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The Effect of Calcium Sulfate on Hard-Tissue Healing After Periradicular Surgery

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The purpose of this study was to determine the effect of calcium sulfate (CS) on cementum deposition and osseous healing after periradicular surgery. The root canals of 24 mandibular premolars in four 2-yr-old beagle dogs were endodontically treated, followed 2 weeks later by periradicular surgery. Mineral trioxide aggregate (MTA) was used as root–end-filling material. The right or left side was assigned at random to receive CS alpha-hemihydrate or no material in the osteotomy sites before closure. The animals were killed after 4 months. Hard-tissue healing was analyzed histomorphometrically. All samples displayed evidence of cementum deposition adjacent to the root-end fillings and bone regeneration in the osteotomy sites. The data was analyzed using the Mann-Whitney *U* test, comparing the scores for cementum and osseous healing of the two groups at significance level of alpha = 0.05. The results indicated that placement of CS in osteotomy sites after periradicular surgery does not significantly affect periradicular healing.

Complete regeneration of hard tissues after periradicular surgery consists of a renewal of the apical attachment apparatus (dentoalveolar healing) and osseous repair of medullary and cortical bone (alveolar healing). The mechanism of dentoalveolar healing is not fully understood. It is thought that undifferentiated mesenchymal cells arise from the periodontal ligament and bone and participate in the healing process. These cells transform into mature fibroblasts, cementoblasts, and osteoblasts and reform the apical dentoalveolar apparatus (1).

In 1972, Andreasen and Rud (2) studied 70 human, periapical-tissue biopsies from 1 to 14 yr after periradicular surgery. Their histological observations revealed three types of healing: healing with reformation of the periodontal ligament; healing with a fibrous scar; and moderate-to-severe inflammation without scar tissue.

Torabinejad et al. (3, 4) found that dentoalveolar healing adjacent to MTA root-end fillings is unique because it results in regeneration of the periapical tissues, including apical cementogenesis. These results were elucidated in animal studies using dogs and monkeys.

Harrison and Jurosky (5) in 1992 created osseous wounds in the mandibles and maxillas of rhesus monkeys and evaluated the alveolar healing histologically. They found that in these "excisional" wounds, the initial blood clot was replaced by granulation tissue emanating from the endosteal tissues. The alveolar healing progressed from the endosteal surfaces toward the external surfaces of the bony wound, resulting in fill with woven bone at 14 days and a functioning periosteum at 28 days.

Pecora et al. (6) in 1997 suggested placement of calcium sulfate as an osteoconductive barrier in periapical osteotomy sites. They suggested that the use of calcium sulfate in this way might result in enhanced osseous healing. They cited several advantages of calcium sulfate, including low cost, ease of application, biocompatibility, and complete absorption of the material over time.

Coetzee (7) in 1980 used calcium sulfate in 110 patients to fill bony defects after various types of surgery in his practice of otorhinolaryngology. Over 90% of these patients had complete bone fill after the first procedure and the remainder of the patients developed bone after a subsequent procedure.

Kim et al. (8) in 1998 used calcium sulfate to fill one- and two-wall periodontal defects in dogs. They found that significantly more bone and cementum was found in the defects filled with calcium sulfate compared with unfilled controls. Kim et al. attributed the bone regeneration to the space-maintaining properties of calcium sulfate.

In 1997, Pecora et al. (9) showed that prehardened calcium-sulfate disk barriers were able to block connective tissue ingrowth in experimental through-and-through defects created in the mandibular angles of rats. They demonstrated that these defects filled more rapidly with bone if calcium sulfate was used. Based on these results, Pecora et al. (6) proposed placement of calcium sulfate in osteotomy sites as a space-filling barrier to enhance osseous healing after periradicular surgery. As an example of success, they cited case reports demonstrating radiographic evidence of bone fill after application of calcium sulfate in periradicular osteotomy sites.

Kim et al. (10) discussed the use of calcium sulfate as a hemostatic agent in periradicular surgery in 1997. They asserted that it could be placed after root-end resection and before root-end preparation and filling, providing a biocompatible hemostatic barrier.

In contrast to the reports of positive effects of calcium sulfate on bone healing, other investigations have cast some doubts on the effect of this substance. In 1965, Radentz and Collins (11) im-

planted calcium sulfate in the alveolar process of dogs. Their histologic analysis showed that calcium sulfate initially prevented the down growth of epithelium. However, in the more mature wounds, calcium sulfate and controls demonstrated similar bony healing.

Calhoun et al. (12) in 1967 removed the mandibular fourth premolars along with a block of surrounding bone and placed a block of calcium sulfate in the surgical defects. They found no advantage in bony healing with calcium sulfate compared to those sites without this substance.

Schaffer and App (13) in 1971 implanted calcium sulfate in infrabony periodontal defects in humans. Calcium sulfate did not seem to slow wound healing and was well accepted by the tissues. They found no radiographic evidence that new bone had formed. They confirmed this finding with surgical reentry of these defects.

Clokie et al. (14) in 2002 used a variety of bone substitutes to close critical size defects in the parietal bones of rabbits. They found that when these defects were filled with calcium sulfate or remained unfilled, they healed with a fibrous scar.

Investigations into the use of calcium sulfate as a promoter of bone healing after surgery have produced mixed results. At this time, it is unknown whether calcium sulfate enhances the alveolar and dentoalveolar healing of periradicular surgery sites. The purpose of this study was to determine the effect of calcium sulfate on hard-tissue healing after periradicular surgery in dogs.

MATERIALS AND METHODS

A total of 24 roots of mandibular second, third, and fourth premolars from four 2-yr-old beagle dogs were used in this experiment. All endodontic procedures were performed under general anesthesia. Induction was provided by an initial intramuscular injection of 10 mg/kg of tiletamine HCl and zolazepam HCl (Telazol®, Fort Dodge Animal Health, Overland Park, KS) and 0.04 mg/kg of atropine sulfate (Abbot Labs, Abbot Park, IL). The animals were then intubated and maintained on inhalation anesthesia with 1% to 3% isoflurane (Forane®, Ohmeda, Liberty Corner, NJ) and 1 to 2 l/min of oxygen for the balance of the procedure. Local anesthesia was provided with a buccal infiltration of 1.8 ml of 2% lidocaine (Xylocaine®, Astra Pharmaceuticals, Wilmington, DE) with 1:50,000 epinephrine.

After gaining occlusal access to the pulp chambers of each tooth, the pulps were extirpated and the root canals were cleaned and shaped using Flex-o-Files® (Dentsply Maillefer, Tulsa, OK) to the apical stop of the each canal. The apical 5 mm of each canal was obturated with warm vertical compaction of gutta-percha. The remaining portion of each root in this group was obturated with MTA. The gutta-percha was placed in the apical segment of the canals to facilitate easy ultrasonic apical preparation during periradicular surgery.

Two weeks after the root-canal procedures, the dogs underwent periradicular surgery on the mandibular right and left quadrants. After obtaining anesthesia, a full-thickness mucoperiosteal buccal flap with two releasing incisions (mesial of the first and distal of the fourth premolars) was reflected. This allowed access to the periradicular tissues in the mandibular second, third, and fourth premolars. The cortical bone over the root ends was removed using a #6 round bur in a high-speed handpiece using copious saline irrigation. The root ends in both groups were resected with a fissure bur approximately 3 mm from the apex at approximately a 60-degree angle to the long axis of the root. Root-end preparations were made to a depth of 3 mm with a Vista P5 ultrasonic unit (Vista Dental, Racine, WI) and S12/90 ultrasonic tip. Root-end cavities were filled with MTA.

The right or left side was assigned systematically to the experimental or control group. In the experimental group, the osteotomy sites were then filled with calcium sulfate alpha-hemihydrate (CAPSET, Lifecore Biomedical). In the control group the sites were allowed to fill with blood.

The mucoperiosteal flaps were sutured with 4-0 silk sutures. All animals were then given 0.01 mg/kg of buprenorphine (Buprenex®, Reckitt and Coleman Pharmaceuticals, Richmond, VA) subcutaneously for pain control and 300,000 units of penicillin (Bicillin C-R®, Wyeth-Ayerst Laboratories, Philadelphia, PA) intramuscularly to prevent infection. After surgery, the animals were placed on a soft diet.

The animals were killed by barbiturate overdose 16 weeks after the second surgical procedure (Euthasol®, Western Medical Supplies, Arcadia, CA). After perfusion with 10% buffered formalin, mandibular block sections containing the premolar teeth and surrounding tissues were resected. These specimens were demineralized in 5% formic acid and then dehydrated in 30%, 70%, and 100% alcohol. After embedding in paraffin, serial buccolingual sections of 7-μm thickness were cut through the center of the apical foramen along the long axis of the teeth. Selected sections were stained with hematoxylin and eosin and evaluated under a light microscope.

The dentoalveolar healing was assessed by measuring the area of cementum in the section adjacent to the root-end filling divided by the apical diameter of the root-end filling. This measurement, termed the dentoalveolar-healing quotient, was used to compare the two groups. This method was used to compensate for any discrepancies in the size of root–end-cavity preparations.

Digital photomicrographs at a magnification of 111× were made of the areas of interest in each slide. Image-Pro Plus® software (version 1.0, Media Cybergenics, Silver Spring, MD) was used to measure the data directly from the photomicrographs. Images were made to document the apical diameter of the root-end fillings and the dimension was recorded in micrometers. Cementum adjacent to the MTA was photographed with the digital system. After digitally removing all parts of the image except the cementum, the area of the cementum adjacent to the MTA was measured in square micrometers (Fig. 1, area is depicted to the left of arrow *A*).

The healing of bone adjacent to the root end was measured as follows. A rectangular field within the section measuring 1620 × 2164 μm was selected. Its longest side was parallel to, centered on, and slightly buccal to the resected root end (Fig. 1, area is depicted to the left of arrow *B*). In this field, the area occupied by bony trabeculae was calculated using ImagePro Plus® software, similar to the area of cementum. The percentage of the entire field occupied by bone was then calculated. This percentage was used as a measure of bone density. The alveolar healing was measured as the percentage area of the designated portion of the section adjacent to the root-end filling that was occupied by bony trabeculae. A single examiner without knowledge of the designation of the groups made all measurements.

RESULTS

Healing was uneventful in both groups. During histologic analysis, it was noted that all 12 roots in each group formed cementum

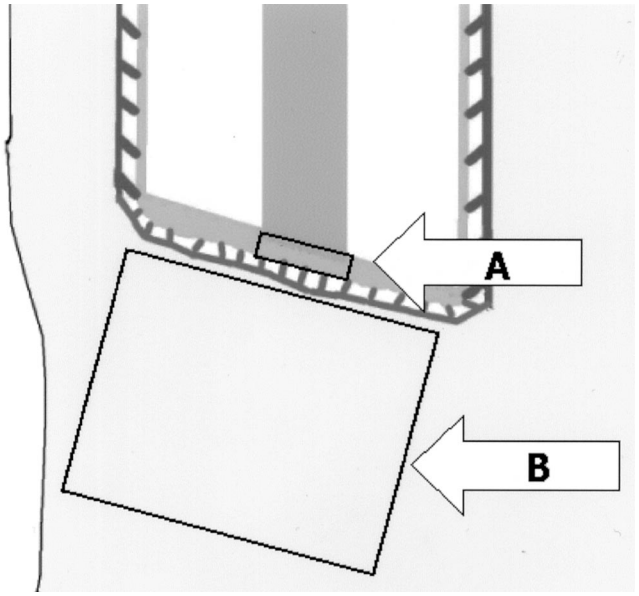

FIG 1. Measurement sites for dentoalveolar (*A*) and alveolar (*B*) healing.

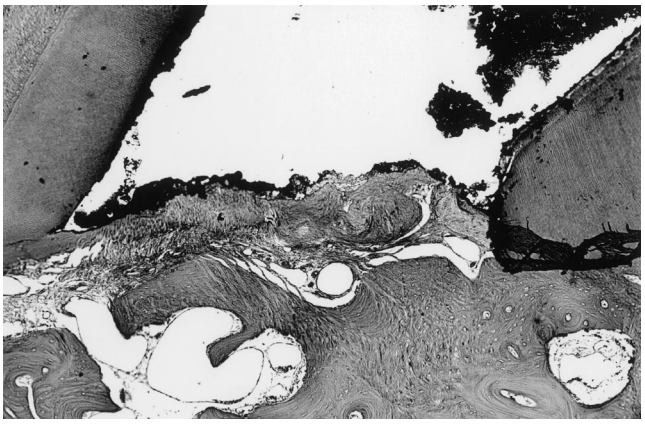

FIG 2. Dentoalveolar healing. MTA root-end filling without calcium sulfate. Cementum is noted directly adjacent to MTA (original magnification ×111).

adjacent to the root-end fillings. Inflammation was noted in the vicinity of three MTA root-end fillings without calcium sulfate and one with calcium sulfate, although not directly adjacent to the MTA.

The cementum adjacent to the MTA appeared to be of varying thickness. The presence of calcium sulfate did not seem to have an effect on this process. The bone density was relatively comparable for both groups. Representative photomicrographs demonstrating dentoalveolar healing are shown in Figs. 2 and 3. Photomicrographs demonstrating alveolar healing are shown in Figs. 4 and 5.

The Mann-Whitney *U* test was used to compare the dentoalveolar healing quotients and the bone densities of the two groups at significance level alpha = 0.05. The mean healing quotients of the two treatments as expressed in the area of cementum (μm^2) in the section adjacent to the root-end filling divided by the apical diameter (μm) were 49.54 μm^2 cementum/μm apical diameter for the retrograde-MTA filling and 40.71 μm^2 cementum/μm apical diameter for the retrograde-MTA filling with calcium sulfate. No

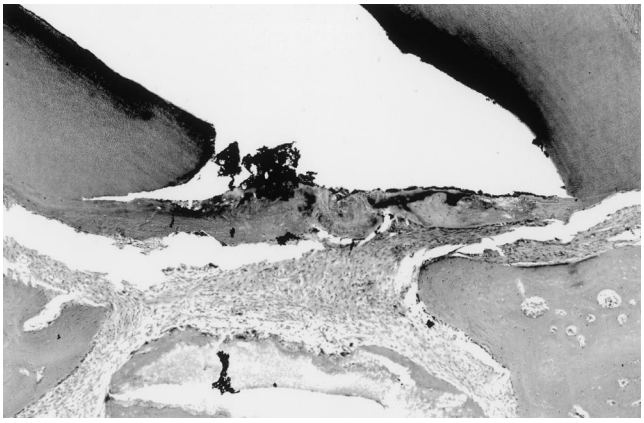

FIG 3. Dentoalveolar healing. MTA root-end filling with calcium sulfate. Cementum is noted directly adjacent to MTA (original magnification ×111).

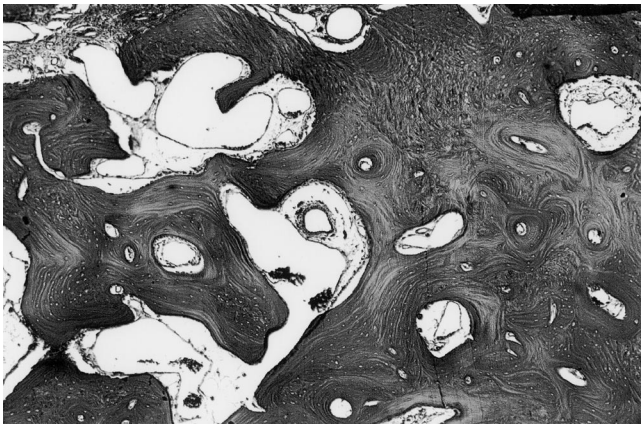

FIG 4. Alveolar healing of osteotomy site without calcium sulfate. Note presence of new bone (original magnification ×111).

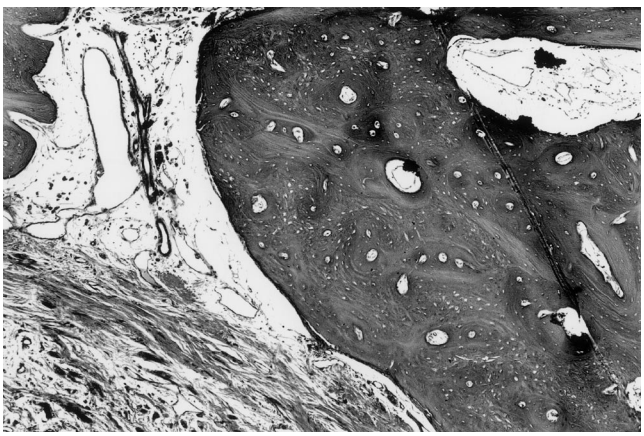

FIG 5. Alveolar healing of osteotomy site with calcium sulfate. Note presence of new bone (original magnification ×111).

statistically significant difference was found between the two groups ($p = 0.608$).

The mean bone densities opposite the root-end fillings were 53.08% for the retrograde-MTA filling group and 54.63% for the retrograde-MTA filling with calcium sulfate group. These results

were not statistically significantly different from each other ($p = 0.799$).

DISCUSSION

The results of this investigation indicate that placement of calcium sulfate in osteotomy sites after periradicular surgery does not affect dentoalveolar or alveolar healing. Therefore, when used as a hemostatic agent in periradicular surgery, it may remain in place without negative effects.

Previous studies have demonstrated the biocompatibility of MTA (15–18). Based on our histological assessment, it seems that placement of calcium sulfate in osteotomy sites after periradicular surgery does not significantly alter the properties of MTA.

The results of this study agree with the findings of Radentz and Collins (11) in 1965, Calhoun et al. (12) in 1967, and Schaffer and App (13) in 1971, who placed calcium sulfate in defects in the mandibles of dogs and examined them histologically. Clokie et al. (14) in 2002 measured the healing of critical size defects in the calvariae of rabbits clinically, radiographically, and histomorphometrically. Schaffer and App's investigation in 1971 involved the use of human patients but was similar because the results were not based solely on radiographs. They gained direct observations of the sites of placement of calcium sulfate through surgical reentry. These studies found that the use of calcium sulfate in surgical defects did not enhance bony healing.

The results of our investigation do not agree with those of several previous studies. The differences could be caused by differences in methods of evaluation: histologic versus radiographic (6, 7). Also, differences could be attributed to the differences in the length of observation. Two investigations demonstrated some beneficial effect of calcium sulfate in the short-term, but little long-term effect (8, 9). Other studies (6, 7) were case reports without controls.

Based on our findings, it seems that the potential of MTA to induce the regeneration of apical hard tissue is not degraded or enhanced when calcium sulfate is placed in the osteotomy sites. In addition, it seems that calcium sulfate does not enhance alveolar or dentoalveolar healing after periradicular surgery.

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