Beneficial Effect of Pretreatment and Treatment Continuation with Risedronate and Vitamin K2 on Cancellous Bone Loss after Ovariectomy in Rats: A Bone Histomorphometry Study

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Summary The purpose of the present study was to examine the effect of pretreatment with risedronate and/or vitamin K2 and treatment continuation with reduced dosing frequency of the drugs on the early cancellous bone loss induced by ovariectomy (OVX) in rats. Eighty female Sprague-Dawley rats, 4 mo of age, were randomized by the stratified weight method into eight groups (n=10 in each group); rats subjected to OVX, but not sham-operated rats, were treated with vehicle, risedronate, vitamin K2 (menatetrenone), or risedronate+vitamin K2 for 4 wk before the surgery, and the treatment was either discontinued (pretreatment groups) or continued after the surgery (treatment continuation groups) for 2 wk. Sham-operated rats (controls) were treated with the vehicle throughout the experimental period. During the 4 wk prior to the surgery (pretreatment), risedronate and vitamin K2 were administered five times a week either subcutaneously at a dose of 2.5 μg/kg body weight (risedronate) or orally at the dose of 30 mg/kg body weight (vitamin K2). During the 2 wk after the surgery (treatment continuation), the dosing frequency of the drugs was reduced to twice a week. Risedronate and vitamin K2 had an anti-resorptive effect on the bone. Pretreatment with risedronate alone, but not vitamin K2 alone, prevented the loss of the cancellous bone volume/total volume (BV/TV) of the proximal tibial metaphysis after OVX. Treatment continuation with vitamin K2 alone prevented the loss of the cancellous BV/TV after OVX, while treatment continuation with risedronate alone increased the cancellous BV/TV to beyond the values in controls. Pretreatment with risedronate+vitamin K2 had a more beneficial effect in increasing the cancellous bone mass than pretreatment with risedronate alone. Treatment continuation with risedronate and/or vitamin K2 appeared to have a more beneficial effect in increasing the cancellous bone mass than the respective pretreatment. Neither the total tissue area nor the cortical area of the tibial diaphysis was affected by any treatment. The present study demonstrated that pretreatment with risedronate had a beneficial effect on the early cancellous bone loss after OVX in rats, with a more beneficial effect when combined with vitamin K2. Moreover, even though the dosing frequency of the drugs was reduced after OVX, treatment continuation appeared to be more beneficial than pretreatment for increasing the cancellous bone mass.

Key Words ovariectomy, cancellous osteopenia, risedronate, vitamin K2, pretreatment

Osteoporosis primarily affects postmenopausal women, because estrogen deficiency during menopause increases bone turnover and induces marked bone loss. Bisphosphonates and vitamin K2 are widely used for the treatment of postmenopausal osteoporosis in Japan. Risedronate has been shown to increase the lumbar and femoral neck bone mineral density (BMD) and to reduce the incidence of vertebral and nonvertebral fractures in postmenopausal women with osteoporosis (1–3). On the other hand, vitamin K2 (menatetrenone) has been reported to maintain the lumbar BMD and reduce the incidence of osteoporotic fractures in patients with postmenopausal osteoporosis (4–6).

Several preclinical studies have reported the efficacy of risedronate or vitamin K2 for cancellous osteopenia using a rat model of postmenopausal osteoporosis. Risedronate suppresses bone resorption and prevents cancellous bone loss in ovariectomized rats (7, 8). Although the long-term efficacy and safety of risedronate has been established (9), stopping risedronate treatment and reducing the dosing frequency of risedronate should be considered to maintain both the efficacy and safety of the drug. On the other hand, vitamin K2 suppresses bone resorption and prevents or attenuates cancellous bone loss in ovariectomized rats (10–13). However, very few studies have demonstrated the
efficacy of combined treatment with risedronate and vitamin K\(_2\) on cancellous osteopenia in ovariectomized rats.

A majority of the cross-sectional and longitudinal studies that have evaluated BMD during premenopausal years suggest that some bone loss does occur even prior to menopause, after attainment of peak bone mass (14). Rapid rates of bone loss are seen during the transitional period beginning 2–3 y prior to the onset of menopause (15), suggesting that premenopausal impairment of ovarian function can also lead to bone loss. Thus, prevention of bone loss during the perimenopausal period is an important issue that should be considered. However, the efficacy of pretreatment to prevent bone loss in postmenopausal women has not been established. The main purpose of the present study was to examine the effect of pretreatment with risedronate and/or vitamin K\(_2\) and treatment continuation with reduced dosing frequency of the drugs after surgery on cancellous bone loss in ovariectomized rats.

**MATERIALS AND METHODS**

_Treatment of animals._ Eighty female Sprague-Dawley rats, 4 mo of age, were purchased from Charles River Lab (Wilmington, MA, USA). The animals were housed under local vivarium conditions (temperature 23.8°C and 12-h on/off light cycle), and were fed a pelleted standard chow diet (Purina Mills Prolab RMH 2500 Rodent diet #5P14, ON, USA), with free access to water. After allowing 1-mo adaptation to the new environment, the rats, 4 mo of age, were randomized by the stratified weight method into eight groups of 10 rats each (Fig. 1). Rats subjected to ovariectomy (OVX), but not sham-operated rats, were treated with vehicle, risedronate, vitamin K\(_2\), or risedronate+vitamin K\(_2\) for 4 wk prior to the surgery. After the surgery, treatment was either discontinued (pretreatment groups) or continued (treatment continuation groups) for 2 wk. The effects of pretreatment on the skeleton were examined by analyzing the data in the groups in which the treatment was discontinued after the surgery. The sham-operated rats (controls) were treated with only the vehicle throughout the experimental period. During the 4 wk prior to the surgery (pretreatment), risedronate (Aventis Pharma, Tokyo, Japan) was dissolved in 0.1 mL of sterile saline and administered subcutaneously five times a week at the dose of 2.5 \(\mu\)g/kg body weight, and vitamin K\(_2\) (menatetrenone; Eisai Co., Ltd., Tokyo, Japan) was suspended in 0.1 mL of a 1,2-propanediol and glycerol solution at a concentration of 12 mg/0.1 mL and administered by gavage deep into the mouth five times a week at a dose of 30 mg/kg body weight. During the 2 wk after the surgery (treatment continuation), the dosing frequency was reduced to twice a week. The dose of risedronate was determined so as to avoid masking of the additive effects of risedronate and vitamin K\(_2\) by the use of high doses, in accordance with previously published data (16, 17). The effective dose of vitamin K\(_2\) was selected in accordance with previously published data (10–13, 18). The body weight of the rats was monitored weekly and the total experimental period was 6 wk. The study was carried out at Texas Tech University Health Sciences Center, and the animals were maintained according to the National Institutes of Health (NIH) Guidelines for Care and Use of Laboratory Animals. All animal protocols

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline</th>
<th>4 wk (Presurgery)</th>
<th>Surgery</th>
<th>6 wk</th>
<th>2 wk (Post surgery)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V-ShV</td>
<td>Vehicle</td>
<td></td>
<td>Sham</td>
<td>Vehicle</td>
<td></td>
</tr>
<tr>
<td>V-OvV</td>
<td>Vehicle</td>
<td></td>
<td>OVX</td>
<td>Vehicle</td>
<td></td>
</tr>
<tr>
<td>R-OvV</td>
<td>Risedronate</td>
<td></td>
<td>OVX</td>
<td>Risedronate</td>
<td></td>
</tr>
<tr>
<td>R-OvR</td>
<td>Risedronate</td>
<td></td>
<td>OVX</td>
<td>Risedronate</td>
<td></td>
</tr>
<tr>
<td>K-OvV</td>
<td>Vitamin K(_2)</td>
<td></td>
<td>OVX</td>
<td>Vehicle</td>
<td></td>
</tr>
<tr>
<td>K-OvK</td>
<td>Vitamin K(_2)</td>
<td></td>
<td>OVX</td>
<td>Vitamin K(_2)</td>
<td></td>
</tr>
<tr>
<td>RK-OvV</td>
<td>Combination</td>
<td></td>
<td>OVX</td>
<td>Vehicle</td>
<td></td>
</tr>
<tr>
<td>RK-OvRK</td>
<td>Combination</td>
<td></td>
<td>OVX</td>
<td>Combination</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Grouping of animals. Eighty female Sprague-Dawley rats, 4 mo of age, were randomized by the stratified weight method into eight groups (\(n=10\) in each group). During the 4 wk prior to the surgery (pretreatment), risedronate was administered five times a week subcutaneously at the dose of 2.5 \(\mu\)g/kg body weight, and vitamin K\(_2\) (menatetrenone) was administered five times a week by gavage deep into the mouth at the dose of 30 mg/kg body weight, with the drug dissolved in 0.1 mL of sterile saline. During the 2 wk after the surgery, risedronate and vitamin K\(_2\) (menatetrenone) were administered at the same doses, but only twice a week. OVX: ovariectomy.
were approved by the Laboratory Animal Care Committee of Texas Tech University Health Sciences Center.

Preparation of specimens. All rats were labeled with 10 mg/kg of calcein (Sigma Chemical, St. Louis, MO, USA) injected intramuscularly 10 d and 3 d before they were sacrificed. The animals were anesthetized with ketamine (80 mg/kg) injected intraperitoneally, together with xylazine (12 mg/kg), and sacrificed by exsanguination. The right femurs and the right tibiae were collected.

The femurs were used for the measurement of bone mineral content (BMC) and BMD, as described below. The tibiae were used for the static and dynamic bone histomorphometric analyses. The bones were fixed overnight in 40% cold ethanol, and then cut into three parts using an Isomet saw (Buehler, Lake Bluff, IL, USA). The proximal tibial metaphysis and tibial diaphysis with the fibular junction were stained with Villanueva Osteochrome Bone Stain (Polyscience, War- rington, PA, USA) for 5 d. The specimens were then dehydrated sequentially in ascending concentrations of ethanol (70%, 95%, and 100%) and xylene, and then embedded in methyl methacrylate (EM Science, Gibbstown, NJ, USA) at 4°C in accordance with the method of Erben (19). Cross-sections of the tibial diaphysis just proximal to the tibiofibular junction were sectioned at 40-μm thickness using a diamond wire Histosaw machine (Delaware Diamond Knives, Wilmington, DE, USA), and the thickness of each cross-sectional specimen was confirmed with an Inspectors' Dial Bench Gauge (L.S. Starrett, Athol, MA, USA). Frontal sections of the proximal tibial metaphysis were cut at 5-μm thickness using a microtome (Leica RM2155; Leica Inc., Nussloch, Germany), transferred onto chromium-gelatin-coated slides, dried overnight under pressure at 42°C, and coverslipped with Eukitt mounting medium (Calibrated Instruments, Hawthorne, NY, USA) for static and dynamic histomorphometric analyses.

Femoral BMC and BMD. The BMC and BMD of the whole right femur were determined by dual energy X-ray absorptiometry (DXA) using a Hologic QDR-2000 Plus (Hologic Inc., Bedford, MA, USA). The instrument was adapted for an ultra-resolution mode, with a line spacing of 0.0254 cm, resolution of 0.0127 cm, and collimation diameter of 0.9 cm. The bone was placed in a Petri dish, and to simulate soft-tissue density, tap water was poured around the bone to a depth of 1 cm. The BMC and bone area were measured, and the BMD of this area was calculated by dividing the BMC by the bone area. The coefficient of variation of these measurements at our laboratory was less than 1.0% (20).

Bone histomorphometric analysis of the tibia. A digitizing morphometric system was used to measure bone histomorphometric parameters. The system consisted of an epifluorescence microscope (Nikon E-400, Osteometrics, Atlanta, GA, USA), an Osteomeasure High Resolution Color Subsystem (Osteometrics) coupled to an IBM computer, and a morphometry program (Osteometrics). The measured parameters for cancellous bone included total tissue volume (TV), bone volume (BV), bone surface (BS), eroded surface (ES), single- and double-labeled surfaces (sLS and dLS, respectively), and interlabel width. These data were used to calculate percent cancellous bone volume (BV/TV), trabecular number (Tb N), trabecular thickness (Tb Th), trabecular separation (Tb Sp), ES/BS, mineralizing surface (MS)/BS [(sLS/21dLS)/BS], mineral apposition rate (MAR), bone formation rate (BFR)/BS, and BFR/BV in accordance with the standard nomenclature proposed by Parfitt et al. (21). In the present study, the region of cancellous bone measured was 1-4 mm distal to the lower margin of the growth plate in the proximal tibial metaphysis, which consists of secondary spongiosa. In addition to the measurement of above parameters, interlabel width beneath the growth plate was used to calculate longitudinal growth rate (LGR). The measured parameters for cortical bone were total tissue area (Tt Ar), cortical bone area (Ct Ar), endocortical ES, and periosteal and endocortical BS, sLS, dLS, and interlabel width. These data were used to calculate marrow area (Ma Ar), endocortical ES/BS, and periosteal and endocortical MS/BS [(sLS/21dLS)/BS], MAR, and BFR/BS.

Statistical analysis. All the data were expressed as means and standard deviation (SD). Multiple comparisons of data among the groups were performed by analysis of variance (ANOVA) with Fisher's protected least significant difference (PLSD) test. All statistical analyses were performed using the Stat View J-5.0 program on a Macintosh computer. A significance level of p<0.05 was used for all the comparisons.

RESULTS

Body weight and femoral BMC and BMD

Table 1 shows body weight and femoral BMC and BMD. The initial body weight did not differ significantly among the eight groups. OVX increased body weight, and pretreatment and treatment continuation with

<table>
<thead>
<tr>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>BMC (mg)</th>
<th>BMD (mg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V-ShV 274±3</td>
<td>279±1</td>
<td>395±19</td>
<td>226±6</td>
</tr>
<tr>
<td>V-OvV 274±3</td>
<td>286±9¹</td>
<td>388±17</td>
<td>220±6*</td>
</tr>
<tr>
<td>R-OvV 277±3</td>
<td>292±26¹</td>
<td>410±22b</td>
<td>234±5b</td>
</tr>
<tr>
<td>K-OvV 275±3</td>
<td>283±26³</td>
<td>374±29a</td>
<td>220±6⁰</td>
</tr>
<tr>
<td>RK-OvV 277±3</td>
<td>300±8³</td>
<td>433±19b</td>
<td>237±6b</td>
</tr>
<tr>
<td>R-OvR 278±4</td>
<td>292±26³</td>
<td>417±24b</td>
<td>239±6b</td>
</tr>
<tr>
<td>K-OvK 278±8</td>
<td>291±7³</td>
<td>383±16</td>
<td>224±6</td>
</tr>
<tr>
<td>RK-OvRK 276±5</td>
<td>296±8³</td>
<td>419±30b</td>
<td>240±9b</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD. ANOVA with Fisher's PLSD test was used to compare the data among the groups.

*Significantly different from V-ShV. †Significantly different from V-OvV. ‡Significantly different from K-OvV.

BMC: bone mineral content, BMD: bone mineral density.
Fig. 2. Cancellous bone mass and bone turnover of the proximal tibial metaphysis. Data are expressed as mean±SD. ANOVA with Fisher’s PLSD test was used to compare the data among the groups.

*Significantly different from V-ShV. †Significantly different from V-OvV. ‡Significantly different from R-OvV. §Significantly different from K-OvV. ¶Significantly different from RK-OvV. BV/TV: bone volume/total volume, BFR: bone formation rate, BS: bone surface, ES: eroded surface.

Table 2. Bone histomorphometric analysis of the cancellous bone of the proximal tibial metaphysis.

<table>
<thead>
<tr>
<th></th>
<th>Tb N (1/mm)</th>
<th>Tb/Tb (μm)</th>
<th>Tb Sp (μm)</th>
<th>MS/BS (%)</th>
<th>MAR (μm/d)</th>
<th>BFR/BV (%/y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V-ShV</td>
<td>3.3±0.5</td>
<td>53.9±3.8</td>
<td>363±57</td>
<td>24.2±4.0</td>
<td>0.98±0.06</td>
<td>268±33</td>
</tr>
<tr>
<td>V-OvV</td>
<td>3.1±0.4</td>
<td>46.2±5.9</td>
<td>407±66</td>
<td>36.9±4.9</td>
<td>1.10±0.08</td>
<td>539±80</td>
</tr>
<tr>
<td>R-OvV</td>
<td>4.0±0.3</td>
<td>45.9±3.3</td>
<td>298±29</td>
<td>26.5±3.4</td>
<td>0.96±0.05</td>
<td>340±61</td>
</tr>
<tr>
<td>K-OvV</td>
<td>3.1±0.4</td>
<td>46.2±4.1</td>
<td>402±57</td>
<td>36.1±6.0</td>
<td>1.07±0.05</td>
<td>510±87</td>
</tr>
<tr>
<td>RK-OvV</td>
<td>4.1±0.7</td>
<td>49.6±5.0</td>
<td>289±49</td>
<td>27.7±4.0</td>
<td>1.01±0.04</td>
<td>343±45</td>
</tr>
<tr>
<td>R-OvR</td>
<td>4.2±0.4</td>
<td>51.0±4.7</td>
<td>275±35</td>
<td>21.6±3.4</td>
<td>0.93±0.04</td>
<td>240±38</td>
</tr>
<tr>
<td>K-OvK</td>
<td>3.9±0.5</td>
<td>42.2±7.3</td>
<td>315±38</td>
<td>31.9±3.3</td>
<td>1.07±0.05</td>
<td>508±109</td>
</tr>
<tr>
<td>RK-OvRK</td>
<td>4.2±0.7</td>
<td>53.9±5.5</td>
<td>276±50</td>
<td>20.7±3.2</td>
<td>0.91±0.04</td>
<td>214±23</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD. ANOVA with Fisher’s PLSD test was used to compare the data among the groups.

*Significantly different from V-ShV. †Significantly different from V-OvV. ‡Significantly different from R-OvV. §Significantly different from K-OvV. ¶Significantly different from RK-OvV.


Risedronate + vitamin K2 enhanced the OVX-induced gain in body weight. O VX decreased femoral BMD, with a percentage femoral BMD loss of 2.7%. Neither pretreatment nor treatment continuation with vitamin K2 had any effect on femoral BMD. However, both pretreatment and treatment continuation with risedronate alone and risedronate + vitamin K2 increased femoral BMD above the V-ShV value.

OVX did not decrease femoral BMC. Neither pretreatment nor treatment continuation with vitamin K2 had any effect on femoral BMC. However, both pretreatment
and treatment continuation with risedronate alone and risedronate+vitamin K₂ increased femoral BMC above the V-OvV value and the V-ShV and V-OvV values, respectively.

**Bone histomorphometric analysis of the cancellous bone of the proximal tibial metaphysis**

Figure 2 and Table 2 show the results of bone histomorphometric analysis of the cancellous bone of the proximal tibial metaphysis. OVX decreased BV/TV and
Tb Th and increased the Tb Sp, as a result of increased bone resorption (ES/BS) and bone formation (MS/BS, MAR, BFR/BS, BFR/BV), with a percentage BV/TV loss of 20.2%. Pretreatment with vitamin K2 did not affect this BV/TV loss, whereas continuous treatment with vitamin K2 prevented BV/TV loss by attenuating the increase in both bone resorption (ES/BS) and bone formation (MS/BS, BFR/BS), with a more significant effect of treatment continuation than pretreatment on bone formation (MS/BS, BFR/BS) and bone resorption (ES/BS). Because only treatment continuation with vitamin K2 had a significant effect on BV/TV, treatment continuation with vitamin K2 was considered to have a more beneficial effect on BV/TV than pretreatment with vitamin K2. On the other hand, pretreatment with risedronate prevented BV/TV loss by attenuating the increase in bone resorption (ES/BS) and preventing an increase in bone formation (MAR, BFR/BS). Furthermore, treatment continuation with risedronate increased BV/TV above the V-ShV value, by suppressing bone resorption (ES/BS) and bone formation (MS/BS, MAR, BFR/BS, BFR/BV), with a more significant effect of treatment continuation than pretreatment on BV/TV, bone formation (MS/BS, MAR, BFR/BS, BFR/BV), and bone resorption (ES/BS). Both pretreatment and treatment continuation with risedronate+vitamin K2 increased BV/TV above the V-ShV value by suppressing bone resorption (ES/BS) and bone formation (MS/BS, MAR, BFR/BS, BFR/BV), with a more significant effect of treatment continuation than pretreatment on BV/TV and bone formation (MAR, BFR/BS, BFR/BV). The benefit of vitamin K2, in addition to the effect of risedronate, was recognized for bone formation and bone resorption: pretreatment with vitamin K2, in addition to risedronate, attenuated the reduction in bone formation (BFR/BS) by risedronate and enhanced the reduction in bone resorption (ES/BS) by risedronate, resulting in a greater effect on BV/TV. Both risedronate and vitamin K2 had an anti-resorptive effect on the cancellous bone, and their effect on BV/TV was associated with improvement of Tb N and Tb Sp. Continuous treatment with risedronate alone or risedronate+vitamin K2 also improved Tb Th.

Bone histomorphometric analysis of the cortical bone of the tibial diaphysis

Figure 3 and Table 3 show the results of bone histomorphometric analysis of the cortical bone of the tibial diaphysis. OVX increased periosteal bone formation (MS/BS, MAR, BFR/BS) and endocortical bone formation (MAR, BFR/BS) and bone resorption (ES/BS). Pretreatment and treatment continuation with risedronate, vitamin K2, or risedronate+vitamin K2 did not affect periosteal bone formation as evaluated by BFR/BS. Pretreatment and treatment continuation with risedronate alone or risedronate+vitamin K2 prevented an increase in endocortical bone resorption (ES/BS), while pretreatment and treatment continuation with vitamin K2 alone did not affect endocortical bone resorption (ES/BS). According to preventing an increase in endocortical bone resorption (ES/BS), an increase in endocortical bone formation as evaluated by BFR/BS was prevented by pretreatment and treatment continuation with risedronate alone and pretreatment with risedronate+vitamin K2. However, treatment continuation with risedronate+vitamin K2 sustained the increase in endocortical bone formation (BFR/BS), which was the benefit of treatment continuation with vitamin K2 in addition to the effect of treatment continuation with risedronate. Despite alteration in periosteal and endocortical bone formation or resorption, OVX did not affect the Ct Ar, Ti Ar, or Ma Ar, and pretreatment and treatment continuation with risedronate, vitamin K2, or risedronate+vitamin K2 did not affect any of these parameters.

DISCUSSION

The present study was conducted primarily to examine the effect of pretreatment (treatment prior to OVX followed by discontinuation) with risedronate and/or vitamin K2 and treatment continuation with reduced dosing frequency of the drugs on cancellous bone loss after OVX in rats. The focus of the discussion was 1) whether pretreatment with risedronate or vitamin K2 alone would be effective to prevent early cancellous bone loss after OVX, 2) whether treatment continuation with risedronate or vitamin K2 alone would be more beneficial for the cancellous bone mass than pretreatment with risedronate or vitamin K2, respectively, and 3) how beneficial pretreatment and treatment continuation with a combination of risedronate and vitamin K2 would be for the cancellous bone.

Pretreatment and treatment continuation with risedronate alone had an anti-resorptive effect on the cancellous and endocortical bone, and treatment continuation with vitamin K2 alone on the cancellous bone, with a more potent anti-resorptive effect of risedronate compared with vitamin K2. Risedronate is known to inhibit osteoclast-mediated bone resorption (22). On the other hand, vitamin K2 is known to have an anabolic action on bone; regulation of bone formation by vitamin K2 may involve its action on the γ-carboxylation of osteocalcin and/or may be mediated by the steroid and xenobiotic receptor (SXR) (23–27). However, vitamin K2 has also been reported to suppress bone resorption in vitro (28–30). Thus, vitamin K2 could regulate bone formation and bone resorption, which is supported by the results of numerous studies using various rat models of osteoporosis (10–13, 31–41).

Although the efficacy of risedronate or vitamin K2 for bone mass, bone strength, and bone resorption in OVX rats has been reported (7, 8, 10–13, 18), the efficacy of pretreatment with risedronate or vitamin K2 for reducing bone loss after OVX remains to be established. The present study demonstrated that pretreatment with risedronate alone prevented the cancellous bone loss observed 2 wk after OVX. One possible explanation for this effect is that risedronate, which has a high affinity to bone, administered before OVX might be retained in the bone and then might act on the osteoclasts after OVX. Stopping risedronate treatment may not promptly
reverse its benefit because of the long residence time of risedronate in the bone. Another possibility is that pre-
treatment with risedronate might increase the cancel-
loss of bone mass prior to OVX. Because it has been
shown that osteoclastic bone resorption significantly
increases 3 d after OVX, peaks between 2 and 3 wk, and
then declines thereafter in the cancellous bone of the
proximal tibial metaphysis of 4-mo-old female Sprague-
Dawley rats (42), our results suggest the efficacy of pre-
treatment with risedronate against early cancellous
bone loss in ovariectomized rats.

On the other hand, pretreatment with vitamin K2
alone had no effect on the cancellous bone, probably
because of its modest effect on the bone in intact rats
prior to OVX. However, the suppression of bone resorp-
tion by risedronate was enhanced and the suppression
of bone formation by risedronate was attenuated when
combined with vitamin K2, resulting in a more benefi-
cial effect of pretreatment with risedronate+vitamin K2
on the cancellous bone mass compared with pretreat-
ment with risedronate alone. The reason for the bene-
fit of vitamin K2 in addition to the effect of risedronate
for cancellous bone, despite no significant effect of vitamin
K2 alone, remains uncertain. However, pretreatment
with vitamin K2 and risedronate may have a synergistic
effect on the cancellous bone mass in OVX rats.

Treatment continuation with risedronate alone
increased the cancellous bone mass above the values in
controls, which was more effective than pretreatment
with risedronate, despite the reduced dosing frequency
of the drug after OVX. These results suggest that inter-
mittent administration of risedronate, without increas-
ing the dose of the drug, is acceptable for increasing
 cancellous bone mass after sufficient pretreatment with
risedronate.

On the other hand, treatment continuation with vitami-
K2 alone prevented cancellous bone loss after OVX,
suggesting the efficacy of the continuous intermittent
administration of vitamin K2 for preventing cancellous
bone loss after OVX. Thus, long-term treatment with
vitamin K2 may be encouraged to maintain the efficacy
of vitamin K2 alone for cancellous bone mass in OVX
rats. The benefit of continuous treatment with vitamin
K2 combined with risedronate for cancellous bone was
not observed, probably because the small additive effect
of vitamin K2 might be masked by relatively high-dose
risedronate, which was both retained and stored in the
bone by pretreatment and administered after OVX by
treatment continuation. However, treatment continued
with risedronate+vitamin K2 increased the can-

cellous bone mass above the control values, with treat-
ment continuation having a greater beneficial effect
than pretreatment on the cancellous bone mass and
bonet turnover, even though the dosing frequency of
the drugs was reduced.

No significant cortical bone loss was observed in OVX
rats. However, OVX induced an increase in endocorti-
cal bone turnover and periosteal bone formation. Pretreat-
ment and treatment continuation with risedronate
alone or risedronate+vitamin K2 could prevent an
increase in bone resorption on the endocortical surface
like on the cancellous bone surface, both of which are
remodeling envelopes. The benefit of vitamin K2 in addi-
tion to the effect of risedronate was recognized for the
ductoral bone: treatment continuation with vita-
mK2 in addition to risedronate counteracted the sup-
pression of endocortical bone formation by risedronate.
Pretreatment and treatment continuation with rised-
ronate alone or risedronate+vitamin K2 did not affect
periosteal bone formation as evaluated by BFR/BS, sug-
gesting that risedronate may not inhibit OVX-related
cortical expansion.

A study conducted by Iwasaki et al. (43) demon-
strated the effect of a combined treatment using vita-
mK2 and a bisphosphonate on the cancellous bone in
tail-suspended rats; the bisphosphonate incadronate
treatment attenuated the cancellous bone loss by sup-
pressing bone turnover, and combined treatment with
incadronate and vitamin K2 was more beneficial to
attenuate the cancellous bone loss because of increased
bone turnover, compared with that following incad-
ronate treatment alone. In the present study, the benefit
of vitamin K2 in addition to the effect of risedronate was
also demonstrated to be the attenuation of suppressed
cancellous bone formation by risedronate and preven-
tion of suppressed endocortical bone formation by
risedronate. These findings suggest that the use of vita-
mK2 in combination with bisphosphonates may be
effective not only in increasing the bone mass, but also
in maintaining bone formation in terms of improving
bone quality, which is determined by microarchitecture,
microfracture, bone turnover, and mineralization (44).

Pretreatment and treatment continuation with
risedronate+vitamin K2 enhanced the OVX-induced
gain in body weight. The reason for this remains uncer-
tain. However, higher cancellous bone mass might
partly contribute to heavier body weight in the groups
of pretreatment and treatment continuation with
risedronate+vitamin K2. Further studies are needed to
clarify this issue.

In conclusion, the present study demonstrated that
pretreatment with risedronate had a beneficial effect on
early cancellous bone loss after OVX in rats, with a
more beneficial effect when combined with vitamin K2.
Moreover, treatment continuation, even though the
dosing frequency of the drugs was reduced after OVX,
appeared to be more beneficial for increasing cancellous
bone mass.

REFERENCES

1) Fogelman I, Ribot C, Smith R, Rihden D, Sod E, Regin-
steer JY. 2000. Risedronate reverses bone loss in post-
menopausal women with low bone mass: results from a
multinational, double-blind, placebo-controlled trial. J
Clin Endocrinol Metab 85: 1895–1900.

2) Harris ST, Watts NB, Genant HK, McKeever CD, Han-
gartner T, Keller M, Chesnut CH II, Brown J, ErikEn EF,
Hoseyni MS, Axelrod DW, Miller PD. 1999. Effects of
risedronate treatment on vertebral and nonvertebral
fractures in women with postmenopausal osteoporosis:
a randomized controlled trial. JAMA 282: 1344–1352.


Pretreatment with Risedronate and/or Vitamin K$_2$


