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Ultrasound stimulation attenuates resorption of tooth root induced by experimental force application

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ABSTRACT

Root resorption is an adverse outcome of orthodontic tooth movement. However, there have been no available approaches for the protection and repair of root resorption. The aim of this study was to evaluate the effects of low-intensity pulsed ultrasound (LIPUS) on root resorption during experimental tooth movement and the effects of LIPUS in the RANKL/OPG mechanism in osteoblasts and cementoblasts *in vitro*. Twenty four Wistar strain male rats of 12-week-old were used in this study. The upper first molars were subjected to experimental movement in the mesial direction for 1–3 weeks. Through the experimental periods, the right upper first maxillary molar was exposed to LIPUS (LIPUS group) every day for 1, 2 or 3 weeks. The nature of root resorption was observed and then quantified by histomorphometric analysis. In the 2 weeks period, significantly greater amount of tooth movement was observed in the LIPUS group ($p<0.05$). In addition, LIPUS group showed less root resorption lacunae and lower number of odontoclasts. In the period of 3 weeks, LIPUS group presented significantly shorter length of root resorption lacunae and smaller amount of root resorption area ($p<0.01$). The number of odontoclasts and osteoclasts was also significantly lower in the LIPUS group ($p<0.01$ and $p<0.05$, respectively). However, no significant differences could be found regarding the amount of tooth movement. It is shown that LIPUS exposure significantly reduced the degree of root resorption during tooth movement without interrupting tooth movement. *In vitro* experiments showed that MC3T3-1 constitutively expressed higher levels of RANKL and RANTES mRNA comparing to OCCM-30. However, OPG mRNA expression was much higher in OCCM-30. LIPUS stimulation significantly increased the mRNA expression of RANKL in MC3T3-E1 at 4 ($p<0.01$) and 12 h ($p<0.05$), although OPG mRNA expression was not affected by LIPUS. In contrast, the expression of RANKL and OPG mRNAs were both significantly increased by LIPUS in OCCM-30 at 12 h ($p<0.01$). Moreover, LIPUS application suppressed the up-regulation of RANKL mRNA induced by compression force in OCCM-30, but no similar effect could be observed in MC3T3-E1. In conclusion, it is suggested that LIPUS exposure significantly reduces root resorption by the suppression of cementoclastogenesis by altering OPG/RANKL ratio during orthodontic tooth movement without interfering tooth movement. LIPUS may be an effective tool to prevent root resorption during tooth movement and is applicable to clinical use in near future.

Introduction

Root resorption is an adverse outcome of orthodontic tooth movement. A certain degree of root resorption occurs in most treatment cases, as is reported that 93% of treated adolescents experienced some form of root resorption [1]. Approximately 15% exhibited moderate to severe apical root resorptions [2] and 10–20% of cases had severe resorption of >3 mm [3]. In most cases, only the surface layers of cementum are resorbed after orthodontic tooth movement, and later repaired by cementoblasts. However, when root resorption exceeds the reparative capacity of cementum, the apical resorption of the root occurs and root shortening is evident [4–6]. Root resorption during orthodontic treatment is well understood as a multifactorial event. Several biological and mechanical factors have been identified as the causes to increase susceptibility to root resorption. Age, gender, nutrition, genetics, type of appliance, amount of force, orthodontic treatments with or without extractions, duration of treatment, and the distance the teeth are moved all have influences on root resorption [5–7]. In general, root resorption is associated with extensive tooth movement and heavy force application. However, a conclusive cause still remains unclear [5–8].

In animal experiments, several trials have been performed to prevent root resorption during tooth movement. Loberg and Engstrom [9] reported that administration of thyroxin reduced root resorption.

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Bisphosphonates have been reported to decreased root resorption [10]. Reduction of root resorption with echistatin, a RGD-containing peptide has been reported [11]. Several other pharmacological agents on root resorption also have been explored to prevent or minimize the incidence of root resorption [12–15]. Unfortunately, no clinically acceptable modalities have been established for the treatment of root resorption.

Mechanical stresses have been reported to activate both osteoclasts and osteoblasts, leading to bone remodeling. Various types of mechanical stimuli have been shown to promote proliferation and differentiation of osteoblasts. Among them, ultrasound (US) stimulation has shown to be very effective in this regard. US is a form of mechanical energy which can be transmitted into living tissues as high frequency acoustic pressure waves, resulting in biochemical events at the cellular level [16]. US therapy has been consistently reported to promote bone formation [17], being clinically employed to promote healing of bone fractures [18]. Different intensities of pulsed US have distinct biological effects on mineralization process [19,20]. It is commonly recognized that low-intensity pulsed US (LIPUS) (30–150 mW/cm^2) is very effective in healing of bone fractures *in vivo* [21,22] and anabolic biophysical effects on several cell types *in vitro* studies [23–25].

Interestingly, recent studies have provided evidence that US also plays an important role in the metabolisms of periodontal tissue [26–28]. El-Bialy et al. [29] demonstrated that LIPUS can prevent tooth root resorption during experimental tooth movement in humans, although its mechanism was not examined in detail. We conducted *in vitro* studies on the effects of LIPUS on mature cementoblast metabolism and demonstrated that LIPUS was effective in increasing calcium content, up-regulation of alkaline phosphatase (ALP) activity, collagen synthesis and mineralization capacity [30,31]. LIPUS exposure also promoted the differentiation of immature cementoblasts [32].

Despite the increasing interest on the role of mechanical stimulation on cementoblast metabolism, literature still lacks information on the effect of LIPUS stimulation on preventing and/or repairing resorbed root surfaces. Thus the aim of this study was to assess the effect of LIPUS stimulation on root resorption caused by experimental tooth movement in rats and to investigate the role of LIPUS in the RANKL/OPG mechanism in osteoblasts and cementoblasts *in vitro*.

Material and methods

Animal experiments

24 Wistar strain male rats of 12-week-old, weighing 300–350 g, were used both for control andLIPUS groups. Then, they were equally divided into 3 groups for different experiment periods (1, 2 and 3 weeks). The protocol was approved by the Animal Care and Use Committee of Hiroshima University. Each animal was anesthetized with intra-abdominal injections of sodium pentobarbital (Nembutal, Dinabott, Osaka, Japan) at a dose of 50 mg/kg b.w. Mini-screws of 1.4 mm in diameter and 2.0 mm in length (Dual-top Anchor Screw, Jeil Medical, Seoul, Korea) were implanted bilaterally 1 mm distally to the maxillary insicors. Ni–Ti coil spring (Rocky Mountain Morita Co., Tokyo, Japan) was used to create 50 gf traction to move mesially the upper first molar by use of the mini-screws as the anchorage (Fig. 1A). These procedures were performed under sterile conditions.

All animals were fed on conventional solid diet (Crea Japan, Tokyo, Japan) and water *ad libitum*. After 1, 2, and 3 weeks force application, the upper jaws were resected en bloc under ether anesthesia (Nakalai Tesque, Kyoto, Japan).

Ultrasound exposure machine (OSTEOSONIC, ITO, Tokyo, Japan) was employed in this study. This system is equipped with transducers of 9.6 cm^2 circular surface area. The sound head of this device has an average beam non-uniformity ratio (BNR) of 3.2–3.6:1 and an effective radiating area (ERA) of 90%.

The rats were kept immovable without any use of anesthesia or sedation, and the ultrasound transducer was placed in contact with one side of the face, in the region corresponding to the upper first molar. The target region had the fur shaved and coupling gel was constantly in place in order to optimize penetration of the ultrasound waves into the tissues. Aiming to exclude a possible side-related effect, half of the experimental rats received ultrasound stimulation on the right side of the face and the other half received ultrasound stimulation on the left side. Control group refers to the sham-exposure side of the rats. A pulsed US signal was transmitted at a frequency of 1 MHz (the pulse repetition frequency = 100 Hz), with a spatial-average intensity of 150 mW/cm^2, and pulsed 1: 4 (2 ms on and 8 ms off). The stimulation

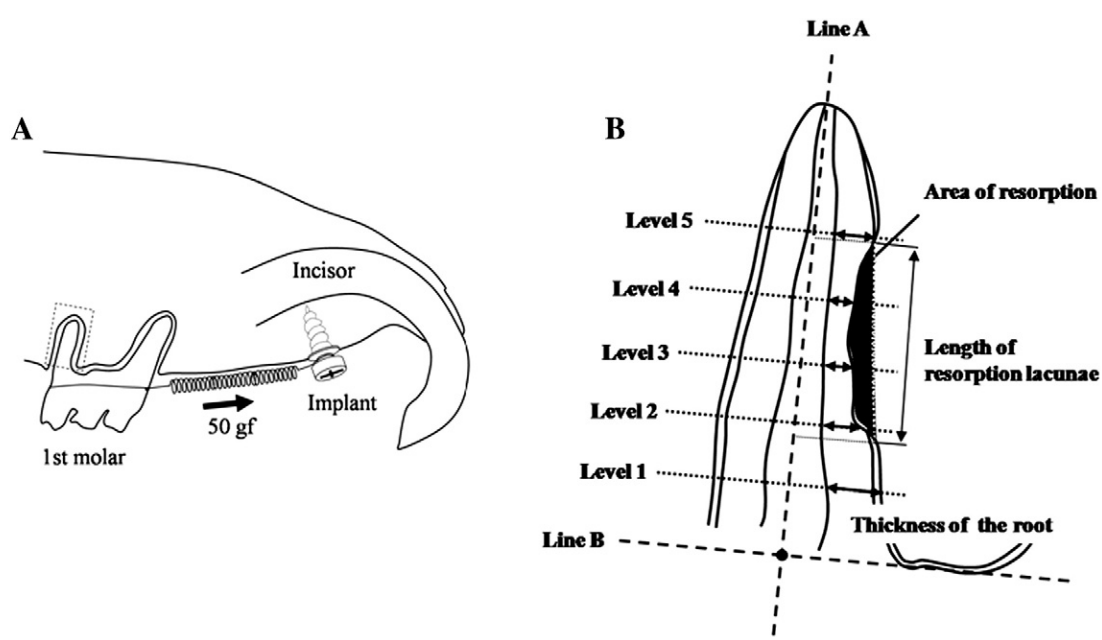

Fig. 1. Schematic representation of the appliance for tooth movement. (A) Schematic representation of the appliance for tooth movement. The maxillary first molar was protracted using 50 gf activated Ni–Ti coil spring. (B) Schematic representation of histomorphometrical analysis. Line A indicates the long axis of the distal root, and line B is perpendicular to line A through the dentine-cementum junction of the furcation area. The thickness of the root and cementum were measured at five apico-gingival levels from apical to the insertion of the two lines.