Response of Serum Carboxylated and Undercarboxylated Osteocalcin to Risedronate Monotherapy and Combined Therapy with Vitamin K₂ in Corticosteroid-Treated Patients: A Pilot Study

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Abstract

Objective The aim of this study was to investigate the responses of serum osteocalcin (OC), undercarboxylated osteocalcin (ucOC) and N-terminal telopeptide of type I collagen (NTx) to corticosteroids, and to examine the effects of risedronate therapy with or without vitamin K₂ supplementation on bone metabolic markers in corticosteroid-treated patients.

Methods Sixteen patients on corticosteroid therapy for neuromuscular disorders were assigned randomly to 2 groups (A: risedronate monotherapy, n=8; B: combined risedronate and vitamin K₂ therapy, n=8) and treated for 1 year. Another 6 patients who received intravenous steroid pulse therapy were assigned to group C for investigation of the effects of corticosteroids on OC and ucOC 1 month after pulse therapy.

Results Serial measurements revealed that significant decreases of OC, ucOC and NTx persisted with a similar time course profile during 1 year of treatment in groups A and B, and between-group analysis failed to demonstrate any additional effects of vitamin K₂ on risedronate therapy. Intravenous steroid pulse therapy induced a transient depression of OC and ucOC within 1 week in group C.

Conclusion These results indicate that serum concentrations of OC and ucOC become consistently low during corticosteroid administration despite risedronate therapy with or without vitamin K₂ supplementation, and the serum ucOC level may not be a reliable indicator of vitamin K status under corticosteroid administration.

Key words: vitamin K₂, risedronate, corticosteroid, osteocalcin, undercarboxylated osteocalcin, N-terminal telopeptide of type I collagen


Introduction

Vitamin K is a cofactor of γ-carboxylase that mediates the conversion of undercarboxylated osteocalcin (ucOC) to osteocalcin (OC) by transforming glutamyl (Glu) residues of OC to γ-carboxyglutamic acid (Gla) (1, 2). Recent in vivo and in vitro studies showed that vitamin K and its analogues have a therapeutic effect on bone metabolism. In particular, menatetrenone, vitamin K₂ with four isoprene units, has been reported to be effective in promoting bone formation in postmenopausal women (3, 4), improving remodeling in hemodialysis patients (5), and preventing the occurrence of new fractures in osteoporotic patients (6). These reports led us to expect that vitamin K₂ may also prevent corticosteroid-induced osteoporosis (7, 8). In fact, in vivo experiments using rats have suggested that vitamin K₂ may prevent prednisolone-induced bone loss (9, 10).

In 2004, the Japanese Society for Bone and Mineral Research established guidelines on the management and treat-
ment of corticosteroid-induced osteoporosis, and recommended vitamin K2 as a second-line therapy to be used in combination with bisphosphonates as the first-line therapy (11). However, there have been few attempts to evaluate the effect of vitamin K2 on bone metabolism in corticosteroid-treated patients.

In the present study, we performed serial measurements of serum bone metabolic markers such as OC, ucOC and N-terminal telopeptide of type I collagen (NTx) to investigate the additive effects of vitamin K2 on bone metabolism, when used in combination with risedronate in corticosteroid-treated patients with neuromuscular disorders.

**Patients and Methods**

**Patients**

Between March 2004 and September 2007, we enrolled 23 patients for a long-term observation study and 6 patients for a short-term observation study at Sapporo Medical University Hospital. This study was approved by the local ethical committee, and written informed consent was obtained from all participants.

**Long-term observation**

Twenty-three patients were enrolled in the long-term observation. The inclusion criteria were: 1) patients with corticosteroid-treatable neuromuscular diseases; 2) patients who had never received corticosteroid therapy; 3) patients who would receive corticosteroid treatment for at least one year after initiation of the observation; and 4) patients who agreed to receive prophylactic therapy for corticosteroid-induced osteoporosis according to the Japanese guidelines (11). During the study period, 7 of 23 patients were excluded from observation because of difficulty in oral intake (n=3), gastrointestinal problems (n=3) or liver dysfunction (n=1). Eventually the data of 16 patients who completed the long-term observation were analyzed. Their neuromuscular disorders included polymyositis (n=2), polymyalgia rheumatica (n=1), neuralgic amyotrophy (n=4), myasthenia gravis (n=3), and multiple sclerosis (n=2).

Of 16 patients, 8 (3 males and 5 females) aged 29 to 77 years (mean, 54.9 years) were assigned randomly to receive risedronate monotherapy (group A) and 8 (2 males and 6 females) aged 34 to 73 years (mean, 53.6 years) were assigned to receive combined risedronate (2.5 mg/day) and menatetrenone (45 mg/day) therapy (group B). Treatment with risedronate and/or menatetrenone was started within 8 days after corticosteroid therapy was initiated, and continued for 1 year.

Serial measurements of serum OC, ucOC and NTx were performed before, at 1 week, 1 month, 3 months and 1 year after the start of treatment with corticosteroid. The ucOC/OC ratio was calculated from the OC and ucOC levels. The percent changes of these markers from the baseline at each measurement were calculated and compared between groups A and B.

**Short-term observation**

This study was performed to evaluate the short-term effects of corticosteroids on serum OC and ucOC levels, because it is known that serum OC concentrations are significantly decreased within one week after the start of oral steroid intake (12). Six patients (3 males and 3 females) aged 20 to 80 years (mean, 44.1 years) were assigned to group C, and received intravenous steroid pulse therapy (methylprednisolone 1,000 mg/day for 3 days) to treat demyelinating diseases of the central nervous system, such as multiple sclerosis, without any prophylactic therapy for osteoporosis. No tapering corticosteroid therapy was given after the steroid pulse therapy. Serial measurements of serum OC and ucOC were performed before, at 1 week, and 1 month after the completion of one cycle of steroid pulse therapy. Percent changes of OC and ucOC from the baseline were calculated at each time point.

**Measurement of bone metabolic markers**

Blood samples were centrifuged at 3,000 rpm for 10 minutes at 4°C. The separated serum samples were stored frozen at -80°C or lower until analysis. Measurements of bone metabolic markers were performed using an immunoradiometric assay (BGP; Mitsubishi Chemical Medience, Tokyo, Japan) for OC, an electrochemiluminescence immunoassay (Picolumi; Sanko Junyaku, Co., Ltd., Tokyo, Japan) for ucOC, and an enzyme-linked immunosorbent assay (Osteomark; Inverness Medical, Tokyo, Japan) for NTx.

**Statistical analysis**

Results are expressed as mean ± SE. Wilcoxon matched-pairs signed ranks test was used to determine whether there was a significant change compared to the baseline occurred at each observation time within a group. Between-group differences were assessed by the Mann-Whitney U test. A probability <0.05 was recognized as statistical significance. The JMP statistical program (SAS Institute Inc., Cary, NC) was used for data analysis.

**Results**

**Baseline characteristics**

Table 1 lists the baseline characteristics and biochemical parameters of the patients. There were no significant differences in baseline age, dose of prednisolone, bone turnover markers and bone mineral density (BMD) between groups A and B in the long-term study.

In the long-term observation, the mean time of initiation of risedronate and/or menatetrenone was 3.5 days in group A and 2.3 days in group B. We tried to start the prophylactic therapy as early as possible to assess the effect of risedronate and/or menatetrenone on changes in bone metabolic
Table 1. Characteristics of the Patients at Baseline

<table>
<thead>
<tr>
<th></th>
<th>Long-term observation</th>
<th>Short-term observation</th>
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<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54.7±5.4</td>
<td>53.6±5.6</td>
</tr>
<tr>
<td>Prednisolone (mg/day)</td>
<td>16.8±2.2</td>
<td>15.8±2.3</td>
</tr>
<tr>
<td>Serum OC (ng/mL)</td>
<td>5.89±1.04</td>
<td>6.16±0.79</td>
</tr>
<tr>
<td>Serum ucOC (ng/mL)</td>
<td>2.10±0.40</td>
<td>2.02±0.43</td>
</tr>
<tr>
<td>Serum NTx (nmol BCE/L)</td>
<td>17.50±2.12</td>
<td>15.14±1.06</td>
</tr>
<tr>
<td>ucOC/OC</td>
<td>0.39±0.03</td>
<td>0.31±0.05</td>
</tr>
<tr>
<td>Lumbar (L2-L4) BMD (g/cm²)</td>
<td>1.060±0.072</td>
<td>0.945±0.067</td>
</tr>
<tr>
<td>Total femur BMD (g/cm²)</td>
<td>1.035±0.055</td>
<td>0.898±0.068</td>
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All the data are expressed mean ± SE. There were no significant differences between groups A and B on the basis of their background data.

OC, osteocalcin; ucOC, undercarboxylated osteocalcin; NTx, N-terminal telopeptide of type I collagen; BMD, bone mineral density.

Figure 1. Oral prednisolone dose after starting risedronate monotherapy (group A; closed circles) or combined risedronate and vitamin K2 therapy (group B; open circles). Data are mean±SE. Between-group analysis showed no significant differences at all time points although the mean values were relatively high in group A up to 3 months.

Figure 2. Percent changes of serum osteocalcin (OC) level after starting risedronate monotherapy (group A; closed circles) or combined risedronate and vitamin K2 therapy (group B; open circles). Data are mean±SE. The asterisks indicate significant differences compared with baseline (p<0.05).

Changes of prednisolone dose in the long-term observation

The prednisolone doses (mg/day) at 1 week, 1 month, 3 months and 12 months were 36.3±5.0, 28.6±4.2, 16.6±3.7 and 9.4±3.2, respectively, in group A, and 32.2±3.8, 21.3±3.4, 16.6±2.8 and 11.0±4.5 in group B (Fig. 1). There were no significant differences between the two groups at all time points throughout the entire observation period.

Changes of BMD in the long-term observation

The lumbar and femur BMD (g/cm²) at 12 months after treatment were 1.068±0.075 and 1.075±0.063, respectively, in group A, and 0.935±0.061 and 0.901±0.060 in group B. In both groups, there were no significant changes in lumbar and femur BMD compared to baseline values (Table 1).

Changes of OC and ucOC in the long-term observation

The percent changes of OC from the baseline at 1 week, 1 month, 3 months and 12 months were -68.2±3.9, -70.0±4.5, -48.2±10.9 and -36.9±10.2%, respectively, in group A, and -59.1±6.4, -48.4±12.0, -39.7±14.5 and -32.3±12.5% in group B (Fig. 2), while the corresponding values of ucOC were -71.1±10.1, -74.9±6.3, -63.8±9.8 and -38.9±16.6% in group A, and -65.2±8.2, -72.8±6.3, -64.3±14.3 and -64.2±12.5% in group B (Fig. 3).

A significant decrease of OC persisted until 12 months in both groups (Fig. 2). On the other hand, the significant change of ucOC disappeared at 12 months in group A, although a significant decrease below -60% persisted throughout the entire course of study in group B (Fig. 3).

The percent changes of ucOC/OC ratio at 1 week, 1
Figure 3. Percent changes of serum undercarboxylated osteocalcin (ucOC) level after starting risedronate monotherapy (group A; closed circles) or combined risedronate and vitamin K2 therapy (group B; open circles). Data are mean±SE. The asterisks indicate significant differences compared with the baseline (p<0.05).

Figure 4. Percent changes of the ucOC/OC ratio after starting risedronate monotherapy (group A; closed circles) or combined risedronate and vitamin K2 therapy (group B; open circles). Data are mean±SE.

Figure 5. Percent changes of serum type I collagen cross-linked N-telopeptide (NTx) level after starting risedronate monotherapy (group A; closed circles) or combined risedronate and vitamin K2 therapy (group B; open circles). Data are mean±SE. The asterisks indicate significant differences compared with baseline (p<0.05).

Figure 6. The effect of intravenous steroid pulse therapy on percent changes in serum osteocalcin (OC; closed circle), undercarboxylated osteocalcin (ucOC; open circle) and type I collagen cross-linked N-telopeptide (NTx; gray circle) levels. Data are mean±SE. The asterisks indicate significant differences compared with baseline (p<0.05).

Changes of bone metabolic markers in the short-term observation

Steroid pulse therapy led to significant changes in OC (-47.4±14.0%) and ucOC (-56.6±15.0%) from the baseline values after 1 week (p<0.05). After 1 month, the percent changes of OC and ucOC increased to -18.2±11.2% and 28.5±23.8%, respectively, from the baseline (Fig. 6). On the other hand, there were no significant changes in NTx from the baseline after 1 week (-2.9±17.8%) and 1 month (-9.7±13.2%).

Discussion

Osteocalcin is one of the most abundant noncollagenous proteins produced by osteoblasts in bone matrix (14, 15). Since a part of the OC enters the bloodstream, the serum OC concentration is used as a marker of bone forma-
tion (16, 17). On the other hand, the serum ucOC concentration is used as an indicator of the status of vitamin K supplementation to promote bone formation in osteoporotic patients, because vitamin K mediates the conversion of ucOC to OC by transforming Glu residues of OC to Gla. Several studies have reported that the circulating level of vitamin K is decreased in osteoporotic patients with bone fractures (18-21) and that a high serum concentration of ucOC is related to the risk of fracture in elderly women or osteoporotic patients (22-26). However, ucOC is not a reliable indicator to estimate the status of vitamin K in corticosteroid-treated patients because a significant decrease of ucOC concomitant with OC reduction is induced by corticosteroids early after administration regardless of vitamin K supplementation, as shown in the present study.

Another striking finding in this study is that serum concentrations of OC and ucOC follow a very similar time course (27). The precise turnover of OC and ucOC in human serum has not been reported in the literature, although a study in rat models reported that one-half of the administered bone Gla protein may be cleared from serum in less than 5 minutes (17). Various studies have demonstrated that corticosteroids depress bone formation with a decrease in osteoblastic activity that is generally attributed to the direct inhibitory effect of corticosteroids on bone formation (28-30). This would explain the significant decrease in OC during corticosteroid therapy as was also reported previously (12). However, this is the first report in the literature of significant decreases in ucOC and OC immediately after corticosteroid administration. One possible explanation of why ucOC decreased significantly with a similar time course profile as OC is the facilitation of γ-carboxylation of serum ucOC following a steroid-induced reduction of serum OC. Another possibility is an as-yet unrecognized effect of steroid that reduces serum OC and ucOC simultaneously in the early stages of corticosteroid intake. The present results indicate the latter possibility, and also suggest that the reduction of ucOC by corticosteroids may be stronger than that by vitamin K alone according to previous studies (4, 31). In the long-term observation, the mean ucOC/OC ratios up to 3 months were relatively lower in group A than in group B in spite of combined therapy with vitamin K in group B (Fig. 4). This paradoxical result may be explained by the relatively high dose of prednisolone used in group A compared with group B, although the dose difference did not reach a statistical significance (Fig. 1). Further measurements of serum OC and ucOC concentrations in the earlier periods immediately after steroid administration (eg. within several hours) are required to resolve whether or not steroids have a direct effect on serum ucOC.

Consistent with a previous report (32), the present study also showed that risedronate significantly reduced NTx in corticosteroid-treated patients, and the magnitude of reduction observed was similar to that previously reported in osteoporotic patients (33, 34). Since NTx has been used as a bone resorption marker in several reports (35, 36), our result suggests that risedronate can induce a significant reduction of bone turnover against the increased osteoclast-mediated bone resorption induced by corticosteroids (37). Although past studies have shown that vitamin K exhibits two contradictory functions; activation (38) and inhibition (39, 40) of osteoclasts, our results indicate that vitamin K may not influence NTx when used concomitantly with risedronate in corticosteroid-treated patients.

In summary, vitamin K, when used in combination with risedronate as prophylactic therapy for corticosteroid-induced osteoporosis had no additive effects on bone metabolic markers compared to risedronate alone (41). Since OC and ucOC were equally depressed shortly after corticosteroid treatment, ucOC is not a reliable indicator to estimate the status of vitamin K under corticosteroid administration. Although the bone metabolic markers were changed according to the administration of corticosteroids, treatment with risedronate and/or menatetrenone appears to be effective to maintain the BMD level for at least 12 months. This study examined a small number of subjects and did not assess the incidence of osteoporotic fracture. These are the limitations of this study and further studies are needed to clarify whether vitamin K supplementation in risedronate therapy provides additional benefits in the prevention of corticosteroid-induced osteoporosis.

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References

8. Lukert BP, Raisz LG. Glucocorticoid-induced osteoporosis: Patho-

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