Journal of Hard Tissue Biology 16[2] (2007) p71-74
© 2007 The Society for Hard Tissue Regenerative Biology
Printed in Japan, All rights reserved.

Original

Periodontal Tissue Reaction to Mechanical Stress in Mice

Takehiro Watanabe[1)], Norimasa Okafuji[1)], Keisuke Nakano[1,2)], Takako Shimizu[2], Rina Muraoka[1)], Saburo Kurihara[2)], Kazuhiro Yamada[1)] and Toshiyuki Kawakami[1,2)]

[1]Department of Hard Tissue Research, Matsumoto Dental University Graduate School of Oral Medicine, Shiojiri, Japan
[2]Matsumoto Dental University Institute for Oral Science, Shiojiri, Japan
(Accepted for publication, May 28, 2007)

Abstract: In this paper, we examined the periodontal tissue reaction course of mice to mechanical stress according to the Waldo method, before examination of the transcription factor profile change. In the examination group, the arrangement of the periodontal ligament was irregular on specimen day 1. The extension and compression sites were observed at the opposite side of the roots. In day 1 and 3 specimens, the osteoclasts appeared in the compression sites. In day 7 specimens, the number of osteoclasts was reduced in number to less than that of day 3. Immunohistochemical examination revealed that the expression patterns of Runx2 and Msx2 were clearly dynamic changed compared to the control specimens. These results suggest that the appearance of transcription factors related to cell differentiation of periodontal ligament, which was due to the mechanical stress of insertion of elastic separator, happened within 24 hours.

Key words: Periodontal ligament, Tissue reaction, Osteoclasts, Osteoblasts, Mechanical stress

Introduction

Periodontal ligament is a soft connective tissue interposed between tooth roots and the inner wall of their alveolar sockets. This tissue is characterized by rapid turnover and high remodeling capacity, which give it adaptability, maintaining a constant width despite being exposed to rapidly changing physical forces such as mastication, speech and orthodontic treatment. During orthodontic treatment, the periodontal tissue reacts after receiving the physical forces due to the treatment causing bone absorption by osteoclasts and bone formation by osteoblasts. In the course of orthodontic treatment, differentiation and activation mainly of osteoclasts and osteoblasts happens due to the related transcriptional factors (1-3). The expression patterns of these factors, the peptides and genes, are very important for recognition of periodontal tissue reaction. At first, we want to establish an animal experiment model using mice. Therefore, in this preliminary study, we examined the periodontal tissue reaction course of mice using the Waldo method.

Materials and Methods

Animal Experiment

60 male ddY mice (8th neonatal week) were secured from Japan SLC Co. (Hamamatsu, Japan). The animals were kept in plastic cages under controlled air conditioning with running water and Picolab Rodent Diet 20 (Japan SLC Co., Hamamatsu, Japan). Under anesthetized conditions with intraperitoneal injection of sodium pentobarbital solution, an elastic module was inserted interproximally between the upper first and second molars on the right side (Figure 1). The same portion of the left side was used as a control.

Correspondence to: Toshiyuki Kawakami, PhD, Professor, Hard Tissue Pathology Unit, Matsumoto Dental University Graduate School of Oral Medicine, 1780 Hirooka-Gobara, Shiojiri, 399-0781 Japan. Phone and Fax +81 - (0) 263 -51- 2035, E-mail: kawakami@po.mdu.ac.jp

Histopathology

One, 3, and 7 days after the elastic insertion, the maxillary bones with surrounding tissues were dissected under anesthetized conditions. The removals were then immediately fixed in 4% paraformaldehyde fixative solution. The specimens were demineralized with 10% ethylenediamine tetraacetate (pH 7.4). Specimens were processed and embedded in paraffin. Horizontally-cut sections 5μm in thickness were processed with a microtome (Figure 2). After consecutive sectioning, the specimens were examined by histopathology (stained with hematoxylin and eosin: HE) and histochemistry (stained with tart rate-resistant acid phosphatase: TRAP).

Immunohistochemistry

Using day 1 specimens, immunohistochemical (IHC) examinations were conducted using an anti-Runx2 monoclonal antibody (PEBP2αA-m-70, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA; 1/10) and an anti-Msx2 monoclonal antibody

(4G1, 1/10), with a Dako EnVision+Kit-K4006 (Dako, Glostrup, Denmark). The 4G1 antibody was developed by Liem TM and Brenner-Moton S (4, 5). It was obtained from the Developmental Studies Hybridoma Bank maintained by The University of Iowa, Department of Biological Science, Iowa City, Iowa, under contact NO1-HD-7-3236 from the National Instiurte of Child Health and Human Development. Diaminobendizine was applied for the visualization of immunohistochemical activity. Samples were then counterstained with hematoxylin. Immunohistochemical staining using phosphate buffered saline in place of the primary antibody was included as a negative control.

Results

Histopathology (HE)

The histopathological view of a horizontal section of a control specimen is shown in Figure 3. The maxillary first, second and third molars were shown as M1, M2 and M3 having two to three cross sectioned roots, as indicated by the dotted circles. The location of the elastic separator is also indicated in the center. In this examination, we observed mainly the medial root of the maxillary second molar, which is indicated by the square in Figure 3.

In control specimens, the arrangement of periodontal ligament, including periodontal collagen fibers, osteoblasts and alveolar bone, was orderly. We observed no absorption of the alveolar bone or osteoblast proliferation in the area (Figure 4 a).

In the examination group, the arrangement of the periodontal ligament was irregular in the day 1 specimens. The extension and compression portions were observed on the opposite side of the roots. In day 3 specimens, the absorption of bone was noted. Osteoclasts appeared in the absorption sites (Figure 4 b). In day 7 specimens, the number of osteoclasts was reduced to fewer than in day 3 specimens.

Histochemistry (TRAP)

In control specimens, there were almost no TRAP-positive osteoclasts around the roots examined, both in the extension portions and especially in the compression portions.

In experimental group, we observed few osteoclasts in the day 1 specimens, especially at the compression portion of the roots (Figure 5 a). Osteoclasts increased in number according to the time course (Figure 5 b). However, as observed in day 7 specimens, TRAP positive-osteoclasts decreased in number.

Immunohistochemistry (IHC)

In the control group, cell arrangement of the periodontal ligament was orderly in manner. Periodontal ligament cells weakly reacted to both Runx2 and Msx2. Strong activity was seen in the periodontal ligament fibroblasts and osteoblasts, especially in the nuclei of osteoblasts. In contrast, Runx2 appeared weakly in the whole area of the periodontal ligaments of control specimens (Figure 6).

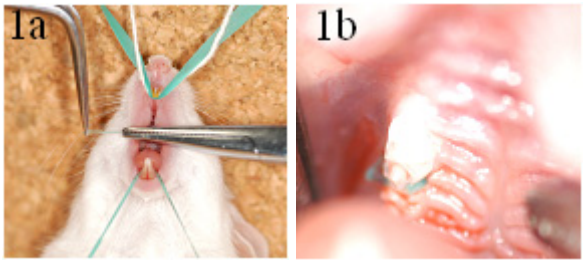

Figure 1. View of the animal experiment (a) and the intra oral appearance (b).

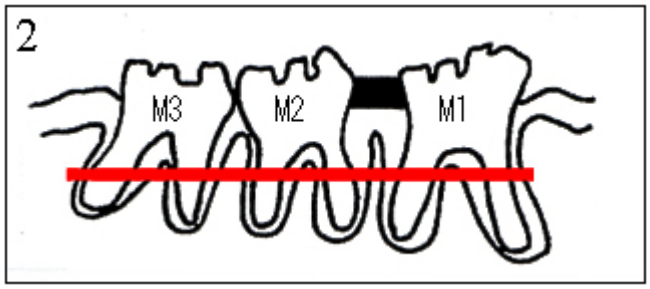

Figure 2. A manufactured part of a histological specimen.

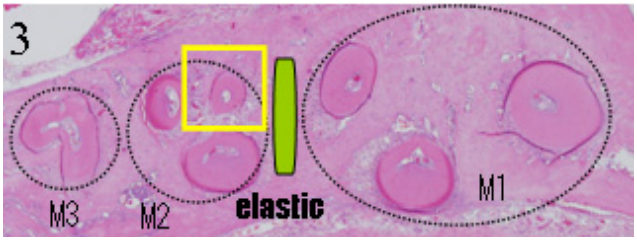

Figure 3. Macroscopic histological view of control specimen.

As for Msx2 in the experimental group, weak immunostaining was observed similar to that in the control specimens. Especially in the extension site, there were comparatively strong immunoreactions to Msx2 (Figure 7).

Discussion

Little is known about the characteristics of the periodontal ligament connective tissue according to the literature (6, 7). Yoshizawa et al. (8, 9) reported the characteristics of the nature and function markers and cell lines of periodontal ligaments. In the report, they established a cell line, PDL-L2, which is distinguishable from periodontal ligament fibroblasts in terms of gene expression profile. The PDL-L2 cells share with osteoblasts the expression of genes for type I collagen, Runx2, and periostin but not the genes for bone sialoplotein or osteocalcin. This profile of gene expression in PDL-L2 cells exactly matches that of the reported periodontal ligament fibroblasts (8). Furthermore, high expression of Msx2 was observed in PDL-L2 cells. Yoshizawa et al. concluded that endogenous Msx2 prevents ligament fibroblasts from undergoing osteoblastic differentiation by repressing Runx2 transcriptional activity.

We thought that the orthodontic tooth movement due to

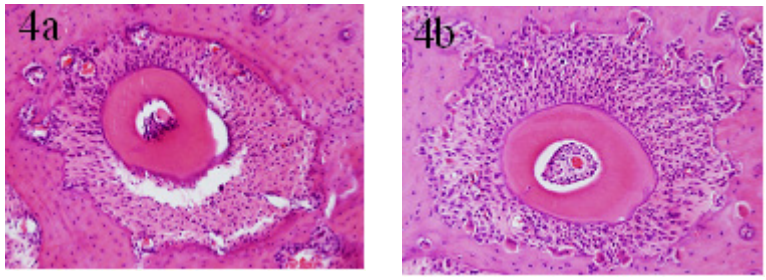

Figure 4. Histological view of control (a) and experimental day 3 (b) specimens.

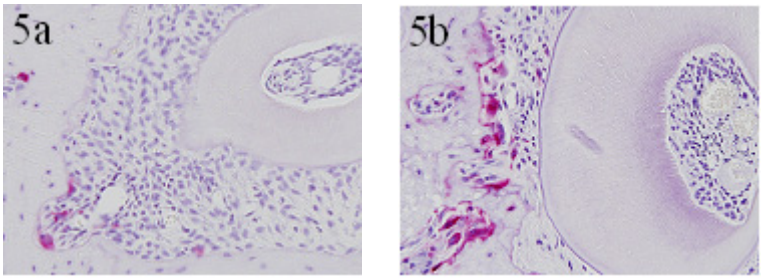

Figure 5. TRAP-positive osteoclasts in experimental day 1 (a) and 3 (b) specimens.

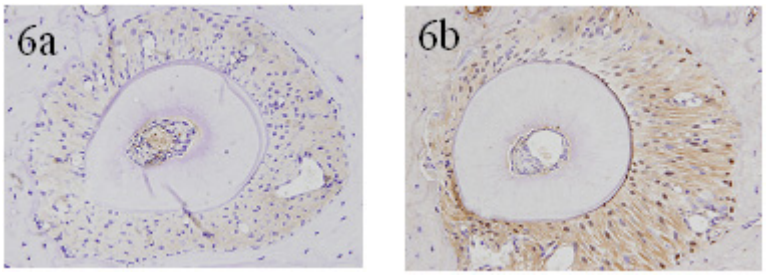

Figure 6. Runx2-activity in control (a) and experimental day 1 (b) specimens.

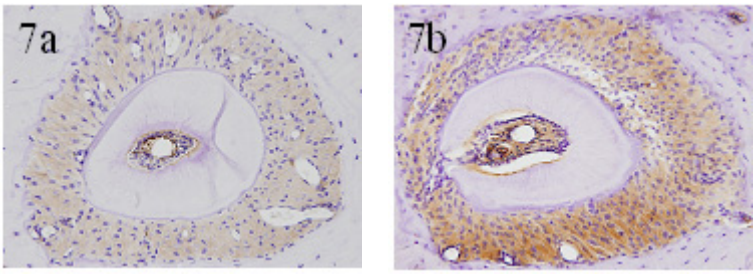

Figure 7. Msx2-activity in control (a) and experimental day 1 (b) specimens.

mechanical stress might be closely related to changes in the expression pattern of the transcription factors mentioned above. Therefore, in this examination, we undertook an examination of the periodontal tissue reaction course in mice using the Waldo method, before experimental of these transcription factor profile changes.

The results of the histopathological examination show that the tissue reaction to the mechanical stress due to the insertion of the separator occurred in the compression site of day 1 specimens. The compression site of the periodontal ligament of the related tooth was narrow due to tissue degeneration. Furthermore, TRAP specimens clearly showed the presence of osteoclasts. This showed that the osteoclastic cytodifferentiation, which appeared in the compression site due to the mechanical stress of this examination, occurred within only 24 hours, whereas there were no data of osteoblastic cell differentiation in this examination. Thus, we believe that osteoblastic cell differentiation also occurred within 24 hours after the receiving of the mechanical stress. The tissue reaction continued throught the examination periods. However, the tissue reaction gradually weakened according to the observation of the histopathological (HE) and histochemical (TRAP) specimens.

Next, we experimented with the immunohitochemical profiles of the related transcription factors, Runx2 and Msx2. In control specimens, the weak expression profiles of Runx2 and Msx2 in the periodontal ligaments of these experiments suggest that these changes maintain the homeostasis of the periodontal membrane. In contrast, in the examintal specimen results, the immunohistochemical reactivities of Runx2 and Msx2 had clearly changed in comparison with the control specimens. There were dynamic changes that occurred in the experimental specimens. According to the results, we thought that the cell differentiation of periodontal ligament cells, due to the mechanical stress of insertion of a separator, occurred within a short time. It is important to examine the expression profile of the related transcription factors. Therefore, in our next examination, we plan to study this profile and the results will be reported in near future date.

Acknowledgements

This research was partially supported by a Grant-in -Aid for Scientific Research #19592374 from the Japan Society for the Promotion of Science and a Grant-in-Aid for Scientific Research # 18791572 from the Ministry of Education,Culture, Sport, Science and Technology of Japan.

References

1. Kanzaki H, Chiba M, Shimizu Y and Mitani H. Dual regulation of osteoclast differentiation by periodontal ligament cells through RANKL stimulation and OPG inhibition. J Dent Res 80: 887-891, 2001
2. Kawamoto S, Ejiri S, Hoshi K, Nagaoka E and Ozawa H. Immunolocalization of osteoclast differentiation factor in rat peridontium. Arch Oral Biol 47: 55-58, 2002
3. Oshiro T, Shintani A, Shibasaki Y and Sasaki T. Osteoclast induction in periodontal tissue during experimental movement of incisors in osteoprogerin-deficient mice. Anat Rec 226: 218-225, 2002
4. Liem JrKF, Tremml G, Roelink H and Jessel TM. Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm. Cell 82: 969-979, 1995
5. Lui Y, Helms AW and Johnson JE. Distinct activities of Msx1 and Msx3 in dorsal neural tube development. Development 131: 1017-1028, 2003
6. Kawarizadeh A, Bourauel C, Gotz W and Jager A. Early responses of periodontal ligament cells to mechanical stimulus in vivo. J Dent Res 84: 902-906, 2005
7. Shiotani A, Shibasaki Y and Sasaki T. Localization of receptor activator of NFkB ligand, RANKL. In periodontal tissues during experimental movement of rat molars. J Electron MIcrosc 50: 365-369, 2001
8. Saito Y, Yoshizawa T, Takizawa F, Ikegame M, Ishibashi O, Okuda K, Hara K, Ishibashi K, Obinata M and Kawashima H. A cell line with characteristics of the periodontal ligament fibroblasts is negatively regulated for mineralization and Runx2/Cbfa1/Osf2 activity, part of which can be overcome by bone morphogenetic protein-2. J Cell Sci 115: 4191-4200, 2002
9. Yoshizawa T, Takizawa F, Iizawa F, Ishibashi O, Kawashima H, Matsuda A, Endo N and Kawashima H. Homeobox protein Msx2 as a molecular defence mechanism for preventing ossification in ligament fibroblasts. Mole Cell Biol 24: 3460-3472, 2004