Wound Healing Process of Injured Pulp Tissues with Emdogain Gel

Hikaru Kaida, DDS, Takafumi Hamachi, DDS, PhD,* Hisashi Anan, DDS, PhD,† and Katsumasa Maeda, DDS, PhD**

Abstract

This study aimed to investigate the wound healing process of injured pulp tissues with Emdogain gel (EMD). Pulpotomy was performed for the first molars of the mandibles in rats. EMD or Vitapex (VIT)-containing calcium hydroxide was applied to the exposed pulp tissues. The treated teeth were extracted after 7, 14, and 28 days and prepared for histologic examination. In the VIT-treated group, the number of interleukin-1β (IL-1β)–expressing macrophages initially increased, followed by that of transforming growth factor–β1 (TGF-β1)–expressing macrophages. The number of cells expressing bone morphogenetic proteins (BMPs) gradually increased with reparative dentin formation. Meanwhile, in the EMD-treated group, cells expressing IL-1β or TGF-β1 were few. However, the number of BMP-expressing cells, partly macrophages, increased in the early phase, and large amounts of reparative dentin were observed. This study demonstrated that different healing processes existed for EMD and VIT. BMP-expressing macrophages might play important roles in reparative dentin formation. *(J Endod 2008;34:26–30)*

Key Words

BMP, dental pulp, Emdogain, macrophage, wound healing

From the *Department of Periodontology, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, Fukuoka, Japan; and †Section of Operative Dentistry and Endodontology, Department of Odontology, Fukuoka Dental College, Fukuoka, Japan.

Address requests for reprints to Dr Takafumi Hamachi, Department of Periodontology, Faculty of Dental Science, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. E-mail address: thamachi@dent.kyushu-u.ac.jp.

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Various materials have previously been used in pulp capping and pulpotomy procedures. Among them, calcium hydroxide has been conventionally used in clinics (1). However, it has been reported that pulp tissues become strongly irritated, and a necrotic layer forms as a result of the alkaline action of calcium hydrate (2). Although reparative dentin is newly formed by calcium hydroxide, its structure is porous (3). The formation of pores increases the risk of bacterial infection (4). Moreover, although the majority of studies with calcium hydroxide–based materials reported hard tissue bridging, Olsson et al (5) claimed that these did not give satisfactory results. For these reasons, the development of biocompatible materials that induce a dentin/pulp complex is preferred.

Recently, many studies have reported that new biocompatible materials such as bone morphogenetic proteins (BMPs) (6), osteogenic protein–1 (OP-1) (7), demineralized dentin (8), and mineral trioxide aggregate (MTA) (9) can induce the formation of reparative dentin. Recent research has suggested that Emdogain gel (EMD) (BIORA AB, Malmö, Sweden), which is often used in periodontal regenerative therapy, also has the capacity to induce rapid reparative dentin formation in pulpotomized teeth (10, 11).

EMD is made from an enamel matrix derivative secreted from Hertwig's epithelial sheath during porcine tooth development (12, 13). This enamel matrix derivative contains amelogenin as its major component in addition to other enamel matrix proteins such as enamelins, tuftelin, amelin, and ameloblastin (14–17). It serves as an important regulator of enamel mineralization (18) and plays an important role during periodontal tissue formation (12, 13). It also stimulates the regeneration of periodontal tissues including the acellular cementum, periodontal ligaments, and alveolar bone by mimicking tooth development. However, the precise mechanisms of periodontal tissue regeneration with EMD remain unknown. Furthermore, the mechanisms of wound healing in the injured pulp tissues with EMD as a pulp-capping material have hardly been demonstrated.

Therefore, the purpose of this study was to investigate the wound healing process of injured pulp tissues with EMD. Because it was reported that EMD enhanced alkaline phosphatase (ALP) activity and the expression of bone matrix proteins in osteoblasts (19), ALP staining and immunostaining against dentin matrix protein–1 (DMP-1), which was one of the bone matrix proteins identified from dentin, were initially performed to investigate the status of the injured pulp and reparative dentin. Moreover, we investigated the behavior of inflammatory cells, the expressions of inflammatory cytokine, anti-inflammatory cytokine, and hard tissue formation–activated factor by using immunohistochemical methods. These are considered to be important for bone tissue remodeling (20). Macrophages and T cells, interleukin-1β (IL-1β), transforming growth factor–β1 (TGF-β1), and BMPs were selected as markers. To elucidate the effect of EMD on wound healing of the injured pulp tissues, the results obtained with EMD were compared with those of Vitapex (calcium hydroxide and iodoform paste; Neo-Dental, Tokyo, Japan) (VIT)-containing calcium hydroxide.

Materials and Methods

Surgical Procedure

A total of 90 mandibular first molars from 45 male Sprague-Dawley rats (age, 5 weeks) were used in this study. All experimental procedures were conducted in accordance with the animal experimental guidelines of Kyushu University. The animals un-

derwent general anesthesia with pentobarbital sodium. After the first molars were cleaned and disinfected with 3% hydrogen peroxide followed by swabbing of the mouth with 0.2% chlorhexidine gluconate, pulpotomy was performed with a #1/2 round bur rotated by a low-speed electric engine. The size of the cavities was 1 mm in depth and 1 mm in diameter. During cavity preparation, the tooth and cutting instrument were irrigated with sterile saline to prevent impairment from heat. Bleeding was controlled with sterile saline irrigation and the use of cotton pellets. All the procedures resulted in pulp exposure of uniform size.

The exposed pulp tissues were covered with Emdogain gel (EMD-treated group, 30 teeth) or Vitapex (VIT-treated group, 30 teeth). The cavities were subsequently sealed with glass ionomer cement (Fuji IX; GC, Tokyo, Japan). Moreover, some teeth were only sealed with glass ionomer cement without the pulp-capping material as control (GIC-treated group, 30 teeth). Each group was randomly divided into 3 additional groups, and the animals were killed at 7, 14, and 28 days after treatment. The number of teeth analyzed at each stage in each group was 10.

Section Preparation

Each animal was fixed by intracardiac perfusion of periodate-lysine-paraformaldehyde (3% paraformaldehyde, 0.01 mol/L $NaIO_4$, and 0.075 mol/L lysine in 0.025 mol/L phosphate buffer, pH 7.3). The mandibular first molars were dissected with the surrounding jaw bones and post-fixed with the same fixative at 4°C for 12 hours. The samples were then demineralized with 10% ethylenediaminetetraacetic acid in a 7.5% polyvinylpyrrolidone solution (Sigma Chemical Co, St Louis, MO) at 4°C for 14 days. The decalcified specimens were then washed with 0.01 mol/L phosphate-buffered saline (PBS) and freeze-embedded in OCT compound (Miles Scientific, Naperville, IL).

Frozen specimens were serially step-sectioned (5 μm) parallel to the long axis of the tooth. Subsequently, enzyme activities and immunohistochemical reactions were investigated in the serial sections.

Enzyme Histochemistry

Nonspecific ALP staining was performed by the method described by Burstone (21), with naphthol AS-BI phosphate (Sigma) as the substrate and Fast Red Violet LB salt (Sigma) as the coupler. The sections were counterstained with methyl green (Sigma).

Immunohistochemistry

Frozen sections were first incubated with 0.3% H_2O_2 in methanol for 30 minutes to remove endogenous tissue peroxidase. After rinsing in PBS, the sections were incubated with appropriate primary antibodies such as mouse monoclonal antibody to rat macrophages, ED1 (Serotec Co, Indianapolis, IN); mouse monoclonal antibody to rat T cells, CD5 (Seikagaku Co, Tokyo, Japan); rabbit polyclonal antibodies to rat IL-1β (Endogen, Woburn, MA); rabbit polyclonal antibodies to TGF-β1 (Promega Co, Madison, WI); goat polyclonal antibodies to BMP-2 (Santa Cruz Biotechnology Inc, Santa Cruz, CA); goat polyclonal antibodies to BMP-4 (Santa Cruz); and mouse and rabbit polyclonal antibodies to DMP-1 (Takara Bio Inc, Shiga, Japan). Immunoreactivity was developed by using a streptavidin-biotin peroxidase staining system (Nichirei Co, Tokyo, Japan). Throughout the staining, the sections were washed 3 times for 5 minutes each with PBS between each incubation step. Finally, the sections were exposed to DAB (Nichirei) until the color was developed and counterstained with methyl green.

In addition, double staining was performed to investigate the relationship between ED1-positive macrophages and the expressions of IL-1β, TGF-β1, BMP-2, or BMP-4. Frozen sections were first incubated with anti–IL-1β, –TGF-β1, –BMP-2, or –BMP-4 antibody, and the color was developed by using DAB (Nichirei), as described above. After washing with PBS, the anti-ED1 antibody was placed on the sections and left to react. Immunoreactivity was developed by using Histofine Simple Stain AP (M) (Nichirei). Tetramisole hydrochloride (Sigma) was used to block endogenous ALP activity. After the color was developed, the sections were counterstained with methyl green.

Histometric Analysis

Histometric analysis was performed with serial sections taken from near the central part of injured pulp tissues. The width of the necrotic layer and the area of reparative dentin formation were measured with the computer software Scion Image (Scion Co, Frederick, MD). ED1-positive mononuclear cells, CD5-positive cells, IL-1β–, TGF-β1–, BMP-2–, and BMP-4–expressing cells were counted in a field determined in an ocular grid (7 mm × 7 mm), which was placed right under the necrotic layer by using a ×400 magnification objective lens (Fig. 1). The average values of 10 sections taken from 10 teeth were used as reported results for each stage in each group. Statistical analysis was performed with the Student *t* test.

Results

Histochemical and Immunohistochemical Findings

VIT-treated Group

Throughout the experimental period, strong ALP activity was observed, and CD5-positive T cells were scattered in the injured pulp tissues. Moreover, the area of the necrotic layer was significant.

Seven days after treatment, many neutrophils, ED1-positive macrophages, and IL-1β–expressing cells were observed below the necrotic layer. TGF-β1– and BMP-expressing cells were few; and reparative dentin was hardly seen (Figs. 2*a* and 3*a*).

Fourteen days after treatment, many neutrophils, ED1-positive macrophages, and IL-1β–expressing cells were still observed below the necrotic layer. However, TGF-β1–expressing cells increased below the necrotic layer. DMP-1–positive reparative dentin was added to the surface of the root canal wall, and the number of BMP-expressing cells was increased near the capillaries and the reparative dentin (Figs. 2*b*, 3*b*, and 4*a–f*).

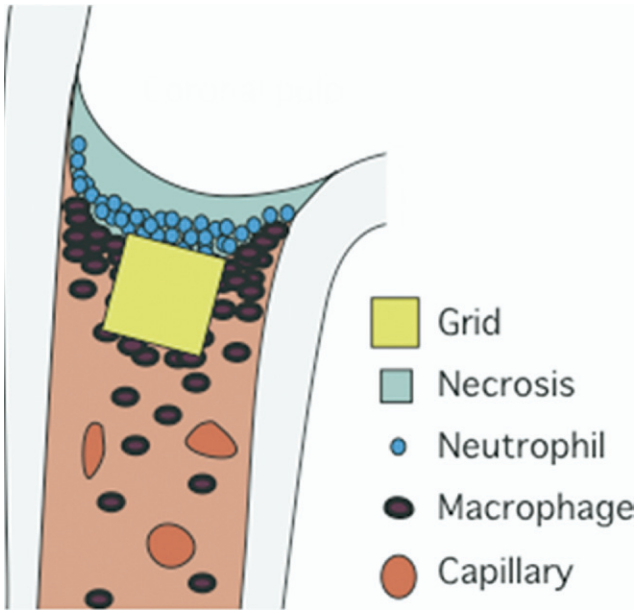

Figure 1. Method of histometric analysis. Each immunopositive cell was counted in a field determined in an ocular grid, which was placed right under the necrotic layer by using a ×400 objective lens.