Microvascular Reviews and Communications

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Vol.2 No.1

Microvascular changes after experimental pulpitis in dog 2. application of metronidazole against inflammation

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Abstract

To treat pulpitis, a pulpectomy, which surgically removes dental pulp, is generally used. Recently, antibiotic therapy has also been utilized. In the present study, the effect of metronidazole, incident to experimental pulpitis, was observed by the resin cast method and a scanning electron microscope (SEM). Beagle dogs with healthy periodontal tissue served as the experimental animals. A standard experimental cavity (2mm in diameter x 2 mm depth) was prepared on the surface of the tooth. Experimental cavities were exposed in the oral cavity for 1 day or for 7 days. Then, one side of the experimental cavities was filled with glass ionomer cement containing 3% metronidazole and observed after 30 days. The remaining side of the cavities was filled with glass ionomer cement as a control. After experimental cavity preparation, leaking resin was observed under the capillary network. In the metronidazole-treated group, the leakage of resin decreased. The vascular structure was regenerated in a manner quite similar to normal pulp. However a circulatory disturbance was caused in the pulpal vasculature.

These results indicate that constricted vessels and metronidazole work together to recover the microcirculation of dental pulp, suggesting that metronidazole can be an effective treatment for pulpitis.[MVRC 2 (1) : 18-23 ,2008]

Key Words: corrosion cast, dental pulp, metronidazole, SEM

Received 2007/3/28, Accepted 2008/1/15

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Introduction

The dental pulp is fibrous connective tissue surrounded by the hard tissue of the tooth. A network of blood vessels and nerves exists in the dental pulp. Blood flow in the dental pulp is supplied from the jawbone through the root apex foramen. The surfaces of dental pulp vessels consist of a dense capillary network (CN) along the odontoblast layer.¹⁾ Pulpitis, an inflammation of the dental pulp caused mainly by dental caries, causes vasculature changes in the CN. Generally, a pulpectomy, which surgically removes the dental pulp, is used as a clinical therapy to treat pulpitis. Recently, antibiotic therapy has also been utilized.²⁾ Metronidazole is an antimicrobial agent that targets anaerobic bacteria, which are thought to comprise 80% of the bacteria in inflamed areas of dental caries.³⁾ A drug delivery system mixing metronidazole with filling material has been described.⁴⁾

The microvascular resin cast method involves the injection of low-viscosity synthetic resin into the blood vessels, dissolution of the peripheral tissue with acid and alkaline, and observation of the blood vessels.⁵⁾ This method has long been used to construct vascular models for macroanatomy studies. A clear three-dimensional image can be obtained through the complete infusion of a synthetic resin to the level of the capillaries and subsequent observation using a scanning electron microscope (SEM) with deep focus. Moreover, the intravascular surface structure is imprinted accurately on the surface of the cast. The diameter of the cast allows the distinction of microcirculatory structures such as arteries, capillaries, and veins.⁶⁾ In dentistry, this method has been used mainly to observe the vasculature of periodontal tissue.⁷⁾

In the present study, the effect of metronidazole application after experimental cavity formation was observed by the vascular cast method and SEM.

Materials and Methods

Surgical procedures. The surgical procedures are illustrated in Fig. 1. The experimental procedures were performed on both sides of the lower premolars (P1 to P4) in six beagle dogs with healthy periodontal tissue. The use of experimental animals was in accordance with the Animal Care Committee guidelines of our institution. A straight diamond bur (Diamond point FG311, Syofu, Kyoto, Japan) and dental turbine (MIJET-T Yoshida, Tokyo, Japan) were used to create a standard cylindrical experimental cavity (2 mm in diameter x 2 mm in depth) on the surface of the tooth with a sufficient amount of water spray to avoid heat stimulation.

The experimental cavities were exposed for 1 day (CP1D) or for 7 days (CP7D) on one side. The experimental cavities were filled with glass ionomer cement (GF) containing 3% metronidazole (MN) and observed after 30 days (group 1: CP1D/MN30D, group 3: CP7D/MN30D). The remaining side of the experimental cavities was filled only with glass ionomer cement as a control (group 2: CP1D/GF30D, group 4: CP7D/GF30D).

Morphological procedures. The dogs received vascular resin injections on the day of the procedure and were anesthetized (25 mg/kg of pentobarbital sodium per

body weight, intravenously) prior to all experimental procedures. The common carotid arteries were cannulated, and Ringer's solution containing 0.2% heparin was perfused until the jugular veins were cleared of blood. After the perfusion, 2% glutaraldehyde phosphate buffer solution (pH 7.4) was injected into the carotid arteries for fixation. Following fixation, we resected the coronoid processes to expose the inferior alveolar arteries, into which the synthetic resin (Mercor[®], Dai Nippon Ink, Japan) was manually injected. Peripheral tissue was decalcified by 10% hydrochloric acid followed by 20% potassium hydroxide to dissolve the tissue. All of the specimens were washed thoroughly with 40°C tap water and freeze dried. After being ion-coated with platinum-palladium, the specimens were examined under an SEM (JSM6301F, JEOL Ltd., Tokyo, Japan).

Results

Fig. 2a shows the entire image of the dental pulp 1 day after experimental cavity formation (CP1D). Remarkable resin leakage was observed in the pulp horn (PH) area, but the capillary network (CN) was seen in the area beneath the cavity (*). Fig. 2b shows the dental pulp in Group 1 (CP1D/MN30D) which was administered 1 day after the experimental cavity preparation (CP1D/MN30D). The surface of the dental pulp was covered by the CN. Fig. 2c shows the vasculature of the dental pulp in Group 2 (CP1D/GF30D). The vascular network in the apex of the root pulp is a dense arrangement. However upper part of the root and coronal pulp (arrow) created the rough arrangement.

Enlargement of the dental pulp (Fig. 3a) revealed tall vascular loops (VL) in the pulp horn area. Along the margin of the cavity, the CN was observed. In a further enlarged image (Fig. 3b), leaking resin was observed under the CN. After metronidazole application (group 1), the tall VL were transformed into low VL (arrows) in the CN (Fig. 3c). Leakage of resin decreased, and the CN was rearranged with low loops (arrows; Fig. 3d). In the coronal pulp of group 2, thin diameter of the main vessels (MV) turned to a bundle (Fig. 3e). The tall vascular loops (VL) are project outwards from the MV for the surface layer. The enlarged images which showed the circulatory disturbance was observed. The tip of the VL breaks off, and traces of erythrocytes (ER) are observed at the tip end. Then the vascular lumen is denatured, and the cast surface becomes rugged (Fig. 3f). Traces of a large number of erythrocytes were observed on the surface of the venules of the main vessels (Fig. 3g).

Fig. 4a shows the entire image of the dental pulp vasculature 7 days after experimental cavity formation (group 3). The CN of the pulp horn (PH) had disappeared, and the main blood vessel was revealed. Along the margin of the cavity, leakage of resin was observed (*). Fig. 4b shows the dental pulp 30 days after metronidazole application, which was administered 7 days after experimental cavity formation (group 3). The surface of the dental pulp was covered by the CN. Fig. 4c

shows the vasculature of the dental pulp in Group 4. The blood vessel of the root pulp is observed, but the vascular cast of the coronal pulp (arrow) is not seen.

In an enlarged image of the dental pulp after CP 7D (Fig. 5a), a resin leakage was observed beneath the CN. The constricted vessel (*) ran along the main artery (A) and vein (V) in the axis direction (Fig. 5b). In the dental pulp of group3, the CN and main vessel (MV) were observed, but neither resin leakage nor constricted

vessels were found (Fig. 5c). A cross-sectional view of the pulp (Fig. 5d) reveals a structure quite similar to that of the normal one. The part in which the vascular cast remained in group 4 was enlarged. A part of the CN remained in the upper side of the root pulp. The CN was transformed to a vascular loop along the MV(Fig5e). Traces of erythrocytes (ER) were observed on the end of the VL (Fig. 5f).

Fig. 1. Surgical procedures.

After experimental cavity preparation(CP), cavities were filled with glass ionomer cement (GF) or w/3%/MN.

group 1: CP1D/MN30D

group 2: CP1D/GF30D

group 3: CP7D/MN30D

group 4: CP7D/GF30D

Fig. 2.

CP1D: Remarkable resin leakage was observed in pulp horn (PH) area (a).

group 1: Surface of dental pulp was covered by capillary network (CN) layer (b). periodontal ligament (PDL).

group 2: Vascular network was rough in upper part of pulp (c). periodontal ligament (PDL). junctional epithelium (JE). coronal pulp (arrow)

Fig. 3.
 CP1D: Pulp horn area. Capillary network (CN), Vascular loops (VL) (a).
 Leaking resin (*) (b).
 group 1: CN was rearranged with low vascular loops (arrows). Leakage of resin (*) (c,d).
 group 2: Tall vascular loops (VL) are prominent (e). Traces of the erythrocyte (ER) were observed on VL(f) and Venules(g).

Fig. 4.
CP7D: CN of pulp horn (PH) had different arrangement (*a).
group 3: CN of PH had regenerated (b). periodontal ligament (PDL).
group 4: blood vessel of coronal pulp was not seen (c). periodontal ligament (PDL).

Fig. 5.
CP7D: Leakage of resin was observed under CN (a). Constricted vessel (*) was observed (b).
group 3: No constricted vessels or leakage of resin under CN (c,d).
group 4: Almost all blood vessels of coronal pulp disappeared.
CN changed to VL (e). ER were observed on the end of VL (f).

Discussion

The dental pulp exists inside the hard tissue of the tooth. Therefore, the dental pulp has limited circulation and blood flow from the outside. The regeneration ability of dental pulp after inflammation is low. To treat pulpitis by using a drug delivery system,⁸⁾ it is necessary to understand the relationship between the vascular system and the inflammation defense mechanism of the dental pulp. The blood vessels of dental pulp have a three-layered structure: the main blood vessel (MV) consisting of arteries and veins in the central layer, the arteriole and venule in the intermediate layer, and the CN in the surface layer.⁹⁾

In another report using the resin cast method, leakage of resin during pulpitis was observed in the intermediate layer.¹⁰⁾ Increased permeability of the venule of this layer is a consequence of pulpitis. A typical inflammation image shows that the resin leaked from the vessel instead of the blood plasma.¹¹⁾ In the first report of the present series, this phenomenon was mainly observed 4 hours to 1 day after experimental cavity formation. A similar image was shown in the present study, but the leakage was minimal after application of metronidazole because metronidazole targets anaerobic bacteria in the inflamed area. After metronidazole application, a venule in the intermediate layer that returned from the CN to the MV was observed. This result suggests that blood circulation out of the dental pulp was recovered. After recovery of microcirculation, the pulp tissue can be regenerated.

Constricted vessels, which appear to be lymphatic vessels, are present 7 days after experimental cavity formation.¹²⁾ A similar result was shown in the present study, but leaked resin or constricted vessels were not observed in the intermediate layer after the application of metronidazole.

Substantial resin leakage was observed in the pulp horn. It has been reported that lymphatic vessels do not exist in the pulp horn.¹³⁾ For this reason, the absorption of resin seems to have been retarded in the pulp horn. In this study, constricted vessels were not observed in this area.

Thirty days after metronidazole application, the inflammation disappeared. The vasculature was rearranged similar to the control. During this period, the metronidazole and constricted vessels worked against the inflammation. In the absence of metronidazole

application, necrosis occurred in the control pulp 30 days after treatment with glass ionomer cement filling alone.

In conclusion, metronidazole work together to regenerate the microcirculation of the dental pulp. These results suggest that metronidazole can be an effective treatment for pulpitis.

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