Original Article

Influence of Occlusal Stimuli on the Microvasculature in Rat Dental Pulp

Naoki Shibutani[a]; Jun Hosomichi[b]; Yuji Ishida[c]; Kunimichi Soma[d]

ABSTRACT

Objective: To examine the influence of occlusal stimuli on the vasculature in the dental pulp, using an occlusal hypofunction model.

Materials and Methods: Twenty 7-week-old male Sprague-Dawley rats were divided into two groups. To produce occlusal hypofunction, the appliances were attached to the maxillary and mandibular incisors. Untreated rats served as controls. Serial horizontal paraffin sections of the mandibular first molar were processed by conventional methods. To evaluate the microvasculature in the dental pulp, sections of each specimen were stained with hematoxylin-eosin.

Results: In the experimental group, the arterioles in the tooth pulp tissue ran convergently, and their inside diameter was significantly smaller than that of the control group.

Conclusion: This study suggests that occlusal stimuli influence the periodontal ligament throughout the microvasculature of the dental pulp. (*Angle Orthod* 2010;80:316–321.)

KEY WORDS: Dental pulp; Hypofunction; Microvasculature; Occlusal stimuli; Periodontal tissue

INTRODUCTION

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 displaced canine examples of hypofunctional teeth. Atrophic changes in the periodontal ligament (PDL) have been reported to be associated with loss of occlusal function,[1,2] such as narrowing of the periodontal space, disorientation of collagen fibers,[3] vascular constriction, and deformation of the mechanoreceptor structure.[4–6] Few studies have examined the histologic changes in the dental pulp seen in occlusal hypofunctional conditions, and the exact mechanism of dental pulp homeostasis is not yet clear.

In occlusal hypofunction, the amount of external root resorption in rat molars is significantly larger than in normal conditions.[7] Clinical reports have revealed that orthodontic treatment may induce pulpal necrosis of occlusal hypofunctional teeth.[8] During orthodontic tooth movement, various growth factors increase in the dental pulp, which changes the morphology of blood vessels[9–12] and nerve fibers.[13] Occlusal stimuli play an important role in periodontal organization.

The periodontium and dental pulp tissue are closely connected by blood vessels, accessory blood vessels, and nerve fibers through the apical foramen; they exert an influence on each other. Atrophic changes in the PDL of occlusal hypofunctional teeth may rapidly spread to the pulp throughout the microvasculature.

However, many uncertainties remain regarding the relationship between homeostasis of the dental pulp and occlusal stimuli. The aim of this study was to investigate the effects of occlusal stimuli on the vasculature in the dental pulp, using an animal model with occlusal hypofunction.

[a] Graduate Student, Orthodontic Science, Department of Orofacial Development and Function, Division of Oral Health Science, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan.

[b] Assistant Professor, Orthodontic Science, Department of Orofacial Development and Function, Division of Oral Health Science, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan.

[c] Clinical Fellow, Orthodontic Science, Department of Orofacial Development and Function, Division of Oral Health Science, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan.

[d] Professor, Orthodontic Science, Department of Orofacial Development and Function, Division of Oral Health Science, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan.

Corresponding author: Dr Naoki Shibutani, Orthodontic Science, Department of Orofacial Development and Function, Division of Oral Health Sciences, Graduate School, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Japan
(e-mail: n.shibutani.orts@tmd.ac.jp)

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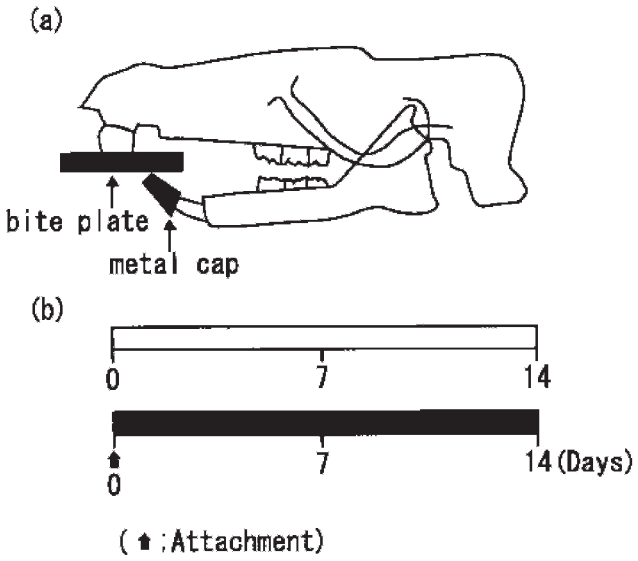

Figure 1. Experimental model and time schedule. (a) Hypofunctional conditions were achieved by the attachment of an anterior biteplate to the maxillary and a metal cap to the mandibular incisors. (b) In the hypofunctional group, rats were killed at 7 and 14 days after attachment of the appliances.

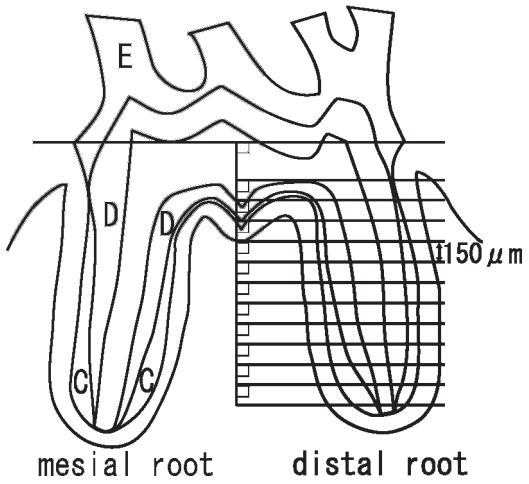

Figure 2. Area of investigation. An area was selected every 150 μm from the root canal orifice of the distal root of the mandibular first molar located superiorly from the middle of the root length. E, enamel; D, dentine; C, cementum.

MATERIALS AND METHODS

Experimental Model

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

In the experimental group, 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.[14] All rats were fed a powdered diet and were given water ad libitum throughout the experimental period. These rats were sacrificed at 7 and 14 days after appliances were attached in the control and experimental groups. 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.

Histologic Preparation

After anesthesia with inhaled diethyl ether and intraperitoneal injection of chloral hydrate (400 mg/kg), animals were perfused intracardially with 4% paraformaldehyde in 100 mM sodium phosphate buffer, pH 7.4. The mandibles were separated immediately and fixed with the same fixative at 4°C for 1 day, decalcified in a 10% ethylenediaminetetraacetic acid (EDTA) solution, pH 7.4, at 4°C for 4 weeks, and finally were embedded in paraffin using conventional methods. Serial horizontal sections of 5 μm thickness were cut (RM2155; LEICA Co Ltd, Nussloch, Germany) vertically to the long axis of the distal root of the mandibular first molar, as in Figure 2, and were mounted on poly-L-lysine-coated glass slides (Matsunami Co Ltd, Osaka, Japan). For histologic observation, sections of each specimen were stained with hematoxylin-eosin (HE).

HE Staining

For descriptive purposes, pulpal vessels were classified as arterioles (thick-walled vessels with a diameter of 10–50 μm); venules (vessels with thin or absent muscular layers, with a diameter of 10–40 μm); capillaries (small vessels, usually with an undetectable lumen, and a diameter of 4–10 μm); or lymphatics (irregularly shaped vessels, 20–50 μm in diameter, and displaying numerous abluminal endothelial projections).[15–17]

Histologic and Quantitative Analysis

The observation area was defined by the depth from the root canal orifice every 150 μm, photographed by a light microscope (200× magnification; Nikon Microphoto-FXA; Nikon, Tokyo, Japan) equipped with a digital camera (DXm1200; Nikon).

Statistical Analysis

Comparisons between the control and hypofunctional pulp groups were performed with the Mann-

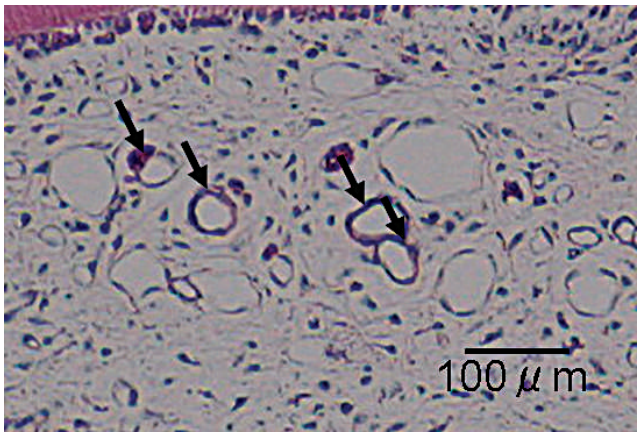

Figure 3. HE staining in the pulp. An area 450 μm from the root canal orifice of the distal root of the mandibular first molar of C7, 7-day control group. Bar = 100 μm; original magnification 400×. *Arrow*, arteriole as thick-walled vessel.

Whitney *U*-test using statistical analysis software (Statvew 5.0; SAS Institute, Cary, NC, USA).

RESULTS

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

Arterioles were identified as thick-walled vessels (Figure 3). After 7 days of induction of hypofunctional conditions (H7), changes in the morphology of the arterioles in the dental pulp were observed (Figures 4 through 6). A decrease in luminal area of the arterioles was observed in H7 owing to the tendency of the arterioles to converge. Similar changes were observed after 14 days in the hypofunctional group (H14) (Figures 5 and 6). Moreover, in the hypofunctional group, the arterioles converged toward the center of the pulp at all depths, unlike those in the control group.

DISCUSSION

The present study was designed to investigate the influences of occlusal stimuli on the expression of vascular changes in the tooth pulp. We established experimental hypofunctional conditions in the molar region using the bite-raising technique of Suhr et al.[14] This method made it possible to reestablish the occlusion after removal of the appliances. Previously, a number of different models[3] have been used to produce occlusal hypofunction, but this has resulted in difficulty in recovering normal occlusion. In our experimental model, occlusal hypofunction was induced at the molar area, which caused hypofunctional changes in the dental pulp.

Our results show that changes in occlusal conditions affected the microvasculature of the dental pulp. This might be explained by the complex connection between the blood vessels of the PDL and dental pulp. Reports have indicated that the PDL blood vessels are subject to various influences, such as vascular constriction and changes in vasodilatory factors.[4,6,18] It has been reported that normal chewing forces may displace sufficient fluid out of the dentine to excite putative mechanoreceptors somewhere inside the dentine/pulp complex.[19] Occlusal stimuli may induce changes in the inner pressure of the dental pulp, which may have an effect on the pulpal blood vessels.

A clinical radiographic study has shown that the severity of root resorption in open-bite teeth is greater than in deep-bite teeth before and after orthodontic treatment.[20] In rats, the amount of external root resorption is significantly greater in teeth with occlusal hypofunction of the periodontium than in those with normal periodontium.[21] Occlusal stimuli affect the metabolism of PDL and dental pulp, which results in internal resorption during tooth movement.[22]

It is thought that the change that occurred in day 7 is an acute alteration, and the change on day 14 is a chronic change. Therefore, natural recovery might not appear over the long term. It is thought that the dental pulp tissue recovers by recovering occlusal function as a clinical significance. In our future research, we will use experimental long-term models and occlusal recovery models to make it clearer.

In summary, this study demonstrates that occlusal stimuli influenced the morphology of the microvasculature within the dental pulp. It is important in orthodontics that nonoccluding teeth with atrophied periodontal tissues recover their physiologic structure and function by retaining occlusal stimuli. In the field of tissue engineering, recent advances have occurred in the study of regeneration of dental pulp and dentin in endodontics.[23] Vasculature is important in tissue homeostasis. Therefore, research into occlusal stimuli is important for the study of pulp regeneration.

CONCLUSIONS

- Loss of occlusal stimuli altered the morphology of arterioles within the dental pulp.
- Results suggest that occlusal stimuli exert influences on the microvasculature within the dental pulp.

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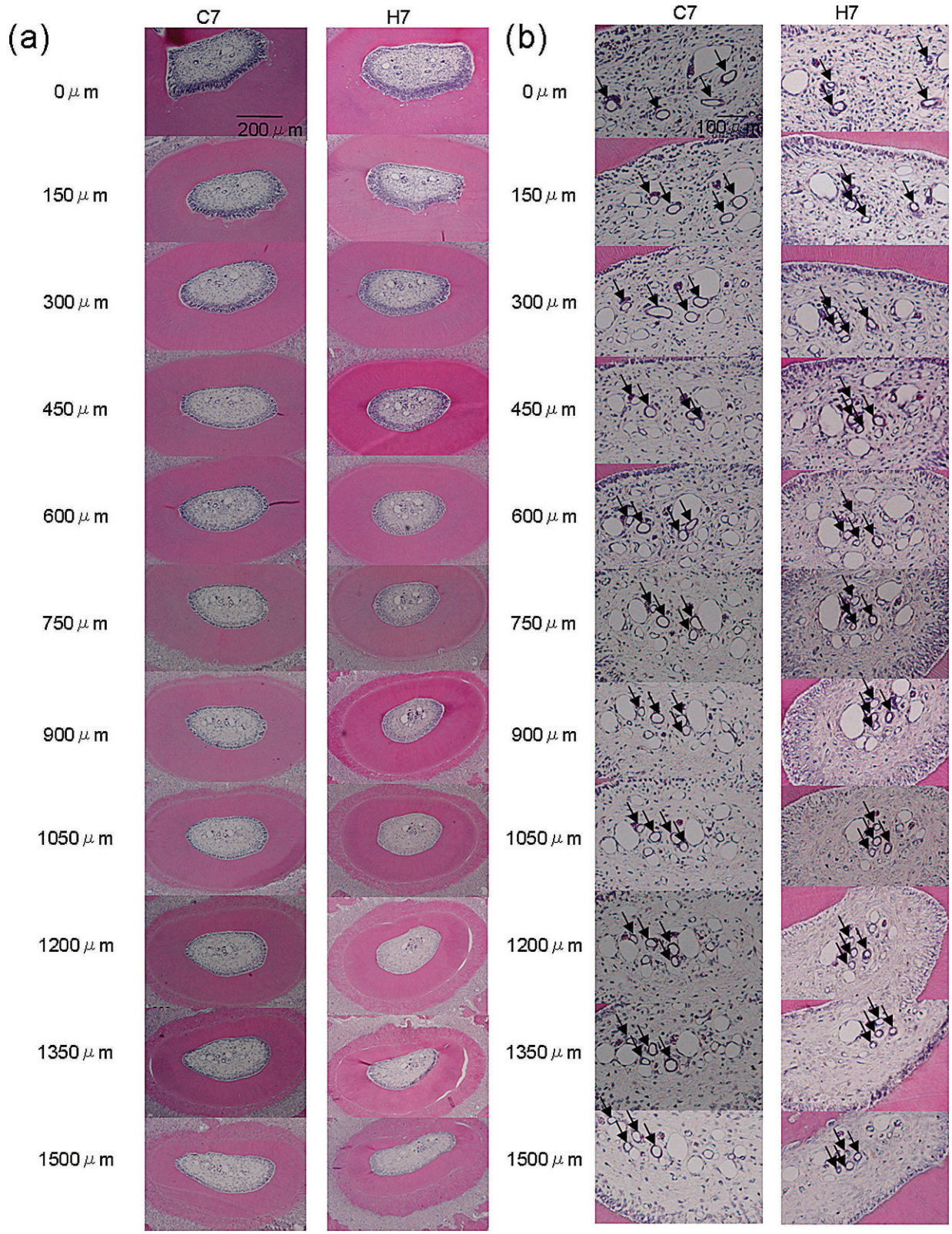

Figure 4. HE staining in the pulp. (a) Lower magnification of the distal root of the mandibular first molar. C7, 7-day control group; H7, 7-day hypofunctional group; bar = 200 μm; original magnification 200×. (b) Higher magnification of the pulp. Bar = 100 μm; original magnification 400×. *Arrow*, arteriole.

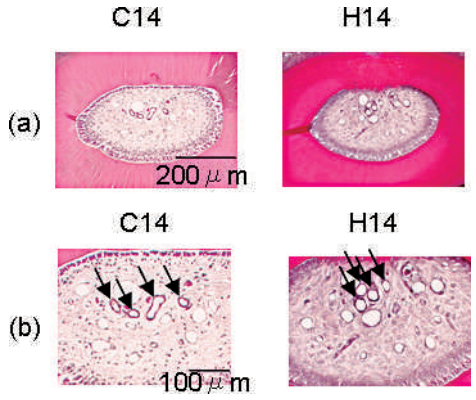

Figure 5. HE staining in the pulp. (a) Lower magnification of the distal root of the mandibular first molar of an area 450 μm from the root canal orifice. C14, 14-day control group; H14, 14-day hypofunctional group; bar = 200 μm; original magnification 200×. (b) Higher magnification of the pulp. Bar = 100 μm; original magnification 400×. *Arrow*, arteriole.

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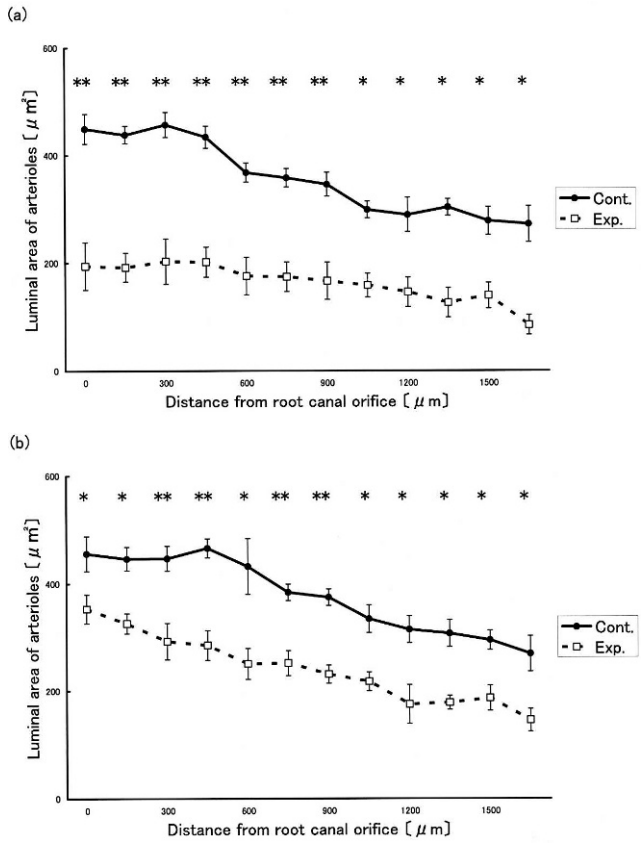

Figure 6. Quantitative analysis of the luminal area of arterioles in the distal root of the mandibular first molar in normal and hypofunctional periodontium groups (* $P < .05$; ** $P < .01$). Upper graph (a) shows 7 days; lower graph (b) shows 14 days.

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