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Occlusal Stimuli Influence on the Expression of IGF-1 and the IGF-1 Receptor in the Rat Periodontal Ligament

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ABSTRACT

Objective: To test the hypothesis that hypofunction/recovered occlusal function has no effect on the changes in insulin-like growth factor-1 (IGF-1) and IGF-1 receptor expressions and cell proliferation of periodontal ligament (PDL) cells.

Materials and Methods: To produce occlusal hypofunction, the appliances were attached to the rats' maxillary and mandibular incisors. Subsequently, occlusal contact of the molar area was thoroughly recovered by removal of the appliances.

Results: In periodontal sections, localization of IGF-1, the IGF-1 receptor, and proliferating cell nuclear antigen (PCNA) immunoreactive cells was significantly more expressed in the control group compared with the hypofunctional group ($P < .01$). In addition, after the recovery of the occlusion, IGF-1, IGF-1 receptor, and PCNA were detected significantly much more than in the hypofunction group ($P < .01$).

Conclusion: The hypothesis was rejected. This study suggests that occlusal stimuli induce cell proliferation of PDL cells by increasing IGF-1 and IGF-1 receptor expression.

KEY WORDS: Periodontal ligament, Occlusal stimuli, IGF-1, IGF-1 receptor, Cell proliferation.

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INTRODUCTION Return to TOC

Mechanical loading upon occlusion is an important regulatory factor in periodontal tissue homeostasis. In orthodontic treatment, there are diverse types of malocclusion such as open-bite malocclusion and high canine position, a stand for hypofunctional teeth. Atrophic changes in the periodontal ligament (PDL) have been reportedly associated with loss of occlusal function,[1,2] such as narrowing of the periodontal space, disorientation of collagen,[3,4] vascular constriction, and deformation of the mechanoreceptor structure.[5,6] However, there are many ambiguities in the mechanisms of PDL homeostasis.

The regulation of periodontium homeostasis involves the proliferation, differentiation, and apoptosis of various cells in periodontal tissue and is modulated by several hormones and growth factors such as insulin-like growth factors (IGFs). IGF-1 plays an important role in the positive regulation of cellular proliferation as an autocrine or paracrine-acting polypeptide growth factor in multiple tissues. The biological action of IGF-1 is mediated by IGF-1 receptor (IGF-1R), a transmembrane heterotetramer with tyrosine kinase activity.[7–9] Regarding periodontal tissue, IGF-1 has been shown to have mitogenic and chemotactic effects on PDL cells in vitro[10,11] and to enhance periodontal tissue healing in vivo.[12,13]

Moreover, IGF-1 has been demonstrated to act as a mitogenic agent for PDL fibroblasts, likely via high-affinity cell surface receptors.[14] Recently, Gotz and his coworkers[15,16] have investigated the distribution pattern of IGF system components in human permanent and deciduous teeth, of which many were localized in the periodontium.

However, the influence of occlusal hypofunction and its recovery on the expression of IGF-1 and/or IGF-1R in the PDL is still obscure. In addition, it remains unclear whether occlusal stimuli induce cell proliferation in the PDL by increasing IGF-1 and IGF-1R expression in an animal. The aim of this study was to investigate the effects of occlusal stimuli on the changes in IGF-1 and IGF-1R expressions and cell proliferation of PDL cells using a hypofunction/recovered occlusal function model.

MATERIALS AND METHODS Return to TOC

Experimental Model

Twenty-five male Sprague-Dawley rats (7 weeks old) were maintained under pathogen-free conditions and divided into control (n = 5) and experimental (n = 20) groups consisting of a hypofunctional (n = 10) and a recovery (n = 10) group. Untreated animals of the same age were used as controls.

In the experimental groups, an anterior metal bite plate and a metal cap constructed from stainless band material (0.180 × 0.005 inches; Rocky Mountain Morita, Tokyo, Japan) were attached to the maxillary and mandibular incisors, respectively (Figure 1a) using light-curing composite resin (Clearfil Liner Bond II; Kuraray Co Ltd, Okayama, Japan), according to the method developed by Suhr et al.[17] In the hypofunctional group, the rats were killed at 3 (H3) and 7 (H7) days after attachment of the appliances. In the recovery group, after a 7-day hypofunctional period, the appliance was removed, and the rats were killed at 3 (R3) and 7 (R7) days after appliance removal. All procedures were carried out under the guidelines of the Animal Ethics Committee of Tokyo Medical and Dental University. The experimental time schedule and procedures are summarized in Figure 1b.

Histological Preparation

After administration of inhalant anesthesia with diethyl ether and intraperitoneal injection of chloral hydrate (400 mg/kg), animals were intracardially perfused with 4% paraformaldehyde in 100 mM sodium phosphate buffer, pH 7.4. The mandibles were immediately removed and fixed with the same fixative at 4°C for 1 day; decalcified in a 10% EDTA solution, pH 7.4 at 4°C; and finally embedded in paraffin by a conventional method. Serial sagittal sections of 5-μm thickness were cut (RM2155; LEICA Co Ltd, Nussloch, Germany) parallel to the long axis of the mesial and distal root of the mandibular first molar (M1), as in Figure 2.

Immunohistochemistry

Nine serial sections from each animal were selected for best orientation and freedom from artifacts. Then, 3 serial sections were stained with each antibody to IGF-1, IGF-1R, and proliferating cell nuclear antigen (PCNA). The deparaffinized sections were incubated with antibodies against IGF-1 and IGF-1R (diluted at 1:50 and 1:200; Santa Cruz Biotechnology, Santa Cruz, Calif) overnight at 4°C, visualized using the biotin-streptavidin method (Histofine MAX-PO Kit; Nichirei, Japan), and counterstained with hematoxylin. For PCNA, sections were incubated with the PCNA antibody (clone PC10, diluted at 1:200; DAKO, Gloserup, Denmark) and visualized using EnVision peroxidase antimouse (DAKO). Finally, sections were stained with 3,3′-diaminobenzidine and counterstained with hematoxylin.

Histological and Quantitative Analysis

An area comprising 100 × 200 μm of the distal root mesial surface of the mandibular first molar, located superiorly from the middle of the root length, was used for investigation. This was because the mandibular first molar distal root is the largest root of the largest mandibular tooth upon which the greatest occlusal function can be exerted. Indeed, the functional structural stability of the PDL fibers at the mesial surface of the upper half of the distal root was also indicated.[3]

The observation area was photographed with a light microscope (200× magnification, Nikon Microphoto-FXA; Nikon, Tokyo, Japan) equipped with a digital camera (DXm1200; Nikon). Each section was measured twice, yielding 6 measurements. The average value for each animal was derived and then calculated according to the following formula: Ratio of immunoreactive cells (%) = (number of immunoreactive cells/ number of total cells) × 100.

Data are expressed as the mean ± standard deviation and analyzed by analysis of variance followed by the Bonferroni post hoc test ($P < .01$) using SPSS 14.0 software (SPSS for Windows, version 14.0; SPSS Inc, Chicago, Ill).

RESULTS Return to TOC

In all the experimental groups, the body weight of the animals increased during the study period, but there was no significant difference in the mean body weight between the control and experimental groups (data not shown).

Hematoxylin and Eosin Staining

In the control group, a longitudinal section of PDL showed high tissue cellularity. The fibroblast-like cells are arranged perpendicularly to the tooth surface in the upper region, while apically, they are arranged more obliquely. The PDL fiber bundles are oblique in orientation (Figure 3a).

After 3 days to induce hypofunctionality (H3), the fibroblast-like cell density decreased, and narrowing of the ligament was observed (Figure 3b). At 7 days in the hypofunctional group (H7), the PDL space decreased to about half of the PDL space of the control group, and thin and disorientated PDL fibers were observed (Figure 3c).

After 3 days of occlusal recovery (R3), there was an increase in PDL cellularity and reorientation of the fibers (Figure 3d). In the 7-day recovery group (R7), the PDL space was wider than it was in the 7-day hypofunction group; the PDL reverted to an almost normal appearance, and functionally oriented fibers running obliquely were noted (Figure 3e).

IGF-1

The IGF-1 immunoreactivity appeared all over the extracellular matrix and PDL cells (Figure 4A, upper panel). Most immunoreactive cells in the PDL were mononuclear and fibroblast-like cells (Figure 4A, black arrowhead). In the control group (Figure 4A [a]), the PDL immunoreactive cells were indicated more dramatically than in the hypofunctional groups ($P < .01$; Figure 4A [b, c] and B [a]). In contrast, after the removal of the appliances, the immunoreactive cells presented at a higher level than the hypofunctional groups ($P < .01$; Figure 4A [d, e] and B [a]).

IGF-1R

Cell types expressing IGF-1R corresponded to those that expressed its ligand (Figure 4A, white arrowhead). In the control group, the PDL cells revealed significant immunoreactivity (Figure 4A [f]) compared with that of the hypofunctional group ($P < .01$; Figure 4A [g, h] and B [b]). In the recovery group, fibroblast-like cells led to an increase in the immunoreactive cell numbers (Figure 4A [i, j] and B [b]). The pattern of IGF-1R immunoreactivity was identical to that noted for IGF-1.

PCNA

The nuclei of the PDL cells were stained immunohistochemically with the PCNA antibody (Figure 5A, black arrowhead). The PCNA-immunoreactive cells in the control group were distributed throughout the ligaments and were more dramatically revealed than in the other experimental groups ($P < .01$; Figure 5A,B). The PCNA-immunoreactive cells in both the H3 and H7 groups gradually decreased ($P < .01$; Figure 5A [b, c] and B). Conversely, the number of PCNA-immunoreactive cells in the PDL in both the R3 and R7 groups increased after the recovery of occlusion ($P < .01$; Figure 5A [d, e] and B).

DISCUSSION Return to TOC

The present study was designed to investigate the influences of occlusal stimuli on the expression of IGF-1 and IGF-1R. In addition, we examined the relationship between the IGF-1 system and cell proliferation of PDL cells in response to occlusal stimuli. We established an experimental hypofunctional periodontal condition at the molar region with the bite-raising technique according to Suhr et al.[17] This method made it possible to reestablish the occlusion after the removal of the appliances. Previously, a number of different models[3,4,18] were used to produce the occlusal hypofunctional condition, but they had difficulties in reestablishing normal occlusion.

In this study, we demonstrated that IGF-1 and its receptor can be detected by immunohistochemistry in the rat PDL. IGF-1 is synthesized in multiple tissues throughout the body in response to growth hormone, so the animal's age is critical because circulating IGF-1 increases during prepuberty and the early puberty period.[19,20] Our experiment was started from 7 weeks of age and designated as late pubertal,[19] suggesting that our results did not correspond with the circulating IGF-1. Consequently, both IGF-1– and IGF-1R–immunoreactive cells in the control group with rats of a different age were immunohistochemically indistinguishable.

This study demonstrated a pattern of extensive reduction in the production of cellular IGF-1 and IGF-1R corresponding to the loss of

occlusal function. In the control group, both IGF-1– and IGF-1R–immunoreactive cells showed significantly greater expression than in the hypofunctional groups, and after occlusal recovery, the expression of both IGF-1 and IGF-1R increased ($P < .01$). This suggests that the biological action of IGF-1 and IGF-1R in PDL was activated under the stimulation of the occlusal stimuli.

There are many reports indicating that mechanical force induced the expression of IGF-1 and IGF-1R in multiple cell types and that it may cause proliferation of cells in an autocrine and paracrine manner[21–24] owing to the contributions of IGF-1. A previous study demonstrated the upregulation of the IGF-1 receptor in PDL cells of orthodontically treated teeth by immunohistochemical investigation.[23] Our findings indicate the analogous IGF-1 and IGF-1R expression patterns in the rat PDL, which is consistent with another report that IGF-1 upregulates its own receptors,[25] initiated by occlusal stimulation.

IGFs are believed to behave as proliferative factors for PDL fibroblast cells[26,27] and increase PDL cell DNA synthesis, proliferation, and chemotaxis.[11,14,28] We examined the PCNA expression in PDL tissues to determine whether the occlusal force provoked cell proliferation. From our results, the PCNA-immunoreactive cells were significantly more abundant in the control group than in hypofunctional groups, and after occlusion reestablishment, the PCNA-immunoreactive cells gradually increased ($P < .01$). In accordance with the evidence of IGF-1 and IGF-1R expression, our results suggested that occlusal stimuli may lead to cell proliferation in PDL following the upregulation of the IGF-1 system. Many studies have demonstrated that IGF-1 and IGF-1R stimulate cell proliferation in vitro, especially PDL cells.[11,21,27] Furthermore, there are indications that IGF-controlled autocrine and paracrine pathways regulate PDL cell survival, proliferation, and matrix turnover.[14,21,28]

Our findings indicate the effect of occlusal stimuli on the regulation of PDL cell proliferation by intensification of IGF-1 and IGF-1 receptors. It is still unclear as to how the PDL can regulate the cell population to maintain a regular space. It has been surmised that both cell proliferation and cell death participate in periodontal ligament changes to maintain periodontium homeostasis throughout life. This study suggests that occlusal stimuli play an important role in the regulation of the periodontal ligament homeostasis by upregulation of IGF-1 and IGF-1R.

CONCLUSIONS Return to TOC

- Occlusal stimuli applied to periodontal tissues induced the expression of IGF-1 and IGF-1R in PDL cells.
- The increase in proliferative cells is in parallel with the expression of IGF-1 and IGF-1R, which suggests the effect of IGF-1 and/or IGF-1R on cell proliferation in PDL.
- These results emphasize the importance of occlusal stimuli in promoting the proliferative action of the IGF-1 system to maintain periodontal tissues homeostasis.
- These findings suggest that the use of an occlusal splint before starting orthodontic treatment may be a beneficial tool for the improvement of periodontal tissue in a hypofunctional tooth.

ACKNOWLEDGMENTS

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FIGURES Return to TOC

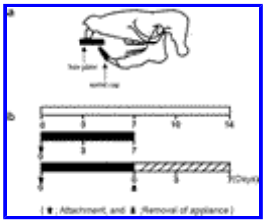

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Figure 1. Experimental model and time schedule. (a) Experimental method. Hypofuntional condition was achieved by the attachment of an anterior bite plate to the maxillary incisors and a metal cap to the mandibular incisors. (b) Time schedule of the experiment. In the hypofunctional group, rats were killed at 3 and 7 days after attachment of the appliances. In the recovery group, the rats were killed at 3 and 7 days after the removal of the appliances

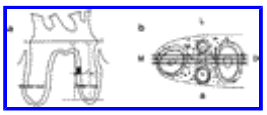

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Figure 2. Area of investigation. (a) An area of 100 × 200 μm of the distal root mesial surface of the mandibular first molar located superiorly from the middle of the root length was used for observation. (b) Serial sagittal sections were cut parallel to the long axis of the mesial and distal root of mandibular first molar. M indicates mesial; D, distal; B, buccal; and L, lingual

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Figure 3. Hematoxylin and eosin staining in the periodontal ligament. (a) Control group, (b) 3-day hypofunctional group, (c) 7-day hypofunctional group, (d) 3-day recovery group, (e) 7-day recovery group. Alv indicates alveolar bone; PDL, periodontal ligament; and Ce, cementum. Bar = 100 μm; original magnification 200×

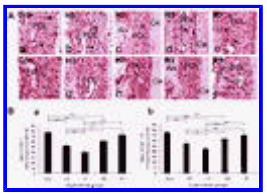

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Figure 4. (A) Immunostaining of insulin-like growth factor-1 (IGF-1) and the IGF-1 receptor (IGF-1R) in the periodontal ligament. The upper panel (a–e) shows the distribution of IGF-1, and the lower panel (f–j) shows that of IGF-1R. IGF-1: The IGF-1–immunoreactive cells (black arrowhead) in the control group (a) presented significantly more than in the hypofunctional groups (b, c). After recovery of occlusion, the immunoreactive cells dramatically increased as compared with the hypofunctional groups (d, e). IGF-1R: IGF-1R–immunoreactive cells (white arrowhead) in the control group (f) were dramatically more numerous than the other groups, especially in the hypofunctional groups (g, h). The immunoreactive cells in the recovery groups increased as compared with the lack of occlusal contact (i, j). Alv indicates alveolar bone; PDL, periodontal ligament; and Ce, cementum. Bar = 50 μm; original magnification 400×. (B) Quantification of cells labeled by immunohistochemistry for IGF-1 and IGF-1R. Ratio of IGF-1 (a) and IGF-1 receptor (b) immunoreactive cells in the periodontal ligament. Data are presented as the mean ± standard deviation from 5 rats in each group. Con indicates control group; H3, 3-day hypofunctional group; H7, 7-day hypofunctional group; R3, 3-day recovery group; and R7, 7-day recovery group. * $P < .05$; ** $P < .01$

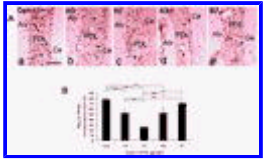

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Figure 5. (A) Immunostaining of proliferating cell nuclear antigen (PCNA) in the periodontal ligament. Representative results of PCNA-immunoreactive cells (black arrowhead) show the difference among the control group (a), hypofunctional groups (b, c), and recovery groups (d, e). In the control group, PCNA expression was significantly more noticable than in the hypofunctional groups, and the PCNA expression increased after 3 days' and 7 days' of occlusal recovery. Alv indicates alveolar bone; PDL, periodontal ligament; and Ce, cementum. Bar = 100 μm; original magnification 200×. (B) Quantification of cells labeled by immunohistochemistry for PCNA. Ratio of PCNA-immunoreactive cells in the periodontal ligament. Data are presented as the mean ± standard deviation from 5 rats in each group. Con indicates control group; H3, 3-day hypofunctional group; H7, 7-day hypofunctional group; R3, 3-day recovery group; and R7, 7-day recovery group. * $P < .05$; ** $P < .01$

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