Comparative Effects of Milk and Soymilk on Bone Loss in Adult Ovariectomized Osteoporosis Rat

By

Hirotaka TAGUCHI, Huayue CHEN, Ryuichiro YANO* and Shizuko SHOUMURA

Department of Anatomy, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu, 501-1194, Japan
*Department of Obstetrics and Gynecology, Gifu Municipal Hospital, 7-1 Kashimacho, Gifu, 500-8513, Japan

– Received for Publication, May 17, 2006 –

Key Words: Ovariectomy, Osteoporosis, Milk, Soymilk, Scanning electron microscopy

Summary: The present study was designed to investigate the comparative effects of milk and soymilk on the bone morphology of ovariectomized rat model with postmenopausal osteoporosis. Forty 12-week-old female Sprague-Dawley rats were randomly divided into the following 5 groups: intact control (control), sham-operated (sham), ovariectomized (OVX), ovariectomized and fed milk (milk), ovariectomized and fed soymilk (soymilk) groups. Rats of OVX, milk and soymilk groups were bilaterally ovariectomized. Milk or soymilk of 3 ml was administered by gavage via an intragastric tube per day for 6 weeks. The distal femoral metaphysis was studied morphologically, together with bone mineral density (BMD), serum parathyroid hormone (PTH) and estradiol levels. In the OVX group, the trabecular bone volume was significantly reduced. The percentage area of resorbing surface was high. In the soymilk group, the trabecular bone volume was 68% higher and the percentage area of resorbing surface was 18% lower as compared with the OVX group. The serum estradiol level rose 48% and the serum PTH level fell 26% in the soymilk group. In the milk group, the trabecular bone volume had the tendency to be high, but there was no significant change of the bone morphology. The present study provides certain evidence that the soymilk has beneficial effects in preventing bone loss in rats induced by ovarian hormone deficiency.

Osteoporosis is a multifactorial skeletal disease characterized by bone loss and structural deterioration of bone tissue, results from an imbalance between bone formation and bone resorption. Osteoporosis is becoming a major public health problem in all developed countries. Despite numerous investigations, the pathogenesis, treatment and prevention of osteoporosis remain unsolved problems.

There are several investigations on the effects of milk and soymilk on human and animal bones (Recker and Heaney, 1985; Omi et al., 1994; Kikuchi-Hayakawa et al., 1998). However, the results are controversy. It is well documented that milk is an excellent calcium source and contains several profitable components for calcium absorption in the intestine, such as lactose and phosphopeptides, formed by the proteolytic digestion of milk casein (Wasserman, 1964; Lee et al., 1980). Milk is also considered to affect bone metabolism directly because milk has a functional role in the growth of newborn animals and is an excellent source of nutrients for human health. Milk might be expected to decrease osteoporotic bone loss and fracture risk. Evidence from clinical trials and case-control studies has been confusing and several observational studies found no decrease in the bone fracture risk with higher consumption of milk (Recker and Heaney, 1985; Feskanich et al., 1997). The survey of the literature concluded that there is no clear benefit of milk intake on bone mass or fracture risk in women (Weinsier and Krumdieck, 2000; Feskanich et al., 2003).

The potential roles of dietary soymilk in the prevention and treatment of chronic diseases have long been known. Many types of soy food are consumed throughout the world. Soymilk is an aqueous extract of whole soybeans. Soymilk contains isoflavones, which are thought to have beneficial effects on the bone (Setchell and Lydeking-Olsen, 2003). It was reported that soymilk was effective for increasing bone mineral density (BMD) and mechanical bone strength, and enhancing intestinal...
calcium absorption (Omi et al., 1994). On the other hand, the action of dietary isoflavones during the premenopausal and perimenopausal periods appears to be variable. Wangen et al. (2000) observed no significant effects of isoflavones on bone turnover clinically, whereas Alekel et al. (2000) reported that there was a slightly beneficial effect on the bone. Here we present a comprehensive study of the effects of milk and soymilk on bone loss in rats induced by ovariectomy, using scanning electron microscopy and histomorphometry.

Materials and Methods

Twelve-week-old female Spraque-Dawley rats were randomly divided into 5 groups of 8 animals each. Intact control (control), sham-operated (sham), ovariectomized (OVX), ovariectomized and fed milk (milk), ovariectomized and fed soymilk (soymilk) groups. All animals were maintained under conventional conditions and had free access to tap water and commercial diet (CE-2, CLEA Japan). The diet contained 1.18% calcium and 0.85% phosphorus.

Rats of the OVX, milk and soymilk groups were bilaterally ovariectomized with sodium pentobarbital deep anesthesia. After operation, milk (Japan dairy farming co., Ltd., Osaka, Japan) or soymilk (MARUSAN-AI Co., Ltd., Aichi, Japan) was administered by gavage via an intragastric tube. A daily dose of 3 ml per rat was given for 6 weeks. The compositions of milk and soymilk are shown in Table 1. Milk and soymilk contained the similar amount of carbohydrates and calories. Milk had greater levels of lipids and calcium, while soymilk included higher amounts of proteins and isoflavones of 22 mg/100 ml (Table 1).

After the experiment, the femurs were removed under ether anesthesia and processed for scanning electron microscopy (SEM). The distal parts of the femurs were trimmed in the sagittal plane. They were treated with 5% sodium hypochlorite solution to expose the epiphyseal and metaphyseal trabecular bone. The bones were then dehydrated in acetone and critical-point dried, mounted on stubs and coated with gold/palladium using an ion sputter. The bones were examined with a Hitachi S-3500 N SEM. Trabecular measurements of the distal femur were done from a 4 mm² area in the central metaphysis 1.0 mm proximal from the growth plate-epiphyseal junction. The trabecular bone volume per tissue volume (BV/TV), trabecular thickness (Tb.Th) was measured using adobe Photoshop, according to the standard nomenclature for bone histomorphometry (Parfitt et al., 1987). The percentage area of resorbing surface on the trabecular bone was also estimated as reported previously (Chen et al., 2004).

The bone mineral density (BMD) of the whole body was measured by Dual Energy X-ray Absorptiometry (DXA) using a type 2000 Toyo Medic QDR. The blood was taken from the heart under ether anesthesia. The serum calcium level was determined by the standard colorimetric method, as described previously (Chen et al., 2002). The serum total cholesterol and triglyceride levels were determined by enzymatic assays (Richmond, 1973; Tamakori et al., 1982). The serum PTH and estradiol levels were measured using immunoradiometric assay kit as reported before (Chen et al., 2005).

All data are presented as mean ± SEM. The statistical analysis was done using StatView J-4.5 (Abacus Concepts). Differences between groups were assessed by one-way analysis of variance (ANOVA). Fisher’s PLSD test was used for post hoc multiple comparisons. Significance was defined as p < 0.05.

Results

There was no significant difference in any measured parameter between the control and sham groups, indicating that the animals were unaffected by the operation as such. Rats in all 5 groups had similar initial body weights (Table 2). At the end of the experiment, however, the animals of the OVX, milk and soymilk groups had a significantly higher body weight than control and sham groups (Table 2). There were no significant differences among the OVX, milk and soymilk groups regard to the final body weight.

The serum calcium, total cholesterol, triglyceride, PTH and estradiol levels are shown in Table 2. In the OVX group, the serum estradiol level was significantly low, the serum total cholesterol level was

| Table 1. The components of milk and soymilk (/100 ml) |
|-----------------|-----------------|
|                 | Milk            | Soymilk         |
| Calories        | 64.0 kcal       | 59.0 kcal       |
| Proteins        | 3.1 g           | 3.7 g           |
| Lipids          | 3.6 g           | 2.4 g           |
| Carbohydrates   | 4.8 g           | 4.5 g           |
| Na              | 58.0 mg         | 57.0 mg         |
| Ca              | 104.0 mg        | 41.0 mg         |
| Isoflavone      | 22.0 mg         | 21.0 mg         |
| Mg              | 0.5 mg          | 0.4 mg          |
| Fe              |                 |                 |
| Zn              |                 |                 |
Table 2. Body weight, serum calcium, cholesterol, triglyceride, PTH and estradiol levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Calcium (mg/100 ml)</th>
<th>Total cholesterol (mg/100 ml)</th>
<th>Triglyceride (mg/100 ml)</th>
<th>PTH (pg/ml)</th>
<th>Estradiol (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>238.6 ± 4.0</td>
<td>269.3 ± 7.3</td>
<td>10.4 ± 0.3</td>
<td>70.9 ± 4.4</td>
<td>74.3 ± 6.2</td>
<td>7.9 ± 1.5</td>
<td>6.6 ± 0.8</td>
</tr>
<tr>
<td>Sham</td>
<td>231.3 ± 6.3</td>
<td>272.3 ± 8.0</td>
<td>10.4 ± 0.4</td>
<td>73.9 ± 3.0</td>
<td>83.3 ± 10.2</td>
<td>8.5 ± 1.5</td>
<td>7.3 ± 1.3</td>
</tr>
<tr>
<td>OVX</td>
<td>232.3 ± 3.6</td>
<td>304.0 ± 19.3</td>
<td>10.3 ± 0.2</td>
<td>98.2 ± 5.5</td>
<td>59.9 ± 7.4</td>
<td>10.1 ± 1.8</td>
<td>2.7 ± 0.6</td>
</tr>
<tr>
<td>Milk</td>
<td>232.8 ± 6.2</td>
<td>298.8 ± 17.7</td>
<td>10.4 ± 0.4</td>
<td>92.5 ± 1.8</td>
<td>60.7 ± 13.0</td>
<td>9.4 ± 3.1</td>
<td>3.4 ± 0.5</td>
</tr>
<tr>
<td>Soymilk</td>
<td>237.6 ± 4.7</td>
<td>304.5 ± 26.0</td>
<td>10.2 ± 0.5</td>
<td>84.8 ± 2.6</td>
<td>50.3 ± 8.4</td>
<td>7.5 ± 1.5</td>
<td>4.0 ± 0.4</td>
</tr>
</tbody>
</table>

* p value < 0.05 vs control group; † p value < 0.05 vs sham group; ‡ p value < 0.05 vs OVX group.

Table 3. Whole body BMD, trabecular bone volume, trabecular thickness and resorbing area

<table>
<thead>
<tr>
<th>Group</th>
<th>BMD (mg/cm²)</th>
<th>Trabecular bone volume (%)</th>
<th>Trabecular thickness (μm)</th>
<th>Resorbing area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>138.4 ± 6.2</td>
<td>21.74 ± 1.19</td>
<td>86.17 ± 3.99</td>
<td>3.94 ± 0.31</td>
</tr>
<tr>
<td>Sham</td>
<td>130.7 ± 5.6</td>
<td>19.59 ± 1.06</td>
<td>86.59 ± 3.51</td>
<td>4.08 ± 0.30</td>
</tr>
<tr>
<td>OVX</td>
<td>129.1 ± 5.1</td>
<td>3.25 ± 0.48</td>
<td>74.76 ± 2.75</td>
<td>7.26 ± 0.41</td>
</tr>
<tr>
<td>Milk</td>
<td>125.7 ± 3.5</td>
<td>4.37 ± 0.38</td>
<td>79.01 ± 3.89</td>
<td>6.60 ± 0.51</td>
</tr>
<tr>
<td>Soymilk</td>
<td>129.1 ± 5.1</td>
<td>5.46 ± 0.54</td>
<td>81.36 ± 2.68</td>
<td>5.92 ± 0.38</td>
</tr>
</tbody>
</table>

* p value < 0.05 vs control group; † p value < 0.05 vs sham group; ‡ p value < 0.05 vs OVX group.

Discussion

The ovariectomized rat has been proven historically effective for evaluating estrogen agonists and...
Fig. 1. Scanning electron micrographs of the femoral distal metaphyses in rat. A: sham group; B: OVX group; C: milk group; D: soymilk group. As compared with the OVX group, the trabecular bone in the central part of the femoral distal metaphysis is higher in the soymilk group. Bar = 1 mm.
antagonists in female reproductive or nonreproductive axes, including bone parameters (Kalu et al., 1991). Furthermore, the bone loss in the ovariec-
tomized rat parallels the skeletal changes observed in the postmenopausal women and responsiveness to hormone replacement therapy. In the present
study, we investigated the effects of milk and soymilk on the bone morphology of ovariecotomized rat model with postmenopausal osteoporosis.

It was reported that ovariecotmy induced an increase in the plasma cholesterol level of rats (Kikuchi-Hayakawa et al., 1998). A reduction in cholesterol was observed with soy protein in experi-
mental animals and human subjects (Carroll and Kurowska, 1995). The fermented soymilk had a favorable effect on the plasma cholesterol level without an increase in steroid excretion. The soymilk was also effective in preventing the cholesterol elevation induced in rats by ovarian hormone defi-
ciency (Kikuchi-Hayakawa et al., 1998). The cho-

lesterol metabolic changes were observed following soy protein feeding in a variety of animal models (Nagata et al., 1982; Carroll and Kurowska, 1995).

In the present study, no significant changes of the serum triglyceride level were observed in the ovar-
iectomized, milk or soymilk groups. However, we found that the ovariecotmy induced the increase in the serum total cholesterol level and that soymilk could inhibit the rise of the cholesterol level in rats.

We consider that soymilk could prevent the increase of the serum cholesterol level induced by ovariecotmy in rats.

In the present study, we found that the tra-
becular bone volume was markedly reduced in the ovariecotomized rat. Animals fed with milk for 6 weeks after the ovariecotmy seemed to have some favora-
able effects on the bone loss induced by ovariecotmy in rats, but no significant changes were observed with the serum analysis and the bone his-
tomorphometry. Milk is recommended as an excel-
lent calcium source for bone health and is consid-
ered an important determinant of peak bone mass.

Toba et al. (2000) studied the effects of milk basic protein on bone metabolism in ovariecotomized rats fed with low-calcium diet (0.3%). They found that milk basic protein could prevent the bone loss induced by ovariecotmy. In the present study, all animals were fed with the normal calcium diet (1.18%). So we think that the diet calcium level is enough for maintaining the normal bone metabo-
lism (Chen et al., 2002).

It is considered that milk intake early in life may decrease the risk of osteoporotic fractures later. Using the milk history questionnaire, many inves-
tigators have conducted the retrospective cross-
sectional studies and have determined a positive
effect of milk intake in childhood and adolescence on BMD in adult. By contrast, a recent study in-
dicated that an adequate vitamin D intake is associ-
ated with a lower risk of osteoporotic hip frac-
tures in postmenopausal women (Feskanich et al., 2003). While milk does not appear to substantially reduce the risk of hip fracture, perhaps because of other nutrients in the milk, such as vitamin A, that do not support bone health (Feskanich et al., 2003).

Soymilk contained rich phytoestrogens, includ-
ing isoflavones. There has been considerable in-
terest in the use of phytoestrogens as substitutes for traditional estrogen replacement therapies in menopausal women. Some evidence suggests that these compounds may provide tissue-selective ef-
fects. Evidence from epidemiological studies of el-
derly women in Asian nations suggests that high consumption of isoflavones from soy products may be protective against fractures in comparison with Western populations. It was reported that soymilk is effective for increasing BMD and the mechanical bone strength by acceleration of the intestinal cal-
cium absorption in ovariecotomized rats (Omi et al., 1994).

Isoflavones are thought to benefit the bone in estrogen-depleted menopausal women relates to the actions of isoflavones on estrogen receptors and other cellular pathways of bone cells. Studies using an ovariecotomized animal model have clearly demon-
strated significant positive skeletal effects of soy protein at doses comparable to the effects of physi-
ological doses of estrogenic molecules in young estrogen-depleted rodents. The effects of isofla-
vones on the estrogen receptors of bone cells may also exert additional actions that generally enhance osteoblastic cell functions and reduce osteoclast-
mediated bone resorption. In the present study, we found that the soymilk could prevent the bone loss induced by ovariecotmy in rats. Although we did not analyze the bone formation, the bone resorp-
tion of the trabeculae was significantly decreased after soymilk treatment. We consider that the soymilk preserved the bone structure by inhibiting the increased activity of the bone resorption. Further-
more, estrogen and PTH are important factors in regulating the bone metabolism. In the present study, we observed that soymilk could enhance the serum estrogen level and reduce the serum PTH level, which may play an important role in sup-
pression of the elevated bone resorption.

In conclusion, the present study showed that the soymilk has some beneficial effects in preventing the bone loss in ovariecotomized rats by inhibiting the augmented bone resorption. Soymilk could also have positive effects on the serum estrogen and PTH levels, which involve in the bone metabolism.
References


Fig. 2. Scanning electron micrographs of the femoral distal metaphyses in rat. A: sham group; B: OVX group; C: milk group; D: soymilk group. There is a considerable resorbing surface on the trabeculae of the femoral distal metaphysis in the OVX and milk groups. Less amount of the resorbing surface is observed in the soymilk group. Asterisks: resorbing surfaces. Bar = 0.1 mm.