Effects of hPTH (1-34) and Gosha-jinki-gan on the Trabecular Bone Microarchitecture in Ovariectomized Rat Tibia

By

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Summary: Although human parathyroid hormone (1-34) [hPTH (1-34)] was reported to improve osteoporotic bone loss, little is known about the anti-osteoporotic effect of the traditional Chinese medicine, Gosha-jinki-gan (GJG). The purpose of this present study was to clarify and compare the effects of hPTH (1-34) and GJG on trabecular bone microarchitecture in ovariectomized (OVX) rat tibia by using microcomputed tomography (micro-CT). Thirty 12-week-old Sprague-Dawley female rats were underwent ovariectomy (OVX) or sham operation. Four weeks later, the ovariectomized rats were further divided into OVX, OVX + PTH, and OVX + GJG groups. hPTH (1-34) was administered subcutaneously at a dose of 20 μg/kg, 3 times/week, and OVX + GJG group received 0.05% aqueous solution of GJG as the only drinking fluid for 8 weeks respectively. The three-dimensional (3D) trabecular microarchitecture of the bone in the proximal tibial metaphysis was evaluated by micro-CT. In the OVX + PTH group, trabecular bone volume (BV/TV), number (Tb.N) and thickness (Tb.Th) were significantly increased, structure model index (SMI) and trabecular bone pattern factor (TBPf) decreased when compared with the OVX group. In comparison to the OVX group, BV/TV and Tb.N were significantly greater, while SMI and TBPf had no marked changes in the OVX + GJG group. These results suggest that the administration of hPTH (1-34) restore the trabecular bone volume and improve the microstructural property as well, while GJG reduce the bone loss without affecting its microstructural property in ovariectomized rats.

Osteoporosis is a disease characterized by a progressive loss of bone mass, particularly in trabecular bone, and deterioration of the bone microarchitecture, which lead to bone fragility and increased susceptibility to fractures¹–³. Osteoporosis is roughly classified into three types. Type I, known as postmenopausal osteoporosis, occurs in women after menopause. Type II, senile osteoporosis, is related to the aging process. Type III, secondary osteoporosis, involves either disease process or drug-related effects that cause the development of osteoporosis. The primary cause of postmenopausal osteoporosis is an estrogen deficiency resulting in the decrease in bone mass⁴.

A number of medications have been reported to be effective for curing osteoporosis based upon the results obtained from animal studies. Currently, several agents such as estrogen and selective estrogen receptor modulators (SERMs), calcitonin (CT), bisphosphonates and parathyroid hormone (PTH) are clinically employed to prevent or treat postmenopausal osteoporosis. Traditional Chinese medicine, characterized as fewer side effects and suitability for long-term administration, has recently been reevaluated in the clinical field⁵. Gosha-jinki-gan (GJG), a traditional herbal combined prescription, has been described as being useful in the treatment of many subjective symptoms, including lumbago, cold in the extremities, leg numbness and pain, copious urine with thirst⁶. GJG is considered to be a useful approach for the improvement of subjective symptoms such as lumbago⁷, sensation of cold and pain in the legs associated with osteoporosis⁸. However, there have been few studies on the effect of GJG on the three-dimensional (3D) trabecular bone microarchitecture in osteoporosis.

The present study was undertaken firstly to evaluate and compare the effects of hPTH (1-34) and GJG on the 3D trabecular microarchitecture in
osteoporosis. We examined whether the herbal combined prescription could inhibit the progression of bone loss and the deterioration of the trabecular bone microarchitecture induced by ovariectomy in rats.

Materials and Methods

Traditional Chinese medicine and chemical

Dried extract powder of traditional Chinese medicine, GJG, was supplied by Tsumura & Company (Tokyo, Japan). The herbal constituents and contents are shown in Table 1. The parathyroid hormone (Human, 1-34) [hPTH (1-34)] was purchased from Peptide Institute, Inc. (Osaka, Japan).

Animals and treatments

The experimental animals were female Sprague-Dawley rats (SLC Japan, INC., Shizuoka, Japan). They were approximately 90 days of age and weighed average 249 g at the beginning of the study. They were maintained at the Life Science Research Center of Gifu University Graduate School of Medicine and had free access to water and commercial diet (CE-2, CLEA Japan) throughout the experiment. All animal experiments were undertaken in accordance with the guidelines for care and use of laboratory animals, Gifu University Graduate School of Medicine.

Under anesthesia, eight rats were subjected to sham operation and twenty two rats were performed bilateral O VX using a dorsal approach. Four additional weeks were allowed to pass before initiation of treatment to permit significant bone loss to occur in the O VX animals. The group of sham-operated control rats (n = 8) and the first group of O VX rats (n = 6) took tap water ad libitum and were kept untreated. The second group of O VX rats (n = 8) received 0.05% aqueous solution of GJG as the only drinking fluid for eight weeks. The daily fluid intake averaged 40 ml. The remaining group of O VX rats (n = 8) were subjected to hPTH (1-34) consisting of subcutaneous injections 3 days/week at a dose of 20 μg/kg body weight.

Measurement of the 3D microarchitecture of the tibia

All rats were sacrificed after eight weeks treatment. At necropsy, the success of ovariectomy was confirmed by failure to detect ovarian tissue and by observation of marked atrophy of the uterine horns. The left tibia from each animal was sampled and cleaned off soft tissue. The proximal metaphysis of the tibiae was then scanned by Micro-CT system (MCT-CB100MF; Hitachi Medical Corporation, Tokyo, Japan) for 512 slices with 15 μm slice thickness at a tube voltage of 50 kV and tube current of 100 μA.

After scanning, image data was transferred to a workstation, and structural indices were calculated using a 3D image analysis system (TRI/3D-BON; Ratoc System Engineering Co. Ltd., Tokyo, Japan). The volume of interest was defined as the 100 slices with 1 mm away below the most distal growth plate (Fig. 1). The gray-scale images were segmented using a median filter to remove noise and a fixed threshold to extract the mineralized bone phase. The isolated small particles in marrow space were removed. Subsequently, cortical and trabecular bone were separated and the structural indices were calculated. Trabecular bone volume (BV; mm³) was calculated using tetrahedrons corresponding to the enclosed volume of the triangulated surface. Total tissue volume (TV; mm³) was the volume of the whole sample examined. The trabecular bone volume fraction (BV/TV; %) was then calculated from these values. Trabecular thickness (Tb.Th; μm) was determined according to the method described by Hildebrand et al.12. Trabeular number (Tb.N; /mm) and trabecular separation (Tb.Spac; μm) were estimated based on the plate model13. In addition to the computation of metric indices, values of nonmetric indices were calculated to describe the 3D nature of the trabecular bone sample. The SMI14, which represents the ratio of trabeculae with a platelike structure to those with a rodlike structure, is a parameter of trabecular morphology. All trabeculae have a platelike structure when the SMI is zero, and all have a rodlike structure when the SMI is three. TBPI15 is the ratio of convex structures to concave structures on the trabecular surface, and the connectivity of trabeculae is considered to be greater when TBPI is smaller.

Table 1. Composition of Gosha-jinki-gan

<table>
<thead>
<tr>
<th>Component</th>
<th>% (w/w)</th>
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</thead>
<tbody>
<tr>
<td>Rehmanniae radix</td>
<td>17.9</td>
</tr>
<tr>
<td>Achyranthis radix</td>
<td>10.7</td>
</tr>
<tr>
<td>Corni fructus</td>
<td>10.7</td>
</tr>
<tr>
<td>Dioscoreae rhizoma</td>
<td>10.7</td>
</tr>
<tr>
<td>Plantaginis semen</td>
<td>10.7</td>
</tr>
<tr>
<td>Alismatis rhizoma</td>
<td>10.7</td>
</tr>
<tr>
<td>Hoelen</td>
<td>10.7</td>
</tr>
<tr>
<td>Moutan cortex</td>
<td>10.7</td>
</tr>
<tr>
<td>Cinnamomi cortex</td>
<td>3.6</td>
</tr>
<tr>
<td>Aconiti tuber</td>
<td>3.6</td>
</tr>
</tbody>
</table>
Statistical Analysis

All data were presented as mean ± SEM. Statistical analysis was done using StatView J-4.5 (Abacus Concepts). Significance of the results was determined by one-way analysis of variance (ANOVA) and Fisher’s PLSD test. A p value < 0.05 was considered statistically significant.

Results

Body weight changes

As shown in Table 2, the rats started with similar mean body weights. OVX significantly increased weight gain of rats by 15% (p < 0.05 vs. sham) at three months after surgery despite the fact that all the animal groups were pair-fed. GJG did not alter weight gain in OVX rats, while hPTH (1-34) treatment reduced the weight gain in OVX rats (p < 0.05 vs. ovx). These results indicated that GJG did not behave as hPTH (1-34) in reducing body weight gain in OVX rats.

3D images alterations

The 3D reconstructed images representing each group are shown in Fig. 2. The microarchitecture was deteriorated in each osteopenic model compared to the sham-operated control group. In the OVX untreated group, the platelike structure partially resolved into a rodlike structure, with many of the connecting rods missing, whereas in the OVX+hPTH (1-34) (op) and OVX+GJG (og)
groups this loss of trabecular bone mass and of connectivity was prevented.

Effects on trabecular microarchitecture in the tibiae

Of the tibial trabecular bone indices (showed in Fig. 3), OVX caused a marked deterioration in microarchitecture of the proximal tibiae, manifested as significant decreases in BV/TV, Tb.N, Tb.Th, and increases in SMI, Tb.Spac and TBPf compared to the sham-operated group. hPTH (1-34) reversed this microstructural deterioration significantly in comparison with the OVX group in BV/TV, Tb.N, Tb.Th and also in Tb.Spac, TBPf, SMI. And in the og group, GJG increased BV/TV by 51.4% and Tb.N by 53.8%, while decreased Tb.Spac by 37.1% compared to the OVX control group. In addition, BV/TV, Tb.Th, SMI, and TBPf were significantly different between the OVX+hPTH (1-34) and OVX+GJG groups. The trabecular bone loss and microstructural deterioration highly inhibited in the hPTH (1-34)-treated group, while GJG reduced the loss of trabecular bone without improving its microstructural property in ovariectomized rats.

Discussion

OVX rat model, which artificially produces the depleted state of estrogen, has been widely used for the study of postmenopausal osteoporosis because of the similarities in the way that trabecular bone is remodeled, although there are some differences in the effects of estrogen depletion on the skeletons.
between humans and rats\textsuperscript{16}. It appears that OVX rat may be a useful model for evaluating the effects of various compounds in the prevention and treatment of osteoporosis\textsuperscript{17}, which is why we have adopted this model.

To our knowledge, this is the first study to assess the influence of GJG on 3D trabecular bone microarchitecture by micro-CT. The salient findings of this study are as follows. First, both hPTH (1-34) and GJG administrations for two months reduce

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**Fig. 3.** Effects of hPTH (1-34) and Gosha-jinki-gan on the trabecular bone microarchitecture in ovariectomized rat tibia. The changes in metric indices (BV/TV, Tb.N, Tb.Th, Tb.Spac) and nonmetric indices (SMI, TBPl) are shown. Data values are expressed as mean ± SEM.

*sham*, sham-operated group; *ovx*, ovariectomized group; *op*, ovx + PTH group; *og*, ovx + GJG group.

See text for abbreviations.
the loss of trabecular bone and decrease the trabecular separation in the proximal tibial metaphysis of ovariectomized rats. Second, hPTH (1-34) changes the surface curvature of the trabecular bone therefore increases the trabecular connectivity and improves its microstructural property compared to GJG.

Gosha-jinki-gan, one of the representatives for compensating the function of the kidney, has been used since ancient times to treat melosalgia, low back pain and numbness\textsuperscript{18}. In Chinese medicine, kidney refers to the urinary and reproductive organs, a part of endocrine and nervous systems in modern medicine. Moreover, kidney has the ability to regulate the function of bones. Theoretically, Gosha-jinki-gan can be used for preventing and treating bone diseases. Ito et al. found that the administration of Gosha-jinki-gan for six months effectively prevented the bone loss in patient with osteoporosis\textsuperscript{19}. As shown in Table 1, GJG is composed of 10 herbal medicines in fixed proportions. Among its components, Achyranthis radix was reported to have inhibitory activity on bone resorption stimulated by parathyroid hormone in bone organ culture\textsuperscript{20}. In addition, ovariectomized rats treated with Achyranthis radix had significantly greater bone mineral density than untreated animals\textsuperscript{21}. However, the actions of the other GJG components on bone remain unclear. Therefore, the action of Gosha-jinki-gan in reducing the bone loss is considered to the activity of Achyranthis radix.

The present study demonstrated that intermittent exposure of PTH for eight weeks in ovariectomized rats restored the bone loss, which agreed with the previous studies\textsuperscript{22–24}. The native hormone synthesized and excreted by the parathyroid gland chief cell is hPTH (1-84), a single-chain polypeptide with 84 amino acids. The first 34 amino acids are the biologically active moiety of mineral homeostasis\textsuperscript{41}. Considering PTH therapy, Forteo (Teriparatide) which is the recombinant human 1-34 amino acid sequence of parathyroid hormone, has been approved in the USA for the treatment of men and postmenopausal women at high risk for osteoporotic fracture and, in Europe for the treatment of postmenopausal women with osteoporosis\textsuperscript{25}. The overall effect of PTH is to raise plasma Ca level and therefore regulates bone metabolism. On a cellular level, PTH enhances the recruitment of preosteoblasts from marrow stromal cells and induces the maturation of lining osteoblasts\textsuperscript{26}. Recently, evidence has emerged that PTH reduces osteoblastic apoptosis, prolonging osteoblast survival and possibly improving its differentiated function in collagen synthesis\textsuperscript{23,27}.

Micro-CT provided satisfactory noninvasive assessment of consecutive slice images corresponding reasonably well to the histological sections\textsuperscript{27}. A 3D visual evaluation of the provided volume revealed the trabecular microarchitecture in detail, allowing for a better understanding of the morphometric changes in the trabecular bone. The parametric analyses then quantitatively presented the microarchitectural changes, with the quantitative results being in agreement with the rendered images.

In conclusion, our initial study provides certain evidence that intermittent administration of hPTH (1-34) was notably effective in inhibiting the trabecular bone loss and the microstructural deterioration as well, while the traditional Chinese medicine, Gosha-jinki-gan reduced the loss of trabecular bone without improving its microstructural property in ovariectomized rats. And the 3D metric and non-metric indices seem to offer useful information to evaluate trabecular bone microarchitecture.

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References


