β-Blocker and Other Analogous Treatments that Affect Bone Mass and Sympathetic Nerve Activity in Ovariectomized Rats

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Abstract: We investigated whether treatments with beta-blockers or other administrations that have similar actions to β-blockers, such as Chinese herbs or needling, were effective in treating osteoporosis induced by ovariectomy (OVX). Female Wister rats were divided into five groups: a sham-operated control group treated with vehicle (Sham, n = 8), an ovariectomized (OVX) group treated with vehicle (Model, n = 8), an OVX group administered with propranolol (Pro, n = 10), an OVX group administered an ethanol extract of Fructus Citri Sarcodactylis (Fcs, n = 9), and an OVX punctured at Sanyinjiao (SP-6) and Neiguan (PC-6) (Needling, n = 8). The treatment started when rats were 12 weeks old and continued for 24 weeks. Serum osteocalcin and urinary deoxypyridinoline (Dpd) levels were upregulated in rats in response to OVX, together with a significantly decreased BMD and trabecular bone area. The Pro, Fcs and Needling treatment improved the decreased BMD and the trabecular area, increased the trabecular number, lowered the trabecular separation to some extent as well as significantly depressed the urinary Dpd levels (p < 0.05). The bone formation markers, such as the mineralizing surface, mineral apposition rate and bone formation rate were not significantly changed, along with a slightly higher trend of osteocalcin levels when compared with the Model rats. The slower heart rate and lower plasma NE levels in these therapeutic groups were also found. Our results suggested that propranolol, Fcs and needling on Sanyinjiao (SP-6) and Neiguan (PC-6) may improve the bone mass of OVX rats, and it provides an alternative and potential therapy for the prevention of postmenopausal osteoporosis.

Keywords: Osteoporosis; β-blocker; Ovariectomy; Bone.
Introduction

Osteoporosis is accepted as one of the most important chronic diseases with regard to death, and its function and social cost (Riggs and Melton, 1995). Besides the cessation of estrogen production by the ovaries resulting in bone loss, other theories have also been suggested in recent years. Some studies have suggested that the sympathetic nervous system has a catabolic effect on bones (Cherruau et al., 1999; Togari, 2002). In vitro data showed that adrenergic agonists stimulate bone resorption in organ culture of mouse calvaria (Moore et al., 1993). Takeda et al. (2002) further demonstrated that the systemic administration of β-agonists resulted in decreased bone formation and bone loss in mice, whereas the administration of propranolol, a nonselective β-blocker, had the opposite effects. Additionally, other studies suggested that the current use of β-blockers is associated with a reduced risk of fractures and may overcome the loss of bone mass in postmenopausal women (Cock and Auwerx, 2003; Schlienger et al., 2004). These findings led to the suggestion that β-blockers may be a potential therapy for osteoporosis.

For many decades, traditional Chinese treatments, such as herbs and acupuncture, have been studied and used to treat bone diseases (Sakamoto et al., 2000; Zhang et al., 2006), but most of them have demonstrated acting via the estrogen pathway. In order to examine the links of β-adrenergic antagonists and bone mass, oral administrations of propranolol (Pro) and ethanolic extract of Fructus Citri Sarcodactylis (Fcs), as well as needling on the points of Sanyinjiao (SP-6) and Neiguan (PC-6) were adopted in our experiment. Propranolol, a β-adrenergic antagonist, is a commonly and widely used drug to control blood pressure and cardiovascular disease with no major deleterious effects. The crude drug of Fcs, is the dried fruit of Citrus Medica sarcodactylis (Chinese name, Foshou), which has been used in traditional Chinese medicine for over 1000 years. Besides having been proved effective in activities of resolving phlegm and relieving cough or asthma (Jin et al., 2002), the ethanol extract of Fcs has also been reported to have central nervous system depressant actions in mice, and steroline (100 µg/kg), the active ingredient of Fcs, may counteract the cardiac excitability induced by isoprenaline in rats. The effects were nearly comparable to propranolol at 150 µg/kg. These results demonstrate that Fcs should be act as a β-blocker (Wang, 1982; Wang et al., 1997). No further study regarding similar central depressant actions with propranolol up to date stimulated us to explore the role of Fcs in the control of bone mineral metabolism or bone turnover in osteoporotic rats from the point of the sympathetic nervous system. We previously reported that needling the Zusanli (ST-36) and Sanyinjiao (SP-6) points had preventive and therapeutical effects to the ovariectomized osteoporotic rats. This may be through its effects on strengthening the mobility and secretion of the gastrointestinal tract (Zhang et al., 2004). In this study, we chose the pair of Sanyinjiao (SP-6) and Neiguan (PC-6) based on its common use and effective actions in clinic treating mental diseases and general disturbances like menopausal syndrome, insomnia or manic-depression. Thus, the purpose of this study was to see whether the prescription has profitable effects on the bone in osteoporotic rats by regulating the tone of the sympathetic nervous system.
Materials and Methods

Animals and Experimental Protocol

Forty-three 7-week-old virgin female Sprague-Dawley rats weighing 220 ± 5 g were obtained from Japan SLC, Inc. The animals were housed at a room temperature of 22 ± 2°C and a humidity of 55 ± 10% with a 12-hour light/dark cycle in metallic cages.

The animals were assigned into five groups according to the bone mineral density (BMD) averages of their 4th, 5th and 6th lumbar vertebrae: 1) Sham (n = 8); 2) Model (n = 8); 3) Propranolol (Pro, n = 10); Fructus Citri Sarcodactylis (Fcs, n = 9); and 5) Needling (n = 8). At 11 weeks of age, excluding a sham surgery in the Sham group, a bilateral ovariectomy was performed on the other four groups. Treatment was started one week post-OVX to allow them to recover from operation and continued for 24 weeks. Body weight was weighed weekly and BMD was assessed monthly throughout the experiment. For histomorphometry analysis, animals were injected with tetracycline (20 mg/kg) intraperitoneally 3 days and calcein (8 mg/kg) subcutaneously 10 days before death to label the sites of bone formation. The rats were killed and uterine weights were measured for the confirmation of OVX success. Right tibiae and L5 lumbar vertebrae were dissected and processed for the undecalcified specimens.

Oral Administration and Needling Manipulation

Every Pro rat was orally administered 2.8 mg/day propranolol hydrochloride (Sigma), which was selected from the literature, and converted into a daily dosage for rats (Takeda et al., 2002). Fcs (0.9 g/kg) was soaked in 100 ml of 80% alcohol for 48 hours and heated at a temperature of 60°C for one hour. The residue was recovered and heated for a second time. The merged filtrate was dealcoholized by a rotary vacuum evaporator (SE-100N, Shimadzu) at 60°C in a water bath. The alcohol extract was dissolved in 3 ml distilled water and orally administered to rats. Needling was performed on Sanyinjiao (SP-6) and Neiguan (PC-6) points for 15 min using short needles with a diameter of 0.22 mm and a length of 10 mm, manipulating in a balanced reinforcing and reducing manner at a frequency of about 2 Hz (120 turns/min). The principle of choosing points followed the standard for rats recorded in the teaching material on experimental acupuncturology (Lin and Wang, 1999). The Neiguan (PC-6) and Sanyinjiao (SP-6) are located on the palmar side of the forearm, 3 mm superior to the transverse crease of the wrist, and 10 mm directly superior to the tip of the medial malleolus on the hind leg, respectively. In order to contrast with the oral administration groups, an equal amount of distilled water was given orally to all the Sham, Model and Needling rats. All the treatments were done five days a week from Monday to Friday.

Measurement of BMD, Blood Pressure and Heart Rate

BMD was assessed monthly throughout the study by dual energy X-ray absorptiometry (DEXA) with an apparatus for small animals (DCS-600 Type Aloka, Osaka). Analysis was
carried out with a box method for the lumbar spines and a random method for the femora and tibiae. Averages of the L-4, L-5 and L-6 lumbar vertebrae and femora or tibiae on both sides were calculated.

Arterial blood pressure (BP) and heart rate (HR) were determined using a tail cuff system. The rats were lightly supported in a holder made of cloth mesh and maintained at 37 ± 1°C (Model THC-1 Digital Thermo, Softron, Tokyo). The BP from the tail artery was indirectly measured using a tail-cuff apparatus (BP-98, Softron), which was controlled with a personal computer. Values are presented as the average of three separate measurements.

Chemical Metabolism

Urine was collected in a metabolic cage and fasting blood was collected from the ophthalmic veins at 36w immediately before sacrifice. The samples were stored at −80°C for the urine free deoxypyridinoline (Dpd) and plasma noepinephrine (NE) assay at the Shionogi biomedical laboratory. Serum osteocalcine (OC), a bone formation marker, was measured using a Rat Gla-OC Competitive EIA Kit (Takara).

Bone Histomorphometry

The right tibiae and L-5 lumbar vertebrae were fixed in 70% ethanol, dehydrated in graded alcohols, and embedded undecalcified in methyl methacrylate at low temperature, as previously described (Wronski and Yen, 1991). Ten-micrometer-thick midsagittal sections were cut with a saw microdome (SP 1600 LEica, GmbH, Nussloch) on vertebrae. The measurements of the trabecular bone volume (BV/TV) were expressed as a percentage of the total volume. Trabecular bone thickness (Tb.Th), trabecular bone number (Tb.N), and trabecular separation (Tb.Sp) of the secondary spongiosa at distances greater than 0.5 mm from the cranial and caudal growth plates were all carried out on lumbar sections with a semiautomatic image analysis system (Image plus Ver. 4.0, Planetron) (Iwaniec et al., 2002). The tibial cross-sections were cut at the site of the proximal tibiofibular joint and used to observe the cortical bone formation. The total cortical area, percent of marrow cavity area, bone surface (BS), single-labeled perimeter, double-labeled perimeter, and double-labeled width were measured, and then the indexes of the percent of the cortical area (%Ct.Ar), mineralizing surface (MS/BS), mineral apposition rate (MAR) and bone formation rate (BFR/BS) were calculated (Sato et al., 1997).

Statistical Analysis

All results are expressed as the mean ± SD for each group. Statistical differences among multiple groups were evaluated using the Tukey test. p < 0.05 were considered to be significant.
EFFECTS OF β-BLOCKERS ON OSTEOPOROTIC OVX RATS

Results

Body Weight

Weight gain was observed in all the rats during the course of the study, OVX induced a significantly higher body weight compared to the sham group from 14 weeks of age (p < 0.05), the Pro, Fcs and Needling treatment did not change body weight significantly, the Pro rats showed a lower tendency among the OVX rats (Fig. 1).

Bone Mineral Density (BMD) of Lumbar Vertebrae, Tibiae and Femora

Until the 9th week of treatment, the rats were almost at the same level on the lumbar vertebrae. From 16th weeks, the lumbar BMD of the OVX model rats appeared to be significantly lower than the sham group (p < 0.05); however, the Pro, Fcs and Needling groups kept rising gradually from the 20th week, which were 8.2~12.6% higher than the model rats at 32w and 36w, respectively, and showed no difference from the sham rats (Fig. 2A). For the tibiae and femora, the Model rats showed a significantly similar decrease as lumbar vertebrae from 16th weeks. From the 24th week, the BMD in the Fcs and Needling groups tended to be 3.7~9.3% higher than the Model rats, and showed no difference from the Sham group; however, the Pro group was almost at the same level as the Model rats in the tibial and femoral BMD (Figs. 2B, 2C).

Effects on the BP, HR, Plasma Concentrations of NE

The systolic blood pressure (SBP), diastolic blood pressure (DBP) and the mean blood pressure (MBP) showed no differences among the five groups through the OVX-operation or treatments (Table 1, SBP and DBP data not shown). In contrast from the BP, the Pro,
Figure 2. Changes in the BMD of lumbar vertebrae, tibiae and femora. Significant difference (p < 0.05) from the Model, Pro, Fcs and Needling rats are depicted as “+”, “a”, “b”, “c”, respectively.

Table 1. Effects of Treatments on Mean Blood Pressure (MBP, mmHg)

<table>
<thead>
<tr>
<th>Groups</th>
<th>16w</th>
<th>20w</th>
<th>24w</th>
<th>28w</th>
<th>32w</th>
<th>36w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>98.7 ± 9</td>
<td>103 ± 8</td>
<td>102 ± 4</td>
<td>96 ± 10</td>
<td>96 ± 4</td>
<td>99 ± 3</td>
</tr>
<tr>
<td>Model</td>
<td>102 ± 7</td>
<td>99 ± 6</td>
<td>97 ± 6</td>
<td>95 ± 5</td>
<td>99 ± 6</td>
<td>103 ± 7</td>
</tr>
<tr>
<td>Pro</td>
<td>97 ± 5</td>
<td>97 ± 8</td>
<td>99 ± 7</td>
<td>95 ± 9</td>
<td>98 ± 7</td>
<td>96 ± 7</td>
</tr>
<tr>
<td>Fcs</td>
<td>94 ± 5</td>
<td>99 ± 6</td>
<td>100 ± 8</td>
<td>91 ± 4</td>
<td>95 ± 4</td>
<td>97 ± 5</td>
</tr>
<tr>
<td>Needling</td>
<td>97 ± 8</td>
<td>102 ± 5</td>
<td>103 ± 5</td>
<td>98 ± 6</td>
<td>101 ± 5</td>
<td>98 ± 5</td>
</tr>
</tbody>
</table>
EFFECTS OF β-BLOCKERS ON OSTEOPOROTIC OVX RATS

Fcs and Needling groups almost showed a significant decrease from the sham and model groups in heart rate from the 17th week, though it had some variations with the lapse of time. A significant difference between the Sham and Model rats was not found (Table 2).

Norepinephrine (NE), acts as both a hormone and a neurotransmitter, is secreted from the adrenal medulla and the nerve endings of the sympathetic nervous system and released into the bloodstream to cause vasoconstriction and increase in heart rate, blood pressure and blood glucose concentration. The concentration of NE in the model rats showed a significant rise when compared to the Pro, Fcs and Needling groups, but it did not differ from the sham rats. These results paralleled to our findings in HR (Fig. 3).

**Table 2. Effects of Treatments on Heart Rate (HR, b/m)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>16w</th>
<th>20w</th>
<th>24w</th>
<th>28w</th>
<th>32w</th>
<th>36w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>316 ± 24</td>
<td>340 ± 28</td>
<td>336 ± 24</td>
<td>330 ± 20</td>
<td>364 ± 23</td>
<td>345 ± 16</td>
</tr>
<tr>
<td>Model</td>
<td>327 ± 20</td>
<td>324 ± 27</td>
<td>321 ± 21</td>
<td>323 ± 28</td>
<td>356 ± 21</td>
<td>361 ± 23</td>
</tr>
<tr>
<td>Pro</td>
<td>297 ± 20</td>
<td>280 ± 25*</td>
<td>292 ± 25*</td>
<td>278 ± 23*</td>
<td>294 ± 19*</td>
<td>293 ± 17*</td>
</tr>
<tr>
<td>Fcs</td>
<td>309 ± 19</td>
<td>292 ± 30*</td>
<td>303 ± 18</td>
<td>279 ± 16*</td>
<td>289 ± 14*</td>
<td>295 ± 26*</td>
</tr>
<tr>
<td>Needling</td>
<td>313 ± 22</td>
<td>307 ± 23</td>
<td>311 ± 29</td>
<td>286 ± 16*</td>
<td>293 ± 8*</td>
<td>298 ± 15*</td>
</tr>
</tbody>
</table>

Significant differences between the sham and model rats are depicted as “*” and “+”, respectively.

Figure 3. Plasma changes of norepinephrine. +: Significantly different from the model rats.

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**Uterine Weights**

OVX caused significant atrophy of the uterus in rats as anticipated, confirming their estrogen deficiency. The uterine weights in the therapeutical groups showed a higher tendency than the control model rats, which were 9%, 18% and 27% higher than the model rats in Pro, Fcs and Needling groups, respectively (the uterus weights were 0.74 ± 0.09 g, 0.11 ± 0.03 g, 0.12 ± 0.01 g, 0.13 ± 0.03 g, 0.15 ± 0.06 g in the Sham, Model, Pro, Fcs and Needling groups, respectively; Sham vs Model, Pro, Fcs and Needling, p < 0.001).
Bone Turnover Markers

Serum osteocalcin (OC) was measured as a specific product of the osteoblast. The urinary elimination of deoxypyridinoline (Dpd), a collagen breakdown product indicative of osteoclast activity, was determined and normalized to creatinine (Cre). OVX induced a significant rise in the urine Dpd and serum OC at 36 weeks of age compared to that in Sham rats, suggesting an increase in bone turnover rate in the OVX rats (Fig. 4). The upregulation of Dpd levels by OVX was significantly depressed by the treatments of Pro, Fcs and Needling (p < 0.05). Although the OC concentration also tended to be depressed by the same treatments, a significant difference was not found. These results indicated that the three treatment groups had suppressing effects on both bone resorption and bone formation, an unbalance of the former prior to the latter seemed to have a primary role in suppressing bone loss after OVX in our study.

Bone Histomorphometry

As shown in Table 3, OVX induced a significant difference on the cancellous bone volume (BV/TV), trabecular number (Tb.N) and trabecular separation (Tb.Sp) compared to that in Sham rats, while these indexes were largely prevented by Pro, Fcs and Needling treatments.
A significantly higher Tb.N than Model rats in the three groups was seen; however, we did not observe any changes in the trabecular thickness (Tb.Th). In the cortical bone on the tibia, the OVX induced a significantly decreased Ct.Ar (%) than that in the sham rats, which contributed to the lower bone mass as well. There were no significant differences between the sham and the OVX model rats on the MS/BS, MAR or BFR/BS, and neither had a significant promoting bone-formation effect seen among the OVX rats. These histological findings confirmed our lumbar and tibial BMD results and indicated that the relatively improved BMD was not strikingly induced by bone formation.

**Discussion**

Up until the present, many animal and human studies have been conducted to estimate the risk of fractures using β-blockers, and different results have been obtained. Takeda et al. (2002) reported that the β-blocker propranolol increased bone formation in ovariectomized female rats. In a large population-based case-control analysis, it was further evidence that the current use of β-blockers is associated with a statistically and significantly decreased risk of fractures in both men and women taken alone as well as in combination with thiazide diuretics (Schlienger et al., 2004). However, prospective data from the Danish Osteoporosis Prevention Study showed 20% lower serum osteocalcin levels in women treated with β-blockers compared to untreated women and suggest that β-blocker use is linked to an increased risk of fracture and no change in bone density (Rejnmark et al., 2004). A study on bone turnover in normal postmenopausal women using a β-blocker showed that bone densities in the lumbar spine and total proximal femur did not change significantly in either group (Reid et al., 2005). Our study showed that the dosage of propranolol we used might improve the decreased lumbar BMD induced by OVX, but surprisingly, similar improvement on the tibiae and femora was not seen, which did not match Takeda’s findings, for he found a significant decrease in both lumbar and tibial BMD. The Fcs and needling treatments were used and expected to have the same effect as propranolol on the bone on account of their similar sympathetic regulating actions, and as anticipated, the two treatments were effective not only to the vertebrae, but also to the tibial and femoral bone loss which developed due to OVX.

**Table 3. Histological Changes of Cancellous and Cortical Bone on Vertebrae and Tibiae**

<table>
<thead>
<tr>
<th>Groups</th>
<th>BV/TV (%)</th>
<th>Tb.Th (µm)</th>
<th>Tb.N (No./mm)</th>
<th>Tb.Sp (µm)</th>
<th>Ct.Ar (%)</th>
<th>MS/BS (%)</th>
<th>MAR (µm/d)</th>
<th>BFR/BS (µm³/µm²/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>40.2 ± 6.1</td>
<td>36.8 ± 6.4</td>
<td>3.7 ± 0.79</td>
<td>331.5 ± 42</td>
<td>11 ± 2.1</td>
<td>57 ± 14.2</td>
<td>1.9 ± 0.3</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>Model</td>
<td>16.5 ± 4.1*</td>
<td>32.2 ± 7.1</td>
<td>1.1 ± 0.5*</td>
<td>520.2 ± 32*</td>
<td>6.3 ± 1.8*</td>
<td>55.1 ± 8.1</td>
<td>3.2 ± 0.6</td>
<td>2.3 ± 0.6</td>
</tr>
<tr>
<td>Pro</td>
<td>23.4 ± 3.6</td>
<td>31.6 ± 6.2</td>
<td>2.7 ± 0.6*</td>
<td>441 ± 56</td>
<td>8.2 ± 3.5</td>
<td>64.3 ± 7.4</td>
<td>5.3 ± 0.5</td>
<td>3.4 ± 1.5</td>
</tr>
<tr>
<td>Fcs</td>
<td>27.2 ± 2.8*</td>
<td>37 ± 3.6</td>
<td>2.9 ± 0.3*</td>
<td>481.9 ± 24</td>
<td>7.5 ± 1.6</td>
<td>50.5 ± 13.6</td>
<td>3.9 ± 0.3</td>
<td>3.3 ± 0.7</td>
</tr>
<tr>
<td>Needling</td>
<td>25 ± 2.1</td>
<td>34.5 ± 2.9</td>
<td>3.1 ± 0.4*</td>
<td>490.5 ± 29</td>
<td>6.4 ± 2.9</td>
<td>48.2 ± 11.2</td>
<td>3.1 ± 0.9</td>
<td>3.7 ± 0.8</td>
</tr>
</tbody>
</table>

Significant differences between the sham and model rats are depicted as “*” and “+”, respectively.
Lazzarini suggested that estradiol decreases the fat pad weight in part by increasing sympathetic nervous system activity, but not via changes in adipose tissue cytosol estrogen receptors (Lazzarini and Wade, 1991). El-Mas et al. investigated the role of ovarian hormones and sympathetic activity in the modulation of ethanol responses. They found that the reduced hypotensive effect of ethanol in OVX rats was associated with an increase in the sympathetic activity, as indicated by the significant increases in the plasma NE levels (El-Mas and Abdel-Rahman, 2000). Along with the increased bone mass induced by a β-adrenergic antagonist, while the body weight and fat pad weight of the animals remained normal (Takeda et al., 2002), it seems to demonstrate that body weight, estradiol, bone mass and the activity of sympathetic nerves are mutually interrelated at some level, although they are partially responsible for their own actions. Our gross anatomical fat increase in the OVX model on dissection and the significantly increased body weight, as well as the greatly reduced uterine weight in the OVX model rats matched previous results (Li et al., 2002). Estrogen treatment generally suppresses the gain in body weight associated with E2 deficiency and increased uterine weight in OVX rats (Zhang et al., 2006); our findings showed a heavier trend, but not a significant increase in the uterus weight than the model rats. It seems to be inconceivable to conclude that Pro, Fcs and Needling treatments resemble an estrogenic action to the OVX rats. However, the unchanged body weights and increased bone mass due to the treatments suggest that the mechanisms and pathways of the body weight, uterus weight and the bone mass regulation are different. On the other hand, the 23% higher NE concentration than the sham rats also seems to suggest a relatively increased activity of the sympathetic nerves for OVX rats, which matched the previous results as well (El-Mas and Abdel-Rahman, 2000). The HR and plasma NE concentration were decreased significantly by the treatment of Pro, Fcs and Needling. This still seems to suggest central depressant actions on OVX rats. However, the unchanged BP results appear to suggest that it is possible, but not necessary, effect due to the sympathetic hyperactivity.

The mechanism for bone loss is due to a negative imbalance of bone resorption and formation. The significantly higher Dpd and OC levels in the model rats than that in the sham rats are in accordance with other’s findings (Zhang et al., 2006) and suggest that the OVX rat model is characterized by a high bone turnover rate. The significantly decreased BV/TV, Tb.N and increased Tb.Sp findings in the Model rats compared to the sham group also accord with the BMD results described previously. The histological indexes of MS, MAR and BFR, reflecting some dynamic changes of the cortical bone, indicate poor profitable effects on bone formation by treatments. Along with the slightly decreased OC and significantly reduced Dpd levels in the Pro, Fcs and Needling groups, there seems to be evidence that the improved bone mass mainly owes to the function of a bone resorption depression rather than a bone formation. In addition, these preventive effects may be possible through re-establishing bone connectivity by increasing the trabecular number, bridging the marrow space between disconnected trabeculae and advancing the cortical bone area, regardless of the bone trabecular thickness.

Fructus Citri Sarcodactylis is a dried fruit widely used to promote the flow of qi, strengthen the function of the stomach and resolve phlegm. Its actions concerning bone
metabolism have not been studied and reported. Our study is the first to report that the extract of Fcs can inhibit the heart rate, lower the plasma NE level and have a preventive function to the bone loss, which are associated with estrogen deficiency by OVX. In our study, the OVX group developed significant bone loss on the tibiae, femora and vertebrae from 12 weeks of age, and the bone loss was gradually prevented by Pro and Fcs treatments. The possibility that the Fcs extract has almost the same NE concentration and similar actions in reducing the HR as propranolol, but different in the bone mass in our study, might be due to dosage deviation from the propranolol and crude herb, the Fcs ethanolic extraction rate or the experimental period. Therefore, further study will be needed to determine the exact mechanism in the future.

There have been many acupuncture studies on osteoporosis, in which the Sanyinjiao (SP-6) was one of the more frequently chosen points, considering its actions on strengthening the spleen by traditional Chinese medicine, consequently leading an increase in the absorption of dietary calcium (Cai, 2003; Zhang et al., 2005). In our study, we could not eliminate the possibility of this action either. On the other hand, puncturing both the Neiguan (PC-6) and Sanyinjiao (SP-6) has been proved to have generally regulating actions to the tone of the autonomic nervous system through clinical study, such as to treat tachycardia or high blood pressure (Liu and Hyodo, 1997), but its inhibiting sympathetic actions correlating with the bone has not been reported. In our study, the antosteogenic function in suppressing bone loss, as well as its sympathetic hypoactivities in slowing the heart rate and plasma NE level, will provide another alternative to the treatment of autonomic dystonia or osteoporosis.

In summary, the treatment with propranolol, Fcs extract administration and needling points on Sanyinjiao (SP-6) and Neiguan (PC-6), aiming at an inhibition of the sympathetic nervous tone to ovariectomized female rats, has a preventive effect on the loss of bone mass, and would be an alternative therapy for osteoporosis.

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