Effect of Vitamin D Metabolites on Bone Metabolism in a Rat Model of Postmenopausal Osteoporosis

Toshio MATSUMOTO,1 Ikuko EZAWA,2 Keiko MORITA,1 Yumiko KAWANOBE,1 and Etsuro OGATA1

1Fourth Department of Internal Medicine, University of Tokyo School of Medicine, Tokyo 112, Japan
2Department of Food and Nutrition, Japan Women’s University, Tokyo 112, Japan

In normal circumstances, two components of bone remodeling, i.e. bone formation and bone resorption, are tightly coupled, so that total bone mass can be maintained. In osteoporosis, these two processes of bone remodeling are uncoupled and the rate of bone resorption exceeds that of bone formation. Although the pathogenesis of osteoporosis is not fully understood, multiple factors have been shown to participate in the development of the disease. These include reduction in sex steroid hormone and calcitonin levels in postmenopausal women (1), a decrease in the renal synthesis as well as the serum levels of 1,25-dihydroxyvitamin D [1,25(OH)2D] (2, 3) and a resultant reduction in intestinal calcium (Ca) absorption (3, 4). As a consequence, there is a negative mineral balance, both at the level of bone and of total body. Therefore, the ideal treatment of osteoporosis would not only be to reduce bone resorption, but also to increase bone formation and to maintain positive mineral balance. In an effort to achieve this therapeutic goal, a rat model of postmenopausal osteoporosis was introduced and the effects of various hormones on bone mineral metabolism were examined.

Rat model of postmenopausal osteoporosis

As mentioned earlier, there is a reduction in serum estrogen level and intestinal Ca absorption in patients with postmenopausal osteoporosis. In order to simulate these conditions, 4-week-old female Sprague-Dawley rats were ovariectomized (OVX) and fed a low Ca diet containing 0.003% Ca and 0.3% phosphorus (P) for two months. The effects of hormones were examined by treating the animals with various agents during the latter half of the experimental period. Metabolic balance studies were performed during the last week of the experimental period, and at the end of the experimental period, the animals were killed, and blood and bone were obtained.

Table 1 shows the effect of ovariectomy and low Ca diet on body weight gain and food efficiency. Ovariectomy enhanced body weight gain and food efficiency, without affecting food intake of the animals. Low Ca diet itself did not affect these parameters significantly, in either sham-operated or ovariectomized rats (Table 1). As shown in Table 2, although OVX rats on a normal Ca diet (0.47% Ca) showed a significant reduction in bone Ca content compared to sham-operated rats, the effect of ovariectomy was more pronounced when the rats were fed a low Ca diet. Thus, in rats on a low Ca diet, ovariectomy caused a significant reduction in both Ca and P contents, as well as the breaking force of the femoral bone. Therefore, the effects of various hormones on these parameters were examined using OVX, Ca-deficient rats.

Effect of calcitonin

Circulating levels of immunoreactive calcitonin (CT) are reported to be low in patients with postmenopausal osteoporosis (1, 5). Because CT has been shown to inhibit osteoclastic bone resorption (6) and to stimulate 1,25(OH)2D3 synthesis (7), the reduction in CT levels may cause an enhancement of bone resorption and a reduction in intestinal Ca absorption. In order to clarify if CT has any preventive role on the development
Table 1. Effect of ovariectomy and low calcium diet on body weight gain and food efficiency in rats.

<table>
<thead>
<tr>
<th></th>
<th>Body weight gain (g/day)</th>
<th>Food intake (g/day)</th>
<th>Food efficiency (Body weight gain/Food intake)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Ca</td>
<td>1.58±0.15</td>
<td>12.5±0.53</td>
<td>0.13±0.01</td>
</tr>
<tr>
<td>Low Ca</td>
<td>1.44±0.08</td>
<td>12.0±0.43</td>
<td>0.12±0.00</td>
</tr>
<tr>
<td>Ovariectomized</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Ca</td>
<td>2.06±0.17*</td>
<td>13.2±0.36</td>
<td>0.16±0.01*</td>
</tr>
<tr>
<td>Low Ca</td>
<td>1.94±0.08c</td>
<td>12.8±0.36</td>
<td>0.15±0.00c</td>
</tr>
</tbody>
</table>

* The measurements were performed on the last day of the experimental period.  
* Significantly different from the sham-operated rats on the same diet (p<0.05).  
* Significantly different from the sham-operated rats on the same diet (p<0.01).

Table 2. Effect of ovariectomy and low calcium diet on serum levels of calcium and phosphorus, mineral contents and physical properties of femoral bone in rats.

<table>
<thead>
<tr>
<th>Serum concentrations</th>
<th>Bone mineral contents</th>
<th>Physical properties of femur</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca (mg/100 ml)</td>
<td>Ca (% of dry weight)</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Breaking force (×10^6 dyn)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Young's modulus (×10^6 dyn/cm²)</td>
</tr>
<tr>
<td>Sham-operated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Ca</td>
<td>10.2±0.1</td>
<td>25.5±0.3</td>
</tr>
<tr>
<td>Low Ca</td>
<td>9.2±0.2a</td>
<td>17.3±0.5a</td>
</tr>
<tr>
<td>Ovariectomized</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Ca</td>
<td>10.1±0.2</td>
<td>23.9±0.1e</td>
</tr>
<tr>
<td>Low Ca</td>
<td>9.6±0.2</td>
<td>14.3±0.5c6</td>
</tr>
</tbody>
</table>

* Significantly different from the rats on normal Ca diet (p<0.01).  
* Significantly different from the rats on normal Ca diet (p<0.05).  
* Significantly different from sham-operated rats on the same diet (p<0.01).

of postmenopausal osteoporosis, the effect of CT on mineral metabolism and physical properties of bone were examined in OVX, Ca-deficient rats.

Synthetic [Asu 1,7] eel calcitonin (Toyo Jozo Co., Shizuoka, Japan), 5 mU/100 g of B.W., was injected intramuscularly every day during the last month of the experimental period. As shown in Fig. 1, CT treatment increased Ca content, as well as the breaking force of the femoral bone: There were no significant changes in serum levels of Ca or P, with the CT treatment.

Physiological importance of CT has been stressed in relation to the postprandial handling of Ca, especially with the demonstration that plasma CT levels increase after feeding (8). Talmage and Van der Wiel (9) reported a marked increase in postprandial urinary Ca excretion, when the rats were thyroidectomized. These results suggested that the physiological importance of the function of CT is to aid the transfer and storage of dietary Ca into bones. In addition, our recent observations indicate that CT enhances renal tubular re-absorption of Ca in thyroparathyroidectomized rats (10). Thus, CT may be playing an important role in the conservation of body Ca storage, and the maintenance of positive Ca balance by inhibiting bone resorption as well as stimulating 1,25(OH)₂D₃ production and renal tubular Ca re-absorption. The present observations that CT treatment partly prevented the reduction in bone mineral contents and the physical tolerance of femur in OVX, Ca-deficient rats further support the above possibilities, and suggest that CT is effective for the treatment of postmenopausal oste-
Effect of calcitonin, 1,25(OH)\(_2\)D\(_3\) and 24,25(OH)\(_2\)D\(_3\) on mineral contents and breaking force of the femoral bone in rats on a low Ca diet. The data are expressed as changes from the values in ovariectomized rats fed a low Ca diet.

Fig. 2. Effect of 1,25(OH)\(_2\)D\(_3\) and 24,25(OH)\(_2\)D\(_3\) on P balance in rats fed a low Ca diet. Daily excretion of P into feces and urine is expressed as percentage of the total daily intake, and plotted downward from the top of each column. Positive balance is indicated by a clear column above zero line.

oporosis. However, it must be pointed out that CT itself does not stimulate bone formation, and that CT treatment alone may not be enough to sustain long term positive mineral balance of bones.

**Effect of 1,25(OH)\(_2\)D\(_3\) and 24,25(OH)\(_2\)D\(_3\)**

Although the importance of 1,25(OH)\(_2\)D\(_3\) on intestinal Ca absorption is well established, 24,25(OH)\(_2\)D\(_3\) has only minimal effect on intestinal Ca absorption. In contrast, while the direct role of 1,25(OH)\(_2\)D\(_3\) on bone formation still remains elusive, several reports indicate that 24,25(OH)\(_2\)D\(_3\) has a specific effect on bone formation (11, 12). According to Endo et al. (12), the effect of 24,25(OH)\(_2\)D\(_3\) on bone formation is evident when the metabolite is given together with 1,25(OH)\(_2\)D\(_3\) and parathyroid hormone (PTH) in organ cultures of embryonic chick bone. Because serum levels of PTH and 1,25(OH)\(_2\)D\(_3\) are expected to be elevated and that of 24,25(OH)\(_2\)D\(_3\) is suppressed in our rat model of postmenopausal osteoporosis, these animals may be a suitable model in which in vivo effects of 24,25(OH)\(_2\)D\(_3\) can be evaluated. Therefore, the effects of 1,25(OH)\(_2\)D\(_3\) and 24,25(OH)\(_2\)D\(_3\) on mineral balance, bone mineral content and physical properties of bone were investigated using OVX, Ca-deficient rats. Two nanograms of 1,25(OH)\(_2\)D\(_3\) or 1 µg of 24,25(OH)\(_2\)D\(_3\) per 100 g of B.W. was administered orally to the rats every day.

Because the intake of Ca was extremely low in these animals, the effect of the vitamin D\(_3\) metabolites on total body mineral balance was estimated by measuring P balance. As shown in Fig. 2, while OVX rats without treatment showed a negative P balance, 1,25(OH)\(_2\)D\(_3\) turned the P balance of these animals into a positive one. Although 24,25(OH)\(_2\)D\(_3\) also made the P balance positive, the effect was smaller, when compared to 1,25(OH)\(_2\)D\(_3\). The effects of 1,25(OH)\(_2\)D\(_3\) and 24,25(OH)\(_2\)D\(_3\) on mineral contents and physical properties of bone are shown in Fig. 1. Treatment of the OVX, Ca-deficient animals with 1,25(OH)\(_2\)D\(_3\) caused a significant increase in both Ca and P contents of the femoral bone, but no significant effect on breaking properties of bone was observed. In contrast, although 24,25(OH)\(_2\)D\(_3\) rather decreased the mineral contents per dry weight of the femoral bone, it increased the breaking force by 22%, an effect comparable to that seen by CT. There was a tendency that 24,25(OH)\(_2\)D\(_3\) but not 1,25(OH)\(_2\)D\(_3\) increased dry weight of the femoral bone. Neither 1,25(OH)\(_2\)D\(_3\) nor 24,25(OH)\(_2\)D\(_3\) affected serum Ca or P\(_i\) levels significantly (data not shown).

The present observations indicate that 1,25(OH)\(_2\)D\(_3\) makes body mineral balance positive, and increases bone mineral contents, without affecting physical properties of the bone. Although 1,25(OH)\(_2\)D\(_3\) has been shown to be a strong stimulator of bone resorption (13), the role of 1,25(OH)\(_2\)D\(_3\) on bone formation is yet to be clarified. A recent report by Parfitt et al. (14) demonstrated that
continuous 1,25(OH)₂D₃ administration restored all the indices of bone formation, mineralization and growth of vitamin D-deficient rats. In addition, Underwood and DeLuca (15) reported that infusion of Ca and P to vitamin D-deficient rats induced bone growth and mineralization equally to that of the rats which were given vitamin D. Collectively, their results suggest that the effect of 1,25(OH)₂D₃ on bone formation and growth is not a direct one but is mediated by the elevation of serum Ca and P levels through the stimulation of intestinal mineral absorption. The present results are in agreement with those results, in that the effect of 1,25(OH)₂D₃ on bone mineral metabolism appears to be mediated through its effect on intestinal mineral absorption and body mineral balance. However, the observation that physical properties of bone was not restored by 1,25(OH)₂D₃ treatment may suggest that some additional factor(s) are required for the full development of bones in rats. In contrast, 24,25(OH)₂D₃ increased physical properties of bones with minimal effects on bone mineral contents and body mineral balance. These results suggest that 1,25(OH)₂D₃ and 24,25(OH)₂D₃ act differently on the matrix phase and mineral phase of bones, but that they act together to maintain mineral balance and structural integrity of bones. The mechanism of how these vitamin D metabolites affect bone metabolism remain to be clarified.

The present study was supported in part by Grantsin-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan. 1,25(OH)₂D₃ was kindly donated by Chugai Pharmaceutical Co., Tokyo, Japan, and 24,25(OH)₂D₃ by Kureha Chemical Co., Tokyo Japan.

REFERENCES

8) Ross, B.A., Cooper, C.W., Frelinger, A.L., and...


