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Mongolian Gerbil as a Novel Animal Model for Ligature-induced Periodontitis

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Inflammation from chronic and acute infections of distal organs and tissues such as periodontitis is a risk factor for atherosclerotic vascular processes. Recently, a new model of atherosclerosis with vascular pathologies was developed in the Mongolian gerbil. In this study, we attempted to develop a model of ligature-induced periodontitis in gerbils and compared the characteristics of that periodontitis model with that in rats and mice. Each gerbil, rat, and mouse was randomly assigned to groups of control and periodontitis. A thread was placed around the cervix of the right and left first molars in the mandible with knots placed on the mesial side of each molar. At day 14 after the ligation, the animals were sacrificed and their mandibles were dissected. To measure alveolar bone loss along with inflammation, histopathological and micro-CT analyses were carried out. Gerbils showed tooth characteristics of deeper gingival crevice, longer cusp, longer root trunk and shorter root than those of rats and mice. The increased CEJ-ABC distance in distal and PDL area in furcation was also observed in ligated gerbils. An inflammatory response in the connective tissue under the junctional epithelium was also shown in all the animals. As a result, we confirmed the induction of periodontitis by ligature in the gerbils. We therefore consider the gerbil to be a useful model for investigating relationship between periodontitis and vascular disease in the same animal.

Key words: Mongolian gerbil, ligature-induced periodontitis, alveolar bone loss, animal model

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Introduction

Periodontitis is an inflammatory disease in periodontal tissues caused by microorganisms and characterized by progressive alveolar bone loss that leads to tooth loss. Experimental animal models are frequently used for the induction of various periodontal lesions [1,2]. For experimental periodontitis studies, rodents, particularly rats and mice are most commonly used. These animals have several advantages, as they are relatively inexpensive, easy to handle, and require only a minimum amount of space. Ligature-induced periodontitis model has been accepted as a useful experimental method of periodontitis with alveolar bone resorption [3]. A previous study also reported that the biofilm accumulated around ligatures in rats and leading to alveolar bone loss in the ligated tooth presented various bacterial species that were commonly observed in humans [4]. This suggests that ligature effectively induces periodontitis in these experimental animals with the alveolar bone loss by the deposition of periodontopathogens as in humans.

Several researchers have suggested that chronic infections may predispose the individual to cardiovascular disease (CVD) such as atherosclerosis and subsequent ischemic heart

disease [5-7]. In recent years, because chronic inflammation caused by mainly gram-negative bacteria is one of the main characteristics of periodontitis, the relationship between oral health, specifically periodontitis, and CVD has been the focus of research in dental sciences [8,9].

Mongolian gerbils (*Meriones unguiculatus*) belong to the rodent family *Cricetidae*. They are semi-desert rodents with sandy-brown color and are widely distributed in North Africa, Northeastern China, and Eastern Mongolia. Because the gerbils have an incomplete circle of Willis, they display a high susceptibility to infarction of the cerebral hemisphere induced by ligature of only one common carotid artery. By comparison, other animals need to have both carotid and vertebral-basilar circulations ligated in order to induce brain infarction [10,11]. Therefore, gerbils have commonly been used as an experimental model of vascular diseases such as stroke [12,13]. To investigate the relationship between periodontitis and CVD, novel experimental animal models for susceptibility to both periodontitis and vascular disease are required. Until now, periodontitis induction in gerbils has not been demonstrated and in this study, we induced ligature-induced periodontitis in gerbils, rats, and mice and compared the characteristics of their induced periodontitis with one another.

Materials and Methods

Induction of periodontitis in gerbils, rats and mice

Mongolian gerbils, F344 rats, and C57BL/6 mice were purchased from Orient Bio (Gyeonggi-do, Korea) and were acclimated for 1 week. Food and water were provided *ad libitum*. The animals were maintained in a temperature-controlled room (22 ± 2°C) on a 12 h light-dark cycle. Each animal was randomly divided into groups (5-6 animals per group) of control and periodontitis. For periodontitis induction, the animals were anesthetized with a 1:2 mixture of Zoletil 50 (Virbac, Carros, France) and Rompun (Bayer, Ansan, Korea) by intraperitoneal injection (~1.5 μl anesthetic per gram of body weight). Thread was placed around the cervix of the right and left first molars in their mandible and knots were placed to the mesial side of each molar. At day 14 after the ligature, the animals were sacrificed and the mandibles were dissected. During the experimental period, the location of thread, gross appearance, and food and water intake were observed daily. If the ligature was missing after checking the location of the thread, new ligatures were placed. All animal procedure protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Yonsei University (2013-0177).

Histological analysis

For histopathological examination, the dissected mandibles were fixed in 10% neutral-buffered formalin for 2 days and decalcified in 5% nitric acid for 1 week. After embedding in paraffin, sections were cut at a thickness of 4 μm. The sections were selected based on the clear appearance of the dental pulp of the mesial and distal roots of the first molars and were stained with hematoxylin and eosin. The alveolar bone loss in the distal and furcation areas of the first molar was examined with a light microscope (Olympus, Tokyo, Japan; ×100 magnification). Alveolar bone loss in the distal area was examined by measuring the distance from the cementoenamel junction (CEJ) to the alveolar bone crest (ABC) using Image Pro Plus (Media Cybernetics, Silver Spring, MD, USA). Alveolar bone loss in the furcation was estimated by measuring the ratio of the periodontal ligament (PDL) area to the region of interest (ROI) of furcation, as previously described [14]. The height of the ROI was 0.8, 0.8, and 0.2 mm from the top of the furcation in mice, rats, and gerbils, respectively. The PDL area was calculated by subtracting the remaining alveolar bone area from the ROI area. To examine the extent of inflammation, the connective tissue under the junctional epithelium of the distal area of the first molar was observed (×200 magnification).

Micro-computed tomography (micro-CT) analysis

The fixed mandibles were scanned using micro-CT (Skyscan, Antwerp, Belgium) to evaluate alveolar bone loss. After the three-dimensional images of the alveolar bone were reconstructed, the ROI was determined. Similar to histopathological analysis, the reconstructed images were selected based on the clear appearance of dental pulp of the mesial and distal roots of the first molars. Alveolar bone loss in the distal and the furcation areas was examined by measuring the distance from the CEJ to the ABC and a ratio of the PDL area to the region of ROI of furcation, respectively. The ROI was defined same as that used in histopathological analysis.

Statistical Analysis

All statistical analyses were performed using SPSS 23

(IBM, Armonk, NY, USA). One-way analysis of variance (ANOVA) followed by Scheffe's post-hoc test was used to determine significant differences. A *P*-value of less than 0.05 was considered to be statistically significant. Data were expressed as mean ± standard error of the mean (SEM).

Results

Inflammation in the gingival tissue

To evaluate inflammation in the gingival tissue, we observed inflammatory cells such as polymorphonuclear cells (PMNs) and mononuclear cells, which had infiltrated into the connective tissue beneath the gingival epithelium (Fig. 1). In the control group for each animal, there were no histopathological lesions such as presence of vascular dilatation or prominent infiltration of inflammatory cells (upper panels). However, in the periodontitis group, thickening of gingival epithelium and infiltration of inflammatory cells to connective tissue were noted in the gingival tissue. Vascular dilatation and numerous inflammatory cells including PMNs, lymphocytes, and macrophages were observed in the connective tissues (lower panels).

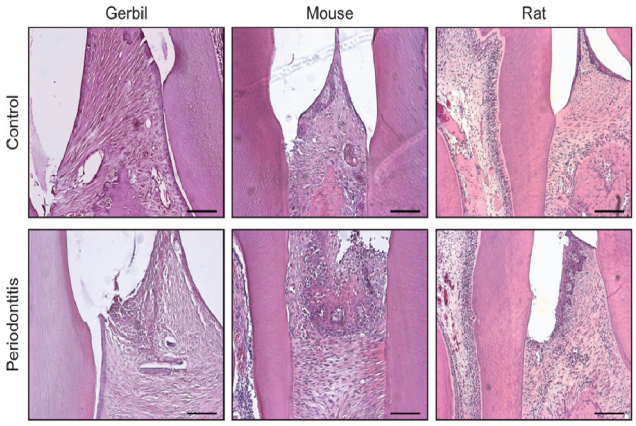

Figure 1. Inflammation in the distal area of ligated first molar in gerbil, mouse, and rat. At day 14 after ligature of the first molar, the animals were sacrificed and inflammation in the distal area of the first molar under the junctional epithelium was observed. The upper and lower images are representative of control and periodontitis groups, respectively. Images were taken at ×200 magnification. Scale bar = 100 μm.

Alveolar bone loss in rats and mice

To determine alveolar bone loss in ligature-induced periodontitis in rats and mice, we measured the CEJ-ABC distance in the distal area (upper panels of Fig. 2A and Fig.

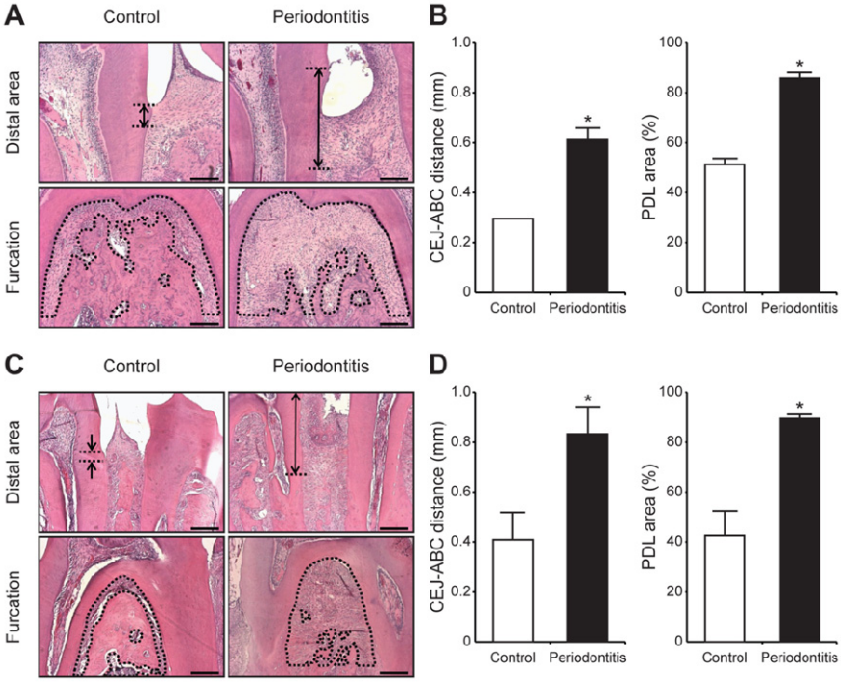

Figure 2. Alveolar bone loss of ligated tooth in rat and mouse. Alveolar bone level in distal area and furcation of first molar was examined via light microscopy (×100 magnification). (A) and (B) The CEJ-ABC distance (arrow bar) in distal and the percentage of PDL area in furcation (black-dotted line) of rat. The height of the ROI was 0.8 mm from the apical side of the furcation and the PDL area was calculated by subtracting the alveolar bone area from the ROI. (C) and (D) The CEJ-ABC distance (arrow bar) in distal and the percentage of PDL area in furcation (black-dotted line) of mouse. The height of the ROI was 0.8 mm. Data are represented with mean ± SEM. * indicates a significant difference at $P < 0.05$. Scale bar = 100 μm.

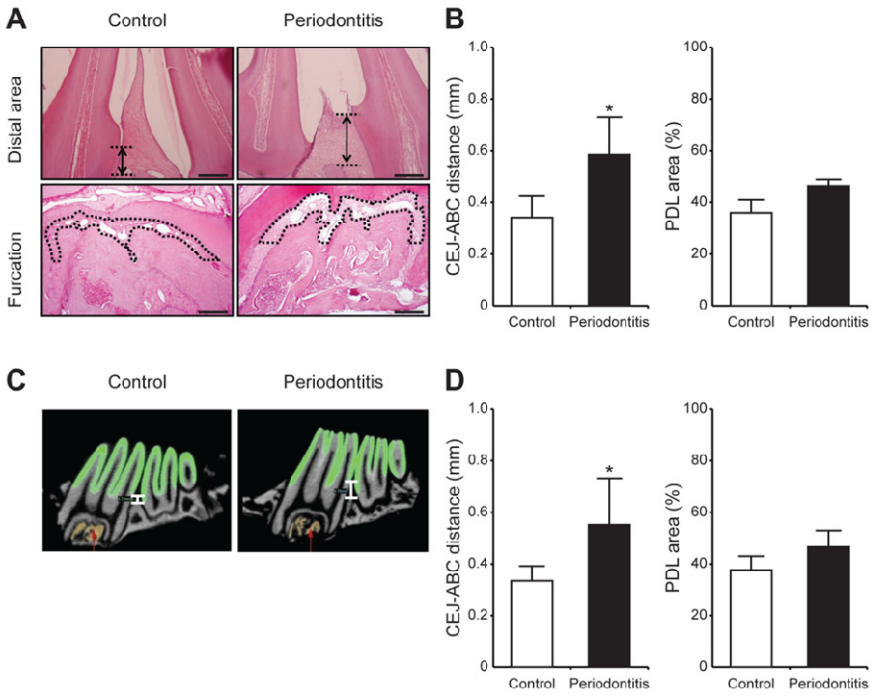

Figure 3. Alveolar bone loss of ligated tooth in gerbil. Alveolar bone level in distal area and furcation of first molar were examined via light microscopy (×100 magnification) and micro-CT. (A) and (B) The CEJ-ABC distance (arrow bar) in distal area and the percentage of PDL area in furcation (black-dotted line) of gerbil. The height of the ROI was 0.2 mm from the apical side of the furcation and the PDL area was calculated by subtracting the alveolar bone area from the ROI. (C) Micro-CT images of gerbil tooth. White bar and yellow area indicate the CEJ-ABC distance in distal area and the percentage of PDL area in furcation, respectively. (D) Results of the CEJ-ABC distance and percentage of PDL area calculation for gerbil using micro-CT. Data are represented with mean ± SEM. * indicates a significant difference at $P < 0.05$. Scale bar = 100 μm.

2C; arrow bar) and the ratio of the PDL area in furcation area (lower panels of Fig. 2A and Fig. 2C; black dotted line). For all rats and mice in periodontitis group, the CEJ-ABC distance showed approximately a 2-fold increase compared to that of the control group (left graphs of Fig. 2B and Fig 2D). The CEJ-ABC distance in rats was 0.30 ± 0.00 mm for control and 0.61 ± 0.05 mm for periodontitis group. Similarly, the CEJ-ABC distance in mice was 0.41 ± 0.11 mm for control and 0.83 ± 0.11 mm for periodontitis group. The ratio of PDL area in periodontitis group of rats and mice showed a similar trend to the CEJ-ABC distance (right graphs of Fig. 2B and Fig 2D). Periodontitis group showed approximately a 1.7-fold increase in rats (51.3 ± 2.3% for control and 86.0 ± 2.2% for periodontitis) and a 2.1-fold increase in mice (42.4 ± 9.9% for control and 89.6 ± 1.6% for periodontitis) compared to those of their control group.

Alveolar bone loss in gerbils

To evaluate the alveolar bone loss by ligature-induced periodontitis in gerbils, we measured the CEJ-ABC distance (upper panels of Fig. 3A; arrow bar) and a ratio of the PDL area (lower panels of Fig. 3A; black-dotted line). Then, we confirmed the level of alveolar bone loss in two areas using micro-CT (Fig. 3C; white bar and yellow area). The CEJ-ABC distance measured by light microscopy was 0.34 ± 0.08 mm for control and 0.59 ± 0.14 mm for periodontitis group, and the same value measured in micro-CT was 0.33 ± 0.06 mm for control and 0.55 ± 0.18 mm for periodontitis group. According to both light microscopy and micro-CT, the CEJ-ABC distance in periodontitis group was significantly increased compared to that of the control group (Fig. 3B). The ratio of the PDL area was measured as 36.0 ± 5.0% for control and 46.4 ± 2.5% for periodontitis group by light microscopy, and 37.7 ± 5.4% for control and 46.6 ± 6.4% for periodontitis group by micro-CT. The ratio of the PDL area in periodontitis group was increased compared to that of control group, but it was not statistically significant. As shown in the micro-CT images, the CEJ-ABC distance and ratio of PDL area in periodontitis group were similar with those with histopathological analysis (Fig. 3D).

Discussion

In this study, we induced periodontitis by ligature in gerbil in 14 days and evaluated inflammation in gingival tissue and alveolar bone loss using histopathological and histomorphometric analyses. In addition, the degree of ligature-induced periodontitis in gerbil was compared to that of the established ligature-induced periodontitis mouse and rat models. During the 14-day experimental period, each gerbil had to have their molars re-ligated two to three times on average, while almost all the threads ligated in mice and rats remained intact. Gerbil has a unique tooth characteristic having a straighter crown shape, a deeper gingival crevice, longer cusps, and shorter roots compared to those of rat and mouse. Because of bulging shape in tooth for rat and mouse, the ligature is well maintained during the 14 days of the experiment. Thus, different tooth contours of each animal species might be associated with the duration of their ligature thread.

Ligature-induced periodontitis showed inflammation and alveolar bone resorption, representative of periodontitis lesions [3]. Periodontopathogens in the biofilm accumulating around ligatures have been proposed to be potential stimulator of bone resorption in periodontitis [4,15,16]. Periodontopathogens induce inflammatory reactions in surrounding tissues, such as recruitment of immune cells, macrophages and lymphocytes and production of proinflammatory cytokines, IL-1β and TNFα, which play major roles in resorption of alveolar bone [17,18]. Tooth-ligated gerbils showed infiltration of inflammatory cells including PMNs, lymphocytes, and macrophages in the connective tissues and in gingival epithelium, as well as vascular dilatations in connective tissues. Mouse and rat with ligature showed significant alveolar bone resorption in both distal and furcation areas. In the gerbils with ligature, significant alveolar bone resorption was shown in the distal area, but not in the furcation area. This might be ascribed to anatomical characteristics of gerbils, which are different from those of mice or rats. The gerbil molars have longer root trunk–the region between CEJ and furcation–than those of mice and rats [19], which makes the distance between ligature and furcation farther than that of the other two animals. Thus, the effect of inflammation induced by accumulated periodontopathogens on ligature might be weaker in the furcation area in gerbils. To further characterize this model, microbial profiling of ligature-induced periodontitis is required.

Gerbils have long been used as an animal model for brain ischemia or stroke studies [10-13]. Previous studies have also suggested an association between periodontitis and cerebral ischemia [20-22]. Although gerbils have different tooth anatomical characteristics from rats and mice, inflammation and prominent alveolar bone loss in the distal areas induced by ligature suggests the gerbil as an alternative animal model for ligature-induced periodontitis. As such, ligature-induced periodontitis in gerbils would be a useful model for studying any links between periodontitis and cerebral ischemia.

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Conflict of interest

The authors declare that they have no conflicting interest.

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