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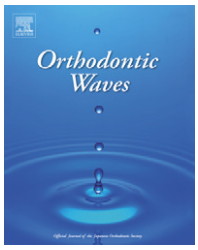

Research paper

ALP activity decreased in compressed PDL cells obtained from severe orthodontically root resorption

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ABSTRACT

Alkaline phosphatase (ALP) plays an important role in periodontal ligament (PDL) itself and alveolar bone in relation of calcification. However, very little is known about the relationship between external apical root resorption (EARR) during orthodontic treatment and ALP.

We examined the effect of compressive force (CF) on ALP activity in human PDL (HPDL) cells from patients with severe orthodontically induced EARR. ALP activity, PGE_2 and IL-1β release were determined. The decrease of ALP and the increase of PGE_2 and IL-1β were greater in the severe root resorption (SRR) group than in the non-resorption (NR) group ($p < 0.001$, two-way repeated measured ANOVA). NS-398, selective cyclooxygenase-2 (COX-2) inhibitor, and/or IL-1β antibody partially prevented the decrease in ALP activity. The presence of both factors together prevented almost completely the decrease in ALP activity to the control level ($p < 0.001$).

Therefore, the decrease in ALP activity was mediated mainly by PGE_2 and IL-1β produced by compressed PDL cells obtained from SRR. These results suggest that the decrease of ALP in compressed HPDL cells may be highly involved in the incidence of severe EARR during orthodontic treatment.

1. Introduction

External apical root resorption (EARR) is an unavoidable pathologic consequence of orthodontic tooth movement. It can be defined as an iatrogenic disorder that occurs, unpredictably, after orthodontic treatment, whereby the resorbed apical root portion is replaced with normal bone. EARR is a sterile inflammatory process that is extremely complex and involves various disparate components including mechanical forces, tooth roots, bone, cells, surrounding matrix, and certain known biologic messengers [1]. Killiany [2] reported EARR of >3 mm to occur at a frequency of 30%, with only 5% of treated individuals found to have >5 mm of root resorption. The etiology of EARR following orthodontic treatment is not fully understood. Harris et al. [3] reported that the sum of the effects of patients' sex and age, and severity of the malocclusion, and the kind of mechanics used accounts for little of the overall variation in EARR. In the last 10 years, interestingly, it was suggested that individual susceptibility [4,5], genetics [6–8], and systemic factors [9] are risk factors for EARR.

Alkaline phosphatase (ALP) is considered to be involved in the process of calcification of various mineralizing tissues [10], and it has long been used as an indicator of osteoblastic

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activity in bone tissue and cell culture systems [11,12]. The ALP activity in the periodontal ligament (PDL) cells is usually higher than that in cells of other soft connective tissues, including the gingiva [13]. Previous studies demonstrated that human PDL (HPDL) cells have higher ALP levels than those in gingival fibroblasts [14,15].

Our previous studies reported ALP activity in human PDL cells was decreased in response to mechanical stress [16]. Thus, the regulation of expression of ALP in PDL may play critical roles in bone remodeling during orthodontic tooth movement. Furthermore, Yamaguchi and Shimizu [17] reported that the decrease in ALP activity was mainly mediated by PGE_2 and IL-1β produced by PDL cells in response to mechanical stress. However, very little is known about the relationship between EARR and these mediators during orthodontic tooth movement.

Thus, this study examined that the effect of compressive force on ALP activity from HPDL obtained from patients with severe EARR and that the relationship between ALP activity, PGE_2 and IL-1β produced by PDL cells in response to compressive force.

2. Materials and methods

2.1. Cell culture

HPDL cells were prepared according to a modification of the method of Somerman et al. [15], as described previously [16]. Briefly, PDL tissues were taken from the roots of premolars extracted from healthy young volunteers during the course of orthodontic treatment, after obtaining informed consent from the donors, and used according to a protocol reviewed by the ethic committee of Nihon University School of Dentistry at Matsudo.

The HPDL tissue specimens were placed in 35-mm tissue culture dishes, and covered with sterilized glass coverslips. The medium used was α-MEM (Gibco, Grand Island, NY, USA), which was supplemented with 100 μg/mL of penicillin-G (Sigma Chemical Co., St. Louis, MO, USA), 50 μg/mL of gentamicin sulphate (Sigma), 0.3 μg/mL of amphotericin B (Flow Laboratories, McLean, VA, USA), and 10% fetal calf serum (FCS, Cell Culture Laboratories, Cleveland, OH, USA). The cultures were kept at 37 °C in a humidified incubator (Forma CO_2 incubator MIP-3326, Sanyo Electric Medica System Co., Tokyo, Japan) in the presence of 95% air and 5% CO_2. When the cells growing from the explants had reached confluence, they were detached with 0.05% trypsin (Gibco) in PBS for 10 min, and then subcultured in culture flasks. The HPDL cells were cultivated in a serial passage culture and were preserved by freezing, and then, after the completion of orthodontic treatment, we conducted an experiment on the preserved PDL cells of five patients in whom severe root resorption of central incisors had taken place. All cases were considered to be Angle class I crowding extraction cases. On the basis of the Malmgren classification [18], root resorption was considered to be severe if at least one-third of the apex of the root had been absorbed. As the control, the cells obtained from five patients with resorption of no more than 2 mm were used.

The PDL cells obtained from severe root resorption and control were as follows—severe root resorption (SRR) group: 2 males, 3 females; 18–25 years old, average: 22.5 years, S.D.: 2.8 years; non-resorption (NR) group: 2 males, 3 females; 17–26 years old, average: 23.2 years of age, S.D.: 3.3 years. For the experiments, HPDL cells from passages six to nine were used.

2.2. Application of compressive forces

In order to reproduce the conditions of pressure during orthodontic tooth movement, we performed the following in vitro experiment, in accordance with the method developed by Kanzaki et al. [19]. HPDL cells were continuously compressed using a uniform compression method as a model of pressure at the site of orthodontic movement. Static compression force is thought to mimic that found *in vivo* during orthodontic treatment. In the present experiments, the cells were only stimulated once. Briefly, a 30 mm in diameter cell disk was placed over a nearly confluent cell layers in the wells of a 6-well plate, on top of which was placed a glass cylinder. Compression force was then controlled by inserting lead granules into the cylinder. Prior to the application of the compression force, the cells were pre-incubated for 1 h in culture medium containing 2% FCS, and then they were subjected to 0.5, 1.0, 2.0, or 3.0 g/cm^2 of compression force for 48 h [20,21].

Next, to clarify whether PGE_2 and IL-1β released by PDL cells of both the SRR and NR groups in response to compressive force (2.0 g/cm^2, 12 h) affect the ALP activity, NS-398 (Cayman Chemical, ML, USA), a selective inhibitor of cyclooxygenase-2 (COX-2), was added to the culture medium for 1 h before the application of compressive force, and/or rabbit anti-human IL-1β polyclonal antibody (10 ng/well, 80% IgG and 20% IgM, Genzyme, Cambridge, MA, USA) was also added to the culture medium 3 h before the end of the experiment for neutralization of IL-1β in the condition medium.

2.3. Assay of ALP activity

The PDL cells were washed three times with PBS on a culture dish, and 1 ml of Tris–HCl containing 5 mM $MgCl_2$ (10 mM, pH 7.4) was added. Then the cells were collected by a cell scraper (Becton Dickinson and Company) and sonicated for 1 min, ALP activity was assayed by the method of Lowry et al. [22]. The amount of *p*-nitrophenol produced was measured at 410 nm. One unit of enzyme was defined as the activity which liberated 1 μmol of product per min at 37 °C, and ALP activity was shown as mU/well.

2.4. PGE_2 and IL-1β determination

The amounts of PGE_2 and IL-1β released from HPDL cells into the culture medium were determined in duplicate using commercially available enzyme-linked immunosorbent assay kits (Quantikine, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Each sample was centrifuged for 10 min at 900 × *g* to discard the cells, after which the supernatants were collected and frozen at −80 °C until the assay for PGE_2 and IL-1β concentrations.