Influence of Selective Immunosuppressive Drugs on the Healing of Exposed Dogs' Dental Pulp Capped with Mineral Trioxide Aggregate

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Abstract

Introduction: Immunosuppressive drugs are used in clinical medicine for a variety of disorders, but their effects on the reparative capacity of the dental pulp are unknown. This study evaluated the influence of selected immunosuppressive drugs on pulpal tissue healing after direct pulp capping of mechanically exposed dog's teeth with mineral trioxide aggregate (MTA). **Methods:** Ten healthy male dogs were assigned into 5 experimental groups: a control group in which no drug was received and 4 experimental groups in which the immunosuppressive drugs prednisone, mycophenolate mofetil, sirolimus, and cyclosporine A were administered 45 days before the operative procedures and until the dogs were killed. Class V cavities were prepared on the buccal surfaces of 12 teeth in each dog. In each cavity, the pulp was exposed and capped with MTA. The pulpal tissue responses to capping material were assessed 65 days postoperatively. **Results:** Compared with the control group, variable responses was recorded in the groups treated with mycophenolate mofetil, sirolimus, and cyclosporine A, which were characterized by moderate to severe inflammatory reactions, tissue necrosis, and total absence of hard tissue bridging. Pulpal tissue responses in the group treated with prednisone were characterized by inflammatory cell infiltration, limited tissue necrosis, as well as partial to complete hard tissue bridging. **Conclusions:** From these findings, it seemed evident that acceptable repair of the dentin-pulp complex, eg, wound healing with hard tissue formation after capping with MTA, is unlikely with mycophenolate mofetil, sirolimus, or cyclosporine A immunosuppressive drug therapy. *(J Endod 2010;36:95–99)*

Key Words

Dogs' teeth, immunosuppressive drugs, mineral trioxide aggregate, pulp capping

Direct pulp capping is a well-established method of treatment in which the exposed dental pulp is covered with a material that protects the pulp from additional injury and permits healing and repair. A final goal of the application of capping materials is to induce the formation of dentin by pulp cells (1).

Despite calcium hydroxide being considered the gold standard for vital pulp therapy, considerable confusion and condemnation of its use persist (2). Recent attempts to develop different pulp capping materials have resulted in the development of mineral trioxide aggregate (MTA) (gray ProRoot MTA; Dentsply, Tulsa, OK) (3), which was first proposed for pulp capping in 1996 (4).

MTA has excellent sealing ability when used as a sealing material on accidental perforations or as a root-end filling material (5). In addition, when MTA was used for direct pulp capping, it showed better interaction with dental pulp tissue than did calcium hydroxide or acid-etched dentin bonding (6). Moreover, MTA has been shown to induce less pulp inflammation and more dentin bridge formation compared with calcium hydroxide cement (7). Karabucak et al (8) used MTA as pulp-capping material after partial pulpotomy to preserve the vitality of the pulpal tissues in 2 cases. Follow-up examinations revealed that the treatment was successful in preserving pulpal vitality and continued development of the tooth. Takita et al (9) investigated the effect of MTA on the proliferation of cultured human dental pulp cells. They reported that the elution components such as calcium ions from MTA had higher proliferation ability of human dental pulp cells than Dycal.

The response to direct pulp capping with dental materials is the formation of a dentin barrier, resulting from the recruitment and proliferation of undifferentiated cells, which might be either mesenchymal stem cells (MSC) or dedifferentiated and transdifferentiated mature cells (10, 11). Once differentiated, the cells synthesize a matrix that undergoes mineralization (10). The extracellular matrix components can induce either reactionary dentin formation (12) or formation of dentin barriers (11). The potential for healing after pulp exposure depends on several factors such as the preoperative and postoperative prevention of bacterial infection, the size of exposure, the efficacy of treatment strategy, and the pulp status (13).

Immunosuppressive drugs inhibit or prevent activity of the immune system. They are used to prevent the rejection of transplanted organs and tissues and to treat autoimmune diseases (eg, rheumatoid arthritis, multiple sclerosis, myasthenia gravis, systemic lupus erythematosus, pemphigus, and ulcerative colitis). They are also used to treat some other inflammatory diseases. The immunosuppressive drugs used in this study affect the immune response at different sites with different mechanisms. These

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effects are of great therapeutic benefits in controlling the immune response to treat disease and conserve transplant organs. Nevertheless, this leads to suppression of the immune system in competing infection and immune surveillance for malignant cells (14, 15). Hoogduijn et al (16) investigated susceptibility of human MSC to tacrolimus, mycophenolic acid, and rapamycin immunosuppressive drugs. They reported that therapeutic concentrations of immunosuppressive drugs affect MSC function.

Experimental data on the effect of the immunosuppressive drugs on the reparative capacity of dental pulp are lacking. Therefore, further investigations of such effect are needed. This study aimed at investigating the influence of selected immunosuppressive drugs on the pulpal tissue healing after direct pulp capping of mechanically exposed dogs' teeth with MTA.

Materials and Methods

The Medical Research Ethics Committee at Mansoura University approved this study. The procedures of this experimental work were performed in the animal house at the Urology and Nephrology Centre, Mansoura University.

Ten healthy male dogs, aged 2.5–3.5 years with intact dentitions, were selected and assigned into 5 equal groups: a control group for no drug administration and 4 experimental groups for oral immunosuppressive drug (prednisone, mycophenolate mofetil, sirolimus, or cyclosporine A) administration 45 days before the operative procedures and until the dogs were killed. In prednisone-treated group, the drug (Hostacortine H; Aventis Pharma, Frankfurt, Germany) was given in a dose of 1 mg/kg/day (17). In mycophenolate mofetil–treated group, the drug (Cellcept; Roche Laboratories, Nutley, NJ) was given in a dose of 10 mg/kg/ day (18). In sirolimus-treated group, the drug (Rapamune; Wyeth, Madison, NJ) was given in a dose of 1 mg/kg/day (17). In cyclosporine A–treated group, the drug (Sandimmune; Novartis, E. Hannover, NJ) was given in a dose of 15 mg/kg/twice daily. Serum samples (taken after 1 week of start) for the determination of trough cyclosporine A levels were taken. Cyclosporine A assay was performed by using a monoclonal fluorescence polarization immunoassay (Abbott Laboratories, Abbott Park, IL). Trough levels were adjusted at the therapeutic range of 200–300 mg/mL (19).

Each animal was sedated with an intravenous injection of ketamine (AMOUN Pharmaceutical Co, El-Obour City, Egypt) in a dose of 1 mg/kg. General anesthesia was induced with an intramuscular injection of 6 mg/kg thiopental sodium (Egyptian Inter Pharmaceutical Industries Co, Tenth of Ramadan City, Egypt). Before the beginning of the experimental procedures, the trachea was intubated, and general anesthesia was maintained by using 1.5–2.5 halothane (Narcotan; PhARCO, Pharmaceuticals, Alexandria, Egypt) in oxygen, delivered through a semiclosed breathing circuit.

Experimental Procedures

Permanent maxillary and mandibular premolars and canines were used as most suitable teeth. All teeth were scaled and polished with a rubber cup on the day of operative procedures. Quadrants of teeth were isolated by using sterile cotton rolls, and saliva was controlled through high-speed evacuation.

Class V cavities (approximately 2.5 mm wide, 3 mm long, 1.5–2 mm deep) were prepared on the buccal surfaces of teeth by using a tungsten carbide pear-shaped bur ISO #500 314 232001 (Komet, Lemgo, Germany) at ultra-high speed, with a copious water spray. A new bur was used on every fourth cavity to avoid excessive heating. The preparations were cut 0.5–1 mm above the free gingiva, parallel to cementoenamel junction. Pulp exposure was performed in the middle of the cavity floor by using a sterile round tungsten carbide bur ISO #500 314 001001 (0.8 mm in diameter; Komet) at high speed and water cooling. A new bur was used for each tooth. The size of pulp exposure produced was 0.8–1 mm. The cavities were washed with sterile saline and dried with cotton pellets. Light pressure was applied so that hemorrhaging could be controlled. In each animal of both groups exposed pulps of teeth were capped with gray MTA (ProRoot1; Dentsply Tulsa, Tulsa, OK). With a sterile metal spatula, MTA powder was mixed with saline in a 3:1 ratio and then placed over the exposure site with a plastic instrument. Zinc oxide–eugenol cement (IRM Ivory, Manufacturer Code #610007; Dentsply Caulk) was used to fill the cavity.

The influence of immunosuppressive drugs on the pulpal tissue behavior to MTA was assessed 65 days postoperatively. At the end of the experimental period, the animals were killed by injecting an overdose of pentobarbital sodium; the teeth were extracted, and their roots were immediately sectioned midway between the cementoenamel junction and the apex. The teeth were fixed in 10% neutral buffered formalin solution for 2 weeks. The mesial and distal proximal surfaces of the teeth were reduced by using a high-speed diamond stone under spray coolant. Then the specimens were demineralized in Morse's solution (50% formic acid, 20% sodium citrate) for 2 months. Finally, the teeth were embedded in paraffin and serially sectioned in buccolingual direction at an average thickness of 6 μm and stained with hematoxylin-eosin and modified Brown-Brenns technique. With an optical light microscope (Zeiss, Goettingen, Germany), specimens were examined as coded slides to avoid possible bias.

Histologic Assessment

The following criteria (20) were used to assess the specimens:

(1) Inflammatory cell response: inflammatory cell infiltration of the pulp tissue was classified as none, absence of inflammatory cells; slight, a few scattered inflammatory cells; moderate, moderate inflammatory cell infiltration around the exposure site; and severe, heavy inflammatory cell infiltration of the coronal pulp or abscess formation.
(2) Tissue necrosis: pulp tissue organization was classified as no necrosis, presence of complete tissue organization in both coronal and radicular pulp; partial necrosis, half or more of the coronal pulp; complete necrosis, complete tissue necrosis of the coronal pulp.
(3) Hard tissue formation: the presence of discontinuous or continuous hard tissue around the exposure site in all the sections examined was characterized as partial or complete calcified bridge formation, respectively.
(4) Bacterial infiltration: the presence of bacteria in the pulp space, the axial floor, or along the cavity walls and within the cut dentinal tubules was characterized as positive bacterial infiltration.

The obtained data were submitted to statistical analysis with the Kruskal-Wallis test and Mann-Whitney *U* test.

Results

The results are shown in Tables 1–3. Results demonstrated that mycophenolate mofetil–treated group, sirolimus-treated group, and cyclosporine A–treated group performed significantly worse than the control group.

All specimens of the control group showed complete hard tissue formation just below the exposure site except 3 specimens that showed partial hard tissue formation with scattered inflammatory cells. Histologic evaluation also showed several large blood vessels without