J. Vet. Sci. (2003), 4(2), 151-154

JOURNAL OF
Veterinary
Science

Studies on the Effects of Biomedicinal Agents on Serum Concentration of Ca^{2+}, P and ALP Activity in Osteoporosis-Induced Rats

Sang-keun Kim, Myung-hun Lee[1] and Man-hee Rhee[2]

College of Veterinary Medicine, Chungnam National University, 220 Gungdong, Yusong-Gu, Daejeon 305-764, Korea
[1]National Veterinary Research and Quarantine Service, Anyang 430-824, Korea
[2]College of Veterinary Medicine, Kyungpook National University, Daegu 702-701, Korea

Received April 3, 2003 / Accepted July 9, 2003

Abstract

The present study was carried out to investigate the effects of biomedicinal agents on Ca^{2+}, P and alkaline phosphatase (ALP) levels in ovariectomized rats. Rats were ovariectomized bilaterally and were fed up with Ca^{2+} and P-free diet during 8~10 weeks to induce osteoporosis. Osteoporosis was determined by the extent of bone density and by lowering the concentrations of serum Ca^{2+}, P and ALP activity every week. Rats in antler, safflower, ipriflavon, or coadminisrated with estrogen groups were administrated with feed supplement for 5 weeks to elucidate the protective and therapeutic effects against osteoporosis. The bone tissue was examined with electron microscope to determine the effects of each treatment on osteoporosis.

1. The levels of serum Ca^{2+} and P in osteoporosis-induced rats, administrated with antler, ipriflavon and estrogen groups, were little higher than those of control rats. However, the levels of serum Ca and P in ovariectomized rats were significantly higher than those of control group ($p<0.05$).

2. The activities of serum ALP in osteoporosis-induced rats, administrated with antler extract, safflower, ipriflavon, or co-admistrated with estrogen, were little increased in comparing with those of control group, but were significantly decreased in with combination of estrogen for 5 weeks. However, The connections were interrupted and the bone matrix was destroyed in the osteoporosis-induced rats.

3. The inter-trabecular connections were examined under electron microscope. The connections were well maintained and bone loss was without in the administration with antler, safflower, and ipriflavon with combination of estrogen for 5 weeks. However, The connections were interrupted and the bone matrix was destroyed in the osteoporosis-induced rats.

Key words: osteoporosis-induced rats, Ca^{2+}, P, ALP levels

Introduction

Osteoporosis is known to be the metabolic disease of bone and the increased sensitivity of bone fracture, compared with normal human being. The decrease of bone density is a major risk factor leading to osteoporosis [1, 6]. Osteoporosis occurs inherently in cats and dogs (e.g., Dachshund), but sometimes it occurs by non-specific causes, such as aging, non-use, and nutritional imbalance [4, 9].

Ohta *et al* [12] have been reported that in osteoporosis the levels of Ca and P was increased. It is conceivable that this increases is due to compensatory refilling of calcium from bone because of the decrease of the secretion of estrogen, the increase of calcium excretion in urine, and the decrease of intestinal absorption of calcium, and finally osteoporosis occurs. There is the increase of bone remodelling and bone loss due to imbalance of absorption and formation of bone in the menopausal osteoporosis. Therefore, ovariectomized and menopausal state lead to bone loss such as decrease of the thickness of trabecular bone and perforation of bone, and lead to fracture [2, 9].

It has been reported that antler-extract has some therapeutic effects such as growth-promoting of white mice, lowering the cholesterol levels, and improving promotion of hepatic function. In addition, antler-extract is known to promotes the antibody production, hematopoiesis of bone marrow, and the phagocytosis and immunity of reticuloendotherial system. Moreover, antler-extract appears to have anti-stress and anti-aging effects, and ameliorate the movement related-osteoporosis [3, 5, 7, 13, 14]. Medical treatments of osteoporosis are divided into the prevention of bone resorption to which estrogen, calcitonin biphosphate, calcium and vitamin D derivatives theraphies belong, and the bone formations to

* Corresponding author: Sang-keun Kim
Dept. of Vet. Med., Chungnam National University, Daejeon 305-764, Korea
Tel: +82-42-821-6754, Fax: +82-42-821-6754
E.-mail: kskkim@cnu.ac.kr

which sodium fluride and PTH theraphies belong.

In present study, we investigated the serum concentrations of Ca^{2+}, P, ALP and histological, electron microscopic observations in ovariectomized rats, which were administrated with antler, safflower, and ipriflavon or combinational administration with estrogen for 5 weeks.

Materials and Methods

Animals

8~10 weeks-old female 120 Sprague Dawley rats (170±20 g) were used. Animals were acclimatized for 2 weeks before use, and had free access to feed and water. Experimental groups were allocated to control group, ovariectomized group, antler group, safflower group, ipriflavon group, antler + estrogene (E) safflower + E and ipriflavon + E groups. The rats were supplied with Ca^{2+} and P-deficient diet (Table 1) for 8~10 weeks after ovariectomy, and then they were administrated with the respective antler, safflower, ipriflavon, antler + E safflower + E and ipriflavon + E groups.

Table 1. Composition of experimental diets(Ca^{2+} and P-deficient diets)

Ingredient	Composition (%)
Crude protein	22.5 Min
Crude fat	3.5
Ca	Not added
P	Not added
Crude fiber	7.0 Max.
Crude Ash	10.0"

Preparation and administration of antler-extracts

The distal one third of each antler of Elk deer (11.25 g), obtained from the National Livestock Research Institute (NLRI), was boiled with 10 times water (v/v) for 4 h, according to the prescription of Oriental Medicine. Thereafter, the antler was extracted, filtrated, and concentrated into a volume of 60 ml. Safflowers, obtained from Korea Oriental Medicine Co. of 60g, were added to 600 ml of 70% ethanol, extracted twice at 75℃ for 3 hr, and precipitated for 30 min, respectively. And resulting supernatant was filtered and concentrated up to 100 ml of volume. Stock solution of ipriflavone (Deovon, Kukje Pharmaceutical Co.), solubilized in the ratio of 200 mg to 10 ml DW, was used for following work. The extracts were administrated PO with stomach tube at the appropriate dose in every other day.

Induction of osteoporosis

The rats were anesthesized with 0.01 ml/g of Avertin solution (Aldrich Co., USA) via i.p. and were incised at one third of midline. Osteoporosis was determined by the extent of bone density and lowering the concentrations of serum Ca^{2+}, P and ALP activity. In addition, osteoporosis was confirmed by electron microscopic observation of bone samples.

Blood collection and serum seperation

The rats were anesthesized with ether. Blood was collected in 1, 2, 3, 4, and 5 weeks of experiments by heart-puncture, collected blood was stored for 30 min at room temperature, and centrifuged with 3,000 rpm for 15 min. The supernatant kept in -20℃ before use.

Measurements of serum Ca^{2+}, P and ALP

The concentrations of serum Ca^{2+} and P were assayed with respective analysis kit (Dry Chemistry Co., Japan). The activity of ALP was with automatic analyser (SM-4000, Biochemical System, Italy).

Statistical analysis

Statistical significances among groups were determined by Duncan's multiple range test with General Linerars Model (GLM) Procedure (SAS ver. 6.12, SAS Institute, 1996).

Results and Discussion

The changes in the concentration of serum Ca^{2+}

The Ca^{2+} levels of serum in antler, safflower, ipriflavone, antler + E safflower + E and ipriflavone + E group showed in Fig. 1. The concentrations of serum Ca^{2+} were 23.24 ± 2.22 ㎍/㎖, 23.45±2.29 ㎍/㎖, 23.20±2.19 ㎍/㎖, 22.12±3.44 ㎍/㎖, 21.65±4.02 ㎍/㎖, 21.22±3.57 ㎍/㎖ for last 5 weeks in osteoporosis-induced rat, which fed with Ca^{2+} and P-free diet for 12 weeks, respectively. The concentrations of serum Ca in all experimental group were higher than those of naive control group (i.e., 20.90±2.45 ㎍/㎖). The recovery effect of treatment on lowering the serum calcium in combinational administration with estrogen is more conspicuous than in single administration (Fig. 1). Especially the co-adminstration of ipriflavon and estrogen have shown the significant decrease of serum Ca^{2+} ($p<0.05$). Those results are simillar with the results of Ohta *et al* [11, 12]. Ohta *et al* [11] have been reported that the concentration of serum Ca^{2+} was increased in the ovariectomy, and it is reported that the concentrations of serum Ca^{2+} and P were increased in the ovariectomy groups. On the other hand, Nyda *et al* [10] have shown that the levels of serum Ca^{2+} was decreased in the ovariectomy. Those results suggest that osteoporosis is induced by the increased bone absorption and Ca^{2+} release from bone, due to the decrease of secretion of estrogen, the increase of excretion of Ca^{2+} in urine and the decrease of intestinal absorption of Ca^{2+} [9, 11, 12].

The changes in the concentration of serum P

Fig. 2 shows the consecutive changes in concentrations of serum P in osteoporosis-induced rats. The concentrations of serum P in antler, safflower, ipriflavone, antler + E., safflower + E and ipriflavon + E groups were 12.92±2.35 ㎍/㎖, 12.41

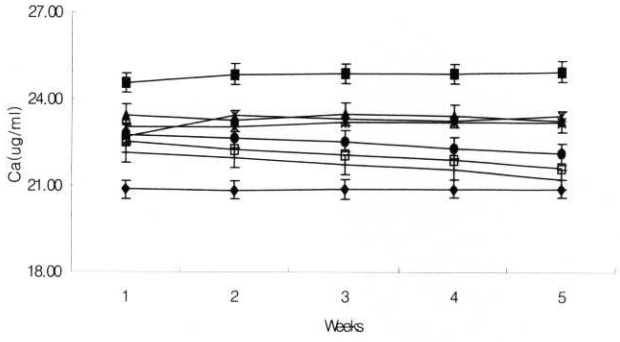

Fig. 1. Effects of antler (▲), safflower (×), ipriflavone (*), antler+E (●), safflower+E (□) and ipriflavone+E (+) on serum Ca^{2+} concentrations in ovariectomized rats.
* Co-adminstration of ipriflavon or safflower and estrogen have shown the significant decrease of serum Ca^{2+} ($p<0.05$).

±2.14 ㎍/㎖, 12.12±2.02 ㎍/㎖, 11.37±2.02 ㎍/㎖, 11.67±1.57 ㎍/㎖, 10.92±1.66 ㎍/㎖, respectively, which is little lower than in osteoporosis-induced group (14.24±1.87 ㎍/㎖). The concentrations of serum P in normal control group were 10.44±1.55 ㎍/㎖, which is much lower than those in other group. Nyda *et al* [10] reported that concentrations of serum P was on the increase in ovariectomy. Whereas Ohta *et al* [11] reported that both concentrations of serum Ca^{2+} and P were on the increase in ovariectomy groups. In our works, the concentration of serum P in ovariectomized group was significantly higher than those in control group. Those increase was partially abolished by the administration of natural product or the combinational administration of natural products and estrogen.

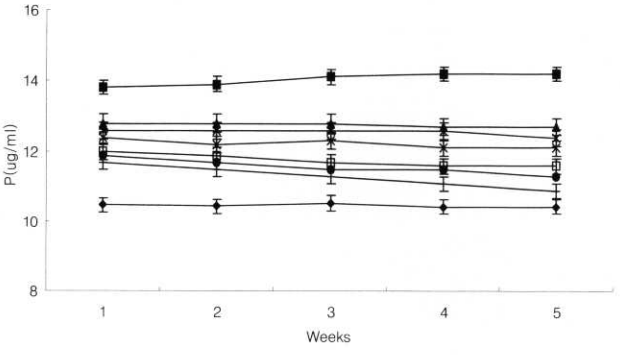

Fig. 2. Effects of various biomedicinal agents on serum P concentrations in ovariectomized rats. Control (◆); Ovariectomy (■); Antler (▲); Safflower (×); Ipriflavone (*); Antler+E (●); Safflower+E (□); Ipriflavone+E (+).

The changes in the activity of serum ALP

The changes in the activities of serum ALP on various biomedicinal agents showed in Fig. 3. The concentrations of serum ALP in antler, safflower, ipriflavone, antler+estrogen, safflower+estrogen, ipriflavon+estrogen groups were 240±11.4 IU/ml, 228±14.3 IU/ml, 228±11.8 IU/ml, 190±10.7 IU/ml, 174±11.5 IU/ml, 165±13.7 IU/ml, respectively, which is little higher than those in control group (158±10.8 IU/ml). However, the activity of ALP in ovariectomized group was 326±10.2 IU/ml, which is much higher than those in natural product-administrated group. In a good line with this, Ohta *et al* [11] have reported that the increase of concentration of serum ALP is due to increasing the exchange ratio of bone by ovariectomy. The serum ALP, a biochemical marker of bone formation, is produced in the osteoblast and the liver, which is various among age, species and menstraul cycle. The levels of serum ALP in fetal stage is higher than those in adults, which is the case in woman after menopause [10, 11, 12].

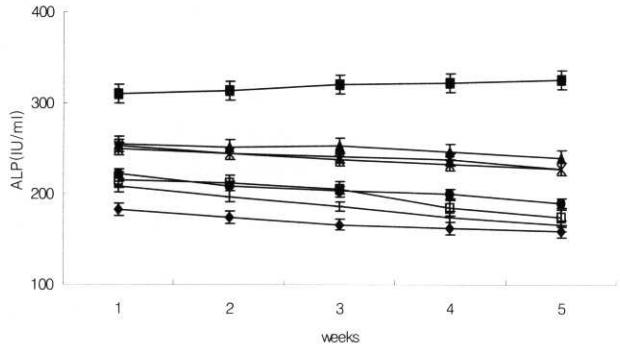

Fig. 3. Effects of various biomedicinal agents on serum ALP concentrations in ovariectomized rats. Control (◆); Ovariectomy (■); Antler (▲); Safflower (×); Ipriflavone (*); Antler+E (●); Safflower+E (□); Ipriflavone+E (+).
* The co-adminstration of ipriflavon or safflower and estrogen have shown the significant decrease of serum Ca^{2+} ($p<0.05$).

Histological changes of bone

In order to study the effects of antler, safflower, ipriflavone, antler + E safflower + E and ipriflavone + E on the bone structure of ovariectomized rats, we performed histological examination. It was found that in control group the connection of intertrabecular bone was maintanined well but in ovariectomized group, trabecular bone was slander and the connection of intrabecular bone was interrupted and the quantity of bone was lost (Fig. 4). However, it was found that in safflower + E and ipriflavone + E -treated group, the trabecular bone was normal and the connection of intertrabecular

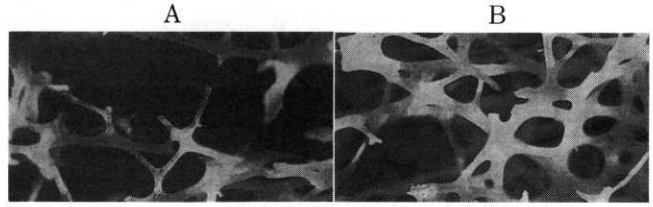

Fig. 4. Osteoporosis-induced group (A) and ipriflavone + E treated groups (B).

bone was maintanined well like in control group (Table 2). In the examination of electronic microscopy, the number of the lacunae of bone was 16, 26, 23, 24, 23, 20, 18 and 18 in control group, in ovariectomized group, in antler, safflower, ipriflavone, antler + E, Safflower + E and ipriflavone + E treated group, respectively (Table 2).

Table 2. Electron microscopic findings on femur bone administrated with various iomedicinal agents in ovariectomized rats

Treatment	Electron microscopic findings No. of lacunae	Changes of trabeculae
Control	16	Not detectable
Ovariectomy	26	Mostly broken &, loss
Antler	23	Slight
Safflower	24	Broken
Ipriflavone	23	Slight
Antler + E	20	Slight
Safflower + E	18	Slight
Ipriflavone + E	18	Slight

It is known that the methods to measure the extent of bone loss are histological and electron microscopic examination, and the examination of bone density. Histological examination allow us to determine the osteoporosis by finding the morphology of trabecular bone, the extent of bone loss, the rate of calcification and bone formation under microscopy [8]. Accordingly it was found that the morphology and connection of trabecular bone in antler and safflower treated group was similar to that in control group. It is suggested that antler extract may play a role in the prevention or treatment of osteoporosis. It is conceivable that the bone loss of ovariectomy is due to the imbalance of the ratio of bone formation and absorption [2, 9, 11, 12].

References

1. **Bemben, D. A.** Exercise interventions for osteoporosis prevention in postmenopausal women. J. Okla. State Med. Assoc. 1999, **92(2)**, 66-70.
2. **Brunelli, M. P. and Einhorn, T. A.** Medical management of odteoporosis: fracture prevention. Clin. Orthop. Tel. Res. 1998, **348**, 15-21.
3. **Elliott, J. L., Oldharm, J. M., Ambler, G. R., Bass, J. J., Spencer, G. S. G., Hodgkinson, S. C., Breier, B. H., Gluckman, P. D. and Suttie, J. M.** Presence of insulin-like growth factor-I receptors and absence of growth hormone receptors in the antler tip. Endocrinology 1992, **130(5)**, 2513-2520.
4. **Ettinger, S. J. and Feldman E. C.** Congenital defects of the dog; In Textbook of veterinary internal medcine, pp. 2115-2129, 4th ed. Philadelphia, W. B. Saunders Co., 1995.
5. **Hattori, M. Yang, X. W., Kaneko, S., Nomura, Y. and Namba, T.** Constituents of the pilose antler of Cervus Nippon var mantchuricus. J. Biopharm. Japan 1989, **43(2)**, 173-176.
6. **Hauselmann, H. J., Kramer, E. and Michel, B. A.** Physical therapy in prevention and treatment of osteoporosis. Ther. Umsch. 1998, **55(11)**, 724-730.
7. **Kim, M. R.** Effects of antler water extract on bone mineral sensity and serum female hormone levels in ovariectomized rats. The Graduate School, Catholic Univ. Ph.D. Thesis 1996, 51-59.
8. **Luis, C. J. and Jone, A. L.** Basic Histology. 5th ed. pp. 305-311. Prentice-Hall International Inc. Philadelphia, 1986.
9. **Meller, Y., Kestenbaum, R. S., Mozes, M., Mozes, G., Yagil, R. and Shany, S.** Mineral and endocrine metabolism during fracture healing in dogs. Clin. Orthop. Rel. Res. 1984. **187**, 289-295.
10. **Nyda, M. J., de Majo, S. F. and Lewis, R. A.** The effects of ovariectomy and physiological doses of estradiol upon body weight, linear growth and fat content of the female albino rat. Bull. Johns Hopkins Hosp. 1948. **83**, 279-287.
11. **Ohta, H., Masuzawa, T., Ikeda, T. and Nozawa, S.** Which is more osteoporosis-inducing, menopause or oophorectomy? Bone Miner. 1992a, **19**, 273-285.
12. **Ohta, H., Makita, K., Suda, Y., Ikeda, T., Masuzawa, T. and Nozawa, S.** Influence of oophorectomy on serum levels of sex steroids and bone metabolism and assessment of bone mineral density in lumbar trabecullar bone by QCT-C value. J. Bone Miner. Res. 1992b, **7(6)**, 659-665.
13. **Seo, B. I.** Effects of the cervi vornu on the aged ovariectomized rat model of postmenopausal osteoporosis. The Graduate School, Kyunghee Univ. Ph.D. Thesis 1998, 18-21.
14. **Zhang, Z. Q., Zhang, Y., Wang, B. X., Zhaw, H. O., Wang, Y. and Zhang, H.** Purification and partial characterization of anti-inflammatory peptide from pilose antler of Cervus Nippon Tetcommninck. J. Pharm. Japan. 1992, **27**, 321-324.