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RANKL expression in rat periodontal ligament subjected to a continuous orthodontic force

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ABSTRACT

Objectives: This study investigated longitudinal changes in receptor activator NF kappa B ligand (RANKL) expression in periodontal ligament (PDL) cells subjected to a continuous orthodontic force.

Design: Fifty-five-day-old male Wistar rats were divided into experimental and control groups. The experimental group had the first molars laterally expanded by a continuous orthodontic force. In each group, the horizontal section specimens were embedded in OTC compound and frozen at 0, 1, 3 and 7 days after the expansion. Sections were observed by immunostaining with anti-RANKL and the tartrate-resistant acid phosphatase (TRAP) staining.

Result: Immunoreaction of RANKL and TRAP-positive cells were observed in the distal region of the controls and on the compressed side of the expansion group in the 3 and 7 days. Immunoreaction of RANKL was also observed after 1 day on the compression side of the expansion group, but here TRAP-positive cells were few.

Conclusions: The experiments have showed that PDL cells are continuously producing RANKL on the PDL pressure side of rats subjected to mechanical stress with a continuous orthodontic force, there was no noticeable the excessive appearance of osteoclasts however. Considering this, it is expected that not only RANKL production but also other cytokines play an important role in the balancing adjustment in the alveolar bone remodeling.

1. Introduction

Alveolar bone is remodelled by resorption and formation under the mechanical stress by orthodontic force.[1] The mechanical stress on a tooth is transduced to the periodontal ligament (PDL), and PDL cells respond to the mechanical stress regulating the resorption and formation of bone matrix by signalling to the surrounding cells.[1] Osteoclasts form on the compressed side of an orthodontically moving tooth and resorb the alveolar bone resulting in the change of position of the tooth.[2,3]

The receptor activator NF kappa B ligand (RANKL) has been identified as an essential factor in osteoclastogenesis and is believed to contribute to the recruitment of osteoclast precursor cells and their differentiation.[4–11] The RANKL is expressed on osteoblasts and stromal cells, and it supports every step of osteoclastogenesis, the differentiation, fusion, survival, and activation.[12] In addition, it has been found that bone resorbing agents such as $1.25(OH)_2$ Vitamin D_3, parathyroid hormone, and interleukin-1, cause the upregulation of the RANKL gene expression in osteoblasts in vitro,[6,13]

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suggesting that RANKL provides a crucial signal for osteoclast formation.

Together with osteoblasts and osteoclasts, PDL cells are thought to play a role in alveolar bone remodeling. The function of osteoclasts is regulated by interaction with these PDL cells, and it is therefore also important to observe the expression of RANKL in PDL cells that could be related to the biological functions of osteoclasts. Investigations have reported the expression of RANKL in PDL cells under conditions with intermittent forces,[14–16] however, bone-remodelling kinetics through RANKL has not been established in PDL cells under a continuous orthodontic force used in standard clinical treatment. This study was designed to investigate the longitudinal changes in RANKL expression in PDL cells subjected to a continuous orthodontic force.

2. Materials and methods

2.1. Subjects

Fifty-five-day-old male Wistar rats were used in this experiment. The continuous orthodontic force was supplied by a Ni–Ti open coil spring (17.6 g) and the upper first molars were laterally expanded (Fig. 1). Rats were anaesthetized with sodium pentobarbital (7 mg/kg, Nembutal, Abbott Laboratories, North Chicago, IL) at 1, 3, and 7 days after expansion and perfused through the heart with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The maxillaes were removed and immersed in 10% EDTA solution for demineralization. The samples were embedded in OTC compound (Sakura Finetek U.S.A., Inc., Torrance, CA) and frozen. The protocol for animal use was reviewed and approved by the animal experiment committee of Hokkaido University Dental School, Sapporo, Japan.

2.2. Cell culture

The PDL tissue was obtained from first molars extracted from 55-day-old male Wistar rats. Immediately after extraction, the teeth were washed three times with phosphate-buffered saline (PBS, pH 7.2). Then PDL tissues from the root surface was detached with a surgical knife and used for cell culture. The PDL tissue was cultured in DMEM (Sigma–Aldrich, Steinheim, Germany) containing 10% fetal bovine serum (GIBCO, Carlsbad, CA), penicillin G (100 units/ml) (GIBCO, Carlsbad, CA), streptomycin (100mg/ml) (GIBCO, Carlsbad, CA), and amphotericin B (0.25 mg/ml) (Cellgro, Herndon, VA) for 7 days. After a confluent monolayer of migrating cells had formed (5×10^5 to 7×10^5 cells/dish), the cells were passaged by trypsinization, and the PDL cells from passage 3 were obtained and treated with 17.6 g/cm^2 of compressive force by the pressure device (Unicontrol Co., TM5SRV, Tokyo, Japan) (Fig. 2) for 24 h at 37 °C in the incubator.

2.3. Immunostaining for cultured cells

The PDL cells in passage 3 were cultured on cover slips in six-well plates for 48 h. After the cultured cells were washed three times with PBS, they were air dried for 30 min and fixed in 30% acetone–PBS for 5 min at room temperature, and reacted with anti-RANKL and anti-vimentin (DAKO Corporation, Carpinteria, CA). Diluted with PBS containing 1% rabbit or goat serum for 8 h at 4 °C. Then the cells were treated with Alexa 568, rabbit anti-goat IgG (H + L) or Alexa 488, goat anti-rabbit IgG (H + L) (Molecular Probes, Inc., Eugene, OR) for 0.5 h at room temperature. The cells were observed by phase-contrast and fluorescent microscope, and the reaction products with second antibodies of Alexa 488, and with Alexa 568, were visualized as fluorescent green yellow and red, respectively.

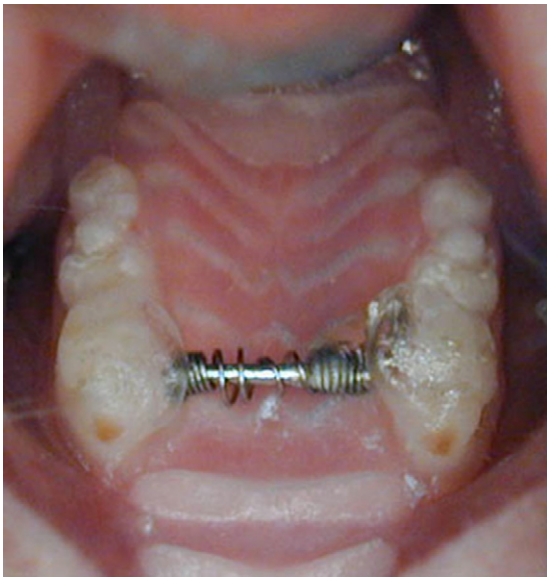

Fig. 1 – Setting of the Ni–Ti open coil.

Fig. 2 – The pressure device in the incubator. PDL cells were continuously compressed by this device.