**INTRODUCTION**

High-dose radiation therapy has been associated with bone loss [1]. The main effect of radiation on bone is atrophy, which involves a reduction in the number of functioning structural components to the tissue without a decline in size. There are several important factors that need to be considered in the pathogenesis of radiation-induced changes in bone, vascular changes, bone matrix, and cellular changes [2]. Such changes are evident early in the development of spontaneous fractures after irradiation [3].

Osteoporosis is a major universal public health trouble that imposes a great financial load to society as well as to families of patients who suffer from related fractures and have reduced functional independence [4]. The design of anti-osteoporotic drugs is based on the processes of bone remodeling. Some agents have been designed to prevent bone resorption (e.g., estrogen, calcitonin, bisphosphonates, calcium, vitamin D, and raloxifene) and other agents mainly encourage bone formation (e.g., fluoride and anabolic steroids) [5].

*Panax ginseng*, also well-known as Korean ginseng, has been used as a broad tonic in long-established Ori-
Irradiation and Korean Red Ginseng treatment

Korea) and allowed 1 wk for quarantine and acclimatization. The Institutional Animal Care and Use Committee at Chonnam National University approved the protocols used in this study, and the animals were cared for in accordance with the Guidelines for Animal Experiments. The animals were then sacrificed using ether anesthesia, and the left tibiae were collected, cleaned of all non-osseous tissue, measured for length and weight, fixed in 10% neutral formalin for 48 h, and stored in 70% ethanol. Tibia length was considered as the maximal distance between the proximal condyles and malleolus. Freshly isolated right tibiae were assessed for their biomechanical strength using the tensile strength testing apparatus. Three-point bending tests were performed using a model3344 apparatus (Instron, Norwood, MA, USA). The lateral surface of the tibia at the tibio-fibular junction was placed on the first point and the proximal tibia on the other. A rounded press head compressed the middle of the tibial shaft until fracture occurred.

Grip strength measurement

Grip strength was assessed as previously described [16] using a grip strength meter (GSM) designed by IWO0-Systems (Seoul, Korea). For testing, mice were gently held so that their back legs were supported with one forelimb lightly restrained. The paw being tested was brought to the bar, the mouse was allowed about 1 s to establish a grip, and then the mouse was gently pulled back in one smooth motion until grip released. Positive grip constituted an immediate grasping of the bar with all fingers and, after release, the paw was relaxed and not clenched. Gripping force was defined as the maximum force recorded on the GSM before the mouse released the bar. Mice were given four trials per session.

Anatomical and biomechanical analysis

The animals were then sacrificed using ether anesthesia, and the left tibiae were collected, cleansed of all non-osseous tissue, measured for length and weight, fixed in 10% neutral formalin for 48 h, and stored in 70% ethanol. Tibia length was considered as the maximal distance between the proximal condyles and malleolus. Freshly isolated right tibiae were assessed for their biomechanical strength using the tensile strength testing apparatus. Three-point bending tests were performed using a model3344 apparatus (Instron, Norwood, MA, USA). The lateral surface of the tibia at the tibio-fibular junction was placed on the first point and the proximal tibia on the other. A rounded press head compressed the middle of the tibial shaft until fracture occurred.

Serum analysis

Immediately after sacrifice, blood samples were collected by vena cava. Serum alkaline phosphatase (ALP)

http://dx.doi.org/10.5142/jgr.2013.37.435
activity was measured on a Dri-chem automatic analyzer (Fuji, Tokyo, Japan) using a diagnostic slide. Serum estradiol (E$_2$) and circulating markers of bone resorption (tartrate-resistant acid phosphatase, TRAP) levels were measured using an estradiol enzyme-linked immunosassay (ELISA) kit (Calbiotech, San Diego, CA, USA) or a TRAP ELISA kit (Usen Life Science, Wuhan, China). The analyses were performed according to protocols provided by the manufacturers.

**Microcomputed tomography analysis**

Morphological measurements, including bone volume density (BV/TV), trabecular thickness/separation/number (Tb.Th, Tb.Sp, Tb.N), structure model index (SMI), cortical bone volume, and mean polar moment of inertia were calculated from the resulting microcomputed tomography (micro-CT) data for each mouse using a model 1172 apparatus (Skyscan, Kontich, Belgium). The regions of interest for analysis were the proximal tibia metaphysis. User-defined contours were outlined on every fifth slice of a 150 slice region extending 2.5 mm distally from the growth plate, starting at the point where the growth plate tissue was no longer visible in the gray-scale computed tomography slice. The proximal 90 slice region was used when analyzing the trabecular bone, and the most distal 60 slices were used when analyzing the cortical bone. For quantification of the trabecular volumetric mineral density (BMD) of tibia, the micro-CT was calibrated using two standard phantoms with a density of 0.25 and 0.75 mg/cm$^3$. The image slices were reconstructed and analyzed using CTan analyzer software (Skyscan).

**Data analysis**

The statistical significance of differences between the results in KRG-treated and untreated groups was determined by two-tailed Student’s t-test by use of the Graph PAD In Plot computer program (GPIP; Graph PAD Software, San Diego, CA, USA). A p-value <0.05 was considered statistically significant.

**RESULTS**

**Anatomical and biomechanical property**

Grip strength, body weight, and uterus weight did not differ among the four groups (Fig. 1). No differences were apparent among the four groups with regard to mechanical property, tibia length, and tibia weight (data not shown).

**Serum biochemical level**

The effects of KRG on serum biochemical markers are summarized in Fig. 2. As compared with the irradiation control group, the serum ALP level was significantly lower in the KRG (i.p.)-treated groups. Mean levels of ALP and TRAP were slightly lower in the KRG (p.o.) group, but they were statistically insignificant. The serum
E₂ levels were not significantly changed in any of the experimental groups.

Microcomputed tomography analysis

Micro-CT images from representative tibia from each group are shown in Fig. 3. Micro-CT revealed that proximal tibial metaphysis from irradiation group had lower trabecular bone compared to the sham group. Compared to the irradiation control group, BV/TV of the KRG (p.o.) group was increased by 28%. The pattern of change of Tb.N and Tb.Sp was similar to that of BV/TV. Consistently, SMI was lower in the KRG (p.o.) group compared to the irradiation control group by about 22%. Trabecular BMD was raised by 56% in the KRG (p.o.) group compared to the irradiation control group.
Intra-peritoneal injection of KRG could only partially improve the radiation-induced bone structural damages in the irradiated mice. No significant differences were apparent between the control and experimental groups with regard to the cortical bone microarchitecture (data not shown).

**DISCUSSION**

The effects of ionizing radiation on osteoclast activity are very unclear, with a preponderance of the literature indicating a decrease in osteoclast numbers and bone resorption activity [17,18]. However, some studies have indicated that an early stimulation of active bone resorption after exposure could contribute to the etiology of radiation-induced bone damage [19,20]. High serum levels of bone turnover markers indicate an increased turnover rate [21] and are related to fast bone loss in untreated osteoporosis. The combination of low BMD and high levels of bone turnover markers are related with an
especially high fracture risk [22]. Clinical application of biochemical bone turnover markers in monitoring the efficacy of antiresorptive therapy in patients with osteoporosis was explored; potential use also includes pres-estimate of rates of bone loss and fracture risk [21]. Some studies verified that ginsenosides appear to inhibit the osteoclastic bone resorption via depression of the new osteoclast formation. These results obviously demonstrated that ginsenosides appear to be the effective component in the osteoclastogenesis inhibition, which has great potential in the treatment of osteoporosis and in bone metastases therapies with less side effects than other treatments [23-25]. In the present study, the effects of KRG extract on bone were evaluated. The administration of KRG extract for 12 wk slightly lowered serum ALP and TRAP levels in irradiated mice, suggesting that KRG extract can reduce the bone turnover rate in mice.

In this study, administration of KRG (p.o.) to the irradiated mice largely prevented trabecular bone loss and the trabecular bone microarchitecture of the proximal tibia in mice. KRG (i.p.) exhibited a slight but not significantly positive effect, suggesting that the treatment with the dose of KRG (i.p.) in this study did not effectively prevent bone loss. Well-designed large studies are needed to determine accurate beneficial doses in vivo and in clinical trials. There was no particular change of the estrogen level between irradiated and sham mice. It means that radiation-induced bone loss has no relation with hormone change by radiation-induced ovary damages. Although some studies have shown significant correlation between grip strength, biomechanical property and BMD [26,27], there was no significant relationship among these markers in this study. The absence of an effect of radiation on cortical bone parameters in the present study is in agreement with earlier findings [28].

In summary, the present study clearly demonstrates the in vivo efficacy of KRG (p.o.) extract to prevent radiation-induced loss of trabecular bone architecture in mice. This study provides evidence that KRG extract is a promising alternative and complementary therapeutic agent for the management of radiation-induced osteoporosis. However, to develop KRG extract as an alternative regime for the treatment of bone diseases, more research will be needed to find the valuable dose and identify the active ingredients in KRG extract. Ginseng is a relatively nontoxic natural product with worldwide distribution, and in addition to its previously known radioprotective properties [11-14], it appears to be a promising radioprotector capable of attenuating the deleterious effects of radiation on bone.

ACKNOWLEDGEMENTS

This research was supported by the 2011 grant from the Korea Ginseng Corporation. The animal experiment in this study was supported by the Animal Medical Institute of Chonnam National University.

REFERENCES

1. Howland WJ, Loeffler RK, Starchman DE, Johnson RG. Postirradiation atrophic changes of bone and related complications. Radiology 1975;117:677-685.
2. Ergun H, Howland WJ. Postirradiation atrophy of mature bone. CRC Crit Rev Diagn Imaging 1980;12:225-243.
3. Chen HH, Lee BF, Guo HR, Su WR, Chiu NT. Changes in bone mineral density of lumbar spine after pelvic radiotherapy. Radiother Oncol 2002;62:239-242.
4. Liu Z, Piao J, Song L, Lu Y, Nan S, Pan Z, Guo Y, Wang X, Li F, Liu J et al. The diagnostic criteria for primary osteoporosis and the incidence of osteoporosis in China. J Bone Miner Metab 2002;20:181-189.
5. Harada S, Rodan GA. Control of osteoblast function and regulation of bone mass. Nature 2003;423:349-355.
6. Nocerino E, Amato M, Izzo AA. The aphrodisiac and adapotogenic properties of ginseng. Fitoterapia 2000;71 Suppl 1:S1-S5.
7. Tang W, Eisenbrand G. Panax ginseng C. A. Meyer. In: Tang W, Eisenbrand G, editors. Chinese drugs of plant origin: chemistry, pharmacology, and use in traditional and modern medicine. London: Springer, 1992. p.711-737.
8. Park JD. Recent studies on the chemical constituents of Korean ginseng (Panax ginseng C. A. Meyer). Korean J Ginseng Sci 1996;20:389-415.
9. Gillis CN. Panax ginseng pharmacology: a nitric oxide link? Biochem Pharmacol 1997;54:1-8.
10. Attele AS, Wu JA, Yuan CS. Ginseng pharmacology: multiple constituents and multiple actions. Biochem Pharmacol 1999;58:1685-1693.
11. Lee HJ, Kim SR, Kim JC, Kang CM, Lee YS, Jo SK, Kim TH, Jang JS, Nah SY, Kim SH. In vivo radioprotective effect of Panax ginseng C.A. Meyer and identification of active ginsenosides. Phytother Res 2006;20:392-395.
12. Lee TK, Johnke RM, Allison RR, O’Brien KF, Dobbs LJ Jr. Radioprotective potential of ginseng. Mutagenesis 2005;20:237-243.
13. Verma P, Sharma P, Parmar J, Sharma P, Agrawal A, Goyal PK. Amelioration of radiation-induced hematological and biochemical alterations in Swiss albino mice by Panax ginseng extract. Integr Cancer Ther 2011;10:77-84.
14. Park E, Hwang I, Song JY, Lee Y. Acidic polysaccharide of Panax ginseng as a defense against small intestinal damage by whole-body gamma irradiation of mice. Acta Histochem 2011;113:19-23.
15. Shin HR, Kim JY, Yun TK, Morgan G, Vainio H. The cancer-preventive potential of Panax ginseng: a review of human and experimental evidence. Cancer Causes Control 2000;11:565-576.
16. Meyer OA, Tilson HA, Byrd WC, Riley MT. A method for the routine assessment of fore- and hindlimb grip strength of rats and mice. Neurobehav Toxicol 1979;1:233-236.
17. Sawajiri M, Mizoe J, Tanimoto K. Changes in osteoclasts after irradiation with carbon ion particles. Radiat Environ Biophys 2003;42:219-223.
18. Vit JP, Ohara PT, Tien DA, Fike JR, Eikmeier L, Beitz A, Wilcox GL, Jasmin L. The analgesic effect of low dose focal irradiation in a mouse model of bone cancer is associated with spinal changes in neuro-mediators of nociception. Pain 2006;120:188-201.
19. Furstman LL. Effect of radiation on bone. J Dent Res 1972;51:596-604.
20. Willey JS, Lloyd SA, Robbins ME, Bourland JD, Smith-Sielicki H, Bowman LC, Norrod RW, Bateman TA. Early increase in osteoclast number in mice after whole-body irradiation with 2 Gy X rays. Radiat Res 2008;170:388-392.
21. Hannon RA, Eastell R. Biochemical markers of bone turnover and fracture prediction. J Br Menopause Soc 2003;9:10-15.
22. Garnero P, Hausherr E, Chapuy MC, Marcelli C, Grandjean H, Muller C, Cormier C, Breart G, Meunier PJ, Delmas PD. Markers of bone resorption predict hip fracture in elderly women: the EPIDOS Prospective Study. J Bone Miner Res 1996;11:1531-1538.
23. He L, Lee J, Jang JH, Lee SH, Nan MH, Oh BC, Lee SG, Kim HH, Soung NK, Ahn JS et al. Ginsenoside Rh2 inhibits osteoclastogenesis through down-regulation of NF-xB, NFATc1 and c-Fos. Bone 2012;50:1207-1213.
24. Cheng B, Li J, Du J, Lv X, Weng L, Ling C. Ginsenoside Rb1 inhibits osteoclastogenesis by modulating NF-κB and MAPKs pathways. Food Chem Toxicol 2012;50:1610-1615.
25. Liu J, Shiono J, Shimizu K, Yu H, Zhang C, Jin F, Kondo R. 20(R)-ginsenoside Rh2, not 20(S), is a selective osteoclastogenesis inhibitor without any cytotoxicity. Bioorg Med Chem Lett 2009;19:3320-3323.
26. Bhattacharya A, Watts NB, Davis K, Kotowski S, Shukla R, Dwivedi AK, Coleman R. Dynamic bone quality: a noninvasive measure of bone’s biomechanical property in osteoporosis. J Clin Densitom 2010;13:228-236.
27. Di Monaco M, Di Monaco R, Manca M, Cavanna A. Handgrip strength is an independent predictor of distal radius bone mineral density in postmenopausal women. Clin Rheumatol 2000;19:473-476.
28. Bandstra ER, Pecaut MJ, Anderson ER, Willey JS, De Carlo F, Stock SR, Gridley DS, Nelson GA, Levine HG, Bateman TA. Long-term dose response of trabecular bone in mice to proton radiation. Radiat Res 2008;169:607-614.