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ORIGINAL ARTICLE

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The effect of methotrexate on bone metabolism markers in patients with rheumatoid arthritis

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Abstract The aim of the present study was to evaluate the influence on urinary excretion levels of N-telopeptide of type I collagen (NTX) and deoxypyridinoline (DPD) as a useful marker for bone resorption, and on serum-bone alkaline phosphate (BAP) levels as a useful marker for bone formation and an early marker of osteoblast differentiation in patients with rheumatoid arthritis (RA) treated with methotrexate (MTX). Thirty patients with RA, diagnosed according to the criteria of the American College of Rheumatology, were involved in this study between March 2003 and January 2005. None of the patients had a history of hormone (estrogen) replacement therapy. All patients were treated with MTX. Methotrexate was administered perorally at a dosage of 4–10 mg/week. All patients underwent general and physical examinations and routine blood and urinary analysis at the baseline, 3 months and 6 months after the initial treatment. Then the levels of NTX and DPD in urine and BAP in serum were measured in all patients. For comparison with the effect of other DMARDs on bone metabolism markers in RA patients, we measured the levels of NTX and DPD in urine and BAP in serum of RA patients, 13 patients treated with salazosulfapyridine (SASP), and 14 patients treated with actarit (ACT). In patients treated with MTX, NTX levels decreased significantly at 3 months after the initial treatment and remained low at 6 months after the initial treatment, and DPD levels significantly decreased at 6 months after the initial treatment. The mean serum BAP levels changed without significant differences from the baseline at 3 months and 6 months. In patients treated with SASP and ACT, all bone metabolism markers had not changed significantly at the three time points. On disease activity erythrocyte sedimentation rate, C-reactive protein, the number of swollen joints and tender joints, and mHAQ score decreased significantly at 3 months after the initial treatment, and remained at low levels at 6 months after the initial treatment with MTX. Methotrexate therapy looks promising in inhibiting generalized bone loss in patients with RA. In addition, NTX is a more sensitive marker than DPD.

Key words Bone metabolism marker · Methotrexate (MTX) · Rheumatoid arthritis

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease that increases the risk of developing bone loss, resulting in juxta–articular and generalized osteoporosis.[1,2] Methotrexate (MTX) is a folate antagonist, commonly used in high doses for the treatment of malignancies, and in lower doses for the treatment of inflammatory diseases such as RA. Some studies[3,4] suggested that long-term use of low-dose MTX in RA patients may aggravate the development of osteopenia and stress fractures. On the other hand, Cranney et al.[5] suggested that low-dose MTX therapy does not have a negative effect on preservation of bone density by evaluating bone mineral density while El Miedany et al.,[6] who evaluated bone metabolism markers, suggested that low-dose MTX may have a bone protective effect.

It is now possible to assess bone turnover using a range of biochemical markers to reflect bone resorption and formation.[7] N-telopeptide of type I collagen (NTX) and deoxypyridinoline (DPD) have been validated as a useful marker for bone resorption, and bone alkaline phosphatase (BAP) as a useful marker for bone formation and an early marker of osteoblast differentiation. Elevated concentrations of bone metabolism markers can reflect several pathophysiologic processes that occur in RA, including activity of the inflammatory process or a high rate of bone turnover, as occurs in osteoporosis, osteoarthritis, and erosive joint destruction.

The aim of the present study was to evaluate the influence on urinary excretion levels of NTX and DPD, and on

serum BAP levels, as bone metabolism markers with MTX in patients with RA. Moreover since NTX, a newer marker of bone resorption, has not been adequately studied, we investigated which of NTX or DPD is the more sensitive marker.

Materials and methods

Subjects

Thirty patients (26 women [21 postmenopausal] and 4 men; age 59.4 ± 4.72 years; mean disease duration 12.9 ± 2.94 years; Steinbrocker's criteria: class I, 8; class II, 14; class III, 6; class IV, 2) with RA, diagnosed according to the criteria of the American College of Rheumatology (ACR),[8] were involved in this study between March 2003 and January 2005. Methotrexate was administered perorally at a dosage of 4–10 mg/week (mean ± SE 6.78 ± 0.31 mg/week; median dose 6.0 mg/week). All patients underwent general and physical examinations and routine blood and urinary analysis at the baseline, and at 3 months and 6 months after the initial treatment. To evaluate disease activity, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), the number of swollen joints and tender joints, Stanford modified health assessment questionnaire (mHAQ)[9] score, and ACR improvement response criteria[10] were measured. Then the levels of NTX and DPD in urine and BAP in serum were measured in all patients as described below. And for comparison with the effect of other disease-modifying antirheumatic drugs (DMARDs) on bone metabolism markers in RA patients diagnosed according to the criteria of the ACR,[8] we measured the levels of NTX and DPD in urine and BAP in serum of 13 patients treated with salazosulfapyridine (SASP) (10 females including 7 postmenopausal women and 3 males; age 64.8 ± 4.2 years; mean disease duration 8.54 ± 1.67 years; Steinbrocker class I, 3; class II, 6; class III, 3; class IV, 1) and 14 patients treated with actarit (ACT) (11 females including 8 postmenopausal women and 3 males; age 58.4 ± 4.14 years; mean disease duration 6.46 ± 2.07 years; Steinbrocker class I, 6; class II, 6; class III, 2; class IV, 0) at the baseline, and at 3 months and 6 months after the initial treatment as described below. For all patients, therapies of other DMARDs excluding MTX, SASP, and ACT were not effective longer than 6 months and MTX, SASP, or ACT were administered for the first time at the commencement of the study. None of the patients had a history of hormone (estrogen) replacement therapy or had used any other bone-sparing drugs or calcium supplements. Thirteen patients treated with MTX, 8 patients treated with SASP, and 6 patients treated with ACT received a constant dose of prednisolone equivalent to 10 mg or less per day (mean ± SE 1.73 ± 0.18, 1.91 ± 0.51, 1.75 ± 0.53 mg/day; median dose 2.5, 2.0, 2.0 mg/day, respectively) at a stable dosage throughout the present study. Informed consent was obtained from all patients. The ethical committee of Hamamatsu University School of Medicine authorized this work.

Measurements of urinary NTX levels, urinary DPD levels, and serum BAP levels

The second void urine samples were collected in the dispensary at approximately the same time in the morning, and stored at –70°C until assayed. N-telopeptide of type I collagen levels were measured using an enzyme-linked immunosorbent assay (ELISA) (Osteomark; Ostex International, Seattle, WA, USA) with a monoclonal antibody against the N-telopeptide to the helix intermolecular cross-linking domain of type I collagen. Deoxypyridinoline levels were determined with commercially available pyridinium crosslinks using a high-performance liquid chromatography kit (EIA; Metra Biosystems, Mountain View, CA, USA). Serum BAP was measured using an AlkaphaseB kit (EIA; Metra Biosystems).

Statistical analysis

All data are presented as mean ± SE. Pretreatment and post-treatment measurements were compared using the Wilcoxon signed-ranks test. Spearman's rank correlation analysis was used to examine the cross-correlation of change rate between markers of bone metabolism and clinical factors in patients with RA. P values of less than 0.05 were considered significant. Analysis was performed on a Macintosh computer using the StatView 5.0 software package.

Results

Urinary NTX levels

In patients treated with MTX, the mean NTX levels decreased significantly from 80.1 ± 16.0 nM bone collagen equivalent (BCE)/mM·Cr at pretreatment to 52.8 ± 9.13 nM BCE/mM·Cr ($P < 0.05$) at 3 months after the initial treatment, and 52.5 ± 5.11 nM BCE/mM·Cr ($P < 0.05$) at 6 months after the initial treatment (Table 1). In patients treated with SASP and ACT, the mean urinary NTX levels did not change significantly at the three time points (Table 1).

Urinary DPD levels

In patients treated with MTX the mean DPD levels were 9.22 ± 0.85 nM/mM·Cl at pre-treatment, 8.12 ± 0.85 nM/mM Cl ($P = 0.062$) at 3 months after the initial treatment, and 7.38 ± 0.65 ($P < 0.05$) nM/mM·Cl at 6 months after the initial treatment (Table 1). Thus, in DPD levels there was no significant decrease at 3 months after the initial treatment but a significant decrease at 6 months after the initial treatment, compared with pretreatment values. In patients treated with SASP and ACT, the mean urinary DPD levels did not change significantly at the three time points (Table 1).

Table 1. Parameters of metabolism markers during therapy with MTX, SASP, and ACT

	Pretreatment	3 Months	6 Months
MTX			
NTX (nM BCE/mM)	80.1 ± 16.0	52.8 ± 9.13*	52.5 ± 5.11*
DPD (nM/M Cl)	9.22 ± 0.85	8.12 ± 0.85	7.38 ± 0.65*
BAP (U/l)	34.6 ± 6.21	31.9 ± 4.76	32.5 ± 5.48
SASP			
NTX (nM BCE/mM)	50.0 ± 4.3	47.1 ± 5.3	42.4 ± 2.8
DPD (nM/M Cl)	7.84 ± 1.08	7.38 ± 1.29	6.80 ± 0.62
BAP (U/l)	26.1 ± 1.8	27.0 ± 1.6	24.9 ± 1.8
ACT			
NTX (nM BCE/mM)	68.1 ± 16.5	65.3 ± 12.5	68.4 ± 14.8
DPD (nM/M Cl)	8.97 ± 1.76	8.98 ± 1.71	9.08 ± 1.83
BAP (U/l)	25.26 ± 2.7	26.3 ± 2.8	26.0 ± 2.6

Mean ± SE
MTX, methotrexate; NTX, N-telopeptide of type I collagen; BCE, bone collagen equivalent; DPD, deoxypyridinoline; BAP, serum-bone alkaline phosphate; SASP, salazosulfapyridine; ACT, actarit
* $P < 0.05$ vs pre-treatment

Table 2. Parameters of disease activity and metabolism markers during therapy with methotrexate

	Pretreatment	3 Months	6 Months
CRP (mg/dl)	2.00 ± 0.64	0.88 ± 0.71*	0.96 ± 0.34*
ESR (mm)	55.55 ± 8.46	45.25 ± 7.67*	43.55 ± 8.62*
No. of swollen joints	9.07 ± 1.19	5.87 ± 1.55*	6.21 ± 1.59*
No. of tender joints	5.12 ± 1.80	2.13 ± 0.75*	2.36 ± 0.56*
mHAQ score	10.1 ± 1.38	4.96 ± 1.25*	4.55 ± 1.44*

Mean ± SE
CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; mHAQ, Stanford modified Health Assessment Questionnaire
*$P < 0.05$ vs pre-treatment

Table 3. Correlation between the rate of decrease on bone resorption markers and disease activity in patients treated with methotrexate

	NTX		DPD	
	Correlation coefficient	*P* value	Correlation coefficient	*P* value
CRP	0.438	<0.05	0.129	<0.05
ESR	0.232	0.199	0.293	0.068
No. of swollen joints	0.397	0.093	0.058	0.795
No. of tender joints	0.150	0.187	–0.180	0.898
mHAQ score	0.446	<0.05	0.472	0.082

Correlation between [BRM (at 6 months) – BRM (at pretreatment)]/[BRM (at pretreatment)] and [CP (at 6 months) – CP (at pretreatment)]/[CP (at pretreatment)] was calculated. Bone resorption markers (BRM) included NTX and DPD, and clinical parameters (CP) CRP, ESR, number of swelling joints, number of tender joints, and mHAQ score

Serum BAP levels

In patients treated with MTX the mean serum BAP levels were 34.6 ± 6.21 U/l at pre-treatment, 31.9 ± 4.76 U/l at 3 months after the initial treatment, and 32.5 ± 5.48 U/l at 6 months after the initial treatment, but without significant differences among the three time points (Table 1). In patients treated with SASP and ACT, the mean serum BAP levels did not change significantly at the three time points (Table 1).

Measurements of disease activity in patients treated with MTX

Patients improved clinically after receiving MTX at 6 months after the initial treatment of MTX; 81% of patients demonstrated an ACR20 response, 38% of them an ACR50 response, and 19% an ACR70 response. C-reactive protein levels, ESR levels, mHAQ score, the number of tender joints, and the number of swollen joints decreased significantly at 3 and 6 months after the initial treatment (Table 2).

Correlation between the rate of decrease on bone resorption markers and disease activity in patients treated with MTX

The rate of decrease of NTX statistically corresponded with the rate of decrease of CRP ($r = 0.438$, $P < 0.05$), and corresponded with the rate of decrease of mHAQ scores ($r = 0.446$, $P < 0.05$) (Table 3).

Discussion

In the present study, within 3 months after the initial treatment patients treated with MTX did not have significantly decreased disease activity, and CRP levels and the markers of bone resorption were virtually unchanged (data is not shown). But at 3 and 6 months after treatment by MTX therapy, patients exhibited controlled disease activity, and CRP and ESR levels had decreased. Furthermore, the level of NTX statistically corresponded with disease activity. Several studies[11–13] have indicated that weekly MTX therapy had improved the markers of bone resorption and MTX therapy might have a bone-protective effect by controlling disease activity. Our results also agree with these reports that bone resorption markers were high in patients with RA and were associated with increased disease activity, and tended to go down with suppression of disease activity by administration of MTX. We consider that the reduction of bone resorption markers may be associated with the reduction of local disease activity around the joints and adjacent joint damage rather than the toxic effect of MTX. In support of this, no effect of MTX on bone resorption markers was found at 3 months after treatment. Rheumatoid arthritis disease activity had a greater effect on bone resorption markers than individual DMARDs, including MTX. The proinflammatory cytokines that are associated with cartilage and bone degradation by synovial inflammation, tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6 have been believed to play an important role in the pathogenesis of RA[14,15]. The bone loss in RA has been believed to occur due to an increased production of receptor activator of the nuclear factor kappa B ligand (RANKL) in the bone mar-

row that activates osteoclasts to resorb bone and cause erosions.[16,17] Furthermore, when osteoblast is stimulated by TNF-α, it guides the manifestation of RANKL; TNF-α directly activates macrophages, which is the antecedent of osteoclast, and finally guides the differentiation of macrophages into the osteoclast.[16,18–20] Since the inhibition of proinflammatory cytokines by suppressing the disease activity may decrease the production of RANKL and prevent the macrophages from guiding the differentiation into the osteoclast, this subsequently may result in a decrease in bone resorption marker levels. With other DMARD therapy, SASP and ACT, the bone metabolism markers did not change significantly. We therefore concluded that MTX therapy is more effective on disease activity than SASP and ACT.

Glucocorticoids are the leading cause of drug-induced osteoporosis.[21,22] Clinically, glucocorticoid-induced bone loss develops rapidly. It has generally been thought that glucocorticoid-induced osteoporosis results from suppressed bone formation[23] and enhanced bone resorption.[24,25] There are two possible mechanisms by which glucocorticoids reduce bone density. The first mechanism is glucocorticoid-induced direct impairment of osteoblast[26] or osteoclast function. The second mechanism is secondary hyperparathyroidism, due to the increased renal excretion and decreased intestinal absorption of calcium.[27] But although glucocorticoid-induced suppression of bone formation has been well documented,[22–25] the effects of glucocorticoids on bone resorption are controversial. Bone resorption was stimulated by glucocorticoids in some studies[25,28] but inhibited in others.[29,30] So in this study we found low-dose prednisolone therapy (mean ± SE 1.73 ± 0.18 mg/day; median dose 2.5 mg/day) has no effect on the bone resorption markers.

Although for bone resorption markers NTX levels showed a significant decrease at 3 months after the initial treatment, DPD levels did not decrease significantly at 3 months after the initial treatment but significantly decreased for the first time at 6 months after the initial treatment. Greenspan et al.[31] and Ravn et al.[32] have indicated that NTX is a more sensitive marker than DPD when measurement of bone-derived type I collagen fragment was done. Furthermore, NTX changes earlier than any other markers of bone resorption after therapy. And because we examined subjects comparatively early after the initial treatment with MTX, DPD showed little significant decrease at 3 months after therapy. In the present study, NTX is considered to be a more sensitive bone resorption marker than DPD in RA treatment.

Bone alkaline phosphate levels were not significantly changed in the present study. In RA, there was conflicting data about the effect on bone formation by the administration of DMARDs. Data on both increased and decreased changes in the levels of bone formation markers in patients with RA after treatment with DMARDs have been reported.[33,34] Although in the present study bone formation was not affected by MTX therapy, because osteoblast activity may have a possibility to change long after the initial treatment with MTX, long-term follow-up is needed.

In conclusion, the present study showed that MTX treatment for RA not only reduces clinical disease activity, as already demonstrated in recent trials, but may also ameliorate bone resorption. In addition, urinary NTX may be a more specific marker of bone-derived type I collagen fragments than urinary DPD.

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