Capping a Pulpotomy with Calcium Aluminosilicate Cement: Comparison to Mineral Trioxide Aggregates

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Abstract

Introduction: Calcium aluminate cements have shown little affinity for bacterial growth, low toxicity, and immunogenicity when used as a restoration material, but calcium aluminate cements have not been tested *in vivo* in pulpotomy procedures. **Methods:** To address this question, a calcium aluminosilicate cement (Quick-Set) was tested along with 2 mineral trioxide aggregates, ProRoot MTA and MTA Plus. These cements were used as a capping agent after pulpotomy. Control rats had no pulpotomy, or the pulpotomy was not capped. Proinflammatory cytokines interleukin (IL)-1β and IL-1α were measured, and histology was performed at 30 and 60 days after capping. The nociceptive response was determined by measuring the lengthening of the rat's meal duration. **Results:** and Conclusions: IL-1β and IL-1α concentrations were reduced in the capped teeth, but no differences were observed among the 3 cements. Dentinal bridging could be detected at both 30 and 60 days with each of the 3 cements, and the pulps were still vital 60 days after capping. Meal duration significantly shortened after placement of the 3 different cements, indicating a nociceptive response, but there were no differences among the materials. Calcium aluminosilicate cement had similar properties to mineral trioxide aggregates and is a viable option for pulpotomy procedures. *(J Endod 2014;40:1429–1434)*

Key Words

Calcium aluminate/aluminosilicate cement, calcium silicate, endodontic, mineral trioxide aggregate, pain, tricalcium silicate

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Calcium aluminate cements show little immunogenicity, toxicity, or affinity for bacterial growth when tested as a restoration material (1–3). Unfortunately, calcium aluminate cements have a higher failure rate than commonly used restoration materials (4–6). Recently, calcium aluminate cements have been tested *in vitro* and *in vivo* as an endodontic material (7–9). In these studies calcium aluminate cement showed little immunogenicity or affinity for bacterial growth, but to date, no study has tested calcium aluminate cements as a pulp-capping material *in vivo*. In addition to studying immunogenicity and bacterial static properties, pain after capping pulpotomies with mineral trioxide aggregates (MTAs) was measured in patients (10). To our knowledge, no study has compared the nociceptive response after capping pulpotomies with different hydraulic cements (a cement that hardens on hydration), and a pain study has not included calcium aluminate cement. To address this knowledge gap, we hypothesized that calcium aluminosilicate cement is a viable pulp-capping material that shows low immunogenicity and little pain and has a high biocompatibility when in contact with tooth pulp.

In this study, a calcium aluminosilicate cement and MTAs were used as a capping material after pulpotomy, and the inflammatory, biocompatibility, and nociceptive responses were measured after placement of these cements. Inflammation was measured by quantifying proinflammatory cytokines interleukin (IL)-1β and IL-1α in the pulp of the treated teeth. Histology was also performed to assess dentinal bridging, the presence of bacteria, and pulp vitality. The nociceptive response was measured by using a behavioral assay, specifically the rat's meal duration. Previous studies have shown that a lengthening of the rat's meal duration correlates to orofacial pain in rats (11–16), and that meal duration has been shown to be significantly longer after pulpotomy (12).

Materials and Methods

Animals

All animal experiments were approved by the Baylor College of Dentistry Institutional Animal Care and Use Committee in accordance with the guidelines of the United States Department of Agriculture, National Institutes of Health Office of Laboratory Animal Welfare, and National Research Council's "Guide for Care and Use of Laboratory Animals." Male Sprague-Dawley rats (250–300 g) were purchased from Harlan Industries, Houston, TX. On arrival, the animals were housed individually in a temperature-controlled room (23°C) and kept on a 14:10 light/dark cycle with lights on at 6:00 AM. The rats were given chow (Harlan Industries, Indianapolis, IN) and water *ad libitum*.

Pulpotomy and Cement Placement

After administering ketamine (90 mg/kg) and xylazine (9 mg/kg), an occlusal pulpotomy on the 6 maxillary molars was completed by a board-certified endodontist by using a ½-round carbide bur. The teeth were immediately capped with 1 of 3 cement materials: ProRoot MTA (Dentsply, Tulsa Dental Specialties, York, PA) mixed 3:1 powder:water, Quick-Set (Avalon Biomed Inc, Bradenton, FL, patent pending) (17), or MTA Plus (Avalon Biomed Inc) mixed at 2.5:1 powder:gel by weight with their respective gels. The mixed cements were placed in the cavity created by the iatrogenic pulpotomy. A self-adhering flowable composite resin (VertiseFlow; Kerr Corporation, Orange, CA) was placed immediately over the cements, and the resin was ultraviolet cured (18). The negative control group had no pulpotomy, and an untreated pulpotomy was used as a

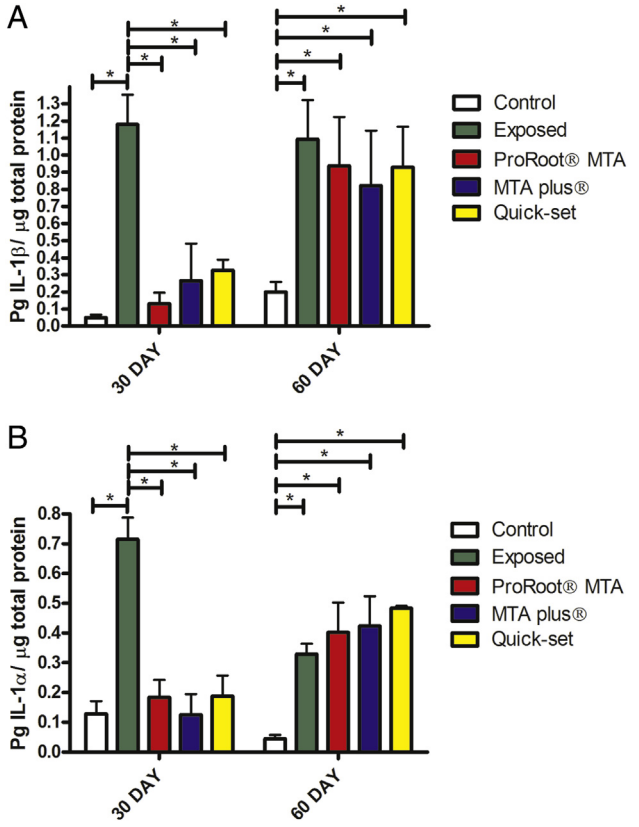

Figure 1. Cytokine levels were analyzed in extracted teeth after pulpotomy and capping with hydraulic cements. A pulpotomy was performed, and the defect was filled with endodontic cement (ie, ProRoot MTA, MTA Plus, or Quick-Set). Control group was anesthetized but did not have the pulp exposed, and exposed group had the pulp exposed but no material was placed. Data for proinflammatory cytokine IL-1β are shown in (*A*), and data for IL-1α are shown in (*B*). *$P < .05$ comparing either exposed or control with other treatment groups within that treatment time period.

positive control. Previous studies had groups with exposed untreated pulp for 28 days or longer (19, 20). Treatment groups in this study included the control, the exposed pulps, the exposed pulps capped with ProRoot MTA, the exposed pulps capped with MTA Plus, and the exposed pulps capped with Quick-Set. Twelve animals were in each of these treatment groups. Six animals in each treatment group were killed at 30 days, and 6 animals were killed at 60 days after capping. Death was completed by exposure to CO_2. After breathing was observed to stop, the animals were decapitated. The maxillae were isolated and fractured along the midline; half of the maxilla was placed in 4% paraformaldehyde and 1 × phosphate-buffered saline for histology, and the other half was placed in liquid nitrogen for storage and later analysis. The maxilla that was either fixed or frozen was randomly chosen for each animal in each treatment group.

Enzyme-linked Immunosorbent Assay

IL-1α was quantified because this cytokine was elevated after pulpotomy, whereas the common proinflammatory cytokine IL-1β was not found to be elevated in some instances (21).

Three to four out of a total of 6 animals from each treatment group were chosen randomly for measurement of the cytokine levels (pg/mL) by enzyme-linked immunosorbent assay (ELISA). To quantitate the cytokines in the pulp tissue, a maxilla from the rat was removed from the liquid nitrogen, and the molars (3 molars per maxilla) were extracted. The teeth were ground and placed in 300 μL T-Per tissue protein extraction reagent containing Halt Protease Inhibitor (Thermo Scientific, Rockford, IL). The lysates were frozen, thawed, vortexed, and centrifuged for 10 minutes at 4°C, and the supernatant was decanted. Total protein in the supernatant was determined in each sample by using a BCA protein assay (Thermo Scientific, Waltham, MA). Quantitation was completed on duplicate 50-μL samples of supernatant by using ELISA (R&D Systems, Minneapolis, MN) following the manufacturer's directions. Values were given as pg IL-1α or IL-1β per μg total protein.

Histology

Histology was performed by using a randomly chosen maxilla from 3 rats per treatment group. First, the maxilla was immersed in 4% paraformaldehyde and 1 × phosphate-buffered saline continuously for 1 week, and then the samples were demineralized in 0.5 mol/L EDTA until radiographic examination revealed an absence of radiopaque structures. The demineralized samples were dehydrated and embedded in paraffin blocks. Serial sections, 6 μm thick, were sliced with a Leitz 1512 rotary microtome (Leica, Buffalo Grove, IL) in a buccolingual longitudinal orientation. Every 20th section was collected and stained with hematoxylin-eosin or a Brown and Hopps stain. Briefly, paraffin was removed with xylene, and the slides were hydrated in a series of ethanol/water treatments. A portion of the slides were immersed in hematoxylin, rinsed, immersed in eosin, rinsed, and dehydrated, and a non-aqueous mounting medium was added. Alternate slides were immersed in crystal violet, rinsed, immersed in Gram iodine, rinsed, and finally immersed in fuchsin and then rinsed and mounted. For the Brown and Hopps stain, gram-positive bacteria were blue, and the gram-negative bacteria were red. Two examiners blinded to tooth position and treatment scored the teeth for immune cell infiltrate, presence of bacteria, or dentinal bridging. Imaging was completed with a Zeiss Axioplan microscope (Thornwood, NY) and an Insight 2 Spot camera (SPOT Imaging Solutions, Sterling Heights, MI). Images were captured and analyzed with Spot Advanced software (SPOT Imaging Solutions).

Bacteria and Pulp Vitality Measurements

Every 20th section was collected for the maxilla of 3 rats in each treatment group, and the sections were stained with either Brown and Hopps stain or hematoxylin-eosin stain. After the Brown and Hopps staining, the bacteria on each slide were counted, and all the bacteria for all the sections of a single maxilla were added together. This total bacteria count per maxilla was reported for each of the 3 rats per treatment group. After the hematoxylin-eosin staining, the percentage of pulp vitality remaining after treatment for each rat was calculated by dividing the area of the vital pulp in the maxillary molars by the average area of vital pulp in the 3 control rats, and then the result was multiplied by 100.

Meal Duration

Meal duration was measured before and after pulpotomy by placing rats in individual, sound-attenuated chambers equipped with computer-activated pellet feeders (Med Assoc Inc, East Fairfield, VT). The feeder units dispensed 45 mg rodent chow pellets (product no. FO 165; Bioserv, Frenchtown, NJ). When a rat removed a pellet from the feeder trough, a photo beam placed at the bottom of the trough was no longer blocked, signaling the computer-controlled system to drop another pellet. The computer recorded the date and time each pellet was dropped, and the computer kept a running tally of the total daily food consumption. A record of the pellets dropped over time established the meal duration. Meal duration was monitored in this study 2 days before and for 9 days after the pulpotomy. Nine days after