



Preliminary report

Subantibiotic dose of azithromycin attenuates alveolar bone destruction and improves trabecular microarchitectures in a rat model of experimental periodontitis: A study using micro-computed tomography



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ABSTRACT

Azithromycin, a macrolide antibiotic, has anti-inflammatory and immunomodulatory activities apart from its antibacterial properties. In this study, we examined the efficacy of subantibiotic dose of azithromycin on ligature-induced periodontitis in rats using micro-computed tomography (micro-CT) imaging and bone parameter analysis. Male Sprague-Dawley rats were allocated to the following four groups: non-ligation (NL) group; ligation-only (L) group; ligation-plus-subantibiotic dose azithromycin (SA) group; and 4) ligation-plus-antibiotic dose azithromycin (AA) group. The rats from Groups L, SA and AA were subjected to periodontitis by placing a ligature around lower right first molar. Immediately after ligation, the rats in SA and AA groups received daily intraperitoneal injections of azithromycin at a dosage of 3.5 or 10 mg/kg body weight, respectively. The ligatures were maintained for 2 weeks at which time the rats had their mandibles hemisected for micro-CT analysis. Subantibiotic dose of azithromycin strongly suppressed reductions in alveolar bone height and bone volume fraction caused by experimental periodontitis. When subantibiotic dosage of azithromycin was administered to rats, ligature-induced alterations in microarchitectural parameters of trabecular bone were significantly reversed. Rats treated with subantibiotic dose of azithromycin presented no significant difference compared to rats with antibiotic dosage in all parameters. While further studies are necessary, subantibiotic dose of azithromycin could be utilized as a host modulator for the treatment of periodontitis.

1. Introduction

Periodontal disease, a chronic inflammatory disorder that occurs within the tissues supporting the teeth, is induced by some gram-negative anaerobic bacterial species that reside in the subgingival area. The inflammatory response of host to periodontal pathogenic bacteria and their products is characterized by an excessive and sustained generation of a variety of inflammatory mediators by the cells of periodontium [1,2], and these mediators ultimately causes tissue destruction in periodontal disease [3–5]. Accordingly, the treatment approach aiming at these destructive mediators may be of great importance in the management of periodontal disease [6].

Macrolide antibiotics have been reported to be effective against various gram-positive and specific gram-negative bacteria [7]. It is well

known that macrolides have a variety of unique biological activities apart from antimicrobial properties, including anti-inflammatory and immunomodulatory characteristics observed both in vivo and in vitro [8,9]. The anti-inflammatory effectiveness of macrolides seemed to be attributed to their suppressive activity on the generation of proinflammatory mediators in various type of cells [10,11].

Azithromycin, a macrolide antibiotic with a 15-atom lactone ring, possesses enhanced antimicrobial property against gram-negative bacteria [12]. There is substantial evidence to support the supplementary use of azithromycin in the therapy of periodontitis [13,14]. In addition, azithromycin also has notable anti-inflammatory and immunomodulatory activities like conventional macrolides due to its suppressive influences on proinflammatory cytokine release by inflammatory cells [15].

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We have previously shown that azithromycin significantly attenuates the generation of IL-6 in macrophages stimulated with LPS isolated from *Prevotella intermedia*, a pathogen related to periodontal disease, implying that it may have possible application as a host response modulator in the treatment of inflammatory periodontal disease [16]. Therefore, this study aimed to assess the influence of subantibiotic dosage of azithromycin on alveolar bone destruction in rats with experimental periodontitis by employing micro-computed tomography (micro-CT) imaging and bone parameter analysis.

2. Materials and methods

2.1. Animals

A total of 19 male Sprague-Dawley rats, 7-week of age and weighing 200–220 g, were obtained from Koatech (Pyeongtaek, Korea). Throughout the experiment, the animals were kept in individual cages under appropriate temperature and humidity and fed standard laboratory food and tap water ad libitum. All of the animal procedures described below were conducted in agreement with the protocols approved by the Institutional Animal Care and Use Committee of Pusan National University.

2.2. Experimental design

The rats were allocated randomly to one of the following four treatment groups: non-ligation (NL) group (n = 5); ligation-only (L) group (n = 4); ligation-plus-subantibiotic dose azithromycin (SA) group (n = 4), in which 3.5 mg/kg body weight of azithromycin was administered; and 4) ligation-plus-antibiotic dose azithromycin (AA) group (n = 6), in which 10 mg/kg of azithromycin was administered. The rats in NL Group were not exposed to ligature-induced periodontitis and served as healthy control. The other rats from Groups L, SA and AA were subjected to experimental periodontitis. Experimental periodontitis was induced by locating a 4-0 silk suture around lower first molar on the right side of each rat, as described in our previously published study [17]. Immediately after ligation, the rats in SA and AA groups received daily intraperitoneal injections of azithromycin at a dosage of 3.5 or 10 mg/kg body weight, respectively, whereas animals in Groups NL and L were given same volume of vehicle solution. The ligatures were maintained for 2 weeks at which time all rats from each group were sacrificed and had their mandible hemisected and fixed in 10% neutral buffered formalin for micro-CT evaluation.

2.3. Micro-computed tomography imaging

We employed a micro-CT system (inspeXio SMX-90CT; Shimadzu, Tokyo, Japan) to scan and image mandibular block specimens. Using appropriate analysis software (TRI/3D-BON; RATOC System Engineering, Tokyo, Japan), three-dimensional (3D) digitized images were produced for quantitative analysis of the alveolar bone, as described in our previously published study [17].

2.4. Linear measurements of alveolar bone loss

All scans were realigned so that both the scan axes and anatomical landmarks aligned uniformly. To determine the extent of alveolar bone height loss, measurements were obtained from the cemento-enamel junction to the crest of alveolar bone on 3D digitized micro-CT images at 4 specific points in the interproximal region between the mandibular first and second molars, according to our previously published method [17].

2.5. Volumetric analysis

Volumetric measurements of the alveolar bone were carried out

following the creation of 3D regions of interest (ROI). The interproximal region between the first and second molars was defined as ROI, following the procedure proposed by Luan et al. [18], beginning coronally by a line drawn immediately below the furcation roofs of the first and second molars, then extending apically for 100 serial scan sections. 2D contours were outlined at even intervals (five data planes each) and the 3D ROI was generated by using the appropriate software (TRI/3D-BON) based on the resultant 2D contours. Bone volume fraction (BVF), the fractions of the residual bone volume (BV) versus total volume (TV) in the created ROI images, was determined with the help of an analysis software package (TRI/3D-BON).

2.6. Analysis of microarchitectural bone parameters

The following characteristics of trabecular microstructure, including trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N), bone mineral density (BMD) and structure model index (SMI), were analyzed in the generated ROIs by using an analysis software (TRI/3D-BON). Tb.Th, Tb.Sp and Tb.N were used to determine the quantity, thickness and organization of trabecular bone. BMD, the volumetric mineral density in the ROI, is calculated with an internal reference in micro-CT units. SMI, which is defined as an important measure of trabecular quality, assesses the plate- versus rod-like properties of the bony trabecular [19].

2.7. Statistical analysis

All data were presented as means \pm SEM. A software package program (SPSS 21.0; SPSS, Chicago, IL, USA) was utilized for the statistical analyses of data. The differences in bone parameters between the groups were evaluated by an analysis of variance (ANOVA) with Tukey *post-hoc* comparisons. A *p* value of < 0.05 was set to indicate a statistically significant difference.

3. Results

3.1. Effect of azithromycin on alveolar bone height

The representative 3D micro-CT images from 2 week specimens are shown in Fig. 1. There was an apparent reduction in the alveolar bone height around the mandibular first molar in Group L (Fig. 1B) compared with Group NL (Fig. 1A). As Fig. 1 reveals, it is obvious that specimens from SA and AA groups displayed dramatic rebound of bone resorption induced by 2 weeks of ligature placement (Fig. 1C, D). Fig. 2 depicts a comparison of the linear distance between the CEJ and alveolar bone crest in the interproximal area between the lower first and second molars. The value for Group L was significantly increased compared with Group NL ($p < 0.01$), demonstrating that placement of ligature resulted in vertical alveolar bone resorption in the 14 days of experimental period (Fig. 2). Compared to Group L, the reduction of alveolar bone height was significantly recovered in Groups SA and AA ($p < 0.05$) (Fig. 2). Notably, bone loss that was induced by ligature was found to be decreased by 65% in Group SA and by 68% in Group AA. Group SA treated with 3.5 mg/kg of azithromycin presented no significant difference when compared with Group AA treated with 10 mg/kg of azithromycin.

3.2. Effect of azithromycin on residual bone volume fraction

The BVF measurement for Group L was significantly reduced than that for Group NL ($p < 0.05$) (Fig. 3). Group L experienced an average 24% decrease in the proportion of the ROI that is occupied by bony structure when compared to that for Group NL. The BVF value found in Group SA was significantly higher than that in Group L ($p < 0.05$) (Fig. 3). When rats were treated with subantibiotic and antibiotic doses of azithromycin, about 83% and 60% of the decrease in the BVF caused

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