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Ganoderma Lucidum Reduces Inflammation-Induced Bone Loss: A Pilot Study in Rats

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Abstract:
Background: Periodontal diseases are characterized by destruction of the tooth-supporting structure and may be a hazard to general health. Ganoderma lucidum (GL) has had a variety of pharmacological activities on joint-derived tissues/cells for decades. However, the therapeutic potential of GL on periodontal destruction has not been investigated. In the present study, we investigated the effects of GL in a rat model of periodontal disease induced by ligature placement.

Materials and methods: Male Sprague Dawley rats were divided into three groups: 1) animals without ligature placement receiving empty vehicle (control); 2) animals with ligature receiving empty vehicle; 3) animals with ligature receiving GL (10 mg/kg/day). The animals were sacrificed on day 10, and tissue samples were prepared for further analysis.

Results: The results demonstrated that the administration of GL ameliorated periodontal and bone destruction. Histomorphological analyses revealed that GL treatment decreased infiltration of polymorphonuclear cells in periodontal tissues.

Conclusion: These findings suggest that GL could constitute a promising therapeutic drug to treat periodontal disease.

Introduction

Periodontitis is one of the most common chronic disorders affecting the world population1. It is defined as a chronic inflammation that drives the destruction of connective tissue and alveolar bone; finally, loss of tooth support are the cardinal signs of periodontal disease[1]. There is an increasing body of evidence supporting that periodontitis is a complex disease in which many factors are associated with the development, progression, and aggressiveness of the disease[1,2]. Although the exact mechanism is not yet clearly elucidated, the most popular view holds that periodontal disease involves intricate interactions of the biofilm with the host immunoinflammatory response and subsequent alterations in bone and connective tissue homeostasis[1,3,4].

Ganoderma lucidum (GL), a medicinal fungus called "Lingzhi" in China, is one of the most highly regarded medicinal fungi in the world[5]. GL contains many bioactive natural components, including triterpenes, polysaccharides, proteins, and unsaturated fatty acids, which could be used in the prevention and treatment of various disorders, such as cancer, hepatopathy, arthritis, hypertension, neurasthenia, and debility because of its most attractive immunomodulatory and antitumor activities[5]. In addition, GL exerts anti-inflammatory and alters bone metabolism properties

both in vivo and in vitro, which has been documented in several studies[6–8].

Based on previous findings, the pharmacological character of GL may involve antagonizing detrimental effects of inflammatory-induced periodontal destruction. Therefore, GL may alter the abnormal bone remodeling process in periodontal tissue. The purpose of this study is to investigate whether GL has the ability to ameliorate inflammation-induced bony destruction in an experimental periodontitis model that may provide solid validation of strategies to treat inflammation-induced bone loss in future.

Materials and methods

Animals

Male Sprague-Dawley rats (250–350 g) were purchased from Biolasco, Inc., Taiwan. The animals were housed and maintained in the National Defense Medical Center, an AAALAC accredited facility, at 21 ± 1 ℃ with a 12-h light/dark cycle. All experiments were conducted in accordance with guidelines for the welfare of experimental animals and were approved by an Institutional Animal Care and Use Committee (IACUC), National Defense Medical Center, Taipei, Taiwan (No. IACUC-16-138).

Experimental design and induction of experimental periodontitis

A flowchart of the study design is illustrated in Figure 1. The rats were randomly allocated into three groups with ten animals per group. The control group did not receive the induction of periodontitis and was given vehicle (normal saline) alone. The ligature group consisted of rats subjected to ligature placement that received the vehicle. For the induction of experimental periodontitis, animals were lightly anesthetized with surgical doses of sodium pentobarbital (35 mg/kg). Sterile, 3-0 (diameter; 0.2 mm) black braided silk thread (surgical silk sutures; UNIK, Taipei, Taiwan) was placed around the cervix of the upper second molars bilaterally and knotted medially as previously described[9,10]. After the rats had recovered from the anesthetic, animals were allowed to eat commercial laboratory food and drink tap water ad libitum. The ligatures were evaluated every other day, gently displaced apically into the gingival sulci to ensure a sub-gingival position, and replaced when dislodged or lost. Animals in GL groups received the same silk ligations as rats in the ligation group and were administered a daily dose of GL (Yeastern Biotech Co. Ltd., New Taipei City, Taiwan; 10 mg/kg in normal saline) by oral gavage. The concentration of GL was determined as in previous in vivo studies with minor modification[11,12]. Body weight of animals was recorded daily. Groups of animals were subjected to microcomputerized tomography (micro-CT) examination at 7 and 10 days after ligature. Animals were sacrificed by carbon dioxide inhalation at 10 days after ligature and jaw specimens were taken and fixed in 4% paraformaldehyde and prepared for histological examination.

Micro-computed tomography (micro-CT) imaging

All jaw specimens that received ligature-induced experimental periodontitis were subjected to micro-CT imaging using a multimodality preclinical imaging system (FLEX Triumph; Gamma Medica Ideas, Northridge, CA, USA) equipped with a CT sub system as previously described[9,10]. The X ray tube was operated at an accelerating potential of 75 kVp (kVp, kilovolts peak, is the maximum voltage across an X ray tube) with a beam current of 120 μA. The field of view for micro CT was fixed at 61.44 mm leading to ×2 magnification of images. The micro CT images were taken under fly mode, with 1024 projections and one frame per projection to achieve a voxel size of 120 μm × 120 μm × 120 μm. Micro CT data were acquired and reconstructed using Triumph XO software (Gamma Medica Ideas) and then visualized and analyzed using VIVID software (Gamma Medica Ideas). This enabled us to observe the morphology around the tooth and dental alveolar

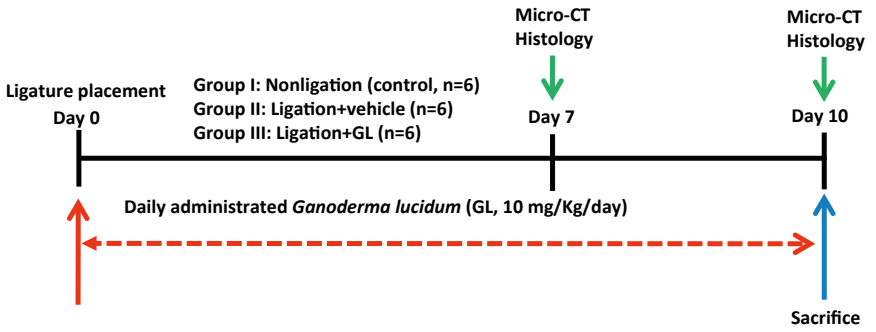

Figure 1
Flow chart and grouping of study design.

bone in all dimensions, including the cemento enamel junction (CEJ), root surface, and dental alveolar crest, as well as the relationships among these areas. Micro CT with reconstructed three dimensional images was also used to assess the distance between the CEJ and the coronal level of the alveolar bone crests (the micro CT bone levels) at both buccal and palatal furcation sites of the right and left maxillary second molars.

Histological and histometric analyses

After the micro CT scanning, the maxillary specimens on day 10 were prepared for histology. The specimens (including gingivae, teeth, and bones) around the molars were dissected, fixed in 10% buffered neutral formalin for 48 h and decalcified in 10% ethylenediaminetetraacetic acid (Sigma, St Louis, MO, USA) for four weeks. Each sample was embedded in paraffin wax and sliced into 4-μm-thick sections in mesiodistal directions. Sections were mounted on glass slides and stained with hematoxylin and eosin . On the mesial surfaces of the second molars in each rat, the following histometric measurements were performed: the distance of the CEJ to the alveolar bone crest (ABC; the alveolar crest bone level) and the area of inflammatory cell infiltrated connective tissue (ICT). The area of ICT was measured in a zone of 0.14 mm^2 of subepithelial gingiva on the mesial surface of the maxillary second molar in each rat, as in previous studies[9,10]. In brief, a grid point intersection analysis was used to estimate the areas of infiltrated and total connective tissue of interdental gingiva at ×120 magnification which has been described previously[9,10].

Statistical analysis

The experimental results were expressed as the mean ± SD (standard deviation). Statistical analysis was performed with Student's t-test for measurements between the distance of the CEJ to the ABC; and the area of inflammatory cell ICT among groups. To assess data reliability, all measurements, including the distances from CEJ to ABC, and the area of ICT were analyzed simultaneously by two certified examiners (P.-H. Huang and M.-C. Hsieh). An intraexaminer and interexaminer calibration were also performed. After calibration, the two examiners separately measured and evaluated the CEJ to the ABC and the area of ICT. The collected data were then input into a statistical package, SPSS for Windows (Version 22.0; SPSS, Inc, Chicago, IL), for statistical analysis. The level of statistical significance was set at $p < 0.05$.

Results

Clinically, at the time of sacrifice, signs of inflammation were observed in gingival tissue around the ligated molars in the ligation group, whereas no marked gingival inflammation

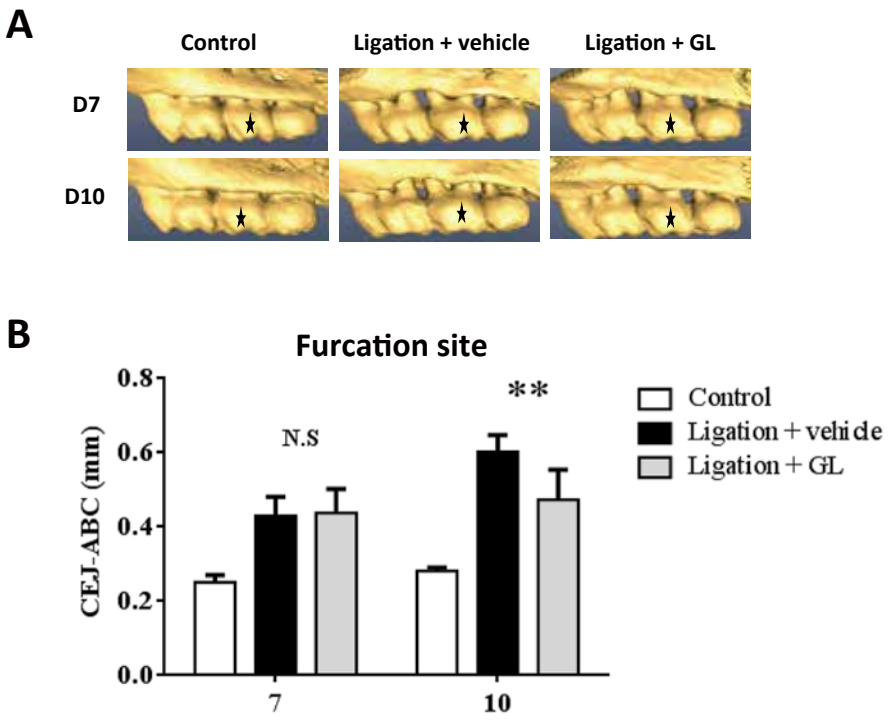

Figure 2

Effect of Ganoderma lucidum (GL) on alveolar bone destruction and tissue damage and quantificatative analysis of the protective effect of GL on alveolar bone destruction and tissue damage. (A) Reconstructed three-dimensional micro-CT images for maxilla second molars alveolar bone level among non-ligation (control), ligation-plus-vehicle, and ligation-plus-GL groups of rats on experimental day 7 and day 10 (asterisks indicate the molars with ligation). (B) Alveolar bone loss quantification was performed from reconstructed micro-CT images through the measurements of the distance between the cemento-enamel junction and the alveolar crest at the buccal "furcation site" of the maxilla second molar with VIVID software (Gamma Medica-Ideas, Northridge, CA, US). Data expressed as the mean ± SD are obtained from 5 animals in each experimental group. *P < 0.05, **P < 0.01, ***P < 0.001 versus ligation-plus-vehicle group.

Figure 3

Effect of GL on periodontal tissue damage. Histological observation (H & E stain) of maxillary intermolar tissue in non-ligation (control), ligation-plus-vehicle, and ligation-plus-GL groups at day 10 (H & E staining, original magnification × 100).

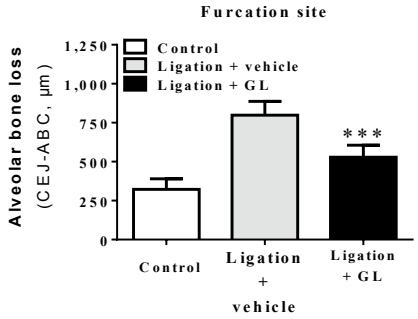

Figure 4

Quantitative analysis of the protective effect of GL on alveolar bone destruction. Alveolar bone loss quantification was performed from histological sections through the measurements of the distance between the cemento-enamel junction (CEJ) and the alveolar crest (ABC) at the buccal "furcation site" of the maxilla second molar (CEJ-ABC, in µm). Data expressed as the mean ± SD are obtained from five animals in each experimental group. *P < 0.05, **P < 0.01, ***P < 0.001 versus ligation-plus-vehicle group.

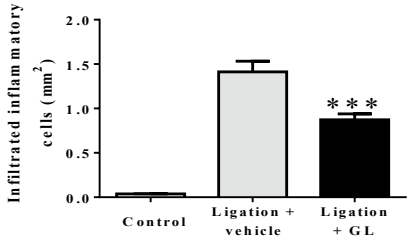

Figure 5

Quantificatative analysis of the protective effect of GL on inflammatory cells infiltration. Infiltrated inflammatory cells was quantitatively measured from series histological sections. Data expressed as the mean ± SD are obtained from five animals in each experimental group. *P < 0.05, **P < 0.01, ***P < 0.001 versus ligation-plus-vehicle group.

was observed in the control and ligation-plus-GL groups (data not shown).

Alveolar bone loss following induction of periodontal disease was assessed in all experimental groups using reconstructed three-dimensional micro-CT images (Figure 2). Apically located ABC at ligation sites in rats from the ligation group were generally observed, whereas no apically located ABC were observed at the same sites in rats from the nonligation (control) group (Figure 2A). At the buccal furcation site of the maxillary second molars, the distance from the CEJ to ABC (CEJ–ABC) was significantly different between the groups ($p < 0.01$, Figure 2A). The placement of ligatures caused a significant increase in CEJ–ABC distance when compared with that in rats without ligatures. For the animals in the GL group, the CEJ–ABC distances were less than the ligation-plus-vehicle group at days 7 and 10 (Figure 2). At day 10 (D10), GL treatment revealed significantly shorter CEJ–ABC distance when compared with rats in the ligation group ($p < 0.01$, Figure 2).

The histopathological analysis of the region between the first and second molars of the control group shows the well-conserved periodontal architecture (Figure 3). At D10, alveolar bone loss was significantly observed in rats subjected to ligature-induced periodontitis (Figure 4). However, a significantly shorter CEJ–ABC distance was measured in the animals receiving GL ($p < 0.001$, Figure 4). A robust inflammatory cell infiltration was also observed in rats subjected to ligature-induced periodontitis when compared with the control group ($p < 0.001$) (Figure 5). Animals that received daily treatment with GL (10 mg/kg) had significantly diminished histological changes induced by experimental periodontitis (Figure 3), which was demonstrated by a recognizable reservation of the tooth-supporting structure ($p < 0.001$, Figure 4), and a significant decrease in the number of infiltrated inflammatory cells was also noted ($p < 0.001$, Figure 5).

Discussion

To our knowledge, this study is the first attempt to investigate the therapeutic effect of GL on periodontal destruction in an animal model. In the present pilot study, we have demonstrated that the administration of GL ameliorates the alveolar bone loss of experimental periodontitis in a rat model which was elicited by ligature placement.

Strategies have been proposed to establish adjunctive therapeutic approaches (i.e., systemic host-modulation therapy) to mediate periodontal breakdown resulting from intricate interactions between bacterial attack and host inflammatory responses[9,10,13,14]. Different kinds of drugs, especially some small molecular drugs (i.e., quercetin, magnolol, curcumin, melatonin etc.), are considered to be capable of managing the periodontal destructive process by means of anti-inflammation,

anti-osteoclastic differentiation properties[9,10,15,16]. These substances may be a potential approach to prevent and treat periodontal diseases in clinical settings[13,14,17]. Based on such background, properties of GL and the character of inflammation-driven alveolar bone resorption in periodontitis elicited by bacterial accumulation in this study, the use of GL was demonstrated as a novel therapeutic regimen to alleviate inflammation-induced periodontal destruction (Figures 3 and 5). In the present study, we provided new evidence of how GL quenches the ligature-induced inflammatory bone resorption (Figure 2). Our work, for the first time, demonstrated that GL inhibits bone resorption in the context of inflammation through in vivo approaches (Figures 2 and 4). In addition, our results demonstrated that the potential protective effects of GL on periodontal destruction are comparable to other studied small molecule drugs[9,10,15,16].

Although it is not easy to extrapolate from a bench experiment to bedside application, these results reveal the potential for therapeutic use of GL for the prevention and treatment of periodontal disease. In view of the increasing public health concerns regarding periodontal health, a therapeutic agent, such as GL, generates much interest by demonstrating antibacterial, anti-inflammatory, and osteogenic properties, and may be particularly useful as a new approach in clinical periodontal therapeutics[14].

In this study, there were some limitations concerning the potential protective effects of GL on inflammation-induced bone loss in vivo. First, in this study, the observation period is 10 days. However, longer follow-up is beneficial to evaluate the healing process and therapeutic potency of GL on experimental periodontitis in rats. Second, to elucidate the pathophysiological findings behind the anti-inflammatory effects of GL, series in vitro or cell-based studies should be encouraged to delineate the possible mechanisms or signaling cascades. Third, further studies on delivery systems for periodontal use, behavioral mechanisms of drug in the diseased periodontal environment in in vivo systems, and other side effects on oral soft and hard tissue remain to be conducted.

Conclusion

In conclusion, GL could contribute to the inhibition of inflammatory and pathological responses during the evolutional changes of experimental periodontitis. This preliminary study suggests that GL may constitute a promising biological therapeutic drug to treat inflammation-induced bone loss.

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Conflicts of interest

The authors declare no financial or commercial conflict of interest.

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