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Effect of different dental burs for experimental induction of pulpitis in mice

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ABSTRACT

Objective: To evaluate the effect of using different dental burs on the development of pulpal inflammation after pulpal exposure in mice.

Design: Eighty-eight C_{57}BL/6 mice were randomly assigned to group A (n = 40), group B (n = 40) and control group (n = 8). The pulps of the maxillary first molars were occlusally exposed using ¼ round burs and polishing burs in group A and B respectively. Animals were sacrificed randomly at 0 h, 4 h, 8 h, 12 h and 24 h after pulpal exposure. Micro-CT scanning was used to determine the success rate of sample preparation. Pulpal tissue changes were evaluated by histopathologic and immunohistochemical analyses.

Results: The success rates of sample preparations were 85% in group A and 90% in group B. The mean maximum diameter of pulpal exposure area was 625.6 ± 30.6 μm in group A and 402.7 ± 18.0 μm in group B ($p < 0.05$). In addition, the mean of the minimum remaining dentine thickness at the marked region of interest was 133.2 ± 29.9 μm in group A and 261.4 ± 16.3 μm in group B ($p < 0.05$). Histopathologic staining demonstrated more signs of inflammation in both groups, as the duration of pulpal exposure increases. However, the rate of inflammatory progress was higher in group A, even spreading to the root pulp tissue within a few hours. For group B, the progress was relatively slow.

Conclusions: Pulpal exposure with different sizes of dental burs affects changes in the development of pulpal inflammation in mice.

1. Introduction

Pulpitis is an inflammatory disease of dental pulp tissue, which may occur secondary to deep caries, wedge-shaped defects, tooth attrition or trauma. Like other injured tissues, the pulp will initially provoke a defense response, depending on the duration and type of the trauma (Pierce, 1998). Besides, as the deep caries progresses, the carious bacterial biofilm composition evolves the pulp tissue (Cooper, Holder, & Smith, 2014). These events can all enable the cells within the pulp tissue to release molecular mediators to result eventually in inflammation. However, the underlying cellular and molecular mechanisms in the development and treatment of pulpitis require further research. It is necessary to establish an acceptable model for further investigation. To date, pulpitis models have been established in rats (Huang et al., 2015), rabbits (Haddad, Lefranc, & Aftimos, 2003), and dogs (Shahravan, Ghoddusi, Eslami, & Rategar, 2010). Mice should be the first choice in research that needs a large sample size or requires crucial vectors for gene knockout. However, due to their small-volume molars and narrow oral space, it is difficult for mice to be used for this purpose. Moreover, a ¼ round bur, which has been used traditionally for establishment of pulpitis in mice, is relatively large for molars and could induce a rapid inflammatory process (He et al., 2017). This extensive inflammation may not benefit subsequent investigations of mechanisms. Thus, we wondered if there was a better instrument for inducing experimental pulpitis in mice.

This research investigated which tool, a ¼ round bur or polishing bur, would be more appropriate for inducing pulpitis in mice. We would like to determine the success rate of using a ¼ round bur and polishing bur to produce experimental pulpitis in mice by pulpal exposure. Also, the similarities and differences of the pulp tissue changes induced by these dental burs were compared histologically with inflammatory parameters and scores.

2. Materials and methods

2.1. Experimental design

Eighty-eight C_{57}BL/6 mice, aged 6–8 weeks and weighing 20–30 g, were purchased from the Medical Laboratory Animal Center of Guangdong Province, China. Their oral conditions were good, without

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dental caries and periodontal diseases. All study protocols were approved by the ethics committee of Stomatology Hospital, Guangzhou Medical University. The mice were randomly distributed into the following three groups: (1) group A (n = 40): the maxillary first molars were drilled occlusally with ¼ round burs (BR-48F, Mani Co., Tochigi, Japan) to make class I cavities, which were deepened until the pulpal tissues were exposed. (2) group B (n = 40): the teeth were prepared in exactly the same way but using polishing burs (TC-21EF, Mani Co., Tochigi, Japan) instead. (3) control group (n = 8): the mice were anesthetized and the teeth were left intact.

The mice were anesthetized by intraperitoneal injection with 1% pentobarbital sodium (0.01 ml/1 g body weight) and fixed in the supine position for surgery. The mouth was opened gently by metal tweezers and sterilized with 75% ethyl alcohol. The bilateral maxillary first molars in groups A and B were drilled occlusally (at medium speed with cooling system from the handpiece) under a microscope (M320 F12, Leica Microsystems, Schweiz) with ¼ round burs and polishing burs, respectively. The exposed pulp tissue was then left open to the oral environment. Eight mice in groups A and B were randomly sacrificed under anaesthesia, by systemic perfusion with 4% paraformaldehyde, at 0 h, 4 h, 8 h, 12 h and 24 h after pulpal exposure. After removal of the soft tissue, the teeth together with maxillae were dissected and fixed in 4% paraformaldehyde at 4 °C for 48 h. In the control group, eight animals were also anesthetized and perfused, and the specimens without pulpal exposure were also collected for Micro-CT and histopathologic analyses.

2.2. Determination for success rate of sample preparation

After being fixed in 4% paraformaldehyde and then washed in running water, each sample was scanned by the micro-CT system (SkyScan1172, Bruker-microCT, Kontich, Belgium) (Morales et al., 2016). The X-ray was operated at a 180° rotation with an X-ray tube setting of 59 kV/0.1 mA for almost 45 min. Next, the scanning results were reconstructed at a resolution of 10 μm voxels using SkyScan's reconstruction software (NRecon v.1.6.3, Bruker-microCT). Endodontic perforation, coronal or root fracture and the overextension of the access hole (more than one hole on the occlusal surface) were considered failure cases and these failures were excluded from the subsequent histological analysis. Then, ten teeth in each group were randomly selected and the mean maximum diameter of pulpal exposure area on the occlusal surface was obtained, which was assessed using an unpaired *t*-test. In addition, the minimum remaining dentine thickness (RDT) was measured at a region of interest of 200 μm in width at the peak of each horn. In each group (n = 10), RDT was obtained at up to 3 pulp horns per tooth at the marked area of interest (Majkut, Sadr, Shimada, Sumi, & Tagami, 2015). The mean of the minimum RDT obtained from both groups was compared, and statistical differences were evaluated. Besides, the total number of the evaluated teeth in each group was eighty and the formula to calculate the success rate is $p = (80\text{-}n_{failure})/80*100\%$. Then, the statistical significance of the success rate was assessed by the chi-square test.

3. Histological analysis

The samples were decalcified in 10% EDTA for 45 days, embedded in paraffin, then sliced serially with a thickness of 4 μm as previously described (Li et al., 2015). The slices were stained with Haematoxylin and Eosin (H & E) and then sealed with neutral balsam. All histopathologic analyses were conducted by a skilled investigator blinded to the treatment groups.

After the failure cases had been excluded, eight sections that represented the maximal coronal pulp tissue at each time point were selected randomly for evaluation. The parameters used in Table 1 were based on previously described criteria (Hebling, Giro, & Costa, 1999; Holland et al., 2005; Leonardo, Barnett, Debelian, de Pontes Lima, & Bezerra da Silva, 2007). Moreover, the parameter was scored 1 to 5, with 1 being the best result and 5 being the most severe. For each parameter in group A or B, the Kruskal–Wallis H tests and Bonferroni tests were performed to identify the differences among six time points and which time points were significantly different from each other. In addition, the differences in groups A and B at the same time point were assessed by Mann–Whitney *U* tests.

Table 1
Parameters and Scores.

Parameters	Scores
Extension of the inflammatory reaction	Absent (score1) Restricted to the exposed site (score2) Up in the coronal pulp (score3) Pervaded in the entire coronal pulp (score4) Involved to the upper part of root pulp (score5)
Intensity of the inflammatory infiltrate	0 to 20 inflammatory cells (score1) 20 to 40 inflammatory cells (score2) 40 to 60 inflammatory cells (score3) Over 60 inflammatory cells (score4)
Extension of the tissue disorganization	Normal tissue (score1) Odontoblastic layer disorganization (score2) Total disorganization of the pulp tissue morphology (score3) Pulp necrosis (score4)

3.1. Immunohistochemical analysis

The specimens (n = 8) collected at 24 h after pulpal exposure in groups A and B were placed in a mixed solution of 3% H_2O_2 and 0.3% TritonX-100 for 30 min and then treated with 0.01 mol/L sodium citrate buffer in a steamer for 15 min. Next, the samples were blocked with normal goat serum for 30 min and incubated with the mouse monoclonal PCNA antibody (dilution 1:5000, Abcam, Cambridge, UK) overnight at 4 °C. After rinsing with PBS, the samples were incubated with secondary antibodies for 1 h at room temperature. Then, the samples were stained visually with DAB and counterstained with haematoxylin to identify nuclei. The results were observed under a microscope (DM4000B, Leica, Germany).

4. Results

4.1. Comparison of the sample preparations

The percentage of the success rates of sample preparations in both groups are shown in Table 2. According to the reconstructed volumes, the pathway of the pulpal exposure seemed like a circular arc in group A and the mean maximum diameter of pulpal exposure area on the occlusal surface was 625.6 ± 30.6 μm (Fig. 1a–c). In group B (Fig. 1d–f), it seemed like a trapezoid and the mean maximum diameter was 402.7 ± 18.0 μm ($p < 0.05$). In addition, ¼ round burs damaged more dental structure in cusp inclination near the pulp horn than did polishing burs. The mean of the minimum RDT at a region of interest of 200 μm in width at the peak of each horn was 133.2 ± 29.9 μm in group A and 261.4 ± 16.3 μm in group B ($p < 0.05$). Moreover, no

Table 2
Success rate of Group A and Group B.

Group	endodontic perforation	fracture	oversize of the access	success rate (%)(n = 80)	P value
Group A	1	5	6	85	> 0.05
Group B	6	2	0	90	

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