Response of Inflamed Pulps of Rat Molars after Capping with Pulp-capping Material Containing Fluocinolone Acetonide

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Abstract

Introduction: Capping inflamed dental pulp tissue is currently a controversial issue. To reduce pulp inflammation and stimulate pulp healing, a pulp-capping material containing fluocinolone acetonide (PCFA) has been developed. This study was aimed to evaluate the inflammatory response and hard tissue formation of inflamed dental pulps of rat maxillary molars after capping with Dycal, mineral trioxide aggregate (MTA), or PCFA. **Methods:** Sixty maxillary rat molars were exposed to the oral environment for 48 hours. The exposed pulps were randomly divided into 6 groups (n = 10) according to pulp-capping materials (Dycal, MTA, or PCFA) and time (8 or 30 days). The cavities were capped and sealed with Fuji II LC. The animals were sacrificed after 8 and 30 days. Histologic specimens were prepared and evaluated for inflammatory response and hard tissue formation. **Results:** Eight days after pulp capping, all experimental groups showed disruption of the odontoblast layer in areas corresponding to the pulpal exposure. Acute inflammation was found in 80%, 60%, and 40% of samples in the Dycal, MTA, and PCFA groups, respectively. PCFA significantly decreased the pulp inflammation compared with Dycal. After 30 days, slight to moderate inflammation was found in all experimental groups. Hard tissue formation was found in 78%, 63%, and 100% of samples in the Dycal, MTA, and PCFA groups, respectively. No significant difference was found among the experimental groups. **Conclusions:** Pulp capping with PCFA reduced the inflammation and stimulated hard tissue formation in the exposed pulps of rat molars. It may be used as a pulp-capping agent in inflamed pulps. *(J Endod 2015;41:508–512)*

Key Words

Calcium hydroxide, dental pulp, fluocinolone acetonide, inflammation, mineral trioxide aggregate, pulp capping

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Direct pulp capping is controversial for cariously exposed pulp because of unpredictable severity of inflammatory condition of underlying pulp tissue and variable long-term clinical success (1–4). Various pulp-capping materials have been used to cap the inflamed dental pulp (3, 5). Calcium hydroxide has been traditionally used as an effective pulp-capping material. Its strong alkalinity promotes antibacterial property and affects vital pulp tissues by the induction of hard tissue deposition (6). The success rate with inflamed pulps is better for calcium hydroxide than for adhesive systems (3, 4, 7). Mineral trioxide aggregate (MTA) and Biodentine are silicate cement, which has been successfully used in vital pulp treatment (8, 9). It has good sealing ability and can stimulate thick dentinal bridge formation, even in cases of irreversible pulpitis (10, 11). In human teeth, similar or better results have been shown when compared with calcium hydroxide (12, 13). However, capping the inflamed pulps with MTA in animal and human studies has demonstrated questionable results (14, 15).

Fluocinolone acetonide (FA) is a synthetic corticosteroid commonly used for topical application in the management of dermatologic disorders and oral vesiculoerosive lesions (16). Interestingly, a wide range of concentrations of FA have a proliferative effect on some types of cells, such as skin fibroblasts and dental pulp cells (17–20). This effect is concentration-dependent; high concentrations inhibit mitotic activity, but low concentrations slightly increase the activity (17). A specific range of concentrations (0.1–10 μmol/L) of FA also stimulates the extracellular matrix and hard tissue formation of human dental pulp cells (19, 20).

Healing of the dental pulp tissue is dependent on the degree of inflammation (21). Thus, to achieve the goal of dental pulp healing in unknown inflammatory conditions, more experiments are needed to discover an effective material that may provide clinicians an additional option for the treatment of caries-exposed pulps. Anti-inflammatory medicament mixed with a drug delivery vehicle may be an effective direct pulp-capping material. Recently, a pulp-capping material containing fluocinolone acetonide (PCFA) has been developed. It is a setting type of calcium hydroxide cement that is able to release hydroxyl ions and specific concentration (0.1–10 μmol/L/mm^2) of FA. The majority of the FA is released within the first week (22). This has been hypothesized to decrease the severity of pulpal inflammation and to stimulate healing and hard tissue formation of inflamed pulp tissue. However, the effects of PCFA have never been elucidated. This *in vivo* study was aimed to compare the effects of Dycal, MTA, and PCFA on the inflammation and hard tissue formation of the inflamed dental pulps in rat maxillary molar teeth.

Materials and Methods

The experiment was modified from the study of Six et al (23). Briefly, the protocol was approved by the Ethics Committee for Animal Use, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand. Thirty 8-week-old male Wistar rats (National Laboratory Animal Center, Mahidol University, Nakhon Pathom, Thailand) were used for this investigation. All animals were cared for with routine husbandry before and after surgery. The animals received an intraperitoneal injection of 50 mg/kg Nembutol (Abbott Laboratories, North Chicago, IL). Mesial aspects of maxillary first molars were selected because of their ease of access. The gingival tissue was cut by electrosurgery. Half-moon, class V–like cavities were prepared at the cervical third of teeth in 1–2 seconds by using 0.6-mm-round tungsten carbide burs with copious sterile saline irrigation. The depth of the cavity was kept constant, approximately half the size of the bur.

TABLE 1. Evaluation Criteria for Rat Pulp Tissue Response Determined by Inflammatory Cells and Hard Tissue Formation

Score	Definition
Inflammatory cell response criteria	
0	No inflammation at or beneath the exposure site
1	Few scattered inflammatory cells at or beneath the exposure site (mild or slight response)
2	General or localized moderate inflammatory cell infiltration in the pulp proper at or beneath the exposure site (moderate response)
3	Severe inflammation and/or abscess formation at or beneath the exposure site (severe response)
Mineralization in the radicular pulp	
0	No trace of mineralization in the radicular pulp
1	Increased deposition of hard tissue along the surface of the remaining pulp tissue
2	The radicular pulp is completely obliterated by a mineralized mass, tubular dentin, or osteodentin

The burs were changed after every fourth cavity. Pulp perforation was established by pressure with the tip of a sharp endodontic explorer (Hu-Friedy Mfg Co, Chicago, IL). The dental pulps were left opened for 48 hours. Then, the animals were re-anesthesized. The cavities were rinsed with sterile saline. Sixty cavities were randomly allocated into 2 groups by date, 8 or 30 days, and 3 groups by materials: Dycal (Dentsply Caulk, Milford, DE), MTA (Dentsply Tulsa Dental Specialties, Tulsa, OK), or PCFA (Chulalongkorn University, Bangkok, Thailand) by stratified randomization. Compositions of PCFA were reported in the previous study (22). The exposed pulps were blot dried and covered with one of the experimental materials. The materials were mixed according to the manufacturer's recommendations and applied directly onto the exposed pulps. The cavities were then primed and restored with Fuji II LC (GC America, Chicago, IL). All procedures were done under dental loupes at ×3 magnification with fiberoptic light (Surgitel, Ann Arbor, MI).

The anesthetized rats were killed by overdose of carbon dioxide gas and confirmed by cervical dislocation. Within a few minutes, the whole maxilla was dissected out, separated into 2 halves, and trimmed into block sections. The sections were fixed in fixative solution, demineralized in 10% EDTA, dehydrated in graded ethanols, and embedded in paraffin. Serial sections of 7-μm thickness were cut through the entire pulps and placed on glass slides. Staining of sections with hematoxylin-eosin was used to evaluate cells and tissue response. Histologic sections were blindly evaluated by one experienced investigator who used an Olympus BX51 microscope (Olympus America Inc, Melville, NY). The criteria and grading system were modified from those of Six et al (24), as summarized in Table 1. Morphometric analysis was used to determine the degree of inflammation and hard tissue formation. The data were analyzed by Kruskal-Wallis and pairwise comparison tests by using IBM SPSS Statistics software version 19 (IBM Corporation, Armonk, NY) at the 95% confidence interval.

Results

From 60 molars of the experimental groups, 3 molars from the 30-day group were excluded from the study, 1 from the Dycal group and 2 from the MTA group. The reasons were due to dislodgement of the restoration and error during tissue processing. In total, 57 molars were included for histologic evaluation. The results of the inflammatory response and hard tissue formation are summarized in Figure 1.

Eight days after pulp capping, acute inflammation was found in 80%, 60%, and 40% of samples in the Dycal, MTA, and PCFA groups, respectively. PCFA significantly reduced the pulpal inflammation compared with Dycal ($P < .05$). Hard tissue deposition was frequently

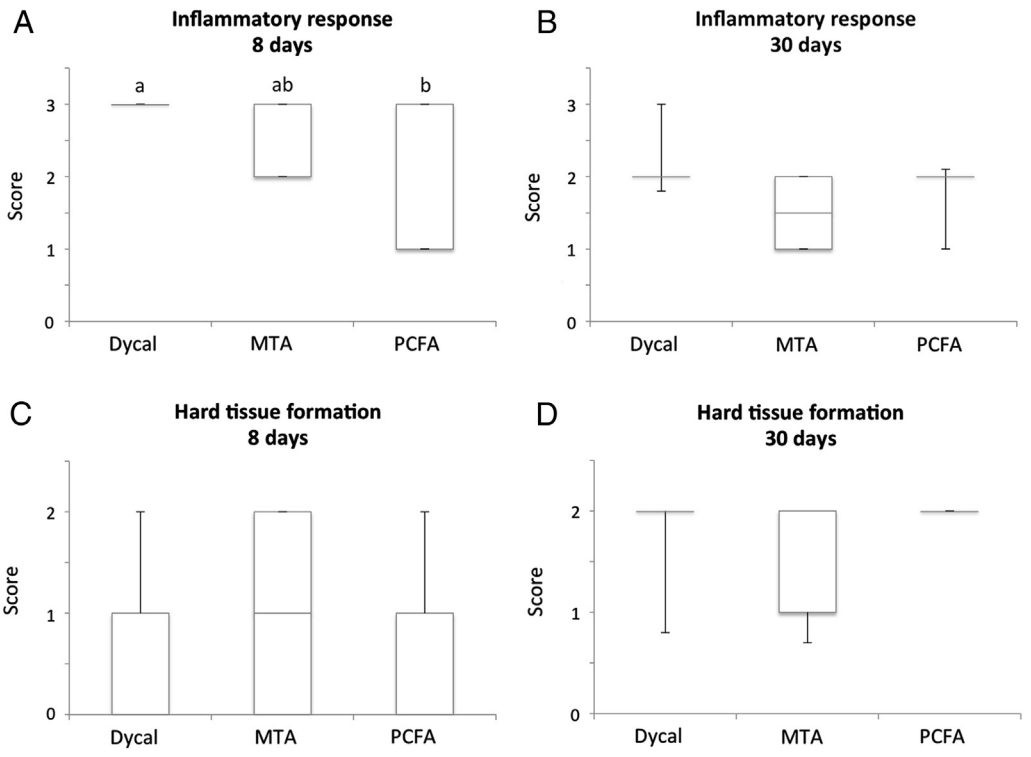

Figure 1. Box plots of data. (*A*) Inflammatory response at 8 days. The letters *a* and *b* indicate statistically significant difference between groups ($P < .05$). (*B*) Hard tissue formation at 8 days. (*C*) Inflammatory response at 30 days. (*D*) Hard tissue formation at 30 days.