INFLUENCE OF NATURAL SOYA ISOFLAVONES AND IPRIFLAVONE ON RATS’ BONE DENSITY

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Received for publication December 30, 2004.

Abstract

Influence of isoflavones and ipriflavone on rats’ mandible, femur and lumbar vertebra mineral density was investigated in experimental model of rats’ postmenopausal hypoestrogenism. Animals were given free access to the standard feed, and specially prepared soya chow with two different contents of soya proteins. After 120 d of the experiment, the mandible, femur and lumbar spine bone mineral density (BMD) was examined. Mandible BMD in group taking high dose of soya proteins differed significantly from that in ovariectomized rats, but no other significant differences between groups taking soya diet and control was observed neither in the femur nor in lumbar vertebra. Also group taking ipriflavone did not differ significantly from ovariectomized and control groups. In each examined bone the differences between ovariectomized and control – sham-operated groups were statistically significant. On the basis of our findings we hypothesize that isoflavones in soya diet can modulate mandible bone metabolism and improve bone quality after ovariectomy.

Key words: rats, teeth, mandible, isoflavones, ipriflavone.

Elapsing of ovarian oestrogen production in peri- and postmenopausal women is a prime cause of osteoporosis. Administering 17β-oestradiol (ERT) and other hormonal drugs including calcitonin, SREM drugs (e.g. raloxifene) with supplementation of calcium are the most popular treatments of osteoporosis in women. Though efficient, ERT increases risk of endometrium and breast cancer (7). Variable responses on the oestradiol treatment due to two different oestrogen receptor (ER): α and β in tissues all over the body, make this drug useless and dangerous in some cases (3).

Phytooestrogens are natural plant substances with oestrogen-like activity. They are obtained from over 300 types of plants: Leguminose, Papilionaceae, Graminae, Cruciferae, Solanaceae, Cucurbitaceae. The main groups of phytooestrogens are isoflavons (genistein, daidzein, glycitein, formononetin), coumestans (coumestrol) and lignans (enterodiol, enterolacton) (6). The main source of isoflavons are soya and red clover, lignans are extracted from corns. Phytooestrogens due to their chemical similarity to oestrogens, have ability to bind oestrogen receptor (ER). Though their affinity to ER is low, concentration of isoflavons can be high enough to bind ER (6).

The aim of this study was to investigate the impact of soya diet and isoflavon derivative (ipriflavone) intake on mandible, femur, lumbar vertebra density, and serum alkaline phosphatase activity in rats’ experimental postmenopausal hypoestrogeny model.

Material and Methods

Wistar female rats, 6 weeks of age, were used in the study. The experiment was conducted according to the animal experimental guidelines approved by Animal Experiment Committee, Skubiszewski Medical University of Lublin.

After two-week adaptation to diet and new environment, 40 rats were bilaterally ovariectomized from an abdominal approach under general anaesthesia with thiopental sodium. These rats were divided into 4 equal experimental groups: OV - receiving standard chow, SA - receiving diet with 2.5% soya content, SB - receiving diet with 25% soya content and IP - receiving 25 mg/rat/d of ipriflavone (Osteofix - Chiesi, Parma, Italy). Ten sham-operated animals, without ovariectomy, constituted the control group - CL.
Table 1
Isoflavones contents in standard and 2.5%, 25% and 100% soya chow (µg of isoflavones/g chow)

<table>
<thead>
<tr>
<th>Isoflavone</th>
<th>Standard chow</th>
<th>2.5% soya chow</th>
<th>25% soya chow</th>
<th>100% soya chow</th>
</tr>
</thead>
<tbody>
<tr>
<td>daidzein</td>
<td>18.4</td>
<td>31.9</td>
<td>89.9</td>
<td>553.5</td>
</tr>
<tr>
<td>genistein</td>
<td>13.8</td>
<td>42.2</td>
<td>131.1</td>
<td>794.9</td>
</tr>
<tr>
<td>glycitein</td>
<td>2.8</td>
<td>13.7</td>
<td>33.3</td>
<td>172.4</td>
</tr>
<tr>
<td>Total</td>
<td>35.1</td>
<td>82.9</td>
<td>247.3</td>
<td>1520.8</td>
</tr>
</tbody>
</table>

Soya diet was specially prepared by “Agropol”-Motycz, Poland. The animals were given free access to the feed and water with diluted calcium lactogluconicum (Calcium- Polfa) (20 mg/100 ml). Before each feeding remaining feed was weighed and average daily intake was calculated. The intake approximated to 20 g of dry chow per rat.

After 120 d of the experiment blood samples were collected from the abdominal aorta and centrifuged immediately. Serum was separated, portioned and frozen at -30°C. Then the rats were anaesthetized with thiopental sodium and the mandible, left femur, and lumbar vertebra were taken. Bones were cleaned from soft tissues and stored at -30°C. Bone mineral density (BMD) was estimated with DPX-A absorptiometry bone densitometer and analysed with Small Animal Software. Manual analysis of each scan allowed estimate BMD (g/cm²) of rats’ mandible, femur, and vertebra. The level of alkaline phosphatase (ALP) was determined by the spectrophotometrical method with 450 nm wave length (2).

Total isoflavones (daidzein, genistein, glycitein) content in standard and soya chow was estimated by chromatography with HPLC-MS – Shimadzu (Table 1) in the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn.

The data obtained were analysed by calculating mean (M) and standard deviation (SD). The significance of differences between groups was determined on the basis of confidence intervals (NIR), which were calculated from variance analysis (ANOVA). Values of P<0.05 were considered to be statistically significant. The Pearson correlation coefficient was also determined for BMD of the mandible, femur, and vertebra.

Results

Statistical analysis revealed significant differences between BMD values in CL and OV among the mandible, femur and vertebra. Only in mandible BMD of group S differed significantly from that of ovariectomized rats. BMD of other groups taking soya diet and ipriflavone did not differ from ovariectomized and sham-operated groups.

Table 2
Daily rats’ isoflavones intake *

<table>
<thead>
<tr>
<th>Isoflavone</th>
<th>Standard chow</th>
<th>2.5% soya chow</th>
<th>25% soya chow</th>
</tr>
</thead>
<tbody>
<tr>
<td>daidzein (mg)</td>
<td>0.37</td>
<td>0.64</td>
<td>1.8</td>
</tr>
<tr>
<td>genistein (mg)</td>
<td>0.28</td>
<td>0.84</td>
<td>2.6</td>
</tr>
<tr>
<td>glycitein (mg)</td>
<td>0.06</td>
<td>0.27</td>
<td>0.6</td>
</tr>
<tr>
<td>Total (mg)</td>
<td>0.71</td>
<td>1.75</td>
<td>5.0</td>
</tr>
</tbody>
</table>

* values calculated on daily chow consumption. Average consumption was about 20 g of dry chow.

Table 3
BMD (g/cm²) *

<table>
<thead>
<tr>
<th></th>
<th>CL</th>
<th>OV</th>
<th>S_A</th>
<th>S_B</th>
<th>IP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mandible</td>
<td>0.337^a</td>
<td>0.306^b</td>
<td>0.318^ab</td>
<td>0.342^a</td>
<td>0.328^ab</td>
</tr>
<tr>
<td>Femur</td>
<td>0.368^a</td>
<td>0.328^b</td>
<td>0.341^ab</td>
<td>0.355^ab</td>
<td>0.345^ab</td>
</tr>
<tr>
<td>Vertebral</td>
<td>0.288^a</td>
<td>0.267^b</td>
<td>0.277^ab</td>
<td>0.278^ab</td>
<td>0.277^ab</td>
</tr>
</tbody>
</table>

* differences between means in the same row are significant when are not designed by using the same superscript.
The correlations between BMD in the mandible, femur, and vertebra in each group were positive, but only between vertebra and femur BMD in groups CL (r=0.57; P=0.017) and OV (r=0.63; P=0.031) were statistically significant.

Table 4
Mean serum ALP activity (U/L)

<table>
<thead>
<tr>
<th>Group</th>
<th>ALP</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>37.11±10.64</td>
<td>c</td>
</tr>
<tr>
<td>OV</td>
<td>65.85±23.08</td>
<td>abd</td>
</tr>
<tr>
<td>S_A</td>
<td>80.28±6.55</td>
<td>d</td>
</tr>
<tr>
<td>S_B</td>
<td>59.57±15.28</td>
<td>bd</td>
</tr>
<tr>
<td>IP</td>
<td>56.00±14.65</td>
<td>abc</td>
</tr>
</tbody>
</table>

* Differences between means are significant when are not designed by using the same superscript. ±SD.

After ovariectomy serum ALP activity significantly increased in comparison with control rats without ovariectomy. Values of ALP in S_A and S_B groups were significantly higher than in CL. Although insignificantly, ALP level had also increased in IP group. Consumption of isoflavones and ipriflavone did not influence serum ALP activity in comparison with OV – control group.

Discussion

Hormone replacement therapy (HRT) is the main method of the treatment and prevention in menopause. The lack of endogenous hormones in menopause is manifested, among other symptoms, as a cardiovascular disorders or osteoporosis. For some reason long supplementation of hormones may be inadvisable regarding oestrogen proliferation boost in the uterus and breast.

Phytooestrogens are thought to have oestrogen activity. Their structural similarity to steroid oestrogens conduces to their ability to bind to ER (7). Arjmandi et al. (1) demonstrated beneficial effect of isoflavones on bones.

Results of this study show that soya isoflavones have moderate influence on the examined bone parameters. Significant decrease in BMD in the bones after ovariectomy was not terminated after supplementation of natural isoflavones and ipriflavone. Only in one case, in group taking high doses of soya isoflavones in chow, significant increase in mandible BMD was observed comparing with ovariectomized control. The mean intake of soya isoflavones from diet was only approximated, our findings about its beneficial impact on bone quality are uncertain. However, in group S_B in spite of ovariectomy mandible BMD increased and was significantly higher than in ovariectomized rats (Table 3) but in other groups (S_A and IP) there was no improvement in BMD level neither in the mandible nor femur or vertebra.

Draper et al. (5) in their study revealed that coumestrol and a-zearalanol prevent or reduce oophorectomy-induced bone loss in skeletally mature rats, but oral isoflavone phytooestrogens had no effect on oophorectomized rats including bone loss at the dose used, probably due to lack of effective absorption. It remains in agreement with our findings (femur and vertebra), nevertheless in their experiment each rat received 131.25 mg dose of active phytooestrogens per week. In our study, group taking high doses of soya isoflavones received only about 35 mg of isoflavones per week. Potter et al. (9) in their clinical study found that daily dose of 90 mg of isoflavones in diet protected against spinal bone loss in women after 6 month treatment. The dose of 55.6 mg did not prevent from bone loss.

Low effect of ipriflavone on BMD level and activity of ALP may be explained by its low affinity to the oestrogen receptors (ER α and β) (6). ERβ mRNA is highly expressed in osteoblasts in rat bone suggesting that there is a distinct mechanism of oestrogen action mediated by ERβ in bone (8). Ipriflavone has weak affinity to ERα and ERβ, genistein has six fold greater affinity to ERβ than to ERα (2). That fact may be pivotal in account of mandible BMD improvement in group S_B.

In the literature, there are few studies about impact of isoflavones intake on bone of the mandible. We tried to find out the differences in BMD between particular bones after ovariectomy and supplementation of isoflavones. In our experiment only the mandible was prevented from bone loss after supplementation of higher doses of isoflavones in diet. In the femur and vertebra we noticed significant bone loss (BMD) without improvement after supplementation of isoflavones in diet.

Serum ALP activity increased after ovariectomy, but soya or ipriflavone intake remained without significant consequence on the enzyme. Nevertheless, in Deyhim et al. (4) study serum ALP was not affected either by ovariectomy or isoflavones diet. Impact of ovariectomy, soya diet, and ipriflavone intake on serum ALP levels was comparable with variability of femur and vertebra BMD observed in the examined groups.

Statistical analysis of correlation revealed significant positive correlation only between BMD of the vertebra and femur in CL group (sham-operated). Among other groups there were also positive correlations, but statistically insignificant. An interesting fact is that in each bone after ovariectomy significant decrease in BMD was noticed, but only in mandible BMD was recovered in the group taking high doses of isoflavones in diet.

Although the differences among examined bones are noticeable, prospective studies have to be performed to explain and confirm or abrogate the hypothesis of specific bone metabolism due to its
placement and function. In animals, the mandible as a part of jaws is being loaded in the act of chewing, but in human also during speaking. Its activity as a part of the body may have influence on different, probably enhanced metabolism.

Acknowledgments: This study was supported by KBN (State Committee of Scientific Research) grant No. 3 PO5E 078 23.

References