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ORIGINAL ARTICLE

Reduction of urinary levels of pyridinoline and deoxypyridinoline and serum levels of soluble receptor activator of NF-kappaB ligand by etanercept in patients with rheumatoid arthritis

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Abstract The effects of soluble TNF-α receptor, etanercept, on bone metabolism were investigated in patients with rheumatoid arthritis (RA). Thirty RA patients were administered etanercept once or twice a week for more than 6 months. We evaluated clinical and laboratory parameters and measured urinary excretion levels of pyridinoline (PYD), deoxypyridinoline (DPD), cross-linked N-telopeptides of type I collagen (NTX), and serum levels of bone alkaline phosphatase (BAP), osteoprotegerin (OPG), and soluble receptor activator of NFκB ligand (sRANKL) at the baseline and at 3 and 6 months after initial treatment with etanercept. Etanercept treatment resulted in an improvement of symptoms due to RA and in a reduction of urinary excretion levels of PYD and DPD as well as serum sRANKL levels, with a significant difference at 6 months, and an increase of serum BAP levels at 3 and 6 months after the initial treatment with etanercept. Urinary NTX and serum OPG levels did not show a significant change at 3 and 6 months after the initial treatment, but serum OPG levels did show a reverse correlation with serum CRP levels, suggesting that the regulation of inflammation in RA may result in an induction of OPG production. Etanercept may have the ability to reduce the levels of bone resorption markers and to increase the levels of a bone formation marker while reducing sRANKL formation in RA patients.

Keywords Deoxypyridinoline · Etanercept · Pyridinoline · Rheumatoid arthritis

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease accompanied by bone loss, resulting in juxta-articular or generalized osteoporosis [1]. The etiology of generalized osteoporosis in RA patients may depend on a variety of factors, including decreased physical activity [2–4], disease activity, and treatment with steroids [5, 6], but the mechanism of this disease has not yet been elucidated. Juxta-articular bone loss is probably due to both a local release of inflammatory agents such as cytokines derived from the rheumatoid synovium [7–10] and articular immobility [1, 9, 10]. The pro-inflammatory cytokines, interleukin 1 (IL-1), and tumor necrosis factor-α (TNF-α), have been believed to play an essential role in the bone metabolism and joint destruction of RA [11, 12].

Infliximab, which is a chimeric human–mouse monoclonal antibody binding soluble and membrane bound TNF-α, has been found to be highly effective in inhibiting bone destruction [13, 14] in RA patients. We have previously reported that infliximab administration to RA patients decreases the levels of bone resorption markers, including deoxypyridinoline (DPD) and urinary cross-linked N-telopeptides of type I collagen (NTX) [15]. Now, we have further studied the effects of a soluble TNF-α receptor, etanercept, on the bone resorption and formation markers measuring urinary pyridinoline (PYD), DPD, NTX, and serum bone alkaline phosphatase

(BAP) in RA patients. PYD is also described to be a bone resorption marker and more sensitive for articular cartilage destruction than DPD and to be more sensitive for disease activity of RA than DPD [16–18].

Osteoprotegerin (OPG) and receptor activator of NFκB ligand (RANKL) system has recently been reported to play an essential role in osteoclast formation and maturation as well as bone destruction in joints with RA patients [19]. RANKL is a member of the TNF ligand superfamily of cytokines that binds to its signal-transducing receptor, receptor activator of nuclear factor κB (RANK) [20–24], and is an essential factor in osteoclast differentiation [25]. OPG is a naturally occurring decoy receptor for RANKL [26]. When bound to RANKL, OPG prevents the binding of RANKL to RANK and thus inhibits the biological activity of RANKL [27]. Blockade with OPG results in protection from bone destruction [19]. The local expression levels of RANKL and OPG (RANKL/OPG ratio) have been described to be an important factor in determining the degree of osteoclast-mediated bone resorption [21, 28–30]. High serum levels of soluble receptor activator of nuclear factor κB ligand (sRANKL) and OPG have been seen in RA patients in comparison with healthy individuals [31], and anti-TNF-α (infliximab) treatment appears to normalize serum levels of sRANKL and OPG in RA patients [31]. Therefore, we also measured the change in OPG and sRANKL levels in RA patients receiving soluble TNF-α receptor (etanercept) treatment.

Materials and methods

RA patients receiving etanercept treatment

Thirty patients (27 women and three men; mean age 61.3±11.2 years; mean disease duration 15.3±7.4 years; Steinblocker's stage I: 1, II: 10, III: 17, IV: 2) meeting the American College of Rheumatology criteria for a diagnosis of RA were evaluated in this study between October 2005 and September 2007. Informed consent was obtained from all participants in accordance with guidelines of the ethical committee of Hamamatsu University School of Medicine. The evaluated patients in this study continued treatment with etanercept for more than 6 months without side effects. Sixteen patients received prednisolone at a constant mean dose of 2.3±2.0 mg/day throughout the study period. Etanercept was injected subcutaneously at a dosage of 25 mg once or twice a week. Twenty patients received etanercept in combination with methotrexate (MTX) perorally at a constant mean dose of 4.5±2.7 mg/week for more than 6 months before etanercept administration. Ten patients were treated with etanercept alone. The patients treated with etanercept were divided into MTX-treated [MTX (+)] and MTX-non-treated [MTX (−)] groups or prednisolone-treated [prednisolone (+)] and prednisolone-non-treated [prednisolone (−)] groups, and their data were evaluated in total and respective groups.

Clinical and laboratory examinations

The erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and anti-agalactosyl IgG antibody (CA-RF) were measured, and the Disease Activity Score of 28 joints combined with CRP (DAS28-CRP) were determined in clinical and laboratory examinations at baseline and at 3 and 6 months after the initial treatment. Serum samples and the second urinary samples in the early morning were obtained just before an initial injection of etanercept at baseline and at 3 and 6 months after the initial treatment with etanercept and stored at −80°C until assayed. Serum CA-RF was also measured with an enzyme-linked immunosorbent assay (ELISA) kit [Ei test, Eisai, Tokyo, Japan, intra-assay coefficient of variance (CV) <10%]. CA-RF is one of rheumatoid factors that have a higher sensitivity to diagnose RA compared with conventional rheumatoid factors. The levels of serum BAP, urinary PYD, DPD, NTX, and serum OPG and sRANKL were measured in all patients as described below.

Measurements of urinary PYD and DPD levels

Takahashi has previously described the methods for measuring urinary PYD and DPD [32]. Briefly, urinary samples were hydrolyzed in 6 M HCl for 20 h. The obtained hydrolysate was filtered and dissolved in distilled water and then subjected to high-performance liquid chromatography. The levels of urinary PYD and DPD were measured. The intra-assay CV was 6.4% for PYD and 6.0% for DPD, and the inter-assay CV was 5.9% for PYD and 6.0% for DPD.

Measurements of serum BAP, urinary NTX, and serum OPG and sRANKL

Serum BAP was measured using an AlkaphaseB kit (EIA, Metra Biosystems, Mountain View, CA, USA). This is an immunoassay utilizing monoclonal antibody made of purified ALP from a human osteosarcoma cell line. NTX levels were measured using an ELISA (Osteomark. Ostex International, Seattle, WA) with a monoclonal antibody against the N-telopeptide to the helix intermolecular cross-linking domain of type I collagen. In this kit, intra-assay CV is 7.6% and inter-assay CV 4.0%.

Statistical analysis

In this study, all data are expressed as the mean±SD. The comparison of values for pre- and post-treatment measure-

ments of the number of swollen and tender joints, CRP, ESR, serum CA-RF, DAS28-CRP, urinary PYD, DPD, and NTX, and serum BAP, OPG, and sRANKL were performed using the Wilcoxon signed rank test. Single regression analysis of these parameters for the total data at the baseline, 3 months, and 6 months was also performed, and the statistical significance of correlation was determined with Pearson's correlation test. In addition, multiple regression analysis between the dependent variables including DAS28-CRP, CRP, and ESR and the independent variables including urinary PYD, DPD, and NTX and serum BAP, OPG, and sRANKL for the total data at baseline, 3 months, and 6 months was also performed to assess the effect on the values of inflammatory parameters by bone metabolism markers.

P values less than 0.05 were considered to be significantly different. This calculation was performed on a Macintosh computer using the Statcel software package.

Results

Clinical and laboratory disease activity

CRP levels in total patients significantly decreased from 3.11±1.80 mg/dl at pretreatment to 0.80±0.85 mg/dl ($P<0.01$) and 0.67±0.88 mg/dl ($P<0.01$) at 3 and 6 months after the initial injection of etanercept, respectively (Table 1). ESR levels, DAS28-CRP, the number of swollen joints, and the number of tender joints, but not serum CA-RF levels, in total patients were also were significantly decreased at 3 and 6 months compared with those at pretreatment. These tendency did not depend on MTX (+) or MTX (−), and prednisolone (+) and prednisolone (−). Three patients were poor responders to etanercept treatment, and their decrease of DAS28-CRP scores at 3 and 6 months compared with the baseline was below 0.6.

Urinary PYD, DPD, and NTX levels

Urinary PYD and DPD levels were corrected with urinary creatinine levels. The mean urinary PYD levels in total patients showed a significant decrease (42.7±18.8 nmol/mmol Cr, $p<0.01$) at 6 months after the initial treatment with etanercept compared with the levels (57.6±41.2 nmol/mmol Cr) at the initial treatment (Table 1). The mean urinary levels of DPD in total patients significantly decreased to 6.60±3.12 nmol/mmol Cr ($p<0.01$) at 6 months compared with 8.17±4.14 nmol/mmol Cr at the initial treatment levels with etanercept. These tendency of the change of urinary PYD and DPD levels did not depend on MTX (+) and MTX (−) or prednisolone (+) and prednisolone (−).

The mean urinary NTX levels in total patients did not show significant differences at 3 and 6 months after the initial treatment compared with the baseline levels. Poor responders to etanercept did not show a significant change of urinary PYD, DPD, and NTX levels (data not shown).

Serum BAP levels

The mean serum BAP levels in the total patients were 27.2±13.9 U/l at pretreatment, 30.7±13.3 U/l ($p<0.05$) at 3 months, and 31.6±14.6 U/l ($p<0.05$) at 6 months after the initial treatment (Table 1) and showed an increase in response to etanercept treatment. The mean serum BAP levels in MTX (+) and MTX (−) groups or in prednisolone (+) and prednisolone (−) groups significantly decreased at 3 months after the initial treatment. Poor responders to etanercept did not show a significant change of serum BAP levels before and after etanercept treatment (data not shown).

OPG and sRANKL levels in serum samples

The mean serum OPG levels (8.30±2.14 pmol/l) in the total patients showed an increase at 6 months after the initial treatment with etanercept compared with the levels at the initial treatment (7.27±2.20 pmol/l). But there were no significant differences between these values. The mean serum sRANKL showed significantly decreased levels (596±623 pmol/l; $p<0.05$) at 6 months after the initial treatment with etanercept compared with the levels at the initial treatment (878±761 pmol/l). The mean serum OPG and sRANKL levels in MTX (+) and MTX (−) groups or prednisolone (+) and prednisolone (−) groups did not show significant differences at 3 and 6 months compared with those at the initial treatment. Poor responders to etanercept did not show a significant change of serum OPG and sRANKL levels (data not shown).

Serum sRANKL (pmol/l)/serum OPG (pmol/l) ratios were calculated in the samples from the same patients. The mean serum sRANKL (pmol/l)/serum OPG (pmol/l) ratios in the total patients were decreased at 3 months (101±101, $p<0.05$) and at 6 months (97±99, $p<0.05$) after the initial treatment with etanercept compared with the levels at the initial treatment (156±135).

Correlation analysis among laboratory and clinical parameters

In the single regression analysis, the correlation coefficient between urinary PYD and urinary DPD was 0.874 ($p<0.0001$), and that between urinary DPD and urinary NTX was 0.518 ($p<0.0001$; Table 2). Thus, there was a significant correlation between bone resorption markers. Reverse correlation coefficients (−0.385, $p<0.05$) were

Table 1 The change in clinical and laboratory parameters, urinary PYD, DPD, and NTX, and serum BAP, OPG, and sRANKL in patients with RA treated with etanercept. It was evaluated at baseline, 3 months, and 6 months after initial treatment with etanercept

		Baseline	3 months	6 months
CRP (mg/dl)	Total	3.11±1.80	0.80±0.85**	0.67±0.88**
	MTX(+)	3.29±1.92	0.85±0.90**	0.68±0.96**
	MTX(−)	2.28±0.71	0.57±0.60**	0.62±0.56**
	Prednisolone(+)	3.27±2.18	0.85±1.03**	0.69±1.03**
	Prednisolone(−)	2.68±0.12	0.67±0.56**	0.62±0.54**
ESR (mm/h)	Total	64.0±26.9	40.5±25.6**	36.1±25.0**
	MTX(+)	62.3±25.4	38.6±24.3**	32.3±23.7**
	MTX(−)	68.6±38.6	50.0±31.9*	51.0±26.4*
	Prednisolone(+)	65.3±24..2	39.1±25.3**	34.5±23.5**
	Prednisolone(−)	62.1±37.0	46.3±29.1*	44.9±29.1*
DAS28-CRP	Total	4.91±0.95	3.20±0.76**	2.99±0.97**
	MTX(+)	5.08±1.15	3.22±0.80**	3.00±1.16**
	MTX(−)	4.71±0.50	2.88±0.50**	2.99±0.51**
	Prednisolone(+)	4.96±1.02	3.31±0.79**	2.99±1.06**
	Prednisolone(−)	4.69±0.50	2.91±0.68**	2.98±0.48*
Serum CA-RF (AU/ml)	Total	395±608	378±615	367±539
Number of swollen joints	Total	7.63±4.11	2.81±2.36**	2.86±2.21**
	MTX(+)	7.73±4.12	3.00±2.27***	2.93±2.15***
	MTX(−)	7.50±3.07	2.00±2.00***	2.25±0.46***
	Prednisolone(+)	8.28±4.60	3.32±2.43***	3.19±2.42***
	Prednisolone(−)	6.60±2.67	1.80±1.62*	2.00±0.53**
Number of tender joints	Total	5.00±5.25	1.38±1.99**	1.80±2.51**
	MTX(+)	5.35±5.40	1.57±1.96***	1.93±2.35***
	MTX(−)	3.25±1.58	1.25±0.21*	1.00±1.07*
	Prednisolone(+)	5.57±6.24	1.25±1.76**	2.17±2.69**
	Prednisolone(−)	4.20±2.57	1.50±2.08**	1.13±0.83**
Urinary PYD (nmol/mmol Cr)	Total	57.6±41.2	50.9±21.4	42.7±18.8**
	MTX(+)	48.0±17.9	47.7±15.1	38.4±6.8*
	MTX(−)	79.2±65.8	58.4±30.9	46.3±29.2*
	Prednisolone(+)	51.8±14.7	50.7±12.9	38.4±6.80**
	Prednisolone(−)	64.6±60.6	51.9±30.1	45.4±27.5*
Urinary DPD (nmol/mmol Cr)	Total	8.17±4.14	8.15±2.90	6.60±3.12**
	MTX(+)	7.27±2.65	7.78±2.21	6.17±2.04*
	MTX(−)	10.20±6.00	9.10±4.00	7.60±4.80*
	Prednisolone(+)	8.10±4.47	8.10±12.9	6.62±3.09*
	Prednisolone(−)	8.26±5.13	8.30±3.6	6.52±3.75*
Urinary NTX (nM BCE/mM Cr)	Total	58.4±37.2	62.6±38.0	53.9±34.5
	MTX(+)	60.4±40.7	67,9±40.7	68.4±45.1
	MTX(−)	46.7±11.1	37.8±13.1	38.6±5.9
	Prednisolone(+)	63.0±44.0	69.9±44.5	71.1±47.4
	Prednisolone(−)	48.5±19.3	46.0±19.3	43.6±8.0
Serum BAP (U/L)	Total	27.2±13.9	30.7±13.3*	31.6±14.6*
	MTX(+)	27.6±14.1	30.0±12.3*	31.0±12.8
	MTX(−)	26.1±9.2	32.0±12.6*	32.3±13.1*
	Prednisolone(+)	30.6±15.2	33.5±13.2*	33.8±14.3
	Prednisolone(−)	22.7±8.4	27.5±10.6*	29.8±10.7*
Serum OPG (pmol/l)	Total	7.27±2.20	7.57±2.24	8.30±2.14
	MTX(+)	7.75±2.12	7.79±1.62	8.86±2.53
	MTX(−)	6.07±2.05	7.60±4.00	7.36±0.93
	Prednisolone(+)	7.54±2.79	7.62±1.89	8.35±2.16
	Prednisolone-(−)	6.97±1.55	7.61±2.39	7.90±2.07
Serum sRANKL (pmol/L)	Total	878±761	679±671	596±623*
	MTX(+)	975±852	697±757	621±638*
	MTX(−)	735±438	635±448	580±344*
	Prednisolone(+)	934±776	649±731	581±555*

Table 1 (continued)

		Baseline	3 months	6 months
	Prednisolone(−)	790±738	644±625	599±580*
Serum sRANKL (pmol/L)/Serum OPG(pmol/L)	Total	156±135	101±101*	97±99*
	MTX(+)	162±152	103±112	92±98*
	MTX(−)	143±91	96±74 *	109±85*
	Prednisolone(+)	160±151	103±114	99±107*
	Prednisolone(−)	140±118	93±87*	93±87*

Data are shown by mean±SD values. Data at 3 and 6 months were compared with those at baseline using Wilcoxon signed rank test. Patients treated with etanercept were divided into MTX(+) and MTX(−) groups or prednisolone(+) and prednisolone(−) groups.
*$p<0.05$ vs. baseline, **$p<0.01$ vs. baseline

seen between CRP and serum OPG, possibly indicating that the regulation of rheumatoid inflammation is related to OPG production. In addition, multiple regression analysis between the dependent variables including DAS28-CRP, CRP, and ESR and the independent variables including urinary PYD, DPD, and NTX, and serum BAP, OPG, and sRANKL was performed to assess the effect of bone metabolism markers on the inflammatory parameters. Urinary PYD, DPD, and NTX, and serum BAP, OPG, and sRANKL levels did not significantly affect on the DAS28-CRP and CRP levels. But urinary DPD levels had significant correlation with ESR levels ($p=0.014$; Table 3).

Discussion

TNF-α has been described as stimulating bone resorption and inhibiting the synthesis of proteoglycans in cartilage [33] as well as playing an important role in the pathogenesis of RA. In the present study, we studied the effects of a soluble TNF-α receptor, etanercept, on bone resorption and formation markers measuring the levels of urinary PYD, DPD, and NTX, and serum BAP in RA patients.

Previous reports have indicated that treatment with infliximab and etanercept increases serum BAP levels and decreases urinary NTX and DPD levels in RA patients [34, 35]. We also have previously reported that infliximab treatment for RA patients decreases urinary excretion levels of NTX and DPD, but does not change serum BAP levels [15]. There are no data, however, regarding total urinary PYD levels in RA patients treated with etanercept. In our study, PYD levels tended to be decreased at 6 months in the treatment with etanercept. DPD was also decreased at 6 months after etanercept treatment. PYD and DPD are derived from collagen breakdown. These cross-links from

Table 2 Single regression analysis among urinary PYD, DPD, and NTX, serum BAP, OPG, and sRANKL, other laboratory parameters, and DAS28-CRP for the total data at the baseline, 3 months, and 6 months was performed, and the statistical significance of correlation was determined with Pearson's correlation test

	Urinary PYD	Urinary DPD	Serum BAP	Urinary NTX	DAS28 - CRP	CRP	ESR/h	Serum CA-RF	Serum OPG	Serum sRANKL
Urinary PYD	–									
Urinary DPD	0.874***	–								
Serum BAP	−0.077	0.019	–							
Urinary NTX	0.290	0.518**	0.106	–						
DAS28-CRP	0.174	−0.075	−0.117	−0.501**	–					
CRP	−0.195	−0.272	−0.279	−0.485*	0.793***	–				
ESR /h	−0.048	0.402*	−0.277	−0.007	0.486*	0.512**	–			
Serum CA-RF	0.083	−0.035	−0.394	−0.254	0.070	−0.060	−0.149	–		
Serum OPG	0.038	0.085	0.067	0.140	−0.286	−0.385*	0.007	−0.298	–	
Serum Srankl	−0.200	−0.054	0.052	−0.045	0.189	0.070	−0.173	0.180	0.078	–

*$p<0.05$, **$p<0.01$, ***$p<0.0001$

Table 3 Multiple regression analysis between the dependent variables including DAS28-CRP, CRP, and ESR and the independent variables including urinary PYD, DPD, and NTX, and serum BAP, OPG, and sRANKL was performed

Dependent variable	DAS28-CRP		CRP		ESR/h	
Coefficients of determination (R^2)	0.416		0.484		0.416	
	Regression coefficient	*P*	Regression coefficient	*P*	Regression coefficient	*P*
Independent variable						
Urinary PYD	0.0473	0.370	0.020	0.763	−0.798	0.489
Urinary DPD	−0.312	0.381	0.134	0.151	7.67	0.014*
Serum BAP	0.0003	0.996	−0.024	0.298	−0.576	0.153
Urinary NTX	−0.023	0.163	−0.0146	0.464	−0.294	0.404
Serum OPG	−0.096	0.325	−0.178	0.151	0.347	0.870
Serum sRANKL	0.001	0.213	0.001	0.677	−0.008	0.283

*$p<0.05$

between the two-collagen fibrils are released unchanged into the urine with the breakdown of collagen. DPD is derived only from type I collagen and is a cross-link originating from several tissues, but mainly from bone [36, 37]. DPD has been validated as a useful marker for bone resorption. DPD has been described to correlate with disease activity of RA and to reflect diffuse bone loss [36]. PYD is the main cross-link between type II collagen fibers in cartilage and is most abundant in bone [36, 37]. PYD is also described to be more sensitive for articular cartilage destruction than DPD and is more sensitive for disease activity of RA than DPD [16–18]. Decreased urinary excretion levels of PYD and DPD were also seen in infliximab therapy, as described by Ostanek et al. [36]. In the present study, a significant correlation in multiple regression analysis between urinary DPD and ESR in RA patients was seen, but that between PYD and the disease activity markers of RA was not seen. Therefore, it is not clear from our study whether a decrease in the urinary excretion levels of PYD by etanercept treatment elucidates the protection of cartilage destruction or of bone destruction by etanercept.

In our study, after the treatment with etanercept for RA patients, NTX did not decrease significantly during 6 months after the therapy. Greenspan et al. [38] and Ravn et al. [39] have indicated that NTX is a more sensitive and bone-specific marker than DPD when measurement of bone-derived type I collagen fragments is carried out. Gorai et al. [40] have indicated that urinary excretion levels of NTX are inversely correlated with the bone mineral density of the lumbar spine and are a more sensitive and specific bone metabolism marker than PYD analogues. Iwamoto et al. [41] has previously shown that NTX levels are correlated with functional class and the HAQ score for lower extremities and that physical inactivity affects bone resorption. Furthermore, NTX changes earlier than any other markers of bone resorption after treatment for osteoporosis. NTX primarily originates from bone tissue, while PYD and DPD exist broadly in other tissue than bone and are derived from inflammatory processes in the joint tissues by RA, as described by Takahashi et al. [32, 42]. Thus, PYD, DPD, and NTX provide different perspectives of bone and cartilage metabolism. Therefore, in our present study, the discrepancy between the change in urinary excretion levels of NTX and that of PYD and DPD may have occurred in RA patients after etanercept treatment.

In the present study, BAP levels increased significantly in etanercept-treated RA patients, although the extent of the increased levels was low. Thus, the increase of a bone formation and osteoblast differentiation marker and the decrease of bone absorption markers by TNF-α blockade by etanercept may presume the possibility of an increase in bone formation. Our previous study of RA patients treated with infliximab indicated that serum BAP levels were not significantly changed after the infliximab treatment [15], which may indicate that etanercept and infliximab have different actions for bone metabolism.

As factors that regulate bone metabolism, the action of OPG and sRANKL system has recently been noticed. Therefore, their serum levels were investigated in RA patients treated with etanercept in our study. RANKL is a member of the TNF ligand superfamily of cytokines that binds to its signal-transducing receptor, receptor activator of NFκB (RANK) [20–24], and plays a critical role in osteoclast differentiation [25]. OPG is a naturally occurring decoy receptor for RANKL [43]. When bound to RANKL, OPG prevents the binding of RANKL to RANK and thus inhibits osteoclast differentiation and consequential bone loss [43–45]. The local expression levels of RANKL and OPG, especially RANKL/OPG ratio, have been described to be essential in determining the degree of osteoclast-mediated bone resorption [46–49].

Ziolkowska et al. [31] have previously reported that RA patients exhibit high serum levels of OPG and sRANKL and that these levels are normalized after anti-TNF-α treatment. In our study, serum OPG levels were increased somewhat at 6 months after initial etanercept administration, without significant difference. Serum sRANKL levels were significantly decreased at 6 months after the initial treatment with etanercept. These results were not consistent with the clinical effects of etanercept, which induces the recovery of bone erosion and destruction. There are some reports that the sRANKL/OPG ratio reflects osteoclast activity in bone destruction in RA [50–53] and that their higher values means a high activity of osteoclast. In our study, sRANKL/OPG ratios were high at the pretreatment of etanercept, but they decreased after treatment with etanercept. Low levels of OPG in synovial fluid from RA patients, compared with osteoarthritis, trauma, and gout patients, have been reported in recent studies [54, 55]. In our present study, serum OPG levels reversely correlated with CRP in single regression analysis. These reports and our results in the present study may suggest the presence of a regulatory mechanism of OPG production in RA patients.

Both TNF-α and IL-1 have been known to increase RANKL and OPG production [56–59]. Therefore, in our study, sRANKL production may have been regulated by the blocking of TNF-α with etanercept. But the levels of OPG production were not significantly changed, indicating the presence of other mechanisms than TNF-α regulation of OPG production in RA. Further study is underway to investigate the mechanism for regulation of OPG production.

In the present study, etanercept was demonstrated to have effects on bone resorption or formation markers and on sRANKL levels in RA patients. In future, the correlation between the change in these marker levels and bone mineral density levels must be investigated to assess the significance of the measurement of these marker levels.

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