Effect of MTAD and Tea Tree Oil Irrigation before Pulp Capping on Healing of Exposed Dental Pulp.

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Abstract: Objective Evaluation of the effect of different irrigating solutions (MTAD, TTO and saline) before calcium hydroxide capping on healing of exposed pulp of dog’s teeth. Materials & Methods The pulps of 144 teeth were exposed. The exposure sites were irrigated with either MTAD, TTO or saline, before application of Ca(OH)2 capping material. Histological examination was done at 1, 4, 8 and 12 weeks using scoring system described by Faraco Junior and Holland. Results showed no difference in degree of inflammation between Saline and TTO groups at 8 weeks, but there was a significant difference between these 2 groups and MTAD group which showed the least degree of inflammation. At 12 weeks MTAD group showed the least degree of inflammation. Concerning the secondary dentin formation, it wasn’t affected by the type of irrigant as there was no significant difference in dentin thickness with different irrigating solutions. Conclusion MTAD irrigation improves the long term pulp reaction to Ca(OH)2 while TTO irrigation has minimal effect on pulp reaction.

Key words: MTAD, tea tree oil, Pulp Capping, exposed dental pulp.

INTRODUCTION

Preserving the dental pulp in a healthy state is important in treating teeth with deep caries. Exposure of the dental pulp can happen as a result of caries, trauma or during cavity preparation. Direct pulp capping is a procedure in which the exposed pulp is covered with a protective wound dressing placed directly over the site of exposure. Ideally pulp healing may occur with the formation of a mineralized tissue barrier called dentine bridge. (Nair et al, 2008)

The potential for recovery after pulp exposure depends on several factors, as the pulp status, pre and post-operative prevention of bacterial infection, size of exposure and the efficacy of treatment strategy. Proper healing of the exposure site requires proper prevention of bacteria as well as proper seal for the exposure site, to prevent leakage. Irrigation of the exposed sites with antibacterial and anti-inflammatory material may enhance the healing potential of the exposed dental pulp. (Murray et al, 2000)

MTAD is a new irrigant introduced by Torabinejad on 2003, It consists of doxycycline, citric acid, and detergent (Tween-80). It is an effective solution for the removal of smear layer when used as final rinse after NaOCl also it has antibacterial activity, as it contains tetracycline, detergent, and has a low pH. (Torabinejad et al, 2003)

The essential oil of Melaleuca alternifolia, Known as tea tree oil (TTO), has been used in medicine for almost 70 years (Carson and Riley, 1995). Tea tree oil contains chemicals known as terpenoids which are believed to give the oil its medicinal properties; as it has antiinflammatory property, as well as antibacterial activity(Hart et al, 2000) (Brand et al, 2001). Terpinene is the ingredient responsible for the healing properties, also it contains another compound, cineol, which contributes the disinfectant properties; it should be less than 10 percent of the oil. (Carson et al, 1995) (May et al, 2000)

Therefore the aim of this study was to investigate the effect of different irrigating solutions (MTAD, TTO and saline) on healing of exposed pulp.

MATERIALS AND METHODS

Twelve adult mongrel dogs were used in this study, with an average weight of 20-30 kg body weight, and of ages ranging from 1 – 1.5 years. All dogs were examined thoroughly before the selection and kept under observation for 2 weeks before being used as experimental animals in the study in order to exclude the diseased animals. The dogs were randomly divided into 4 groups; 3 dogs each, relative to the observation periods tested,
1, 4, 8 and 12 weeks. A Total of 144 teeth were selected for this study (n = 12). The utilized teeth of each dog (12 teeth) were classified into 3 equal groups (n = 4) as follows:

**Group A:** Irrigated with MTAD before application of capping material.
**Group B:** Irrigated with saline before application of capping material.
**Group C:** Irrigated with TTO before application of capping material.

The animals were anesthetized with a mixture of XylazineHCl 1 mg/Kg body weight and Ketamine HCl 5 mg/kg. The anesthesia was maintained through the operative time by venous drip (1/2g Thiopental / 500 ml dextrose 5%).

Prior to operative procedure, teeth were cleansed and polished with pumice paste to remove plaque and calculus. The operating field was disinfected with antiseptic solution. Working area was isolated using sterile cotton rolls and gauze. Animal was covered using sterile towels, exposing only the operative field.

On the facial surfaces of the selected teeth, class V cavities were prepared in a standardized protocol approximately 1 mm coronal to the gingival margin (approximately 3× 5 mm). The cavity was prepared using sterile carbide inverted cone bur under copious sterile water coolant and at rotational speed ranging between 25000 -40000 r.p.m. A new bur was used on every fourth tooth to ensure cutting efficiency. The finished cavities were having proper undercuts at the line angles to retain the filling material.

The pulpal floor of the prepared cavities were finished as close as possible to the pulps until the pink shadow of the pulp became apparent, leaving a thin dentin barrier. A sterile explorer was used to create pulp exposure in the center of the cavity floor. Bleeding of the pulp was controlled using sterile cotton pellets moistened with sterile saline until physiologic haemostasis occurred, and then dried with cotton pellets.

**Preparation of the Irrigating Solutions:**

**MTAD Preparation:**
MTAD is a mixture of doxycycline, an acid (citric acid) and detergent (tween 80). It is supplied in the form of 2 components; powder, and liquid. The liquid was slowly injected into the powder bottle, the combination was gently rocked back and forth for 60 seconds or till the powder was completely dissolved, following manufacturer’s instructions. After that the solution was drawn into the 5 ml delivery syringes. The syringe was removed from the bottle and attached to the endodontic needle.

**TTO Preparation:**
TTO was prepared by adding 5 ml of full concentration of the oil to distilled water and few drops of tween 80 were added, to obtain 5 % concentration of TTO.

Each tooth was irrigated with 5 ml of irrigating solution for 5 minutes. After irrigation of the exposure sites, they were dried with sterile cotton pellets and capped directly with Ca(OH)\textsubscript{2} capping material. All teeth were sealed with intermediate restorative material (IRM), a thick quick setting zinc oxide and eugenol cement. This material was used to exclude bacterial leakage according to the method used by (Brännström and Nyborg, 1973).

The animals were sacrificed after 1, 4, 8 and 12 weeks. Teeth were extracted, fixed in 10% neutral buffered formalin solution and decalcified. The specimens were embedded in paraffin, sectioned at an average thickness of 6 µm, and stained with hematoxyline & eosin for histological examination.

The degree of inflammation and dentin thickness were evaluated using light microscope, according to the criteria that were based on a scoring system described by Faraco Junior and Holland, (2004). The histomorphological evaluation of the intensity of inflammation and the thickness of hard tissue bridging was determined by scores of 1 to 4 as follows:

**Intensity of Inflammatory Reaction of the Dental Pulp:**
(Evaluated at a magnification of 400X)
1. Absent or very few inflammatory cells.
2. Mild: average number less than 10 cells.
3. Moderate: average number of 10- 25 cells.
4. Severe: average number greater than 25 cells.

**Thickness of Hard Tissue Bridge Formation:**
(Evaluated in three different points of the bridge)
1. Partial or absent bridge.
2. From 1 to 149 µm.
3. From 150 to 249 µm.
4. Above 250 µm.
Data was presented as mean and standard deviation (SD) values.

**Results:**

**I. Degree of Inflammation:**

**One Week Observation Period:**
Pulp tissue revealed severe degree of inflammation with all irrigants (score 4) with highly inflamed pulp and extravasated red blood cells. No significant difference in degree of inflammation between irrigants.

**Four Weeks Observation Period:**
Pulp tissue showed severe degree of inflammation (score 4) with all irrigants. No significant difference was found with any of these irrigants ($P$-value = 1.000).

**Eight Weeks Observation Period:**
Saline and TTO irrigants sustained severe degree of inflammation (score 4), while MTAD revealed mild to moderate inflammation (score 2.5). There was a statistically significant difference between mean degree of inflammation of the three irrigant solutions ($P$-value = 0.005). There was no statistically significant difference between saline and TTO; both showed the statistically significantly highest mean scores of inflammation.

**Twelve Weeks Observation Period:**
The results showed a statistically significant difference between mean degree of inflammation of the three irrigant solutions when used before Ca(OH)$_2$ with distilled water capping material ($P$-value = 0.004). Saline sustained severe inflammation (score 4), MTAD showed mild inflammation (score 2), while TTO revealed moderate inflammation (score 3).

**II. Dentin Thickness Scores:**

**One & Four Weeks Observation Periods:**
At these early stages, no effect of the irrigant (saline, MTAD and TTO) on the pulp response to the materials in concern to dentine bridge formation. No evidence of dentine formation was found with any of the irrigating solutions.

**Eight Weeks Observation Period:**
In general dentine thickness started to appear in this examination period with no difference between the irrigants. Minimal dentine thickness was produced with all irrigating solutions during this period.

**Twelve Weeks Observation Period:**
It revealed similar maximum dentin thickness with MTAD and TTO groups (score 2), followed by saline (score 1.5), which showed less dentin thickness. There was no statistically significant difference between MTAD and TTO and saline groups.

![Fig. 1: Bar chart representing mean degree of inflammation with the three irrigant solutions.](image)
Fig. 2: Bar chart representing mean dentin thickness scores of all capping materials with the three irrigant solutions at all tested periods.

Discussion:
Direct pulp capping is a well established method of treatment, in which the exposed dental pulp is covered with a suitable material that protects the pulp from additional injury and permits healing and repair. (American association of endodontists, 1981)

Tea tree oil (TTO) has been reported to have a high antimicrobial activity. This allows immediate haemostatic control and also reduces the number of viable microorganisms near the pulp. The recommended therapeutic concentration for TTO is 2.5-5%, in which it can retain its antibacterial property without any toxic effect.

When TTO was used as an irrigant at the exposure site, the diffusion into the dental pulp and its effect is limited because tissue dissolution of the solution is directly proportion to its concentration and the contact time with the pulp tissue. That’s why when TTO was used as irrigant in this study didn’t cause detrimental effects to pulp inflammation.

In this study evaluation of the influence or effect of the irrigants on the pulp reaction to the capping materials was performed by determining the degree of inflammation of the dental pulp as well as the quality of dentine bridge formation under capping materials. The degree of inflammation was determined by the number of inflammatory cells, while dentine thickness was the determined factor of the quality of dentin bridge formation.

The irrigants were used before application of the capping material as a trial for control of bleeding and infection and promotion of healing. In the present study, three irrigating solutions were compared histopathologically by determining the difference in pulp response. Dogs teeth were selected because of their pulp structural similarity to human teeth, and the experimental conditions can be controlled (Berkovitz et al, 2002). All conditions were standardized, as all irrigating solutions were used in the same dog, and all dogs were chosen to be of the same age and weight to overcome the effect of any external factor.

On comparing the three irrigant solutions used (Saline, MTAD and TTO) after one week there was no significant difference in degree of inflammation between the three irrigants, which showed severe inflammation, presence of large number of inflammatory cells, dilated and congested blood capillaries. This inflammation mostly occur because of the trauma of the pulp during cavity preparation and preparation of the exposure site, inspite of all the precautions taken during the preparation. This is in agreement with Faraco and Holland, (2001).

MTAD resulted in less inflammation at 8 and 12 weeks than saline and TTO group. This may be attributed to its antibacterial effect and its effect on the smear layer, which result in less bacterial leakage and less inflammation. This is in agreement with Fawzi et al (2010).

In this study there was no significant difference in degree of inflammation between saline and TTO group, which showed the highest scores, but there was significant difference between these 2 irrigants and MTAD group which showed the lowest degree of inflammation.
Conclusion:
Based on the results of this study, it can be concluded that MTAD irrigation improves the pulp reaction to Ca(OH)_2, while TTO irrigation has minimal effect on pulp reaction.

REFERENCES