RAPID COMMUNICATION

Serum undercarboxylated osteocalcin level increases with 48 weeks of teriparatide treatment in pre-treated elderly rheumatoid arthritis patients who use anti-resorptive drugs

S. Mokuda, N. Sawada, K. Matoba, A. Yamada, M. Onishi, Y. Okuda, K. Jouyama, Y. Murata, and K. Takasugi

Department of Internal Medicine, Center for Rheumatic Diseases, Dohgo Spa Hospital, Matsuyama City, Ehime, Japan

ABSTRACT. ***Aim:*** **The serum undercarboxylated osteocalcin (ucOC) level, a biochemical bone marker of vitamin K insufficiency, is often affected by anti-osteoporosis drugs. There have been no reports regarding the relationship between ucOC and teriparatide.** ***Subjects and methods:*** **We conducted a prospective observational study of 26 female rheumatoid arthritis (RA) patients. The patients were divided into 3 groups: those who underwent a direct switch from anti-resorptive drugs to teriparatide (12 cases), those who started teriparatide without pre-treatment (5 cases), and the control patients (9 cases). The median age (interquartile range) of the patients in each group was 75 (67-77), 82 (78-84), and 69 (62-80) yr, respectively. All patients, except controls, received 48-week treatments of teriparatide. We analyzed the median 48-week changes from baseline of the serum ucOC levels with the Steel-Dwass method.** ***Results:*** **The median change from baseline in the direct switch group was higher than that in other groups ($p<0.05$).** ***Conclusions:*** **The serum ucOC levels increased with treatment of teriparatide in elderly RA patients, especially when the patients received pre-treatment.**

(J. Endocrinol. Invest. 35: 796-799, 2012)

INTRODUCTION

A relationship between vitamin K intake and the incidence of proximal femur fractures has been reported by some authors (1-3). The key role of vitamin K is to facilitate the binding of osteocalcin (OC) to the bone matrix (4). When vitamin K deficiencies exist, OC binds less well to the bone matrix and enters the bloodstream as undercarboxylated OC (ucOC).

Serum ucOC is a biochemical bone marker of vitamin K insufficiency. The serum ucOC level can be used to predict the possibility for proximal femur fractures in elderly females (5). However, we should keep in mind when interpreting ucOC values that bone resorption inhibitors, such as bisphosphonates, tend to decrease serum ucOC levels (6-8). We have also reported this phenomenon in rheumatoid arthritis (RA) patients (9). Moreover, we have previously reported that serum ucOC levels decrease with glucocorticoid use (9). Schafer et al. has reported that serum ucOC levels increase with PTH(1-84) use (8). We have found no previous reports regarding the relationship between serum ucOC levels and the bone formation stimulator PTH(1-34), teriparatide (TPTD). In the present study, we measured the serum ucOC levels in RA patients who received 48-week treatments of TPTD.

Key-words: Glucocorticoid, osteoporosis, rheumatoid arthritis, teriparatide, ucOC.

Correspondence: S. Mokuda, MD, Department of Internal Medicine, Center for Rheumatic Diseases, Dohgo Spa Hospital, 21-21 Otsu Dohgo-Himezuka, Matsuyama, Ehime, 790-0858, Japan

E-mail: bachblau_since2001@yahoo.co.jp

Accepted September 5, 2012.

SUBJECTS AND METHODS

Design and study population

We conducted a single-institute-based non-randomized prospective observational study of female RA patients with osteoporosis who were treated at Dohgo Spa Hospital. The aim of this study was to investigate whether serum ucOC levels are affected by TPTD. Twenty-six female RA patients were treated with TPTD (20 μg per day) at Dohgo Spa Hospital for 48 weeks. All of the patients were over 59 yr of age and fulfilled the classification criteria of American college of Rheumatology (ACR) 1987 (10). All of the patients received TPTD and calcium compounds (≥1000 mg per day) together. We excluded patients with the following factors: hospital transfer, discontinuation of TPTD, use of warfarin, use of vitamin K preparations (for example, menatetrenone), use of active vitamin D preparations and use of anti-resorptive (AR) drugs such as bisphosphonates or selective estrogen receptor modulators that were concomitant with TPTD. The remaining 17 elderly RA patients were divided into 2 groups: those who underwent a direct switch from AR drugs (over 6 months) to TPTD (Group A) and those who started TPTD *de novo* (Group B, these patients had not been treated with AR drugs during the previous 6 months). Moreover, the RA patients who were unable to use anti-osteoporotic drugs for any reason were included in the control RA group (Group C), which consisted of the patients who did not receive AR drugs, TPTD, warfarin or vitamin K preparations over a period of 1 yr.

Data collection

Informed consent was obtained from all of the patients and the following data were collected: 1) the serum ucOC

levels were measured with an electrochemiluminescence immunoassay (Picolumi; Sanko Junyaku Co., Ltd., Tokyo, Japan). We measured the serum ucOC levels at 0 weeks, 24 weeks, and 48 weeks in Group A and Group B and at 0 weeks and >48 weeks in Group C; 2) the serum amino-terminal propeptide of type I procollagen (PINP) levels were measured with radioimmunoassay. We measured the serum PINP levels at 0 weeks, 24 weeks, and 48 weeks in Group A and Group B; 3) hip dual-emission X-ray absorptiometry scans were performed with the DPX-BRAVO densitometer (GE Healthcare Japan, Co., Ltd., Tokyo, Japan); 4) information about each patient obtained from the medical records, including the patient's age, retrospective disease history, and duration and retrospective history of AR drug use. Each oral glucocorticoid was recorded as the dose of prednisolone (PSL) per day at 0 weeks and 48 weeks. Most patients took oral PSL once or twice a day.

RA disease activity was calculated as DAS 28-CRP according to the formula on the Disease Activity Score (DAS) web site (http://www.das-score.nl/index.html). The estimated glomerular filtration rates (eGFR) were calculated using the following formula devised by the Japanese Society of Nephrology: eGFR (ml/min/1.73 m^2) = $194 \times Cr^{-1.094} \times Age^{-0.287} \times 0.739$.

Statistical analyses

The significance of the differences between 2 groups was determined using the Mann-Whitney U test or Wilcoxon signed rank's test. The differences between 3 groups were estimated using the Kruskal-Wallis test followed by the Steel-Dwass method. To evaluate the relationships between the serum ucOC levels and the corticosteroids, the Spearman rank correlation was used. Data processing and analyses were conducted using Microsoft Excel software.

RESULTS

Characteristics of the patients

Group A (direct switch from AR drugs to TPTD) included 12 patients. Group B (initiation of TPTD *de novo*) included 5 patients. The control group (Group C) included 9 patients. Pretreatment in Group A was as follows: 8 patients received alendronate at a dose of 35 mg per week, 1 patient received minodronate at a dose of 1 mg per day, and 3 patients received raloxifene at a dose of 60 mg per day. Table 1 lists the baseline characteristics of these 3 groups. None of the items had statistical significance among the three groups. The median age [interquartile range (IQR)] of Group A, Group B, and Group C was 75 (67-77), 82 (78-84), and 69 (62-80) yr, respectively. The median duration of RA (IQR) of Group A, Group B, and Group C was 15 (12-31), 27 (25-30), and 19 (12-21) yr, respectively. The median glucocorticoid dose (IQR) (PSL) at 0-weeks was 5.0 (3.0-5.0), 4.0 (3.0-5.0), and 5.0 (4.0-5.0) mg per day, respectively. The median glucocorticoid dose (IQR) (PRL) at 48-weeks was 4.5 (3.0-5.0), 3.0 (3.0-5.0), and 5.0 (4.0-5.0) mg per day, respectively. Wilcoxon signed rank's test revealed that the doses of corticosteroids recorded at 0 weeks and 48 weeks were not statistically significant for any group (p=0.11, 0.32 and 1.0, respectively).

Comparison of the serum ucOC and PINP levels among the three groups

Table 2 shows the mean serum levels of bone formation markers in the 3 groups. In Group A, statistically significant differences were observed the ucOC levels between measured at 0 weeks and those measured at 24 and 48 weeks (p=0.002, 0.003). Moreover, statistically significant differences were observed between the ucOC levels measured at 0 weeks and those measured at only 24 weeks (p=0.043) in Group B. No statistically significant differences were observed between the ucOC levels measured at 0 weeks and those measured at 48 weeks (p=0.441) in Group C. We measured the median serum PINP levels at the same time in Group A and B. In the Group A, no statistically significant differences were observed between the PINP levels measured at 0 weeks and those measured at 24 weeks or 48 weeks (p=0.051, 0.092, respectively). In the Group B, no statistically significant differences were also observed between the PINP levels measured at 0 weeks and those measured at 24 weeks or 48 weeks (p=0.080, 0.893, respectively).

When we compared the serum ucOC levels and the median percent change from baseline, a statistically significant difference were observed between Groups A and B at 24 weeks (p=0.035) (Fig. 1A). The serum ucOC levels were elevated at 24 weeks and the change ratio of Group A was higher than that of Group B. Additionally, the Kruskal-Wallis test showed that a statistically significant difference ex-

Table 1 - *Patients' characteristics.*

	Direct switch from AR drugs to TPTD (Group A) (no.=12)	TPTD without pre-treatment (Group B) (no.=5)	Control (Group C) (no.=9)	K-W p
Age	75 (67-77)	82 (78-84)	69 (62-80)	0.175
Duration of RA (yr)	15 (12-31)	27 (25-30)	19 (12-21)	0.590
eGFR (ml/min/1.73 m^2)	77.6 (67.4-102.3)	100.8 (90.4-109.5)	68.8 (56.6-80.0)	0.052
DAS28-CRP	2.67 (2.12-2.95)	2.36 (2.21-2.54)	1.84 (1.51-2.43)	0.129
Glucocorticoid (prednisolone) (mg/day) (at 0-week)	5.0 (3.0-5.0)	4.0 (3.0-5.0)	5.0 (4.0-5.0)	0.679
Glucocorticoid (prednisolone) (mg/day) (at 48-week)	4.5 (3.0-5.0)	3.0 (3.0-5.0)	5.0 (4.0-5.0)	0.773
Bone density in neck of femur (g/cm^2)	0.605 (0.480-0.648)	0.472 (0.379-0.529)	0.661 (0.522-0.807)	0.148

No statistically significant differences were observed between the 3 groups, as estimated with the Kruskal-Wallis test. Median (interquartile range). AR: anti-resorptive; TPTD: teriparatide; K-W: the Kruskal-Wallis test; RA: rheumatoid arthritis; eGFR: estimated glomerular filtration rate; CRP: C-reactive protein; DAS: disease activity score.

Table 2 - *Bone formation markers at 0, 24, and 48 weeks.*

		0 weeks	24 weeks	*p* (0 *vs* 24 weeks)	48 weeks	*p* (0 *vs* 48 weeks)
Group A	ucOC (ng/ml)	0.89 (0.56-1.79)	3.00 (2.40-7.46)	0.002[a]	2.52 (1.33-6.85)	0.003[a]
	PINP (ng/ml)	45.1 (28.4-66.4)	117.0 (79.7-153.0)	0.051	73.5 (57.0-122.0)	0.092
Group B	ucOC (ng/ml)	2.49 (1.42-3.58)	8.56 (3.46-9.67)	0.043[a]	3.25 (1.65-3.95)	0.225
	PINP (ng/ml)	99.4 (43.9-114)	89.4 (77.8-178)	0.080	63.7 (57.9-79.3)	0.893
Group C	ucOC (ng/ml)	5.23 (3.04-6.95)	-	-	4.59 (2.84-6.63)	0.441

Median (interquartile range). [a]The significance of the difference between the two groups was determined by the Wilcoxon signed rank's test. ucOC: undercarboxylated osteocalcin; PINP: serum aminoterminal propeptide of type I procollagen.

isted among 3 groups (p=0.007). According to the results of the Steel-Dwass method, the change ratio of Group A was higher than that of Groups B and C at 48 weeks (p<0.05 and <0.05, respectively). The serum ucOC levels at 48 weeks was elevated only in the group A. Serum ucOC levels might be elevated by TPTD, especially in pre-treated patients, since there were no differences in the doses of glucocorticoids at 0 weeks and 48 weeks.
On the other hand, when we compared the serum PINP levels and the median percent change from baseline, no differences between Groups A and B were observed at 24 weeks and 48 weeks (p=0.234, 0.282, respectively) (Fig. 1B).

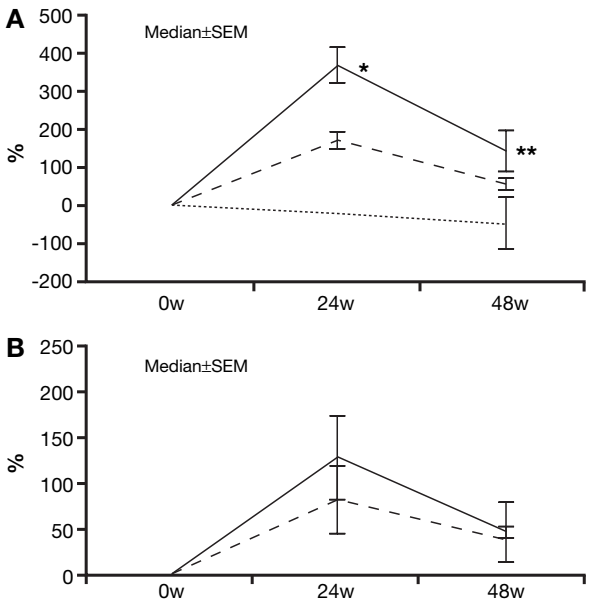

Fig. 1 - *Serum undercarboxylated osteocalcin (ucOC) and serum aminoterminal propeptide of type I procollagen (PINP) levels with the median percent change from the baseline. A) Serum ucOC levels. Difference at 48 weeks among group A (black solid line), group B (black dashed line), and group C (black dotted line) were determined with the Kruskal-Wallis test (p=0.006). The results of the Steel-Dwass method showed that the serum ucOC levels of Group A were higher than those of Groups B or Group C (p<0.05, p<0.05). B) Serum PINP levels. No differences between Group A (black solid line) and Group B (gray dashed line) at 48 weeks were observed. *Mann-Whitney U test (p<0.05). **Steel-Dwass method (p<0.05).*

The relationship between the serum ucOC levels and the doses of glucocorticoids during the use of TPTD

We have previously reported that glucocorticoids affect serum ucOC levels (9). In this study, we examined the effects of glucocorticoids during the use of TPTD. We collected data for patients in Groups A and B (no.=17) at 48 weeks. The Spearman rank correlation test showed that the serum ucOC levels and the glucocorticoid doses were inversely correlated with a correlation coefficient of –0.541 (p=0.031).

DISCUSSION

Many authors have previously reported a relationship between more than 12 months of TPTD use and several bone formation markers, including markers of glucocorticoid-induced osteoporosis (11-18) such as PINP, total OC, bone alkaline phosphatase, *etc.* No authors have reported a relationship between the serum ucOC levels and TPTD. The serum ucOC as level is often used as a biochemical bone marker of vitamin K insufficiency. However, Hozuki et al. have reported that ucOC and OC, a bone formation marker, move in parallel under corticosteroid therapy (7). We previously reported that the serum ucOC levels were affected by glucocorticoids, bisphosphonates and selective estrogen receptor modulators (9). Shiraki et al. reported that the serum ucOC level, a predictive factor of fracture events in primary osteoporotic patients, is lower in those who receive bisphosphonates, than in non-treated patients (19). Teriparatide is a bone formation stimulator and elevates serum OC levels (13, 14, 16). Therefore, we had assumed that serum ucOC levels might increase with TPTD.
RA is a risk factor for osteoporosis. Elderly female RA patients often suffer from severe osteoporosis due to glucocorticoid use, due to the effects of cytokines and inflammation, which are uncontrolled with AR drugs. We experienced that elderly female RA patients require TPTD to treat vertebral fractures. In our study, we discussed that age, sex, disease activity, eGFR, dose of glucocorticoids, *etc.* were not statistically significantly different between the 3 RA groups. The median serum ucOC level in the pre-treated group was elevated at 24 weeks and 48 weeks, and the change ratio was higher in the pre-treated group than in the non-pre-treated group or the control group at 48 weeks. No statistical differences between 0 weeks and 48 weeks glucocorticoid doses were observed. Therefore, there might be few effects of glucocorticoids on serum ucOC levels. We

also measured the serum PINP levels in the 2 groups. PINP is known to be a predictive marker of bone mineral density during TPTD treatment (20). In our data, the PINP levels were not affected by pre-treatment with AR drugs, which differ from ucOC. This phenomenon has already been reported by several authors (16, 20).

We have discussed the relationship between glucocorticoids and serum ucOC levels. We previously reported that there is an inverse correlation between the dose of PSL and the serum ucOC level (9). This inverse correlation is maintained under treatment with TPTD. When we measure the serum ucOC level in a patient taking PSL, the ucOC level decreases. Elderly RA patients often take glucocorticoids, AR drugs, and TPTD. Therefore, we must keep in mind that many drugs affect the serum ucOC levels.

In our preliminary data and unpublished observations, the serum ucOC levels did not increase in patients taking vitamin K2 and teriparatide. However, since we were unable to measure the blood vitamin K concentrations in the present study, we could not describe the relationship between ucOC and vitamin K concentrations. Other limitations included the following: the number of cases was minimal for statistical analysis, we were unable to use a multiple regression analysis since the number of cases was small, and we were not able to measure total OC levels.

ACKNOWLEDGMENTS

Conflict of interest statement

None declared.

REFERENCES

1. Booth SL, Tucker KL, Chen H, et al. Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. Am J Clin Nutr 2000, 71: 1201-8.
2. Apalset EM, Gjesdal CG, Eide GE, Tell GS. Intake of vitamin K1 and K2 and risk of hip fractures: The Hordaland Health Study. Bone 2011, 49: 990-5.
3. Fujita Y, Iki M, Tamaki J, et al. Association between vitamin K intake from fermented soybeans, natto, and bone mineral density in elderly Japanese men: the Fujiwara-kyo Osteoporosis Risk in Men (FORMEN) study. Osteoporos Int 2012, 23: 705-14.
4. Price PA. Vitamin K-dependent formation of bone Gla protein (osteocalcin) and its function. Vitam Horm 1985, 42: 65-108.
5. Vergnaud P, Garnero P, Meunier PJ, Bréart G, Kamihagi K, Delmas PD. Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS Study. J Clin Endocrinol Metab 1997, 82: 719-24.
6. Hirao M, Hashimoto J, Ando W, et al. Response of serum carboxylated and undercarboxylated osteocalcin to alendronate monotherapy and combined therapy with vitamin K2 in postmenopausal women. J Bone Miner Metab 2008, 26: 260-4.
7. Hozuki T, Imai T, Tsuda E, et al. Response of serum carboxylated and undercarboxylated osteocalcin to risedronate monotherapy and combined therapy with vitamin K(2) in corticosteroid-treated patients: a pilot study. Intern Med 2010, 49: 371-6.
8. Schafer AL, Sellmeyer DE, Schwartz AV, et al. Change in undercarboxylated osteocalcin is associated with changes in body weight, fat mass, and adiponectin: parathyroid hormone (1-84) or alendronate therapy in postmenopausal women with osteoporosis (the PaTH study). J Clin Endocrinol Metab 2011, 96: E1982-9.
9. Mokuda S, Okuda Y, Onishi M, et al. Post-menopausal women with rheumatoid arthritis who are treated with raloxifene or alendronate or glucocorticoids have lower serum undercarboxylated osteocalcin levels. J Endocrinol Invest 2012, 35: 661-4.
10. 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: 315-24.
11. Gatti D, Viapiana O, Idolazzi L, Fracassi E, Rossini M, Adami S. The waning of teriparatide effect on bone formation markers in postmenopausal osteoporosis is associated with increasing serum levels of DKK1. J Clin Endocrinol Metab 2011, 96: 1555-9.
12. Tsujimoto M, Chen P, Miyauchi A, Sowa H, Krege JH. PINP as an aid for monitoring patients treated with teriparatide. Bone 2011, 48: 798-803.
13. Miyauchi A, Matsumoto T, Sugimoto T, Tsujimoto M, Warner MR, Nakamura T. Effects of teriparatide on bone mineral density and bone turnover markers in Japanese subjects with osteoporosis at high risk of fracture in a 24-month clinical study: 12-month, randomized, placebo-controlled, double-blind and 12-month open-label phases. Bone 2010, 47: 493-502.
14. Finkelstein JS, Wyland JJ, Lee H, Neer RM. Effects of teriparatide, alendronate, or both in women with postmenopausal osteoporosis. J Clin Endocrinol Metab 2010, 95: 1838-45.
15. Burshell AL, Möricke R, Correa-Rotter R, et al. Correlations between biochemical markers of bone turnover and bone density responses in patients with glucocorticoid-induced osteoporosis treated with teriparatide or alendronate. Bone 2010, 46: 935-9.
16. Keel C, Kraenzlin ME, Kraenzlin CA, Müller B, Meier C. Impact of bisphosphonate wash-out prior to teriparatide therapy in clinical practice. J Bone Miner Metab 2010, 28: 68-76.
17. Cosman F, Wermers RA, Recknor C, et al. Effects of teriparatide in postmenopausal women with osteoporosis on prior alendronate or raloxifene: differences between stopping and continuing the antiresorptive agent. J Clin Endocrinol Metab 2009, 94: 3772-80.
18. Saag KG, Shane E, Boonen S, et al. Teriparatide or alendronate in glucocorticoid-induced osteoporosis. N Engl J Med 2007, 357: 2028-39.
19. Shiraki M, Yamazaki Y, Shiraki Y, Hosoi T, Tsugawa N, Okano T. High level of serum undercarboxylated osteocalcin in patients with incident fractures during bisphosphonate treatment. J Bone Miner Metab 2010, 28: 578-84.
20. Blumsohn A, Marin F, Nickelsen T, et al; EUROFORS Study Group. Early changes in biochemical markers of bone turnover and their relationship with bone mineral density changes after 24 months of treatment with teriparatide. Osteoporos Int 2011, 22: 1935-46.