Pulpal Tissue Inflammatory Reactions after Experimental Pulpal Exposure in Mice

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Abstract

Introduction: The purpose of this study was to establish a stable experimental mice pulpal inflammatory model and to evaluate inflammatory reactions of pulpal tissue after pulpal exposure. **Methods:** Pulpal inflammation was induced in 80 C57BL/6 mice by occlusal exposure of the pulp of the maxillary first molar. The mice were sacrificed randomly at 0, 1, 6, 12, 24, 48, and 72 hours after pulpal exposure. Mice without pulpal exposure served as controls. Maxillary teeth were obtained and prepared for histologic analyses and real-time polymerase chain reaction analyses. **Results:** As the duration of pulpal exposure increases, the inflammatory reaction is exacerbated. Within 6 to 12 hours after pulpal exposure, pulp tissues experienced red blood cell extravasation to the destruction of the odontoblast layer. After 24 hours, necrosis was observed in the pulpal tissue; until 72 hours, necrosis spread to the whole coronal pulpal tissue, and a large number of inflammatory cells were found in the radicular pulpal tissue. The results of histomorphologic scores have the same trend; samples from the 72-hour group possessed the highest score followed by samples from other groups ($P < .01$). The expression levels of inflammatory cytokines increased over the 72 hours, and there was a high rate of inflammatory cytokine expression at 6 and 12 hours after pulpal exposure. **Conclusions:** Our study represents a stable mice model for studying pulpal inflammation *in vivo*. Mouse pupal inflammation progresses rapidly, with dramatic changes evident in just a few hours. *(J Endod 2016;■:1–6)*

Key Words

Animal model, histomorphologic evaluation, pulpal inflammation

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Significance

In the present study, we created a convenient mouse model of pulp inflammation. Our study can provide a theoretical reference for the study of the pathogenesis of pulp inflammation, and allows for better understanding of the development state of pulp inflammation in clinical situations.

Pulpitis is one of the most common oral diseases and is mainly caused by bacterial infection of the dental pulp. The introduction of bacteria into the pulp can easily result in irreversible pulpitis that impairs the spontaneous healing of the pulp tissue, ultimately resulting in necrosis and death of the pulp tissue. During pulp inflammation, dental pulp cells secrete a variety of inflammatory cytokines that participate in the immune response and cause pain (1), leading to periapical inflammation, which exacerbates a patient's suffering.

In recent years, researchers have focused on studying the molecular mechanism of pulpitis. However, the signaling pathways of pulpitis development have not been clarified; therefore, a reasonable and stable animal model is needed to study the development mechanism of pulpitis.

Many investigators have induced periapical and pulpal lesions in mammals *in vivo* (2–4). To date, these investigations have focused on the histologic description of periapical inflammation in mice but not on a description of pupal inflammation.

Also, most of these studies involved large animals, such as monkeys, dogs, and pigs, which have higher similarity to humans regarding teeth anatomy, evolution, and development, or animals that are easier to operate on such as rabbits and rats. Compared with the aforementioned animals, mouse models of pupal inflammation have distinct advantages. For instance, sample sizes can be larger, and models using knockout mice are easy to create.

Therefore, in the present study, we induced pulpal inflammation of mice by pulp exposure at 0, 1, 6, 12, 24, 48, and 72 hours; we used inflammatory parameters and scores for evaluation and observed changes in inflammatory cytokine expression in order to create a convenient model for studying the pathogenesis of pupal inflammation *in vivo*.

Material and Methods

Induction of Pupal Lesion

All animal studies were approved and supervised by the Ethics Committee of Affiliated Stomatology Hospital, Guangzhou Medical University, Guangzhou, China. Eighty C57BL/6 mice (8–10 weeks old) weighing 20–30 g were purchased from the Medical Laboratory Animal Center of Guangdong Province, China, and randomly divided into 8 groups. Seventy mice were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg), and a cavity was prepared with a #1/4 dental round bur on the occlusal surface of the bilateral upper first molars (class 1 cavity) under a surgical microscope (×40). The upper first molar was drilled (medium speed with cooling system) until the pulp was visible through the transparency of the dentin floor of the cavity. Pulp was subsequently exposed using an endodontic hand file (0.15-mm diameter tip, 2% taper, 21 mm). The exposed preparation cavity (Fig. 1*A–C*) was left open to the oral environment. Ten animals without exposed pulp served as the controls.

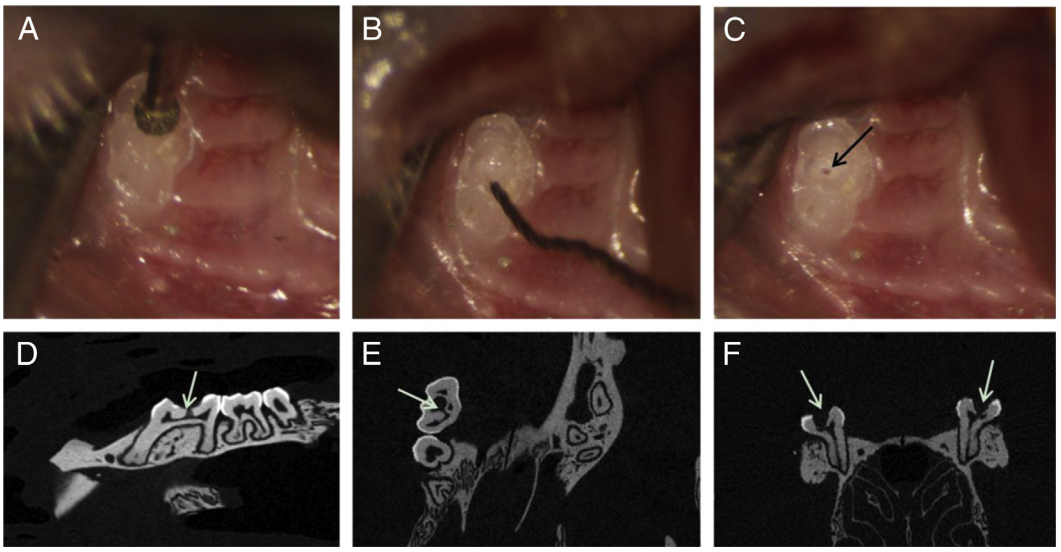

Figure 1. Establishment of an animal model. Pulp exposure and micro-CT images of the upper first molar in a mouse. (*A–C*) The procedure of pulp exposure (×40). (*D*) The sagittal view after pulp exposure. (*E*) The transverse view after pulp exposure. (*F*) The coronal view after pulp exposure. (*Arrows* indicate a pulp lesion.)

Sample Preparation

Ten mice in each group were sacrificed at 0, 1, 6, 12, 24, 48, and 72 hours after pulpal exposure. Five maxillae were dissected free of soft tissues, fixed with 4% paraformaldehyde at 4°C for 48 hours, and then imaged using micro–computed tomographic (micro-CT) imaging. After micro-CT image acquisition, these samples were prepared for histologic analysis. The other 5 maxillae (including 10 teeth that were extracted from maxillae) were used for analysis of the inflammatory cytokines.

Micro-CT Analysis

After being washed in running water, each maxilla with the tooth was dried, mounted on a custom attachment, and scanned in a micro-CT scanner (SkyScan 1172; Bruker-microCT, Kontich, Belgium) operated at 60 kV and 100 mA (0.5-mm Al filter). The scanning was performed by rotating the mounted sample 180° around the vertical axis, with a rotation step of 0.4°. The cross-sectional pixel size and intersection distance were both 9.93 mm. Images were reconstructed (NRecon v.1.6.3, Bruker-microCT) to obtain axial cross sections of each specimen's inner structure. DataViewer v.1.5.1.10 software (Bruker-microCT) was used for 2-dimensional evaluation of the diameter of the pulpal exposure site.

Histopathologic Analysis

The maxilla with the tooth was rinsed and decalcified with 10% EDTA for 6 weeks, dehydrated, and embedded in paraffin; 4-μm thick serial sections were cut in the mesiodistal direction and used for histologic analyses.

Qualitative and quantitative histopathologic analyses were performed on hematoxylin-eosin–stained specimens using the bright-field mode of a fluorescence microscope (Leica/DM4000 B; Leica Microsystems, Heidelberg, Germany). We chose 5 samples at control 0, 1, 6, 12, 24, 48, and 72 hours after pulp exposure, respectively. During each time point, each sample was selected at a 5~6 high microscope field of vision, which has the maximum area of coronal pulp tissue for quantitative analysis. An experienced examiner blinded to the groups conducted all analyses.

The histomorphologic parameters used in this study (Table 1) were based on criteria previously described (5, 6); each parameter was scored 1 to 4 (with 1 being the best result and 4 being the most severe). The study-wide null hypothesis was that the distributions of histologic findings would be the same among the 8 experimental groups for all 5 histomorphologic parameters. To reduce the study-wide type I error rates, multigroup comparisons were performed using a Bonferroni correction alpha of $P = .0018$. For each histomorphologic parameter, a null hypothesis of equal distributions among the 8 groups was tested using the Kruskal-Wallis H test for comparison of multiple independent samples of frequency table data. If P was $<.05$ for differences between the 8 groups, then Mann-Whitney U tests were performed to identify which groups were significantly different from each other.

Real-Time Polymerase Chain Reaction

Teeth were collected from groups of 5 animals at intervals of 0, 1, 6, 12, 24, 48, and 72 hours after cavity preparation. Teeth were also collected from control animals. After removing surrounding tissue, the teeth were stored in liquid nitrogen until used.

TABLE 1. Parameters and Scores Used for Evaluation

Parameters	Scores
Location of inflammatory cells	Absent (score 1) Restricted to the exposed site (score 2) Up to odontoblast layer (score 3) Pervaded in the entire coronal pulp (score 4)
Intensity of the inflammatory infiltrate	0–20 inflammatory cells (score 1) 21–40 inflammatory cells (score 2) 41–80 inflammatory cells (score 3) Over 80 inflammatory cells (score 4)
Inflammatory edema	Absent (score 1) Present (score 2)
Vascular leakage	Absent (score 1) Present (score 2)
Extension of pulp necrosis (coronal pulp)	Absent (score 1) 1%~10% necrotic area (score 2) 11%~20% necrotic area (score 3) Over 20% necrotic area (score 4)