Higher Efficacy of Urinary Bone Resorption Marker Measurements in Assessing Response to Treatment for Osteoporosis in Postmenopausal Women

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Osteoporosis has reached epidemic proportions. This situation has stimulated the development of biochemical markers to assist in assessing osteoporotic risk and monitoring treatment efficacy. Biochemical markers for assessing the level of bone resorption have been developed during the last few decades. One of the most widely used bone resorption markers is cross-linked N-terminal telopeptides (NTX). Measurements of urinary and serum NTX provide indications of the level of bone resorption during osteoporosis treatment. However, it remains unclear whether urinary or serum NTX measurements show better efficacy for assessing osteoporosis treatment effects during the early phase of treatment. Therefore, the primary aim of the present study was to compare the efficacies of urinary and serum NTX measurements for assessing the level of bone resorption during the early stage of osteoporosis treatment. We enrolled 43 postmenopausal Japanese women in an open-label randomized placebo-controlled trial. Overall, 21 women in the osteoporosis treatment group and 19 women in the placebo group completed the study. There was a significant reduction in urinary NTX in the treatment group, which was detectable as early as 4 weeks and maintained until 16 weeks, compared with the placebo group. On the other hand, serum NTX did not show a significant reduction in the treatment group compared with the placebo group until 16 weeks. These results indicate that urinary NTX measurements are more sensitive and show higher efficacy than serum NTX measurements for assessing treatment effect during the early phase of osteoporosis treatment in postmenopausal women.

Biochemical markers for assessing bone turnover, which can be used to diagnose metabolic bone diseases, have been developed and made commercially available during the last few decades (Seibel 2000; Swaminathan 2001). Bone resorption markers comprised of urinary type I collagen degradation products, such as free or total pyridinium cross-linked pyridinoline or deoxypyridinoline and cross-linked N-terminal telopeptides (NTX) and C-terminal telopeptides (CTX), are most widely used.

Serum assays for assessing bone resorption have recently been developed (Clemens et al. 1997; Karmatschek et al. 1997), and include...
assays for NTX and CTX. One potential advantage of serum assays is that they should be less affected by both analytic and biologic variability than urine assays (Fink et al. 2000; Hannon and Eastell 2000; Takahashi et al. 2002). The analytic variability in urine assays is increased by the additional need to measure urine creatinine and calculate excretion as a ratio, while the biological variability in urine assays may be increased by differences in renal clearance, particularly for peptides that could be resorbed and/or degraded in the kidneys. In addition, urine assays show great diurnal variation, which contributes to intra-subject day-to-day variability (Blumsohn et al. 1994; Hannon et al. 1998). Moreover, collecting urinary samples is laborious, and precise timing is required when 2-hr morning samples are used. Therefore, a recently introduced serum assay for NTX may provide advantages over urinary NTX assays (Woitge et al. 1999). In particular, lower short-term and long-term intra-subject variabilities were reported for the serum NTX assay compared to urinary NTX assays (Eastell et al. 2000; Fall et al. 2000). The signal-to-noise ratios for urinary and serum NTX assays were found to be quite similar for patients treated with hormone replacement therapy (Eastell et al. 2000), consistent with other studies for bisphosphonate treatment (Greenspan et al. 1998a, b). However, it remains unclear whether urinary or serum NTX measurements show better efficacy for assessing the level of bone resorption during the early phase of osteoporosis treatment.

Risedronate is a pyridinyl-bisphosphonate that suppresses osteoclastic activity and has been demonstrated to reduce clinical vertebral and non-vertebral fractures in postmenopausal women within 6 months of treatment (Harrington et al. 2004; Roux et al. 2004). Furthermore, risedronate was reported to significantly increase bone mineral density (BMD) in both Caucasian and Asian osteoporosis patients (Fogelman et al. 2000; Fukunaga et al. 2002; Leung et al. 2005).

To clarify whether urinary or serum NTX measurements show better efficacy for assessing treatment effects during the early phase of treatment, we measured urinary and serum NTX in postmenopausal Japanese women and compared their efficacies for assessing treatment effects during the early stage of risedronate treatment.

**Patients and Methods**

**Study population and design**

The present study was an open-label randomized placebo-controlled trial. The study subjects were recruited from an osteoporosis clinic in Yamagata prefecture in Japan. Specifically, the subjects were postmenopausal women with a distal forearm BMD of < 80% of the young adult mean (YAM) value established by the Japanese Society of Bone and Mineral Research (JSBMR) (Iki et al. 2001). Subjects who had used estrogen, estrogen-related drugs, bisphosphonates, fluoride, vitamin D supplements, calcitonin or calcitriol and those with a history of metabolic bone disease, impaired renal and liver function, recent parathyroidism, diabetes mellitus or rheumatoid arthritis were excluded from the study. After receiving written informed consent from each subject according to the ethical guidelines of the Japanese Government, the subjects were randomly assigned to receive either risedronate (Beneto®, Takeda Pharmaceutical Co. Ltd., Tokyo) 2.5 mg daily or a matching placebo in the morning after an overnight fast. The maximum plasma concentration and the area under the plasma concentration-time curve up to 24 hr after oral administration of risedronate were previously reported to be 2-3-fold higher in Japanese subjects than in Caucasian subjects (Ogura et al. 2004). Therefore, the recommended dosage of risedronate is 2.5 mg for Japanese, compared with 5.0 mg for Caucasians. Recently, daily oral risedronate (2.5 mg) was shown to act as an effective therapy for involutional osteoporosis in Japanese patients with good tolerability (Kushida et al. 2004). All the study subjects also received calcium carbonate 200 mg daily and vitamin D 10 IU daily. These doses of calcium carbonate and vitamin D have no significant effects on BMD until 12 months (Shiraki et al. 1999). Subjects who refused randomization due to a preference for either one of the treatment options received the treatment of their choice. The subjects were assessed for their BMD as well as fasting blood and urinary markers of bone turnover. The participation of the subjects was voluntary and they could withdraw from the study at any time in accordance with the Helsinki II declaration. The study protocol was approved by the Committee for Ethics at Yamagata University Faculty of Medicine.
Fig. 1 shows a flowchart of the present study. Informed consent to participate in the study was obtained from 43 of 51 eligible women, while the remaining 8 women were unwilling to participate. Overall, 26 women agreed to be randomized, resulting in the allocation of 13 women to each of the risedronate and placebo groups. The remaining 17 women did not consent to randomization, but agreed to active follow-up measurements (preference group). Among these 17 women, 9 women opted for risedronate treatment and 8 opted for placebo treatment. The follow-up period was 16 weeks between April 2004 and May 2005. Overall, 1 woman in the risedronate group and 2 women in the placebo group were lost to follow-up between the randomization and the follow-up at 16 weeks, due to unwillingness to continue. The subjects who dropped out were similar with respect to weight, height and distal BMD to those who attended the follow-up at 16 weeks (data not shown). Finally, 21 (95.5%) women in the risedronate group and 19 (90.5%) women in the placebo group completed the study.

**BMD measurements**

BMD was measured at the distal forearm by dual-energy X-ray absorptiometry (Osteometer DTX200®, Toyomedic Co. Ltd., Tokyo) at −1, 0, 4, 8, 12 and 16 weeks. The analytic precision was estimated as the mean percent coefficient of variation (%CV) for the measurements. The CV for the DTX200 was less than 0.5%, according to the data provided by Toyomedic Co. Ltd.

**Quantification of urinary and serum NTX**

Fasting blood and second morning void urine samples were collected between 08:00 and 10:00 after an overnight fast twice at baseline (weeks −1 and 0) and then at weeks 4, 8, 12 and 16 in both groups. The urinary samples were stored at −20°C until analysis. The serum was separated from the blood samples and also stored at −20°C until analysis. All specimens were assayed in a single run to eliminate interassay variability. Urinary and serum NTX were measured with an ELISA kit (Osteomark™, Inverness Medical Professional Diagnostics, Princeton, NJ, USA) according to a commercial protocol (Showa Medical Science Co., Tokyo). Serum NTX was expressed as nanomoles of bone collagen equivalents per liter (nM BCE), while urinary NTX was expressed as nM BCE per millimole of creatinine (mM Cr) to correct for urinary dilution. The mean CVs for the measurements were 3.6% for urinary NTX and 6.8% for serum NTX, according to data provided by Mochida Pharmaceutical Co. Ltd. (Tokyo).

**Statistical analysis**

To analyze the changes in the bone turnover markers and BMD, we compared the corresponding percent changes after 4, 8, 12 and 16 weeks of risedronate therapy.
py with the baseline values. To assess the better marker for monitoring the response, signal-to-noise ratios between the size of the treatment response (signal) and the variability (noise) were calculated for the bone markers (Bland and Altman 1986; Eastell et al. 2000). These signal-to-noise ratios were calculated using the percent differences in the mean NTX values between risedronate treatment and placebo treatment as the signal and the long-term CV as the noise. The signal-to-noise ratios for urinary and serum NTX were compared.

The better markers among serum and urinary NTX would show greater percent changes between risedronate treatment and placebo treatment and lower long-term CV values (Eastell et al. 2000). In this context, a signal-to-noise ratio of ≥ 1 indicated a significant decrease in the bone marker level after treatment, while a ratio of < 1 indicated a non-significant response to treatment. Thus, higher ratios indicate more useful markers (Alvarez et al. 2000). To calculate the long-term CVs of the bone markers, we used the difference between the two baseline measurements (weeks −1 and 0) with its respective mean and s.d. of 40 subjects. Comparisons between groups were carried out using the Mann-Whitney U-test. The level of significance was set at p < 0.05. All statistical analyses were performed using StatView 5.0J® for Windows (SAS Institute Inc., Cary, NC, USA).

RESULTS

Baseline characteristics

The baseline characteristics of the two groups are shown in Table 1. Although the serum NTX was significantly higher in the placebo
group than in the risedronate group, there were no significant differences between the two groups for any of the other baseline parameters.

**BMD**

The changes in distal forearm BMD are shown in Fig. 2. There were no significant differences between the two groups at 4, 8, 12 and 16 weeks.

**Biochemical markers**

There was a significant reduction in urinary NTX in the risedronate group, which was detectable as early as 4 weeks and maintained until 16 weeks, compared with the placebo group (Fig. 3). Serum NTX did not show a significant reduction in the risedronate group compared with the placebo group until 16 weeks (Fig. 4). The signal-to-noise ratio of urinary NTX was > 1.0 as early as 4 weeks, and tended to increase up to 12 weeks. In contrast, the signal-to-noise ratio of serum NTX was not elevated to > 1.0 until 16 weeks of treatment, although it showed a tendency to increase in the weeks prior to this time point (Table 2).

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**Fig. 3.** Percent changes in urinary NTX in postmenopausal women during risedronate or placebo treatment over 16 weeks. Error bars represent the SE. *p < 0.05, risedronate vs. placebo.

**Fig. 4.** Percent changes in serum NTX in postmenopausal women during risedronate or placebo treatment over 16 weeks. Error bars represent the SE. *p < 0.05, risedronate vs. placebo.
DISCUSSION

BMD is the most frequently used measurement for diagnosing osteoporosis and monitoring osteoporosis treatments in postmenopausal women (Nohara et al. 2006). However, changes in BMD following osteoporosis treatment only appear after 6 months or longer. Therefore, several bone biochemical markers are under development for earlier detection of the effects of osteoporosis treatments, and several studies have found that changes in biochemical markers at 3 or 6 months are correlated with changes in BMD at 2 years (Chesnut et al. 1997; Bjamason and Christiansen 2000; Fink et al. 2000; Greenspan et al. 2000). Some advantages of using biochemical markers over BMD for follow-up of osteoporosis are as follows: (a) markers have the potential for higher sensitivity; (b) markers are related to systemic events rather than local events; (c) markers respond to treatment more rapidly than BMD to treatment.

Bone resorption markers can be measured in both urine and serum (Fall et al. 2000). Measuring such markers in urine or serum is advantageous, since the samples are easy to collect in the clinic and handle in the laboratory. However, it remains unclear whether urine or serum samples show better efficacy for assessing treatment effects during the early phase of osteoporosis treatment. In the present study, there was a significant reduction in urinary NTX in the treatment group, which was detectable as early as 4 weeks and maintained until 16 weeks, compared with the placebo group. On the other hand, serum NTX did not show a significant reduction in the treatment group compared with the placebo group until 16 weeks. Furthermore, we found that urinary NTX had a higher signal-to-noise ratio than serum NTX from 4–12 weeks of risedronate treatment. Taken together, these findings mean that the effects of variability when using NTX measurements to monitor differences in bone resorption caused by antiresorptive therapy differ between urinary and serum samples. Eastell et al. (2000) reported signal-to-noise ratios for urinary and serum NTX of 2.8 and 2.9, respectively. In that study, the percent difference between the mean 2-month NTX values in postmenopausal women treated with either HRT plus a calcium supplement or the calcium supplement alone was used as the long-term variability for noise. Greenspan et al. (1998a, b) reported signal-to-noise ratios of 3.8 for urinary NTX and 4.0 for serum NTX using the percent difference between the mean 6-month NTX values as the noise (Fall et al. 2000). Therefore, the reported the signal-to-noise ratios for urinary and serum NTX were quite similar in these studies. In contrast, we used the percent difference between the mean 4-month NTX values in postmenopausal women treated with either risedronate plus calcium and vitamin D supplements or the calcium and vitamin D supplements alone as the long-term variability for noise, and found that the signal-to-noise ratio for urinary NTX was > 1.0 as early as 4 weeks, whereas the signal-to-noise ratio for serum NTX required 16 weeks to reach > 1.0. The differences between the signal-to-noise ratios for urinary and serum NTX during the early stage of risedronate treatment observed in the present study have not been proposed by other researchers to date. The differences in treatments and the values used for calculations of the percent differences may play

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<th>CV&lt;sup&gt;a&lt;/sup&gt;</th>
<th>%difference</th>
<th>S/N&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td>Urinary</td>
<td>29.3</td>
<td>37.5</td>
<td>1.28</td>
<td>39.9</td>
<td>1.36</td>
<td>41.4</td>
<td>1.41</td>
<td>29.7</td>
<td>1.01</td>
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<tr>
<td>Serum</td>
<td>20.3</td>
<td>1.27</td>
<td>0.06</td>
<td>5.47</td>
<td>0.27</td>
<td>8.15</td>
<td>0.40</td>
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<sup>a</sup>Long-term %CV.

<sup>b</sup>Signal-to-noise ratio.
important roles in determining noise values, and subsequently affect the signal-to-noise ratios. Another possibility is that the peptide epitopes measured in serum and urine samples may represent different pools of metabolites. For example, rapidly excreted urinary peptides may reflect the initial phase of osteoclast action, whereas the serum pool may reflect a combination of both early and subsequent processing of collagen from resorption pits. Since bisphosphonates continue to suppress the number and action of osteoclasts, the serum NTX levels may lag behind the urinary NTX levels.

Delmas et al. (2003) assessed the effects of physician reinforcement using urinary NTX marker data on persistence with risedronate treatment. They found that patients receiving a message indicating a favorable urinary NTX marker response showed a significantly improved 1-year persistence among postmenopausal women treated with risedronate, and indicated that monitoring of osteoporosis treatments using urinary NTX data should be encouraged. Furthermore, Clowes et al. (2004) reported a 57% improvement in adherence to antiresorptive therapy at 1 year in patients who received nurse monitoring or monitoring involving graphing of the urinary NTX response to treatment compared to patients who did not receive any monitoring. To obtain good adherence to treatment, patients should be provided with a reinforcement message for the treatment as early as possible. Thus, urinary NTX, which can demonstrate efficacy at an early phase of treatment, is a useful bone turnover marker for controlling the adherence of patients.

Based on previous reports that the nadir reduction in NTX reached 35-40% (Hochberg et al. 1999; Shiraki et al. 2002), the degree of NTX reduction observed in the present study is likely to indicate that risedronate is an effective treatment for our study subjects. Furthermore, the present results obtained by NTX measurements confirmed the efficacy of a daily dose of 2.5 mg of risedronate, chosen in accordance with the results of a dose-ranging study in Japanese osteoporotic patients (Shiraki et al. 2002), and indicated that this dose has comparable effects to those obtained in studies of Caucasian postmenopausal women treated with a daily dose of 5.0 mg of risedronate (Harris et al. 1999). Osteoporosis in Japanese women may be adequately treated with smaller risedronate doses than those used to treat Caucasian women (Fukunaga et al. 2002). This difference in the effectiveness of risedronate treatment between two ethnicities may be due to differences in the pharmacokinetics of risedronate (Ogura et al. 2004) and/or genetic factors (Eisman 1999).

A limitation of the present study is that the period of treatment used to evaluate the treatment response was too short to address the efficacy of risedronate on BMD or the relationships between short-term reductions in urinary and serum NTX and BMD. In this respect, a previous study indicated that short-term reductions in bone resorption markers were significantly associated with a subsequent decrease in the risk of fractures in patients treated with risedronate (Eastell et al. 2003). Follow-up studies are required to determine whether early changes in urine and serum NTX after treatment are associated with long-term BMD improvement and fracture prediction for individual patients in the present study. Another limitation of this study is that we did not compare the outcomes between randomized and non-randomized patients because the numbers of study subjects were too small to clarify the effect of bias by a preference (Brewin and Bradley 1989).

In conclusion, the results of the present study demonstrate that urinary NTX measurements are more sensitive and show higher efficacy than serum NTX measurements for assessing treatment effects during the early phase of risedronate treatment in postmenopausal Japanese women. A beneficial effect of using urinary NTX measurements may be to improve persistence in postmenopausal women treated with antiresorptive therapy. However, these preliminary findings need to be validated in follow-up studies to address the efficacy of risedronate on BMD and the relationships between short-term reductions in urinary and serum NTX and BMD.
References


