Effects of daidzein on alveolar bone loss and internal microstructures of bone in a rat model of experimental periodontitis: a study using micro-computed tomography


Background and Objective: Daidzein is an isoflavone abundant in soybeans, kudzu root and red clover, which have been widely studied for its therapeutic potential. The present study was designed to evaluate the effects of daidzein on alveolar bone loss and internal microstructures of bone in a rat model of experimental periodontitis by assessing morphological data obtained from micro-computed tomography (micro-CT).

Material and Methods: Twenty-four male Sprague-Dawley rats were randomly assigned to the following three groups comprising eight animals each: the nonligation (NL) group; the ligation (L) group; and the ligation+daidzein (LD) group. To induce periodontitis, a 4-0 braided silk ligature was tied around the cervical area of the lower-right first molars of rats in groups L and LD. Rats in the LD group were given daily doses of daidzein (10 mg/kg of body weight) by intraperitoneal injection immediately after ligature placement. Two weeks after the placement of ligatures, mandibular block biopsies were scanned using a micro-CT system.

Results: Daily administration of daidzein strongly suppressed the ligature-induced loss of alveolar bone height. In addition, when rats were treated with daidzein, the ligature-induced decrease in the bone volume fraction was significantly recovered. Furthermore, daidzein significantly reversed ligature-induced deteriorations in the microarchitecture parameters of trabecular bone, such as trabecular thickness, bone mineral density, trabecular separation and structure model index.

Conclusion: The study presented here demonstrates, for the first time, that daidzein effectively reduces alveolar bone destruction resulting from experimental periodontitis in rats. Further studies are necessary for the translation of this compound clinically to improve the outcomes of patients diagnosed with periodontitis.
Periodontal disease is a bacterially induced chronic inflammatory condition characterized by irreversible destruction of the tooth-supporting tissues and can lead to tooth loss if the disease is poorly controlled (1). Studies have suggested that periodontal disease may be associated with increased risk for several systemic diseases, including cardiovascular disease, diabetes and stroke, and for preterm low birthweight of infants (2–4).

It has been recognized that the tissue destruction seen in periodontal disease is determined by the host immune-inflammatory responses to pathogenic plaque bacteria and their by-products. Accordingly, various host-modulating strategies, as an adjunct to traditional mechanical periodontal therapies, have been developed (5,6).

Periodontal disease is consistently associated with high levels of proinflammatory mediators, such as nitric oxide (NO), interleukin (IL)-1β, IL-6 and tumor necrosis factor-alpha (TNF-α). The levels of these mediators are clearly raised in diseased sites and they are intimately related to clinical signs of disease (7–11). In addition, a number of studies have suggested that NO, IL-1β, IL-6 and TNF-α have powerful ability to provoke bone resorption in periodontal disease (12–15). Thus, host-modulating agents that block these destructive mediators appear to be of therapeutic value in attenuating tissue destruction in periodontal disease.

Daidzein is an isoflavone found at high concentrations in soybeans, kudzu root and red clover (16). Daidzein has been previously characterized as having anti-inflammatory activity. It has been reported that daidzein could inhibit inducible NO synthase expression and NO production in lipopolysaccharide (LPS)-activated murine macrophages (17,18). Daidzein has also been shown to inhibit expression of IL-6 gene in response to TNF in mouse fibroblasts (19). In addition, daidzein was found to suppress the plasma levels of IL-6 and TNF-α and to attenuate ischemia/reperfusion-induced myocardial damage in rat models (20).

We have previously shown that daidzein strongly down-regulates the production of NO and IL-6 in macrophages activated with LPS from Prevotella intermedia, a pathogen implicated in periodontal disease (21), suggesting that it may have potential use in the treatment of periodontal disease. Therefore, the present study was designed to evaluate the in vivo effects of daidzein on alveolar bone loss and the internal microstructures of bone in a rat model of experimental periodontitis by assessing morphological data obtained from micro-computed tomography (micro-CT).

Material and methods

Animals

Twenty-four male Sprague-Dawley rats (Koatech, Pyeongtaek, Korea), 7 wk of age and weighing 200–220 g, were used in this study. They were kept in plastic cages under standard conditions with standard rat chow and tap water ad libitum. Before the experiment, the rats were allowed 1 wk to adapt to the laboratory environment. All animal procedures described below were conducted with approval from the Institutional Animal Care and Use Committee of Pusan National University.

Experimental design

Rats were randomly assigned to the following three groups comprising eight animals each: the nonligation group (NL group); the ligation group (L group); and the ligation+daidzein group (LD group). At the start of the experiment, the rats showed no clinical signs of gingival inflammation. Periodontitis was induced in the rats of groups L and LD. The rats were anesthetized by intraperitoneal administration of a 1 : 1 mixture of Zoletil 50® (Virbac, Carros Cedex, France) and Rompun® (Bayer, Leverkusen, Germany), at 0.1 mL/100 g of body weight. Then, a 4-0 braided silk ligature was placed subgingivally around the cervical area of the lower-right first molar and knotted at the mesiobuccal side. Rats in the LD group were given with daily doses of daidzein, at a dose of 10 mg/kg of body weight, by intraperitoneal injection immediately after ligature placement. The same volume of vehicle solution was administered to rats in groups NL and L. Two weeks after the placement of ligatures, the rats were killed by carbon dioxide inhalation, and mandibular block biopsies were harvested and fixed in 10% neutral formalin for micro-CT analysis.

Micro-CT analysis

All mandibular block biopsies were scanned using a micro-CT system (inspeXio SMX-90CT; Shimadzu, Tokyo, Japan). The micro-CT parameters were set as follows: image pixel size, 1024 × 1024; voxel size, 10 × 10 × 10 μm³; section thickness, 10 μm; image magnification, 10-fold; voltage, 90 kV; and beam current, 0.1 mA. Three-dimensional (3D) images were generated for each specimen using the 3D reconstruction software, TR1/3D-BON (RATOC System Engineering, Tokyo, Japan), as recommended by the manufacturer, for visualization and quantitative analysis of the image data on a personal computer.

Linear alveolar bone measurements

All scans were reoriented before analysis to uniformly align the scan axes and anatomical positions. To assess alveolar bone loss, the distance from the cemento–enamel junction to the coronal level of the alveolar bone crest on reconstructed 3D micro-CT images was measured at four sites in the interdental region between the mandibular first and second molars (i.e. the distobuccal and distolingual line angles of first molars and the mesiobuccal and mesiolingual line angles of second molars).

Volumetric analysis

Volumetric measurements were performed following the selection of 3D regions of interest (ROI) (Fig. 1). The interdental area between the first and second molars was used as the ROI,
following the criteria defined by Luan et al. (22), starting coronally by a line drawn between the adjacent roofs of the furcations, continuing in an apical direction for 100 continuous scan slices (i.e. 1 mm apical to the roofs of the furcations). Two-dimensional (2D) contours were drawn at regular intervals (every five data planes) (Fig. 1A–C). Then, a 3D ROI was generated using the image-analysis software TRI/3D-BON, based on the resultant 2D contours (Fig. 1D). Bone volume fraction (BVF) in the selected ROI images was assessed using TRI/3D-BON. The BVF value indicates the ratio of the residual bone volume to total volume.

Analysis of microarchitecture parameters of trabecular bone

The microstructural parameters of bone, including trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N), bone mineral density (BMD) and structure model index (SMI), in areas that have bone in the selected ROI images, were assessed using a software package (TRI/3D-BON). Tb.Th, Tb.Sp and Tb.N provide detailed information on the amount, thickness and organization of trabecular bone. BMD is calculated and determined with an internal reference in micro-CT units. SMI, a key measure of trabeculae quality, was calculated based on a differential analysis of the triangulated surface of the structure. SMI was designed to be 0 for perfect plate-like structure, 3 for perfect rod-like structure and 4 for perfect spheres (23).

Statistical analysis

Data are expressed as mean ± standard error of the mean. The data were analyzed using a statistical software package (SPSS 21.0; SPSS, Chicago, IL, USA). The differences in bone parameters between the groups were analyzed using ANOVA with Tukey post-hoc comparisons. p < 0.05 was considered statistically significant.

Results

Effect of daidzein on alveolar bone height

Figure 2 shows a comparison of the linear measurements from the cemento–enamel junction to the alveolar bone crest (the micro-CT bone levels) in the interdental region between the mandibular first and second molars. The value for the L group was significantly larger than the value for the NL group (p < 0.01), indicating that 14 d of ligature placement induced alveolar bone loss in rats (Fig. 2). The loss of alveolar bone height was significantly smaller in the LD group than in the L group (p < 0.01), suggesting that the administration of daidzein suppressed the ligature-induced alveolar bone loss (Fig. 2). Approximately 82% of ligature-induced bone loss was found to be prevented by the daily administration of daidzein. The difference between NL and LD groups was not significant (Fig. 2).

Effect of daidzein on residual bone volume

The ROI highlighted in Fig. 1 was used to determine the BVF. The BVF was significantly smaller for the L group than for the NL group (p < 0.01) (Fig 3). The placement of ligatures caused an average decrease of 32% in the fraction of the ROI occupied by bone tissue (BVF) compared with that in rats without ligatures. The BVF was significantly larger in the LD group than in the L group.
When rats were treated with daidzein, about 57% of the ligature-induced decrease in the BVF was prevented. The value for the LD group was significantly smaller than that for the NL group ($p < 0.01$) (Fig 3).

**Effect of daidzein on microarchitecture parameters of trabecular bone**

The microarchitecture parameters of trabecular bone in the selected ROI are summarized in Fig. 4. Ligature placement caused significant changes in the trabecula of alveolar bone. The Tb.Th (Fig 4A) and BMD (Fig. 4D) values were significantly larger in the LD group than in the L group ($p < 0.01$), whereas Tb.Sp (Fig. 4B) and SMI (Fig 4E) values in the LD group were significantly smaller than those found in the L group ($p < 0.01$).

Daidzein administration reversed ligature-induced alterations in Tb.Th, BMD, Tb.Sp and SMI values by about 39%, 41%, 65% and 72%, respectively. Although rats in the LD group had a larger Tb.N value (Fig. 4C) than rats in the L group, there was no statistically significant difference between both groups.

**Discussion**

Natural compounds, such as flavonoids, may be useful in the host modulation of periodontal disease. Flavonoids, polyphenolic compounds present in a large number of plants and vegetables, possess a wide spectrum of biological activities, including antioxidant, anti-inflammatory, anti-carcinogenic, anti-angiogenic, anti-allergic and antiviral properties (24,25). Isoflavones are a class of bioactive flavonoids that have been shown to prevent various chronic diseases, such as atherogenesis, neurodegenerative diseases and osteoporosis (26–28).

Daidzein, an isoflavone found in a number of plants and herbs, has been widely studied for its therapeutic potential (16). The results of our previous study demonstrated that daidzein strongly suppresses *P. intermedia* LPS-induced production of NO and IL-6 at both gene transcription and translation levels in macrophages (21). Daidzein inhibits the degradation of inhibitory $\kappa$B-$\alpha$ induced by *P. intermedia* LPS (21). In addition, daidzein suppresses nuclear factor-$\kappa$B transcriptional activity by regulating the nuclear translocation and DNA-binding activity of nuclear factor-$\kappa$B p50 subunit and blocks signal transducer and activator of transcription 1 phosphorylation (21). These results indicate that daidzein could be a promising agent for treating inflammatory periodontal disease. The present study was carried out to evaluate the preventive potential of daidzein in a rat model of experimental periodontitis using micro-CT analysis.

Animal models of periodontitis have been used to evaluate the effect of new periodontal treatment modalities. Experimental periodontitis can be induced in several different ways, including a soft diet, introduction of pathogenic bacteria, local LPS injection or placement of ligature around teeth. The well-characterized model of
ligature-induced periodontitis in rats, utilized in the present study, has been extensively used in experimental periodontal research (29–32). This method promotes subgingival plaque accumulation, thereby increasing gingival inflammation and bone loss.

Periodontitis, characterized by alveolar bone loss, affects bone mass and trabecular microarchitecture (33–35). There are several approaches that
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pressive effect of daidzein on ligature-induced alveolar bone destruction are unknown. It can be speculated from our previous in vitro study (21) that the administration of daidzein might inhibit the LPS-induced production of proinflammatory mediators, such as NO, IL-1β, IL-6 and TNF-α, which have strong potential to cause bone resorption in periodontal disease.

In addition, there is evidence indicating that daidzein has the potential to prevent bone loss. Studies have demonstrated that daidzein can stimulate osteogenesis and inhibit osteoclastogenesis (41–43). Daidzein treatment in a rat model of male osteoporosis increased trabecular bone mass and decreased cancellous bone turnover (44). Daidzein also enhanced osteogenic differentiation of human bone marrow-derived mesenchymal stem cells (45).

Taken together, the study presented here demonstrates, for the first time, that daidzein effectively reduces alveolar bone destruction resulting from experimental periodontitis in rats.

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References


