Tissue response to direct pulp capping with calcium hydroxide preceded by corticosteroid or corticosteroid/antibiotic dressing: a histological study in rats

Resposta do tecido pulpar à aplicação da pasta à base de hidróxido de cálcio precedida do emprego do curativo de corticosteróide ou corticosteróide/antibiótico: estudo histológico em ratos

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Abstract

Purpose: This study histologically evaluated the reaction of tooth pulp to treatment with calcium hydroxide in distilled water preceded by a corticosteroid (Decadron) or corticosteroid/antibiotic (Otosporin) dressing.

Methods: Calcium hydroxide solution was applied to exposed tooth pulp for 5 min or for 72 h, using a sample of 120 molars of rats; histological examination was performed 7, 14, 30 and 60 days after treatment.

Results: A mild inflammatory reaction was observed at 7 and 14 days whether the dressings were used for 5 min or for 72 h. When the corticosteroid/antibiotic dressing was used for 5 min, the tissue reaction and the mineralized barrier formed at 30 and 60 days were similar to those of animals in the control group. Use of the corticosteroid dressing for 5 min or 72 h or the corticosteroid/antibiotic dressing for 72 h delayed healing of the pulp tissue.

Conclusion: Temporary dressings resulted in a milder inflammatory reaction during the early postoperative period. The tissue reaction and the quality of the barrier formed when the Otosporin temporary dressing was applied for 5 min were similar in the experimental and the control groups at longer postoperative periods. Temporary dressings of Decadron applied for 5 min or 72 h or Otosporin applied for 72 h caused a slight retardation of the healing process of the tissue that was apparent 30 days after the intervention.

Key words: Corticosteroids; antibiotics; dental pulp exposure; calcium hydroxide

Resumo

Obgetivo: Avaliar a reação pulpar à pasta de hidróxido de cálcio em água destilada, precedida de curativo de corticosteróide (Decadron) ou corticosteróide/antibiótico (Otosporin) por 5 min ou 72 h.

Metodologia: Em 120 polpas de dentes de ratos expostas, a análise histológica foi realizada após 7, 14, 30 e 60 dias de cada tratamento.

Resultados: Nos períodos iniciais observou-se leve reação inflamatória quando os curativos foram aplicados por 5 min ou 72 h. A reação tecidual e a barreira formada sob ação do hidróxido de cálcio após o curativo de Otosporin por 5 min foram semelhantes àquelas do grupo controle aos 30 e 60 dias. Os curativos de demora com Decadron por 5 min e 72 h e Otosporin por 72 h promoveram retardo no processo de reparação.

Conclusão: Os curativos de demora promoveram reação inflamatória mais suave nos períodos iniciais de análise. A reação tecidual e a qualidade da barreira formada sob a ação do Otosporin por 5 min foram semelhantes àquelas do grupo controle nos períodos pós-operatórios mais longos. Os curativos de demora com Decadron por 5 min e 72 h e Otosporin por 72 h promoveram ligeiro retardo no processo de reparação tecidual a partir de 30 dias.

Palavras-chave: Corticosteróides; antibióticos; exposição da polpa dentária; hidróxido de cálcio

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Introduction

Direct pulp capping consists of covering an area of exposed dental pulp with a material that allows dentin barrier formation and the maintenance of the vitality and normal function of the pulp tissue (1). Calcium hydroxide has frequently been used as a stimulating agent for pulpal tissue repair. Drugs have often been used in association with calcium hydroxide to improve its effect; among them, corticosteroids are often employed to control the inflammatory reaction and to reduce postoperative pain (2-4).

Corticosteroids can control increases in intrapulpal pressure that occur after induced trauma; this plays an important role in healing, considering the confinement of pulpal tissue to rigid, non-extensible walls (5). However, some studies (6-8) questioned the beneficial effects of corticosteroids as the definitive capping material, and some practitioners began to use them instead as temporary dressings. Souza & Holland (9) reported improved results when a temporary dressing with corticosteroid/antibiotic was applied for 48 hours prior to the protection of the pulpal stump with calcium hydroxide. However, no improvement in pulp response was found when corticosteroids were used for only 10 minutes prior to the application of calcium hydroxide paste (10).

Because of the anti-inflammatory properties of corticosteroids and the importance of the control of intrapulpal pressure for capping success, the aim of this study was to evaluate the reaction of exposed pulpal tissue of rat teeth to capping with calcium hydroxide paste in distilled water when the capping was preceded by application of a corticosteroid (Decadron) or corticosteroid/antibiotic dressing (Otosporin) for five minutes or 72 hours.

Methods

The first maxillary molars of 60 male rats (*Ratus novergicus*, Albinus, Holtzman) were used. The rats were anesthetized by intramuscular administration of Francotar (Virbac of Brasil Ind. Com. Ltda, Roseira, SP, Brazil) anesthetic at a dosage of 0.08 ml per 100 grams body weight together with a muscle relaxant and analgesic (Virbaxyl, 2%) (Virbac of Brasil Ind. Com. Ltda, Roseira, SP, Brazil) at a dosage of 0.04 mL per 100 g body weight. Following the administration of anesthetic, the animals were placed on an apparatus introduced by Houston (11) and adapted for rubber dam placement.

The teeth were isolated, operating field antisepsis was performed with 70% alcohol, and the cavities were prepared on the occlusal surfaces, using a round ½ and an inverted cone 33½ bur (JET – Carbide Burs, Beavers Dental, Canada) running at slow speed. The pulps were exposed using an explorer (size 1) (Golgran – Ind. Com. Int. Odontológicos Ltda, São Paulo, SP, Brazil), and the dressings were placed, making use of sterile filter papers in sizes compatible with the cavities.

In groups 1 and 2, the teeth received Otosporin (corticosteroid/antibiotic, Wellcome – ZENECA Ltda, Cotia, SP, Brazil) or Decadron (corticosteroid, Prodome Ltda, Campinas, SP, Brazil), respectively, for five minutes. In groups 3 and 4, the dressing (Otosporin and Decadron, respectively) remained for 72 hours; in these animals, a sterile stainless steel disk was placed over the dressing and sealing was carried out using glass ionomer cement (Vitrebond – 3M Dental Products, St Louis, USA).

After temporary dressing removal, calcium hydroxide paste (Labsynth Ltda, Diadema, SP, Brazil) in distilled water was placed over the pulp exposure, which was protected by a stainless steel disk, and the cavities were sealed with silver amalgam (Velvalloy – S.S. White Ltd, Rio de Janeiro, RJ, Brazil).

In group 5, calcium hydroxide paste in distilled water was placed immediately after pulp exposure, without using any previous dressing.

Six teeth were treated with each tested substance for each period of analysis (7, 14, 30 and 60 days).

At the end of each postoperative period, animals were sacrificed by anesthetic overdose. The upper first molars were separated from the jaws, fixed in 10% buffered formalin solution for 48 hours, and subjected to routine histological examination. Serial sections of 6 µm thickness were prepared and stained with hematoxylin/eosin, Masson's trichrome and the Brown & Breen technique for bacterial evaluation. Histological analysis was carried out using an optical microscope.

Results

G_1 – Direct pulp capping with calcium hydroxide paste preceded by Otosporin dressing for five minutes

In Group 1 animals, which received Otosporin dressing for five minutes followed by pulp capping, histological examination of the teeth at seven days post operation showed disorganized and highly cellularized pulpal connective tissue, with newly formed collagen fibers obliterating the exposure (Fig. 1.1). In the deep area of the coronary pulp, a few inflammatory cells could be seen. At 14 days post operation, the fibrous barrier, which was in the initial phase of its formation, was irregular and showed the inclusion of a great number of cells. On its internal surface, the presence of an organized and aligned cell layer resembling odontoblasts could be seen, while immediately below this layer a great concentration of connective tissue cells, mainly fibroblasts, and an intense process of vascular neoformation were observed.

At 30 days, a barrier in the mineralization process was observed, with some fiber disorganization and cells included in the organic matrix. Subjacent to the barrier, the odontoblastic layer was organized and the pulpal connective tissue was largely cellularized, with expanded and hyperemic blood vessels.

At 60 days, a thick mineralized barrier consisting of two clearly distinct layers, one with a large number of collagen fibers and the other with fewer such fibers, was seen. The subjacent soft connective tissue was extremely cellularized, with hyperemic blood vessels (Fig. 1.2).

The pulpal connective tissue was free from bacteria at all postoperative time points examined.

G_2 – Direct pulp capping with calcium hydroxide paste preceded by Decadron dressing for five minutes

In Group 2 animals, which received Decadron dressing for five minutes followed by pulp capping, histological examination of the teeth at seven days post operation showed collagen fiber formation. Odontoblasts in areas near the exposure were disorganized, and the pulpal tissue presented mild inflammation, with large numbers of cells and hyperemic blood vessels (Fig. 1.3).

At 14 days, the barrier was undergoing the process of mineralization and had almost integrally obliterated the pulp exposure. Subjacent to this barrier, there were a high concentration of connective tissue cells and mild inflammation.

At 30 days post operation, the mineralized barrier was incomplete and included degenerating cells within its boundaries. In the subjacent pulpal connective tissue, the presence of cells with slightly enlarged and highly stained nuclei, apparently normal blood vessels, and absence of inflammation were noted. The odontoblastic layer in the exposure area remained disorganized.

At 60 days, the mineralized barrier was incomplete and consisted of one less mineralized and more disorganized layer near the capping material and another more mineralized layer that was in contact with the pulpal connective tissue; a large number of cells were present inside the second layer. The subjacent pulpal connective tissue appeared intensely cellularized, with fibroblasts rich in cytoplasmic processes but an absence of inflammatory cells (Fig. 1.4).

There was no evidence of the presence of bacteria in the pulpal connective tissue in any of the specimens.

G_3 – Direct pulp capping with calcium hydroxide paste preceded by Otosporin dressing for 72 hours

At seven days, a zone of necrotic tissue was seen adjacent to a barrier consisting of a fibrous organic matrix with cellular inclusions. The pulpal connective tissue was richly cellularized, with inflammatory cells and new collagen fibers and hyperemic blood vessels. The odontoblastic layer was disorganized (Fig. 1.5).

At 14 days, the fibrous barrier with cellular inclusions appeared to be beginning the process of mineralization. In the walls bordering the exposure, the odontoblastic layer was disorganized, and there was reactionary dentin formation.

At 30 days, the mineralized barrier had, in its internal extremity, a large number of elongated cells. The subjacent pulpal connective tissue presented a few mononuclear inflammatory cells and was hyperemic with congested blood capillaries.

At 60 days, the mineralized barrier remained irregular, with different degrees of mineralization and cells included in its matrix. The pulpal tissue immediately beneath it still presented abnormal features (Fig. 1.6).

Through the use of the Brown & Brenn staining technique, it was observed that no bacteria were present in the interior of the pulpal connective tissue.

G_4 – Direct pulp capping with calcium hydroxide paste preceded by Decadron dressing for 72 hours

At seven days, there was formation of a fibrous barrier subjacent to the fibrin clot, with inclusion of necrotic tissue beneath the capping material. The subjacent pulpal connective tissue presented hyperemia and congested blood vessels (Fig. 1.7).

At 14 days, an almost complete fibrous barrier in an advanced stage of mineralization, with many cell inclusions, was seen. The subjacent pulpal connective tissue was characterized by the presence of many fibroblasts within an amorphous and fibrous substance and by slightly hyperemic blood capillaries.

At 30 days, the formation of a thick mineralized barrier, which was irregular and incomplete with cells included in its interior, and which presented different degrees of mineralization, was noted.

At 60 days, the mineralized barrier was still incomplete and irregular, with cells included in its interior and apparent alteration of its mineral content. The subjacent pulpal connective tissue was rich in hyperemic blood capillaries and fibroblasts (Fig. 1.8).

G_5 – Direct pulp capping with calcium hydroxide paste (control)

At seven days post operation, necrotic tissue was seen in contact with the capping material. The observed tissue necrosis was associated with severe inflammation of the subjacent pulpal tissue, characterized by multinuclear cells and hyperemic blood vessels (Fig. 1.9). Bordering the pulp exposure area, there was a slight disorganization of the odontoblastic layer and of the connective tissue, as well as collagen fiber neoformation.

The pulpal tissue inflammation persisted at 14 days, and the odontoblastic layer remained disorganized.

At 30 days, a barrier formation with cells included within it was present. The pulpal connective tissue presented hyperemia, inflammatory cells and not very extensive fibrous intercellular substance. Next to the exposed area, there was reactionary dentin formation with irregular contours, and the odontoblasts continued to appear disorganized.

At 60 days, the mineralized barrier appeared thick and irregular. The pulpal connective tissue in contact with the barrier surface showed vascular hyperemia. The odontoblastic layer on the dentin surface lateral to the barrier presented a normal aspect (Fig. 1.10).

In the Brown & Brenn stained histological sections, no bacteria were observed in the pulpal connective tissue at any of the time points analyzed.

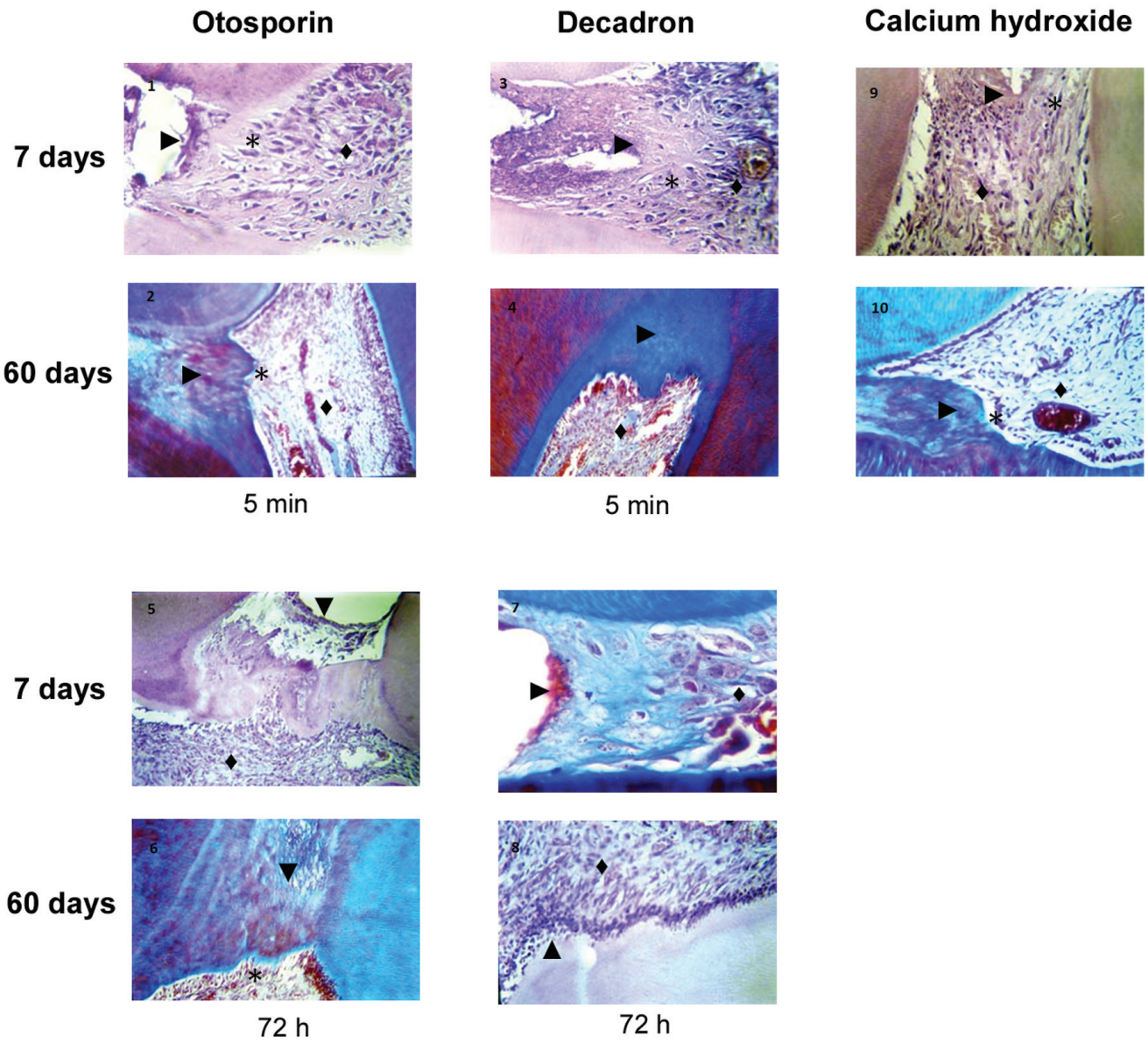

Fig. 1. Histological analysis of the groups treated with Otosporin (Ot), Decadron (D), or calcium hydroxide [$Ca(OH)_2$], as follows:
(1) G_{Ot5min} 7 days. Fibrous barrier subjacent to capping material (►). Disorganized odontoblastic layer (*) and highly cellularized pulpal connective tissue (♦) H.E. 450x.
(2) G_{Ot5min} 60 days. Mineralized barrier (►), odontoblastic layer organized (*) and pulpal connective tissue rich in cells, hyperemic and congested blood vessels (♦) T.M. 200x.
(3) G_{D5min} 7 days. Fibrous barrier subjacent to capping material (►). Disorganized odontoblastic layer (*), pulpal connective tissue rich in cells with mild inflammation and congested and hyperemic vessels H.E. 300x.
(4) G_{D5min} 60 days Mineralized barrier (►), highly cellularized pulpal connective tissue and congested and hyperemic vessels (♦) T.M. 200x.
(5) G_{Ot72h} 7 days. Area of superficial clot necrosis, presence of fibrous barrier with cells included in its interior obliterating almost completely the exposure (►). Highly cellularized pulpal connective tissue (♦) H.E. 250x.
(6) G_{Ot72h} 60 days. Mineralized barrier with cells included in its interior (►), disorganized odontoblastic layer (*) T.M. 200x.
(7) G_{D72h} 7 dias. Fibrous barrier subjacent to capping material with cells included in its interior (♦) and congested and hyperemic vessels (►) T.M.; 700x.
(8) G_{D72h} 60 days. Lateral wall showing disorganized odontoblastic layer (►), thick irregular predentin and connective tissue rich in fibroblast and blood vessels (♦) H.E. 250x.
(9) $G_{Ca(OH)2}$ 7 days. Fibrous barrier subjacent to capping material (►), disorganized odontoblastic layer (*) and connective tissue and highly cellularized (♦) H.E. 300x.
(10) $G_{Ca(OH)2}$ 60 days. Complete mineralized barrier (►), organized odontoblastic layer (*) and subjacent pulpal connective tissue showing hyperemia (♦) T.M. 250x.

Discussion

When capped with calcium hydroxide, pulp exposure healing follows a pattern already described by many authors. Initially, there is a superficial necrotic zone, beneath which an inflammatory reaction can be observed. Later an intense fibroblastic proliferation, which tends to isolate the exposure area, is noted. Subsequently, a mineralized barrier is formed, and the process is concluded by the formation of a dentin bridge (12-16).

The inflammatory reaction observed in the initial periods after pulp exposure and the fact that the dental pulp must be inserted between inextensible hard walls that do not allow it to expand necessitate the use of an anti-inflammatory agent. The use of corticosteroids to reduce the initial inflammatory reaction and prepare the tooth pulp for the application of the capping material is an option. In this study, it was observed that Otosporin (hydrocortisone in combination with polymyxin B sulfate and neomycin sulfate) or Decadron (dexamethasone) applied for 5 minutes or 72 hours resulted in a reduction of inflammatory infiltrate compared to the use of calcium hydroxide in distilled water without a previous dressing. Previous studies (4,17) also reported that corticosteroids control inflammatory reactions in pulpal tissue.

Although Sathler (18) has suggested that corticosteroids applied for long periods inhibit the inflammatory process, constraining the proliferation of blood vessels, fibroblasts and collagen deposition, in the present work, no inhibition of blood vessel proliferation or collagen deposition was observed at 7 or 14 days postoperatively. However, there was a slight retardation of pulpal tissue repair at 30 days postoperatively when the corticosteroid dressing was used for 5 minutes or 72 hours and when the corticosteroid/antibiotic dressing was applied for 72 hours. This retardation resulted in the presence of an incomplete and irregular barrier with osteoid features and a somewhat disorganized odontoblastic layer.

Takayama et al. (8) emphasized the beneficial effects of corticosteroid application for short periods of time, although they noted that when applied for long periods, such dressings had deleterious effects on the pulp. Other studies (18,19) reported that both hydrocortisone and dexamethasone inhibit collagen synthesis in pulpal tissue.

The observation that corticosteroids diminish inflammation but result in deleterious effects on tooth pulp when employed as definitive capping material has led researchers to continue their attempts to determine the best application period for corticosteroids. In this study, when a corticosteroid/antibiotic dressing was applied for 5 minutes, the tissue response and the barrier formed in the 30-day and 60-day postoperative periods were similar to those of the control group in which no dressing was used. These findings corroborate those of Holland et al. (10), who observed that a 10-minute topical application of prednisolone acetate to dogs' teeth contaminated by the environment prior to the use of calcium hydroxide did not alter the results. However, Teixeira & Tancredo (20) reported a higher percentage of success when a temporary dressing was applied for 10 minutes rather than for 7 days.

The therapeutic action of applied corticosteroids depends on the strength of the corticosteroid and on the concentration of the product, as well as on its penetration powder (18). Dexamethasone, present in Decadron, has an anti-inflammatory potential 25 times higher than that of hydrocortisone, which is present in Otosporin.

The anti-inflammatory potential of the dressings tested in this study seems to have minimally interfered with the healing process. The pulps reacted in a slightly different manner when the dressings were applied for 5 minutes instead of 72 hours, and corticosteroid alone promoted the formation of more irregular barriers than did the combination of corticosteroid and antibiotic. Compared with calcium hydroxide alone, either dressing used for 72 hours caused a slight retardation of the healing process observed at 30 days. This delay may be related to the inhibition of the inflammatory response that occurs when the dressing is left for a long period of time in contact with the pulp tissue.

Many studies (6,9,18,21) have demonstrated that the combined use of corticosteroids and antibiotics is more effective than the use of corticosteroids alone, which is likely due to the fact that antibiotic agents act on bacteria that may be present in inflamed pulps. In this study, corticosteroid/antibiotic dressing applied for 5 minutes was the only tested procedure that did not retard healing of the pulpal tissue when compared to calcium hydroxide alone, suggesting that its utilization is unnecessary in intact pulps that are free from contamination and inflammation.

Conclusions

Based on the results of this study, the following conclusions can be drawn:

1. Temporary dressings with corticosteroid (Decadron) and corticosteroid/antibiotic (Otosporin) applied for 5 minutes or 72 hours resulted in a milder inflammatory reaction at early analysis periods than did calcium hydroxide applied without previous dressing.
2. The tissue reaction and the quality of the barrier formed after application of a corticosteroid/antibiotic (Otosporin) temporary dressing for 5 minutes were similar to those observed in the control group at longer postoperative periods (30 and 60 days).
3. Temporary dressings with corticosteroid (Decadron) applied for 5 minutes or 72 hours, as well as corticosteroid/antibiotic (Otosporin) dressings applied for 72 hours, resulted in a slight retardation of the tissue healing process beginning 30 days postoperatively.

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