A comparative study of dentin bridge formation following pulpotomy using calcium hydroxide and mineral trioxide aggregate in young dogs

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Summary

The efficacy of dentin bridge formation of mineral trioxide aggregate (MTA) and calcium hydroxide were compared following pulpotomy in young dogs. Forty-two mandibular permanent premolars teeth of seven 6-month-old dogs were used in this study. Following anesthetizing the animals, rubber dams were applied and the teeth were pulpotomized. Then the wounds were dressed with either MTA or calcium hydroxide, respectively. The animals were perfused with 1 to 4 months later. The jaws containing the pulps and the surrounding dentins were prepared and examined histologically by two independent evaluators blinded to the treatment groups. Dentin bridges were formed in all of the examined pulps with the mean thickness of 246 µm for MTA and 244 µm for calcium hydroxide groups. Tunnel defects and porosities within the dentinal bridges in the calcium hydroxide cases were more evident than those of the MTA-treated cases. In this study, 2 of 33 MTA-treated cases and 11 of 37 calcium hydroxide cases demonstrated acute and chronic inflammation of pulps. It appears that pulp reaction to MTA is more favorable and this material is more suitable than calcium hydroxide for pulpotomy in young permanent teeth.

Key words: Ca (OH), Mineral trioxide aggregate (MAT), Pulpotomy, Dentinal bridge, Dogs

Introduction

The use of calcium hydroxide to maintain pulp vitality and promoting a bridge formation was introduced by Teuscher and Zander (1938). The failures in calcium hydroxide pulpotomy have been long the result of chronic pulp inflammation, internal resorption and canal calcification. At present, pulpotomy is recommended for young permanent vital teeth with incompletely formed apices. Complete pulpectomy and obturation is performed following the closure of the apex in order to forestall the root canal calcification (Hallett and Porteus 1963; Patterson, 1967). Some investigators (Pitt Ford, 1985; Tagger and Tagger, 1985) demonstrated that capping the pulp with calcium hydroxide led to creation of a necrotic zone adjacent to the material. They stated that the necrotic tissue eventually became degenerated and disappeared, leaving a void between the dressing material and the newly-formed dentinal bridge. Further investigations demonstrated that the void between the dressing material and dentinal bridge might become contaminated by bacterial microleakage along the cavity margin resulting in the possibility of pulpal insult (Bergenholtz et al., 1982; Cox et al., 1985). The presence of tunnel defects within the newly-formed hard tissue following calcium hydroxide pulpotomy was another finding demonstrated by some investigators (Cox et al., 1985; Pitt Ford, 1985). Goldberg et al., (1984) suggested that Dentin bridges formed underneath calcium hydroxide following pulpotomy did not function properly because of their porosity that could not prevent bacterial ingrowth along the cavity margin. Jean et al., (1988) have expressed doubt as to whether calcium hydroxide has been the best material for inducing dentin bridge formation. They compared calcium hydroxide with a mixture of 50%
tricalcium phosphate and 50% hydroxyapatite and showed that the latter produced a thicker bridge and a more rapid formation of normal tubular dentin. It is recommended that an effective pulp dressing material should be biocompatible, provide a biological seal, prevent microleakage and have a negligible solubility. Mineral trioxide aggregate (MTA) with good sealing ability (Lee et al., 1993) and high degree of biocompatibility (Torabinejad et al., 1995) has been, therefore recommended and used in endodontic practices. MTA has been reported as an effective material in pulp capping (Pitt Ford et al., 1996), root end filling (Torabinejad et al., 1997), apexification and perforation repairs (Pitt Ford et al., 1995; Shabahang et al., 1999).

The purpose of this study is to compare the efficacy of calcium hydroxide and MTA for dentinal bridge formation following pulpotomy in young dogs’ permanent teeth and to evaluate whether MTA can be a valuable substitute for calcium hydroxide as a vital pulpotomy agent.

Materials and Methods

Seven 5 to 6-month-old dogs were selected for this study. The animals had immature permanent mandibular premolars with open apices according to preoperative radiographs. A total of 42 teeth including mandibular right and left second, third and fourth premolars were used for this study. Each animal was anesthetized subcutaneously with infiltration of 40 mg/kg sodium pentobarbital and 2 mg/kg of zylazine. We isolated the teeth with rubber dams. Access cavities were made and the coronal pulp tissues were eliminated with sharp curettes. Bleeding was controlled with saline solution and strile cotton pellets before placing the pulp dressing materials. In the half of the teeth, including mandibular right premolars, the wounds were covered with MTA (Loma Linda University, Calif., USA) mixed with strile saline at a 3:1 powder:saline ratio. In the rest of the teeth, including mandibular left premolars, the wounds were covered with a paste of pure calcium hydroxide powder (Merk, Darmstadt, Germany) mixed with distilled water. The access cavities in all teeth were then double sealed with Zinc oxide and Eugenol (ZOE) and amalgam. The dogs were re-anesthetized after either 30, 60, 90 or 120 days, and perfused through the left and right carotid arteries by 0.9% saline followed by a fixative consisting of 10% buffered formalin. The lower jaws containing the examined teeth, were then resected and fixed in 10% buffered formalin for 7 days. After fixation, each tooth (with it’s surrounding structures) was excised with a surgical saw. The specimens were demineralized, dehydrated, embedded in paraffin and serially sectioned in an apico-coronal direction into 8-μm-thick sections. Alternative slides were stained with hematoxylin and eosin for evaluation of cell differentiation and masson trichrom for demonstration of connective tissue. All of the samples were independently evaluated by two independent investigators, one endodontist and one pathologist, who were blinded to the study groups. The histological assessment included both quantitative and qualitative analysis of the newly-formed dentins and pulps’ responses.

Results

The number, distribution and pulps’ responses of samples to two materials are given in Table 1.

Nine samples of MTA and five of calcium hydroxide groups were excluded from the study due to the damages to the amalgam fillings and technical problems.

Radiographs of the experimental samples (Fig. 1) showed voids between the filling materials and the newly-formed reparative dentin bridges in all cases treated with calcium hydroxide. In contrast, none of the samples treated with MTA demonstrated such finding.

All of the samples treated with MTA and calcium hydroxide showed dentin bridges under medicaments, averaged 246 μm for MTA and 244 μm for calcium hydroxide in thicknesses (Table 1).

The dentin bridges formed in the MTA samples showed regular tubular dentins, which were continuous with the walls of the dental pulps (Fig. 2). On the other hand, the majority of the samples treated with calcium hydroxide showed dentin bridges consisting
of two distinct zones: Zone 1, in contact with the medicaments, the earlier reparative dentins which were irregular and had numerous lacunae and tunnel defects and zone 2, regular tubular dentins in contact with the radicular pulps (Figs. 3a and 3b).

The results showed that the average thicknesses of the earlier irregular reparative dentin bridges and the presence of tunnel defects and porosities in the cases treated with calcium hydroxide were approximately twice that of cases treated with MTA (P<0.05).

Experimental samples taken after 90 days revealed that 7 of 10 calcium hydroxide cases had inflammatory reactions in the pulps. At the same period, the MTA groups had normal pulp tissues morphologies and no inflammatory cells (Table 1). Comparing the two medicament groups at 120 days, two MTA and four calcium hydroxide cases had both chronic and severe acute inflammatory reactions in the pulps predominated by polymorphonuclear leukocytes (PMNs) which could be related to the presence of tunnel defects in the newly-formed dentin bridges (Figs. 3a, 3b and 4).

Fig. 1: Radiographs two months after pulpotomy and placement of calcium hydroxide (top) and MTA (bottom). Note presence of voids between dentin bridges and restorative materials in calcium hydroxide samples (top)
Fig. 2: Histologic appearance of formed dentin bridge after three months under MTA. Note regular dentin ridge, which is formed continuous with the walls of the dental pulp (D) and organized odontoblastic layer (O), (H&E, ×2.5)

Fig. 3a: Microscopic features of formed reparative dentin bridge under calcium hydroxide after three months (H&E, ×2.5)
Fig. 3b: Higher magnification of rectangular demarcated area in (3a), Note irregular and porosed dentin (ID), regular tubular dentin (R) and presence of tunnel defects (arrow) in dentin coursing toward the pulp (H&E, ×10)

Fig. 4: Pulp reaction to calcium hydroxide after four months. Although reparative dentin has formed, the tunnel defects and porosities within dentin bridge permit irritants to diffuse to the pulp. Note the inflammatory reaction (m), (Masson trichrom, ×2.5)
Table 1: Measured parameters at different times in two experimental groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Examination time (day)</th>
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<tr>
<td></td>
<td>30</td>
<td>60</td>
<td>90</td>
<td>120</td>
<td>MTA</td>
<td>Ca(OH)$_2$</td>
<td>MTA</td>
<td>Ca(OH)$_2$</td>
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<tr>
<td>Number of samples</td>
<td>10</td>
<td>12</td>
<td>3</td>
<td>5</td>
<td>11</td>
<td>10</td>
<td>9</td>
<td>10</td>
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<td>Bridge thickness (µm)</td>
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<td>297</td>
<td>155</td>
<td>184</td>
<td>191</td>
<td>227</td>
<td>514</td>
<td>338</td>
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<tr>
<td>Bridge thickness (%)</td>
<td>10%</td>
<td>25%</td>
<td>100%</td>
<td>100%</td>
<td>27%</td>
<td>90%</td>
<td>66%</td>
<td>100%</td>
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<td>Tunnel porosities (%)</td>
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<td>100%</td>
<td>100%</td>
<td>27%</td>
<td>90%</td>
<td>66%</td>
<td>100%</td>
<td>13 27</td>
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<tr>
<td>Inflammation (%)</td>
<td>70%</td>
<td>2%</td>
<td>4%</td>
<td>70% 22%</td>
<td>40%</td>
<td>13 27</td>
<td>2% 11</td>
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<tr>
<td>Voids between bridges and medicaments (%)</td>
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<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>37</td>
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Discussion

In this study, dentin bridges formation was observed in all samples treated with MTA and calcium hydroxide. The mean thicknesses of the formed dentin bridges at different times are depicted in Table 1. The thicknesses of the bridges in these experimental cases indicate that MTA, like calcium hydroxide, rather than being inert, is irritant and actively promotes hard tissue formation. The findings of the present study, do not fully agree with the results reported by Pitt Ford et al., (1996). They studied the dental pulps responses to MTA and calcium hydroxide as pulp capping materials in monkeys. They found complete dentin bridges in all MTA samples, whereas two of six samples treated with calcium hydroxide revealed dentin bridges after five months.

The formed dentin bridges in the calcium hydroxide samples showed more irregular and porous than those treated with MTA, i.e., un-mineralized areas appearing as tunnel defects which were coursing towards the pulps, were more evident (Fig. 2).

This finding relatively supports the results reported by Cox et al., (1985), Pitt Ford and Roberts, (1991), Pitt Ford et al., (1995; 1996), Torabinejad et al., (1997) and Shabahang et al., (1999). The presence of formed irregular porous dentins in the majority of calcium hydroxide-treated samples indicates that pulps suffered from this material (Fig. 3a and 3b). This is in keeping with the results reported by Jean et al., (1988).

In this study, we observed voids between dressing materials and newly-formed dentin bridges in all samples treated with calcium hydroxide. In contrast, none of the MTA samples demonstrated such finding (Fig. 1). These are in accord to the results reported by other investigators (Stanley and Lundy, 1972; Tronstad, 1974; Tagger and Tagger, 1985; Pitt Ford and Roberts, 1991). They demonstrated that following the pulp dressing with calcium hydroxide, a necrotic pulp tissue zone is formed adjacent to the material. The dentin bridge then forms between this layer and the underlying vital pulp. The necrotic tissue eventually becomes degenerated and disappears, leaving a void between the dressing material and the bridge. We believe that the void formation under calcium hydroxide instead of being attributed to the high pH effect of material on the vital pulp tissue, could be referred to the solubility and non-setting nature of the material. The
reason for supporting this idea is that such finding was not observed in MTA-treated samples.

In this study, 2 out of 33 MTA and 11 out of 37 calcium hydroxide treated samples demonstrated acute and chronic inflammatory cells in the pulps. Our findings suggest that the increasing number of inflamed pulps in the calcium hydroxide-treated cases was the result of voids formation between dentin bridges and medicament interface which facilitated the entrance of irritants through dentin bridges toward the pulps via tunnel defects. Some investigators have demonstrated that bacterial microleakage at restoration margins is among important causes of pulp inflammation (Bergenholtz et al., 1982; Cox et al., 1985; Watts and Paterson, 1987). They established a plausible explanation that amalgam was not an effective sealing agent and would not prevent bacterial ingrowth along the cavity margin. Watt et al., (1987) state that bacteria present in contact with calcium hydroxide could lower the pH of the material by converting it to calcium carbonate and that is why calcium hydroxide seems to disappear. Unlike calcium hydroxide, MTA sets hard within four hours and has a compressive strength and a negligible solubility, so it dose provide a solid barrier against microleakage which gives the pulp tissue an opportunity to reorganize itself (Torabinejad et al., 1995). Therefore, MTA is a more suitable material for vital pulp therapy.

Acknowledgments

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References