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Effect of Korean Red Ginseng on radiation-induced bone loss in C3H/HeN mice

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This study investigated the effects of Korean Red Ginseng (KRG) on radiation-induced bone loss in C3H/HeN mice. C3H/HeN mice were divided into sham and irradiation (3 Gy, gamma-ray) groups. The irradiated mice were treated for 12 wk with vehicle, KRG (per os, p.o.) or KRG (intraperitoneal). Serum alkaline phosphatase (ALP), tartrate-resistant acid phosphatase, estradiol level, and biomechanical properties were measured. Tibiae were analyzed using micro-computed tomography. Treatment of KRG (p.o., 250 mg/kg of body weight/d) significantly preserved trabecular bone volume, trabecular number, structure model index, and bone mineral density of proximal tibia metaphysic, but did not alter the uterus weight of the mice. Serum ALP level was slightly reduced by KRG treatment. However, grip strength, mechanical property, and cortical bone architecture did not differ among the experimental groups. The results indicate that KRG can prevent radiation-induced bone loss in mice.

Keywords: *Panax ginseng*, Korean Red Ginseng, Radiation, Bone loss, Trabecular bone

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-

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ental medicine to augment vitality, health, and longevity, particularly in older people [6,7]. Commercially available ginseng is classified into fresh, white, and Korean Red Ginseng (KRG). To preserve ginseng for an extensive period of time, KRG is made by steaming and drying the fresh ginseng, suggesting chemical alteration by heat [8]. In Oriental medicine, ginseng is extracted with hot water and used for medicinal purposes. Aqueous extracts of ginseng are composed of a mixture of glycosides, ginsenosides, trace minerals, and a variety of complex carbohydrates as well as proteins, peptides, and amino acids [8]. The main pharmacologically active constituents of ginseng are believed to be ginsenosides, which are derivatives of the triterpene dammarane structure [8,9]. The pharmacological effects of ginseng have been confirmed in the central nervous system and in the cardiovascular, endocrine, and immune systems. In addition, ginseng and its constituents have been certified to possess antineoplastic, antistress, radioprotective, and antioxidant activities [9-15].

Despite the many reports concerning the radioprotective effects of ginseng [11-14], there is surprisingly little information in literature relating to the action of KRG in modifying radiation-induced bone loss. The primary aim of the study was to evaluate the effects of KRG in preventing osteoporosis and ameliorating bone loss in irradiated mice. To the knowledge of the authors this is the first study showing prevention of radiation-induced bone damages by KRG.

MATERIALS AND METHODS

Animals

Eight-week-old female C3H/HeN mice were obtained from a specific pathogen-free colony at Orient Bio (Seoul, 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 housed in a room that was maintained at 22±2°C and relative humidity of 50±5%, with artificial lighting from 08:00 to 20:00 h and with 13 to 18 air changes per hour. The animals were housed in groups of three per polycarbonate cage, and were given tap water and commercial rodent chow (Samyang Feed, Seoul, Korea) *ad libitum*.

Irradiation and Korean Red Ginseng treatment

The animals were irradiated with 3 Gy for the experiment using ^{137}Cs-generated gamma-rays (Gamma-cell; Nordion, Montreal, PQ, Canada), at a dose-rate of 2 Gy/min. A total of 24 mice (n=6 per group) were divided into sham control, irradiated control, KRG administered per os (p.o.) in combination with irradiation, and KRG administered intraperitoneally (i.p.) in combination with irradiation. KRG was given (250 mg/kg/d) p.o. from 1 wk before irradiation to 12 wk after irradiation, or was given (50 mg/kg/every other day) i.p. from 3 d before irradiation to 12 wk after irradiation. KRG extract was provided by Korea Ginseng Corporation (Daejeon, Korea). The extract contained Rb1 (0.83%), Rb2 (0.32%), Rc (0.39%), Rd (0.11%), Re (0.26%), Rf (0.16%), Rg1 (0.20%), Rg2 (0.14%), Rg3 (0.01%), and Rh1 (0.10%), and other minor ginsenosides.

Grip strength measurement

Grip strength was assessed as previously described [16] using a grip strength meter (GSM) designed by IWOO-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, 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.

Serum analysis

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

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 immunoassay (ELISA) kit (Calbiotech, San Diego, CA, USA) or a TRAP ELISA kit (Uscn 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).

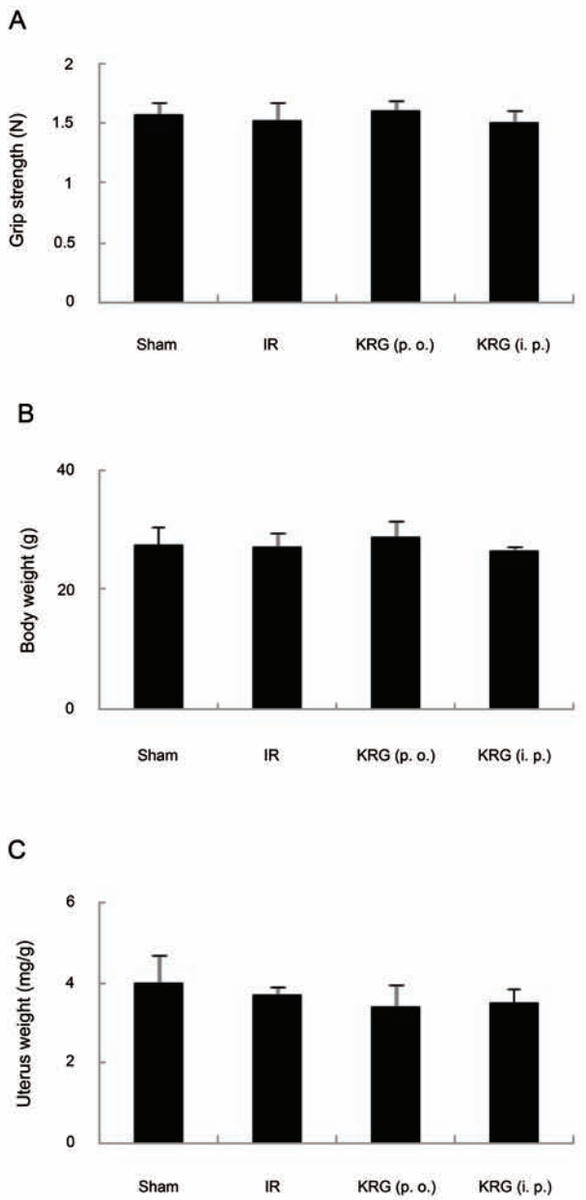

Fig. 1. Effect of Korean Red Ginseng (KRG) on grip strength (A), body weight (B), and uterus weight (C) at 12 wk after whole-body irradiation (IR) with 3 Gy. KRG was given (250 mg/kg/d) per os (p.o.) from 1 wk before irradiation to 12 wk after irradiation. KRG was given (50 mg/kg/every other day) intraperitoneally (i.p.) from 3 d before irradiation to 12 wk after irradiation. Data are expressed as mean±SD (n=6).

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

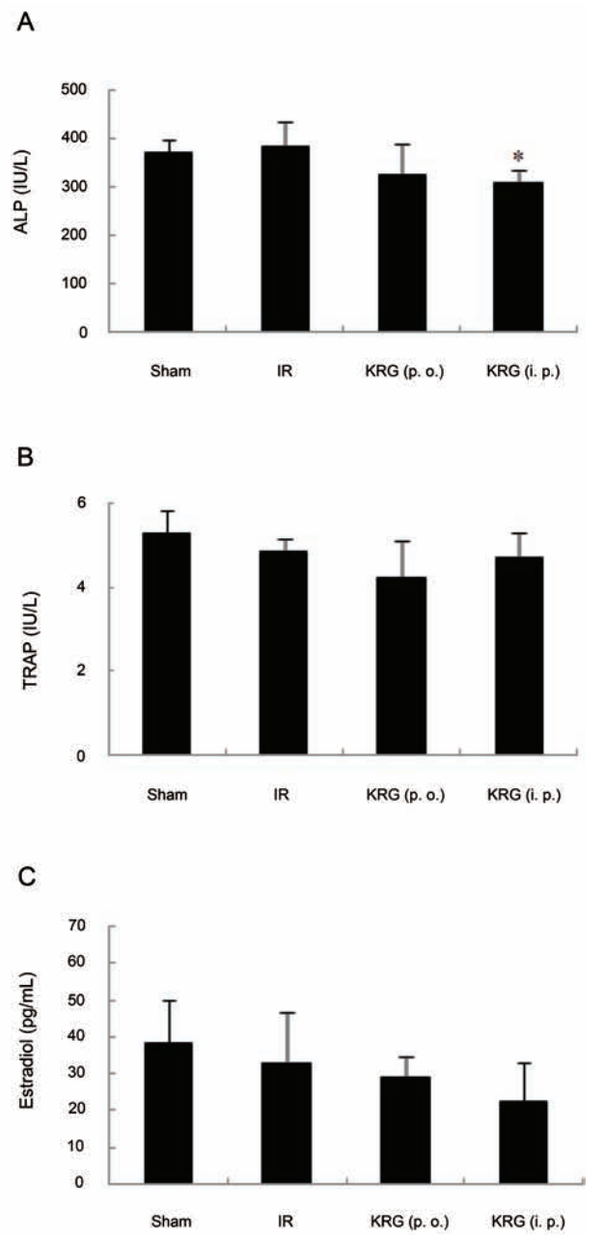

Fig. 2. Effect of Korean Red Ginseng (KRG) on serum biochemical markers at 12 wk after whole-body irradiation (IR) with 3 Gy. Alkaline phosphatase (ALP, A), tartrate-resistant acid phosphatase (TRAP, B), and estradiol (C) levels were measured. KRG was given (250 mg/kg/d) per os (p.o.) from 1 wk before irradiation to 12 wk after irradiation. KRG was given (50 mg/kg/every other day) intraperitoneally (i.p.) from 3 d before irradiation to 12 wk after irradiation. Data are expressed as mean±SD (n=6). *p<0.01 vs. IR group at corresponding parameters.

E_2 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 com-

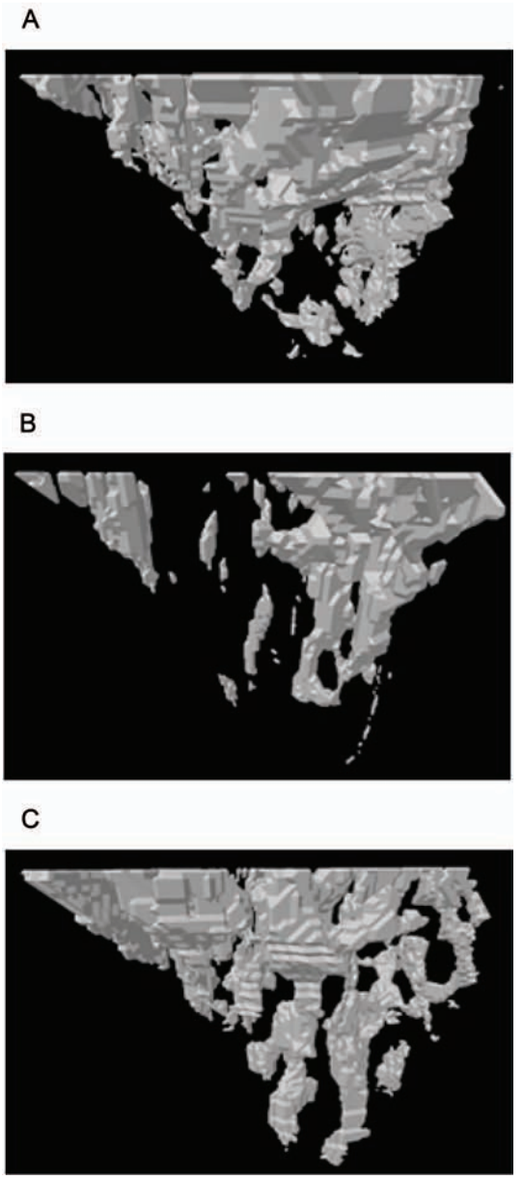

Fig. 3. Representative microcomputed tomography three-dimensional images of trabecular architecture of tibia in (A) sham control, (B) irradiation control and (C) an irradiation + red ginseng (per os)-treated mouse.

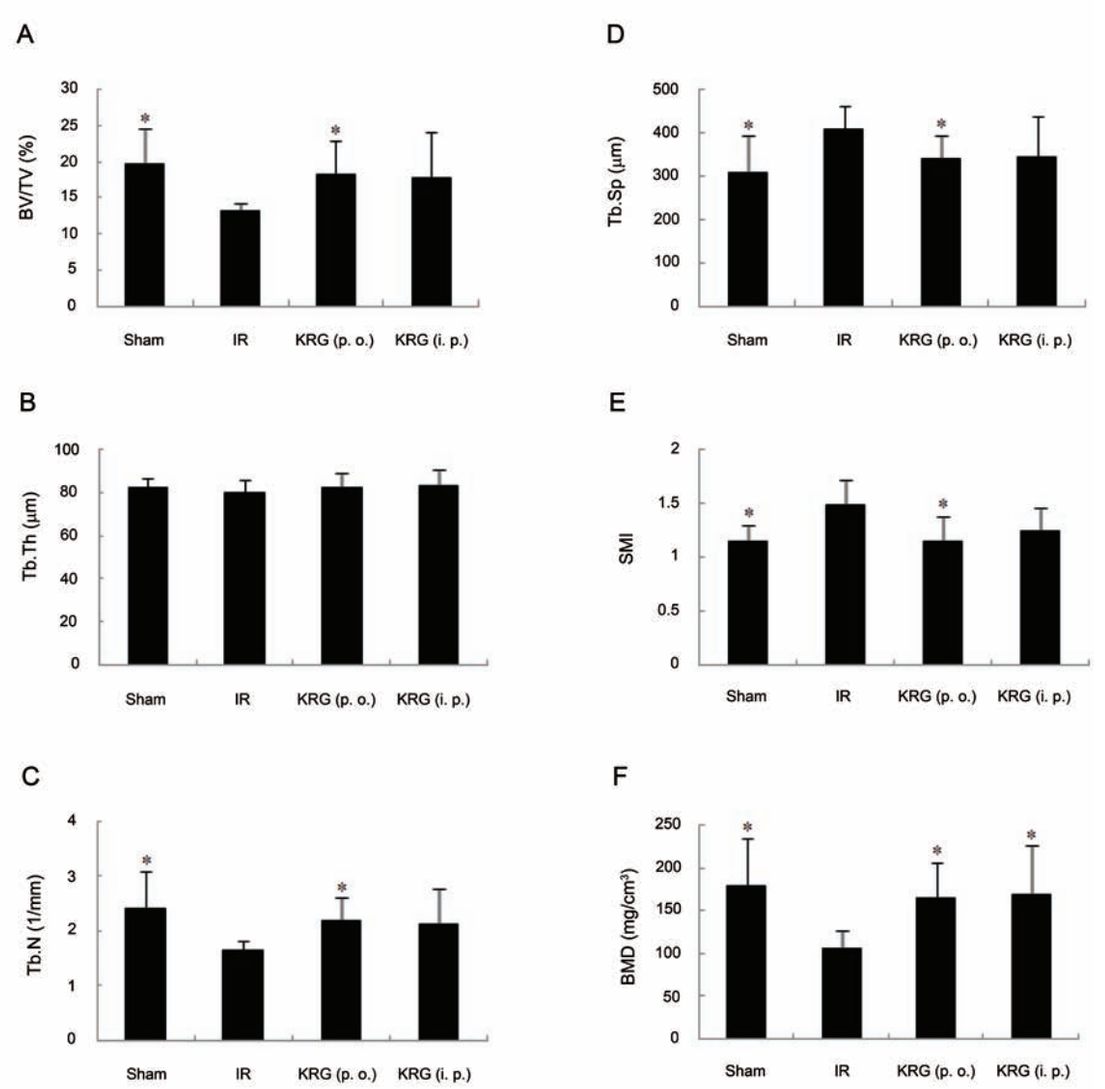

Fig. 4. Effect of Korean Red Ginseng (KRG) on trabecular bone properties in tibia 12 wk after whole-body irradiation (IR) with 3 Gy. Bone volume density (BV/TV, A), trabecular thickness (Tb.Th, B), trabecular number (Tb.N, C), trabecular separation (Tb.Sp, D), structure model index (SMI, E), and trabecular volumetric mineral density (BMD, F) were calculated. KRG was given (250 mg/kg/d) per os (p.o.) from 1 wk before irradiation to 12 wk after irradiation. KRG was given (50 mg/kg/every other day) intraperitoneally (i.p.) from 3 d before irradiation to 12 wk after irradiation. Data are expressed as mean±SD (n=6). *p<0.05 vs. IR group at corresponding parameters.

pared with the irradiation control group (Fig. 4). Intraperitoneal 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 preestimate 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 osteoclastgenesis inhibition, which has great potential in the treatment of osteoporosis and in bone metastases therapeutics 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.

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