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ORIGINAL ARTICLE

The effect of boron on alveolar bone loss in osteoporotic rats

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Abstract *Background/purpose:* The aim of this study is to investigate the effects of systemically administered boric acid on osteoporosis-related bone alterations, alveolar bone loss, receptor activator of nuclear factor kappa-b ligand (RANKL) expressions, and mandibular bone density in experimental periodontitis model in osteoporotic rats.
Materials and methods: Thirty-six male Wistar rats were separated into five study groups: non-ligated control (C, $n = 6$) group; periodontitis (P, $n = 6$) group; osteoporosis (O, $n = 8$) group; osteoporosis + periodontitis (O+P, $n = 8$) group, and osteoporosis + periodontitis with 50 mg/kg/d boric acid (BA50, $n = 8$) group for 15 days. Osteoporosis was created with intraperitoneal injection of 80 mg/kg retinoic acid for 15 days. Silk ligatures (4/0) were placed around the mandibular right first molar teeth to induce experimental periodontitis. After induction of osteoporosis and periodontitis, rats were sacrificed at Day 15. Alveolar bone loss was evaluated with a stereomicroscope by measuring the distance from the cement-enamel junction to the alveolar crest. Density measurements were performed on radiographs. RANKL and tartrate-resistant acid phosphatase (TRAP) staining were performed on histological slides.
Results: Alveolar bone loss was significantly higher in the O+P group than those of the other groups ($P < 0.05$). Boric acid decreased bone loss ($P < 0.05$). TRAP + osteoclast numbers were highest in the P group and lowest in the control group. The differences in TRAP + osteoclast numbers among control, P, O+P, and BA50 groups were significant ($P < 0.05$). There were no significant differences in RANKL expression and mandibular bone density ($P > 0.05$).
Conclusion: Within limitations of this study, we conclude that boric acid may decrease alveolar bone loss in a rat model with periodontitis and osteoporosis.

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Introduction

Osteoporosis is defined as "a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration with consequent increase in bone fragility and susceptibility to fracture."[1] Osteoporosis is not a life-threatening disorder but still affects many people by causing pain, disability, and diminished quality of life.[2] Aging, estrogen deficiency, and inflammatory diseases are the most common factors contributing to the development of osteoporosis. There are some treatment procedures for osteoporosis and these are mainly: calcium and vitamin D reinforcement, antiresorptive agents such as bisphosphonates, selective estrogen receptor modulators, parathyroid hormone, and surgery.[2]

The relationship between osteoporosis and oral health is still a complex problem and the evidences are contradictory. Some studies have suggested an association between low skeletal bone mineral density (BMD) and periodontal bone loss and tooth loss,[3,4] while others have not.[5,6] Furthermore, the mechanisms underlying any potential association between periodontal disease and osteoporosis are not fully understood but experimental studies of osteoporosis and periodontitis suggested a strong relationship.[7,8] In both diseases, there is an increased production of cytokines that stimulate osteoclastic activity.[8] Kobayashi et al[7] showed that alveolar bone mass was reduced by ovariectomy and that estrogen deficiency significantly enhanced the loss of alveolar bone in experimental periodontitis in mice.

One of the most preferred treatment protocols for osteoporosis is antiresorptive agents such as bisphosphonates. These drugs are very effective in preventing bone resorption in long bones and vertebra but have a serious side effect on jaws. Osteonecrosis caused by bisphosphonates makes any surgical dental procedure impossible and it is very hard to treat.[9]

Being directly linked to bone metabolism,[10] boron might help reverse the effects of osteoporosis. Boron is the fifth element in the periodic table and has the characteristics of both metals and nonmetals. Boron interacts with calcium, vitamin D, and magnesium. Boron is not found alone in nature and is abundant in nature as boric acid (BA) and borate. Boron can be obtained in the diet through the consumption of fruits, vegetables (potato and avocado), legumes, nuts, eggs, milk, wine, and dried foods.[11] Many of the foods that contain boron are likely to have beneficial effects on bone.[12] The daily requirement of boron has yet to be defined, but daily multivitamin and mineral supplements contain between 3 mg and 9 mg.[12]

Boron also has been shown to increase bone strength measures in rats and found to be effective on early bone regeneration in rabbits after expansion of midpalatal suture.[13] Hakkı et al[10] showed that boron can induce osteogenesis by regulating RunX2, bone sialoprotein (mRNA expression level), and bone morphogenetic protein-4, -6, and -7 (protein level) in osteoblastic cells *in vitro*. Also, Demirer et al[14] reported that systemically administered BA diminishes alveolar bone loss, decreases inflammatory cell infiltrate, and increases osteoblastic activity in experimental periodontitis in rats. In a previous study, we also demonstrated that 30 mg/kg and 50 mg/kg boric acid decreased osteoclastic activity in diabetic rats.[15]

In an attempt to find an alternative treatment for osteoporosis and based on these favorable aspects of BA, we hypothesized that boron might be a potent suppressor of bone loss in osteoporosis. Therefore, the aim of this study is to investigate the effect of BA on alveolar bone loss and mandibular bone density in osteoporotic rats with periodontitis.

Materials and methods

Thirty-six male Wistar rats, with an average weight of 270—320 g, were used in this study. They were housed in specially designed wire cages and maintained on a 12 hour/12 hour light/dark cycle with a constant room temperature of 23°C. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Cumhuriyet University, Sivas, Turkey. Adequate measures were taken to minimize pain or discomfort in animals. The animals were randomly divided into five groups as follows: nonligated control group (C, $n = 6$); ligated periodontitis group (P, $n = 6$); osteoporosis only group (O, $n = 8$); ligated periodontitis and osteoporosis group (O+P, $n = 8$); and ligated osteoporosis with 50 mg/kg/d BA group (BA50, $n = 8$).

Induction of osteoporosis

Twenty four rats were administered retinoic acid (80 mg/kg). The other rats were administered sham injections. Retinoic acid was mixed with olive oil and 0.5 mL of this mixture was given to the rats via intraperitoneal injection for 15 days.

Induction of experimental periodontitis

After osteoporosis was achieved in the groups, rats in the P, O+P, and BA50 groups received ligature placement performed under general anesthesia using ketamine (40 mg/kg, Eczacibasi Ilac Sanayi, Istanbul, Turkey). A 4-0 silk suture (Dogsan Ilac Sanayi, Istanbul, Turkey) was submarginally placed around both the right and left first molars of mandibular quadrants. The sutures were checked after application, and lost or loose sutures were replaced. All ligatures were placed by the same operator (H.B.Y.). The animals were kept in individual cages and received water and food *ad libitum*. BA was prepared as 50 mg/kg for 0.5-mL distilled water and systemically administered by gastric feeding at a rate of 0.5 mL daily for 15 days. The other rats were administered saline solution. On Day 15, the animals were sacrificed and the tissues were prepared for morphometrical and histopathological analyses.

Measurement of alveolar bone loss

The mandibles were stained with aqueous methylene blue (Merck & Co., Inc., Whitehouse Station, NJ, USA; 1%) to identify the cemento-enamel junction. The alveolar bone height was measured under a stereomicroscope (×25 magnification; Stemi DV4, Carl Zeiss, Jena, Germany) by recording the distance from the cemento-enamel junction to the alveolar bone crest. Measurements were taken at