an Fcγ receptor of macrophages and/or other cells. The present case warns us that etanercept needs to be used with caution in the treatment of patients with AOSD.

REFERENCES

1 Hoshino T, Ohta A, Yang D, *et al.* (1998) Elevated serum interleukin 6, interferon-gamma, and tumor necrosis factor-alpha levels in patients with adult Still's disease. *J Rheumatol* **25**, 396–8.

2 Kawashima M, Yamamura M, Taniai M, *et al.* (2001) Levels of interleukin-18 and its binding inhibitors in the blood circulation of patients with adult-onset Still's disease. *Arthritis Rheum* **44**, 550–60.

3 Maruyama J, Inokuma S (2010) Cytokine profiles of macrophage activation syndrome associated with rheumatic diseases. *J Rheumatol* **37**, 967–73.

4 Efthimiou P, Paik PK, Bielory L (2006) Diagnosis and management of adult onset Still's disease. *Ann Rheum Dis* **65**, 564–72.

5 Bagnari V, Colina M, Ciancio G, Govoni M, Trotta F (2010) Adult-onset Still's disease. *Rheumatol Int* **30**, 855–62.

6 Singh S, Samant R, Joshi VR (2008) Adult onset Still's disease: a study of 14 cases. *Clin Rheumatol* **27**, 35–9.

7 Stern A, Riley R, Buckley L (2001) Worsening of macrophage activation syndrome in a patient with adult onset Still's disease after initiation of etanercept therapy. *J Clin Rheumatol* **7**, 252–6.

8 Zou J, Rudwaleit M, Brandt J, Thiel A, Braun J, Sieper J (2003) Up regulation of the production of tumour necrosis factor alpha and interferon gamma by T cells in ankylosing spondylitis during treatment with etanercept. *Ann Rheum Dis* **62**, 561–4.

Efficacy and Safety of Ketoprofen Patch in Patients With Rheumatoid Arthritis: A Randomized, Double-Blind, Placebo-Controlled Study

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This study assessed the efficacy and safety of ketoprofen patch compared with placebo in patients who had rheumatoid arthritis and persistent wrist pain. Patients (N = 676) who had achieved systemic disease control with a disease-modifying antirheumatic drug and/or systemic corticosteroid, but still had persistent wrist pain, were randomized to a 2-week course of once-daily treatment with application of a 20-mg ketoprofen patch or a placebo patch to the wrist. The primary efficacy end point was the percent change from baseline to the end of treatment in the intensity of wrist pain scored by each patient on a 100-mm visual analog scale. The mean ± SD percent change on the pain intensity scale was significantly larger in patients treated with ketoprofen than in those receiving placebo (31.2% ± 30.3% [95% confidence interval: 28.0-34.4] vs 25.5% ± 31.2% [95% confidence interval: 22.1-28.8]; P = .020). However, the actual difference of the mean pain intensity scale between the 2 groups was small at the end of treatment. The frequency of adverse events was similar in both groups. The ketoprofen patch was more effective than placebo for relieving persistent local joint pain in patients with rheumatoid arthritis. The patch was also safe and well tolerated during the 2-week treatment period.

Keywords: *Rheumatoid arthritis; nonsteroidal anti-inflammatory drugs; clinical trial*

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Rheumatoid arthritis (RA) is a chronic progressive inflammatory disease that affects multiple joints and characteristically causes pain and swelling. The American College of Rheumatology (ACR) guidelines for the management of RA recommend that the goals of treatment be prevention or control of joint destruction, inhibition of loss of function, and relief of pain.[1] To achieve these goals, the guidelines recommend that baseline pharmacological treatment of RA should include disease-modifying antirheumatic drugs (DMARDs) with adjunctive use of nonsteroidal anti-inflammatory drugs (NSAIDs), as well as oral and topical corticosteroids.[1] In Japan, the same approach to the management of RA has been advocated, with an auxiliary role for NSAIDs.[2]

In 1971, Vane[3] found that inhibition of cyclooxygenase (COX) is the primary mechanism mediating the anti-inflammatory effect of aspirin and several other NSAIDs. Subsequent studies identified COX-2 as an isozyme of COX involved in the development of inflammation.[4-6] This led to the release of various selective COX-2 inhibitors with improved gastrointestinal tolerance. However, recent studies have shown that COX-2 inhibitors may increase the risk of cardiovascular events, such as myocardial infarction,[7,8] and some of these drugs have been withdrawn from the market.[9]

A percutaneous NSAID formulation has been shown to deliver the drug to the muscles, tendons, and joints beneath the application site without producing a

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clinically significant blood concentration.[10-12] Such formulations may be a treatment option to relieve joint pain while minimizing unwanted effects on the gastrointestinal system.[13-15] Several percutaneous NSAID preparations have been shown to be effective and safe for the topical treatment of osteoarthritis (OA) of the knee[16-18] and ankle sprain.[19] However, no percutaneous NSAIDs have been demonstrated to show efficacy against joint pain in patients with RA.

With regard to percutaneous ketoprofen formulations, the once-a-day topical patch containing 100 mg of ketoprofen per patch (Keofix, Zambon Group, Italy) is well known. The 100-mg topical patch is composed of a 90-cm^2 (8.2 × 11 cm) backing layer, a matrix layer containing the drug, and a release layer.[14,19] The matrix layer contains 20% ketoprofen suspended in an acrylic pressure-sensitive adhesive.[19] The ketoprofen patch we used in this study was a once-a-day topical patch containing 20 mg of ketoprofen, which was developed by Hisamitsu Pharmaceutical Co (Saga, Japan). This 20-mg ketoprofen patch was composed of a 70 cm^2 (7 × 10 cm) backing cloth and a matrix layer containing 2% ketoprofen in a nonaqueous base. We performed a randomized double-blind controlled study that compared the efficacy and safety of the ketoprofen patch with a placebo patch for the relief of persistent wrist joint pain in RA patients who had already achieved systemic disease control with DMARD and/or oral corticosteroid therapy.

METHODS

Between August 2005 and July 2006, the present multicenter, randomized, double-blind controlled study was conducted at 80 Japanese centers in compliance with the Declaration of Helsinki and the Japanese Good Clinical Practice Guideline. Before the start of the study, the protocol was approved by the institutional review board of each participating center, and all patients who were enrolled gave written informed consent. This study was registered with the Clinical Trials Information registry, a public clinical trials registry in Japan operated by the Japan Pharmaceutical Information Center (registration number JapicCTI-050184).

Patients

Patients who met all of the following requirements were eligible for this study: fulfilling the 1987 ACR Criteria for the Classification of RA[20]; treatment with an oral or intrarectal NSAID without dosage modification for at least 8 weeks; fixed doses of DMARDs and/or systemic corticosteroid for a specified period (≥20 weeks for intramuscular gold salts and ≥8 weeks for any nongold DMARD or systemic corticosteroid); physiotherapy (if any) without modification for at least 8 weeks; wrist joint pain persisting for at least 1 month; and age 20 years or older.

Patients with any of the following conditions were excluded from the study: current or previous treatment with any anti–tumor necrosis factor agent; concomitant or previous aspirin-induced asthma or bronchial asthma; known allergy to benzophenone or related compounds (including ketoprofen); known allergy to any topical preparations or adhesives; any concomitant illness that might affect the local response to the study treatment; any wound or dermatitis affecting the study joint; and confirmed or potential pregnancy, recent delivery, current breast feeding, or a desire to become pregnant during the study period. In addition, patients were excluded if the 100-mm visual analog scale (VAS) pain intensity score for the study wrist joint was less than 20 mm or more than 80 mm before the start of treatment.

Study Design

During the 2 to 4 weeks before enrollment, patients were screened for eligibility with regard to demographic and clinical characteristics. To minimize the variability of VAS pain intensity scores, training in the method of scoring was given to both patients and investigators with an audiovisual guide. Patients received 1 week of training about use of the VAS and then discontinued all nonstudy NSAIDs at least 1 week (short-acting NSAIDs) or 2 weeks (long-acting NSAIDs such as tenoxicam, oxaprozin, and piroxicam) before the treatment period.

Patients also discontinued all topical analgesic or anti-inflammatory preparations applied to both upper extremities (excluding the shoulders) at least 2 weeks before starting the study treatment. A 4-week washout period was required if the patient was receiving intra-articular therapy with sodium hyaluronate for the study joint or injectable corticosteroids at any site.

Patients who were confirmed to be eligible by the randomization center were enrolled and randomized to apply a 2-week course of treatment with either ketoprofen or placebo patch. To ensure objectivity of assessment, all patients and investigators were blinded to the treatment assigned until after completion of the study. In each patient allocated to the ketoprofen group, a 70-cm^2 (7 × 10 cm) patch containing 20 mg of ketoprofen was applied once daily to the more painful wrist joint. Patients in the placebo group

were given placebo patches without ketoprofen with an identical appearance. Each patient scored the intensity of pain in the study wrist joint by using the VAS daily during the screening period and during the 2-week treatment period. Clinical assessment and laboratory tests (hematology tests, biochemistry tests, and urinalysis) were performed at the start (baseline) and end of the 2-week treatment period.

Clinical Assessment

The primary population analyzed for efficacy was the intent-to-treat (ITT) population, which included all patients who received at least 1 dose of the study treatment and were evaluated for efficacy. The per protocol (PP) population was defined as the subset of the ITT population that excluded patients with major protocol violations. The safety population consisted of all patients who received at least 1 dose of the study treatment.

The primary efficacy end point was the percent change in the intensity of wrist joint pain on the VAS from baseline to the end of treatment. Secondary efficacy end points included the percentage of patients with 50% or greater reduction in the intensity of pain,[21] the absolute change of pain intensity from baseline to the end of treatment, and the subjective rating of overall pain by each patient at the end of treatment using 7 categories (markedly improved, improved, slightly improved, unchanged, slightly aggravated, aggravated, and markedly aggravated). The percentage of patients with 20% or greater reduction in the intensity of pain was also assessed by post hoc analysis. Safety was assessed by monitoring adverse events (symptoms and clinical findings) and laboratory data (hematology tests, biochemistry tests, and urinalysis). The study was terminated in patients who developed adverse events if the investigator decided to stop treatment, as well as in patients who withdrew from the study themselves, those who were lost to follow up, and those who had any other condition that led to cessation of treatment by the investigator.

Statistical Analysis

In an exploratory study of the ketoprofen patch in RA patients, the difference of mean VAS percent changes between the ketoprofen and placebo groups was 8.1% and the standard deviation was 35.6%. Given a significance level of .05 (2-sided) and a power of 80%, the number of subjects needed to confirm superiority of ketoprofen over placebo would be 303 per group. Assuming that 10% to 20% of subjects discontinue administration, the target number of subjects was determined to be 350 per group.

The percent change of wrist pain intensity on the VAS from baseline to the end of treatment (the primary efficacy end point) was compared between the 2 groups by the Wilcoxon rank sum test. The percentage of patients with 50% or more and 20% or more reduction of the intensity of their pain and the absolute change of pain intensity from baseline to the end of treatment were compared between the groups by using Fisher's exact test and the Wilcoxon rank sum test, respectively. The subjective ratings of overall pain by the patients were summarized and compared between the 2 groups with the Wilcoxon rank sum test. In the safety population, Fisher's exact test was used to compare the incidence of adverse events between the 2 groups. Differences were considered to be statistically significant at $P < .05$ (2-sided).

When treatment of a patient was ceased for any reason, the VAS pain intensity score and the subjective rating of overall pain were determined at that point.

RESULTS

Demographic Profile and Disposition of Patients

Of the 847 patients screened, 676 patients were enrolled and randomized to apply ketoprofen patch (n = 338; ketoprofen group) or placebo patch (n = 338; placebo group). All of the randomized patients started the study treatment and 652 of them completed the protocol. Among those allocated to apply a study drug, 13 patients withdrew from the ketoprofen group and 11 withdrew from the placebo group. The reason for withdrawal was an adverse event that made further treatment inappropriate according to the investigator in 16 patients (9 and 7 from the ketoprofen and placebo groups, respectively), another condition that made further treatment inappropriate according to the investigator in 4 patients (1 and 3), and the patient's own request in 4 cases (3 and 1) (Figure 1).

Because all of the randomized patients started the study treatment, the safety population was identical to the ITT population. The 2 treatment groups were well matched with regard to the demographic and baseline characteristics when the ITT population was assessed (Table I).[22]

Efficacy

In the ITT population, the mean (± standard deviation) baseline VAS score for the intensity of pain

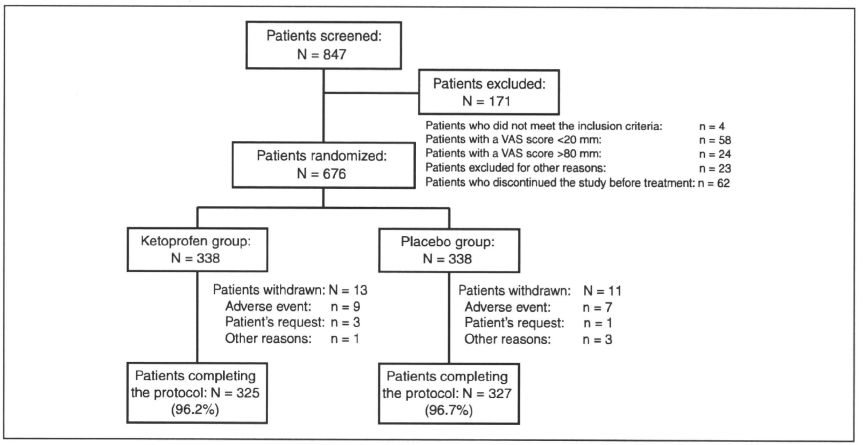

Figure 1. Disposition of the patients. Patients completing the protocol applied the full 2-week course of treatment.

Table I Demographic and Baseline Characteristics of the Intent-to-Treat Population

	Ketoprofen Group (n = 338)		Placebo Group (n = 338)	
Sex, n (%)				
Male	66	(19.5)	50	(14.8)
Female	272	(80.5)	288	(85.2)
Age, y, mean (SD)	58.2	(12.7)	59.2	(11.2)
RA stage, n (%)[a]				
I	32	(9.5)	23	(6.8)
II	91	(26.9)	101	(29.9)
III	122	(36.1)	111	(32.8)
IV	93	(27.5)	103	(30.5)
Functional class, n (%)[a]				
I	75	(22.2)	74	(21.9)
II	223	(66.0)	227	(67.2)
III	39	(11.5)	36	(10.7)
IV	1	(0.3)	1	(0.3)
Study joint, n (%)				
Right wrist	196	(58.0)	190	(56.2
Left wrist	142	(42.0)	148	(43.8)
Wrist joint pain (VAS score), mm, mean (SD)[b]	50.1	(15.1)	49.8	(14.7)

RA = rheumatoid arthritis; VAS = visual analog scale.
a. RA stage and functional class were determined according to Steinbrocker's criteria.[22]
b. Baseline pain at the study joint scored by patients on a 100-mm VAS.

affecting the study wrist joint was 50.1 ± 15.1 mm in the ketoprofen group and 49.8 ± 14.7 mm in the placebo group. At the end of treatment, the mean VAS pain intensity score was 34.4 ± 18.2 mm in the ketoprofen group and 36.5 ± 17.6 mm in the placebo group. There was a significant difference between the 2 groups with respect to the primary efficacy end point, which was the percent change of the VAS pain intensity score from baseline to the end of treatment (P = .020, Wilcoxon rank sum test; Table II). There was also a significant difference in the absolute change of the VAS pain intensity score from baseline to the end of treatment (P = .026, Wilcoxon rank sum test; Table II).

In the ITT population, there was no significant difference in the percentage of patients with 50% or greater reduction of the VAS pain intensity score, but there was a significant difference in the percentage of patients with 20% or greater reduction of the VAS pain intensity score (P = .020, Fisher's exact test; Table II).

In the ITT population, the subjective ratings of overall pain by the patients showed a significant difference between the ketoprofen group and the placebo group (P = .010, Wilcoxon rank sum test; Table III).

Table II Change of Pain Intensity on the Visual Analog Scale (VAS) From Baseline to the End of the 2-Week Treatment Period in the Intent-to-Treat Population

	Ketoprofen Group (n = 338)	Placebo Group (n = 338)	*P* value
Percent change of the VAS score, %			
Mean ± SD	31.2 ± 30.3	25.5 ± 31.2	.020[a]
95% confidence interval	28.0 to 34.4	22.1 to 28.8	
Median	28.6	22.5	
Absolute change of the VAS score, mm			
Mean ± SD	15.7 ± 16.0	13.2 ± 16.4	.026[a]
95% confidence interval	14.0 to 17.4	11.5 to 15.0	
Median	13.0	11.0	
Reduction of pain intensity			
≥50% reduction, % (n)	26.6 (90/338)	20.1 (68/338)	.056[b]
95% confidence interval	22.0 to 31.7	16.0 to 24.8	
≥20% reduction, % (n)	61.2 (207/338)	52.1 (176/338)	.020[b]
95% confidence interval	55.8 to 66.5	46.6 to 57.5	

a. Between-group comparison by the Wilcoxon rank sum test.
b. Between-group comparison by Fisher's exact test.

Table III Patient Subjective Ratings at the End of Treatment in the Intent-to-Treat Population, n (%)

	Rating Categories[a]							
	1	2	3	4	5	6	7	*P*[b]
Ketoprofen group (n = 337[c])	15 (4.5)	103 (30.6)	117 (34.7)	91 (27.0)	10 (3.0)	1 (0.3)	0 (0.0)	.010
Placebo group (n = 337[c])	18 (5.3)	63 (18.7)	137 (40.7)	97 (28.8)	19 (5.6)	3 (0.9)	0 (0.0)	

a. Rating categories were as follows; 1: markedly improved, 2: improved, 3: slightly improved, 4: unchanged, 5: slightly aggravated, 6: aggravated, 7: markedly aggravated.
b. Between-group comparison by the Wilcoxon rank sum test.
c. One patient in each group had no subjective ratings at the end of treatment.

Safety

The overall incidence of adverse events was not significantly different between the 2 groups, being 15.7% (53/338) in the ketoprofen group and 16.0% (54/338) in the placebo group (P = 1.000, Fisher's exact test; Table IV). No serious adverse events occurred in either group.

Adverse events at the application site. Adverse events occurred at the application site in 17 of 338 patients (5.0%) from the ketoprofen group compared with 19 of 338 patients (5.6%) from the placebo group, and there was no significant difference between the 2 groups (Table IV). In the ketoprofen group, contact dermatitis (n = 10) was the most common adverse event affecting the application site, followed by dermatitis, erythema, and pruritus in 2 patients each. In the placebo group, contact dermatitis and pruritus occurred in 5 patients each and were the most common application site adverse events, followed by erythema, swelling, and dermatitis (Table IV).

Other clinical adverse events. Other clinical adverse events occurred in 26 of 338 patients (7.7%) from the ketoprofen group compared with 29 of 338 patients (8.6%) from the placebo group, and there was no significant difference between the 2 groups (Table IV). Individual adverse events also showed a similar frequency between the 2 groups (Table IV).

Laboratory findings. Laboratory adverse events were reported in 15 of 338 patients (4.4%) from the ketoprofen group compared with 13 of 338 patients (3.8%) from the placebo group, with no significant difference between the 2 groups (Table IV). No significant laboratory abnormalities were detected in either group.

DISCUSSION

The present randomized, double-blind, controlled study showed that ketoprofen patch was significantly

Table IV Incidence of Common Adverse Events in the Safety Population

MedDRA SOC, PT	Ketoprofen Group (n = 338)	Placebo Group (n = 338)
Any adverse event[a]	53 (15.7)	54 (16.0)
Clinical adverse events at the application site[a]	17 (5.0)	19 (5.6)
General disorders and administration site conditions	7 (2.1)	14 (4.1)
Application site dermatitis	2 (0.6)	2 (0.6)
Application site erythema	2 (0.6)	4 (1.2)
Application site pruritus	2 (0.6)	5 (1.5)
Application site swelling	0	3 (0.9)
Skin and subcutaneous tissue disorders	10 (3.0)	5 (1.5)
Dermatitis (contact)	10 (3.0)	5 (1.5)
Clinical adverse events outside the application site[a]	26 (7.7)	29 (8.6)
Gastrointestinal disorders	1 (0.3)	2 (0.6)
General disorders and administration site conditions	3 (0.9)	0
Edema (peripheral)	2 (0.6)	0
Infections and infestations	9 (2.7)	10 (3.0)
Nasopharyngitis	7 (2.1)	7 (2.1)
Injury, poisoning and procedural complications	3 (0.9)	1 (0.3)
Contusion	2 (0.6)	0
Investigations (Blood pressure decreased)	0	1 (0.3)
Musculoskeletal and connective tissue disorders	6 (1.8)	9 (2.7)
Arthralgia	3 (0.9)	8 (2.4)
Renal and urinary disorders	0	1 (0.3)
Nervous system disorders	1 (0.3)	0
Respiratory, thoracic and mediastinal disorders	4 (1.2)	5 (1.5)
Upper-respiratory tract inflammation	3 (0.9)	3 (0.9)
Skin and subcutaneous tissue disorders	0	2 (0.6)
Laboratory adverse events[a]	15 (4.4)	13 (3.8)
Alanine aminotransferase increased	3 (0.9)	3 (0.9)
Aspartate aminotransferase increased	1 (0.3)	2 (0.6)
Blood lactate dehydrogenase increased	2 (0.6)	2 (0.6)
C-reactive protein increased	2 (0.6)	2 (0.6)
Blood urine present	1 (0.3)	3 (0.9)
White blood cell count increased	1 (0.3)	2 (0.6)
Protein urine present	2 (0.6)	0

MedDRA, medical dictionary for regulatory activities; SOC, system organ class; PT, preferred term. Figures in the table represent the number of patients with the specified adverse events (incidence [%]). If an adverse event occurred more than once in the same patient during the study, the patient was only counted once. This table lists adverse events (PTs) reported in at least 0.5% of the patients from either group.

a. Between-group comparison using Fisher's exact test gave $P = 1.000$ for the overall incidence of adverse events, $P = .864$ for the incidence of application-site clinical adverse events, $P = .779$ for the incidence of non–application-site clinical adverse events, and $P = .847$ for the incidence of laboratory adverse events.

superior to placebo patch for the relief of wrist pain in RA patients. To the best of our knowledge, this is the first clinical trial that has demonstrated the benefit of a percutaneous NSAID formulation for the management of RA. In some RA patients, clinically significant inflammation or pain persists at certain joints despite adequate control of systemic disease activity. Percutaneous NSAID formulations may offer a safe and useful treatment option for the relief of local pain in such patients because of a low potential for causing systemic adverse effects[13-15] and ease of application. In the present study, the treatment period was relatively short (2 weeks). According to a meta-analysis of studies on chronic musculoskeletal pain,[21] however, the efficacy and safety of topical NSAIDs for chronic musculoskeletal conditions is generally investigated over a 2-week period. Therefore, assessing the efficacy of the patch for local pain after 2 weeks was considered to be reasonable.

In the present study, patients allocated to the 2 treatment groups had similar mean baseline VAS scores for the intensity of pain affecting the study wrist joint, indicating that the baseline severity of wrist pain was comparable between the 2 groups.

The percent change and the absolute change of the VAS score for wrist pain from baseline to the end of treatment in the ITT suggested that the patch was effective in the present study, as mentioned above, but the actual difference of the VAS score between the 2 groups at the end of treatment was only 2.1 mm. In several other studies,[23-25] the minimum clinically significant difference of the VAS score was suggested to be 12 to 19 mm. However, these studies mainly investigated the changes of acute pain in patients from the emergency department using VAS scores and verbal category ratings. We found nothing in the literature concerning the minimum clinically significant difference of the VAS score for pain in RA patients. In general, even oral formulations are less effective for RA than for OA and other diseases, and it is thought to be difficult to observe the efficacy of NSAID treatment because RA patients have severe inflammation. Therefore, we considered it clinically significant that the present study showed significant superiority of the ketoprofen patch compared with the placebo patch. In a recent study of the 100-mg ketoprofen patch (Keofix), the difference of the VAS score for tendinitis[26] or ankle sprain[19] pain between the active patch and the placebo patch was 12 to 13 mm. Unlike these studies, the subjects of our study were patients with RA who presumably had more severe inflammation than patients with tendinitis or sporting injuries. This may be why our study failed to show a marked difference in efficacy. Also, the patch we used only contained 20 mg of ketoprofen, which was one-fifth of the ketoprofen content in the 100-mg patch, so its effect might have been milder.

Regarding the secondary efficacy end point, there was no significant difference in the percentage of patients with a 50% or more reduction of the VAS pain intensity score. However, reanalysis of the data showed that the percentage of patients with a 20% or more reduction of the VAS pain intensity score was significantly different between the 2 groups. Given that a 20% or more reduction of the VAS score is considered to be clinically significant according to ACR,[27] which are the guidelines for clinical assessment of RA released by the American College of Rheumatology, the change of the VAS score observed in the present study can also be assumed to be clinically significant. Regarding the subjective rating of overall pain by the patients, all of the pain categories showed significant differences between the 2 groups, which supports our findings about the primary efficacy end point.

Similar results were obtained by analysis of the primary and secondary efficacy end points in the PP population.

Percutaneous NSAID formulations target drug delivery to the tissues beneath the application site,[10-12] and a percutaneous preparation of ketoprofen has already been shown to be effective for OA[16-18] and ankle sprains.[19] Some investigators have reported similar efficacy of oral and topical NSAID preparations for the treatment of OA.[17,18] Patients with RA generally have more severe arthritis and may not show more benefit from even systemic NSAID treatment than placebo. Considering this fact, the present demonstration that ketoprofen patches are effective for persistent wrist pain in RA patients is clinically important.

The overall incidence of adverse events was not significantly different between the 2 treatment groups. Among the adverse events affecting the application site, contact dermatitis was more frequent after application of ketoprofen patches, whereas erythema, pruritus, and swelling were more frequent in the placebo group. The frequency of gastrointestinal adverse events, which are the most significant adverse reactions to oral NSAIDs, was similar in the ketoprofen and placebo groups. Thus, ketoprofen patch therapy was safe and well tolerated during the 2-week treatment period.

In conclusion, daily application of ketoprofen patches for 2 weeks was more effective than placebo for relieving persistent wrist joint pain in RA patients who had received fixed doses of DMARDs and/or systemic corticosteroid for a specified period.

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REFERENCES

1. American College of Rheumatology Subcommittee on Rheumatoid Arthritis Guidelines. Guidelines for the management of rheumatoid arthritis: 2002 update. *Arthritis Rheum.* 2002;46:328-346.

2. Kawai S. Current drug therapy for rheumatoid arthritis. *J Orthop Sci.* 2003;8:259-263.

3. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature New Biol.* 1971;231: 232-235.

4. Xie WL, Chipmann JG, Robertson DL, Erikson RL, Simons DL. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proc Natl Acad Sci U S A.* 1999;888:2692-2696.

5. Kujubu DA, Fletcher BS, Varnum BC, Lim RW, Herschman HR. TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. *J Biol Chem.* 1999;266:12866-12872.

6. Hla T, Neilson K. Human cyclooxygenase-2 cDNA. *Proc Natl Acad Sci U S A.* 1992;89:7384-7388.

7. Bresalier RS, Sandler RS, Quan H, et al. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. *N Engl J Med.* 2005;352:1092-1102.

8. Caldwell B, Aldington S, Weatherall M, Shirtcliffe P, Beasley R. Risk of cardiovascular events and celecoxib: a systematic review and meta-analysis. *J R Soc Med.* 2006;99:132-140.

9. Kawai S, Kojima F, Kusunoki N. Recent advances in nonsteroidal anti-inflammatory drugs. *Allergol Int.* 2005;54:209-215.

10. Osterwalder A, Reiner V, Reiner G, Lualdi P. Tissue absorption and distribution of ketoprofen after patch application in subjects undergoing knee arthroscopy or endoscopic carpal ligament release. *Arzneimittelforschung.* 2002;52:822-827.

11. Rolf C, Movin T, Engström B, Jacobs LD, Beauchard C, Le Liboux A. An open, randomized study of ketoprofen in patients in surgery for Achilles or patellar tendinopathy. *J Rheumatol.* 1997;24:1595-1598.

12. Rolf C, Engström B, Beauchard C, Jacobs LD, Le Liboux A. Intra-articular absorption and distribution of ketoprofen after topical plaster application and oral intake in 100 patients undergoing knee arthroscopy. *Rheumatology.* 1999;38:564-567.

13. Zacher J, Altman R, Bellamy N, et al. Topical diclofenac and its role in pain and inflammation: an evidence-based review. *Curr Med Res Opin.* 2008;24:925-9250.

14. Mazières B. Topical ketoprofen patch. *Drugs R D.* 2005;6: 337-344.

15. Lin J, Zhang W, Jones A, Doherty M. Efficacy of topical nonsteroidal anti-inflammatory drugs in the treatment of osteoarthritis: meta-analysis of randomised controlled trials. *BMJ.* 2004;329:324.

16. Bruhlmann P, Michel BA. Topical diclofenac patch in patients with knee osteoarthritis: a randomized, double-blind, controlled clinical trial. *Clin Exp Rheumatol.* 2003;21:193-198.

17. Rother M, Lavins BJ, Kneer W, Lehnhardt K, Seidel EJ, Mazgareanu S. Efficacy and safety of epicutaneous ketoprofen in Transfersome (IDEA-033) versus oral celecoxib and placebo in osteoarthritis of the knee: multicentre randomised controlled trial. *Ann Rheum Dis.* 2007;66:1178-1183.

18. Underwood M, Ashby D, Cross P, et al. Advice to use topical or oral ibuprofen for chronic knee pain in older people: randomised controlled trial and patient preference study. *BMJ.* 2008;336:138-142.

19. Mazières B, Rouanet S, Velicy J, Scarsi C, Reiner V. Topical ketoprofen patch (100 mg) for the treatment of ankle sprain: a randomized, double-blind, placebo-controlled study. *Am J Sports Med.* 2005;33:515-523.

20. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum.* 1988;31:315-324.

21. Mason L, Moore RA, Edwards JE, Derry S, McQuay HJ. Topical NSAIDs for chronic musculoskeletal pain: systematic review and meta-analysis. *BMC Musculoskel Disord.* 2004;5:28.

22. Steinbrocker O, Traeger CH, Batterman RC. Therapeutic criteria in rheumatoid arthritis. *JAMA.* 1949;140:659-662.

23. Gallagher EJ, Liebman M, Bijur PE. Prospective validation of clinically important changes in pain severity measured on a visual analog scale. *Ann Emerg Med.* 2001;38:633-638.

24. Bird SB, Dickson EW. Clinically significant changes in pain along the visual analog scale. *Ann Emerg Med.* 2001;38:639-643.

25. Kelly AM. The minimum clinically significant difference in visual analogue scale pain score does not differ with severity of pain. *Emerg Med J.* 2001;18:205-207.

26. Mazières B, Rouanet S, Guillon Y, Scarsi C, Reiner V. Topical ketoprofen patch in the treatment of tendinitis: a randomized, double blind, placebo controlled study. *J Rheumatol.* 2005;32:1563-1570.

27. Felson DT, Anderson JJ, Boers M, et al. American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis. *Arthritis Rheum.* 1995;38:727-235.