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Histologic evaluation of pulpotomy performed with ethyl-cyanoacrylate and calcium hydroxide

Avaliação histológica de pulpotomia realizada com etil-cianoacrilato e com hidróxido de cálcio

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ABSTRACT: The aim of this study was to evaluate histological aspects of the pulp-dentin complex of dogs submitted to pulpotomy and capped with ethyl-cyanoacrylate and calcium hydroxide. Thirty dog teeth were divided into 2 groups of 15 as follows: Group 1 - ethyl-cyanoacrylate; Group 2 - calcium hydroxide. The pulpotomies were carried out following all of the treatment precautions recommended for dogs. After 30 days the specimens were submitted to histological preparation and were then blindly evaluated by a histologist. Data were analyzed statistically by the Fisher exact test, comparing the two groups. After 30 days, the presence of a hard tissue barrier was observed in 83.3% of Group 1, and in 100% of Group 2 (p = 0.478). A continuous hard tissue barrier was observed in 50% of the ethyl-cyanoacrylate group and 75% of the calcium hydroxide group (p = 0.652). It can be concluded that both materials induced hard tissue barrier formation, but Group 2 had a higher percentage than Group 1, with no significant statistical differences; the differences observed between the different barriers (continuous/non-continuous) were not significant between groups and there was no pulpal necrosis in either group.

DESCRIPTORS: Calcium hydroxide; Cyanoacrylates; Pulpotomy.

RESUMO: O objetivo do presente estudo foi avaliar o aspecto histológico do complexo dentino-pulpar de cachorros quando capeado após a pulpotomia com etil-cianoacrilato e hidróxido de cálcio. Trinta dentes de cães foram divididos em 2 grupos de 15 da seguinte forma: Grupo 1 - Etil-cianoacrilato; Grupo 2 - Hidróxido de cálcio. A pesquisa foi realizada tomando-se todos os cuidados recomendados para o tratamento com os cães. Após 30 dias do procedimento realizado os espécimes foram submetidos ao preparo histológico e logo após foram avaliados de forma cega por um histologista. Os resultados foram analisados estatisticamente através do Teste Exato de Fisher. No grupo 1, 83,3% e no grupo 2, 100% dos dentes apresentaram a barreira de tecido duro (p = 0,478). A barreira de tecido duro contínua foi observada em 50% dos casos tratados com etil-cianoacrilato e em 75% dos com hidróxido de cálcio (p = 0,652). Pode-se concluir que tanto o grupo 1 como o grupo 2 induziram a formação da barreira de tecido duro, porém o grupo 2 teve um percentual maior do que o grupo 1, não havendo diferença estatisticamente significante; a diferença observada entre as estruturas das barreiras (contínuas e não-contínuas) não foi significante. A necrose pulpar não foi observada em nenhum grupo.

DESCRITORES: Hidróxido de cálcio; Cianoacrilatos; Pulpotomia.

INTRODUCTION

The dental pulp is a well-vascularized and innervated connective tissue, which is capable of healing in response to various stimuli as well as to local injury. When the coronary pulpal tissue is injured, it can produce an inflammatory response; however, several investigators have demonstrated that the dental pulp has the capacity to heal.

Conservative treatment, for example, pulpotomy, is a procedure that preserves the vitality of the pulp tissue, and for many years calcium hydroxide has been used as a capping material. Because of its proven biocompatibility and mineralized tissue-inducing properties, calcium hydroxide preparation has been the medicament of choice in pulpotomies. For these reasons, and also to maintain pulp vitality, it has been used in pulpotomies since the 1940s, having been pioneered by Teuscher, Zander[11] (1936).

New materials like the mineral trioxide aggregate and Portland cement have been studied. After the research conducted by Menezes *et al.*[9] (2004), it was concluded by the authors that both alternatives are effective as pulp protection following pulpotomy.

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Even though these products have been the best choice up until now, it is important to explore new alternatives that could also present acceptable biological and biochemical properties, be easy to handle and have low cost.

In the search for other materials with a potential application, a group of adhesives known as cyanoacrylates have received increasing attention with a view to improving various surgical procedures. These materials have attracted the attention of researchers in the fields of medicine and dentistry because they present adhesive, haemostatic and bacteriostatic properties, and they also induce more rapid tissue repair by reducing the degree and the time of the inflammatory response[2,4,8]. In addition, Azevedo *et al.*[1] (2003) found in their study that the presence of dividing cells three days after seeding in the group treated with Super Bonder (Loctite Química do Brasil, São Paulo, SP, Brazil) also indicates the preservation of the self-renewal capacity of these cells.

According to Koukoubis *et al.*[8] (1995), cyanoacrylate is an adhesive that results from the chemical reaction between formaldehyde and the esters of cyanoacetate. The monomer can then be modified by altering the ester group of the molecule to obtain compounds of different chain lengths. These products have been used in neurosurgery, otorhinolaryngology, gastrointestinal tract surgery and in oral and maxillofacial surgery. They have also been applied in kidney, liver and bone lesions and on animal and human blood vessels.

Ethyl-cyanoacrylate also has been used in cases with corneal perforation. Felberg *et al.*[5] (2003) relate that the application of this specific adhesive is a safe, easy method and that it is very useful in maintaining ocular integrity.

In dentistry, Cvek *et al.*[3] (1987), encouraged by the low-level irritation response, carried out a study in which it was possible to observe the formation of a hard tissue barrier in monkey dental pulp covered with Isobutyl cyanoacrylate.

Based on the above, the present study was set out to observe the histological response of dog pulps capped with a material that has ethyl-cyanoacrylate in its formula (Super Bonder). As a control group, pulpotomy capped with calcium hydroxide (Vetec Química Fina LTDA., Rio de Janeiro, RJ, Brazil) was carried out.

MATERIALS AND METHODS

The research protocol was previously approved by the University of Pernambuco ethics committee (#211/03).

Thirty upper mature permanent teeth (second and third incisors, canines, second and third premolars) from three healthy male dogs with ages ranging from 2 to 3 years, of no specific race and weighing between 15 and 20 kg, were studied. Initially, the dogs were anaesthetized with a sodium pentobarbital solution (3 mg/kg of weight) administered intravenously. The teeth were isolated with a rubber dam and cleaned with 2% chlorhexidine before the operative procedure. The enamel and part of the dentin were removed with diamond points and exposure of the pulp was performed with burs #1013 (second and third incisors), #3071 (canines), #2214 (second and third premolars) (KG Sorensen Ind. Com. Ltda., Barueri, SP, Brazil), according to the anatomy of the teeth. The coronal portion of the pulp was then amputated using a sterile #2 round bur and a #3 spoon excavator. After these procedures, the dentin debris were removed by irrigation of the cavity with 10 ml of sterile saline solution. The bleeding was controlled with sterile cotton pellets associated with irrigation.

The teeth were then randomly divided into two groups for the following procedures:

- **Group 1 (experimental):** one drop of ethyl-cyanoacrylate was placed on pulps with an explorer probe on five teeth on the left-hand side of each dog maxilla.
- **Group 2 (control):** calcium hydroxide paste (calcium hydroxide/distilled water) was placed over the pulps using a sterile amalgam carrier used for retrograde obturation. The paste was also moulded with light pressure using dry cotton pellets on five teeth on the right hand-side of each dog maxilla. Subsequently, the calcium hydroxide was covered with a calcium hydroxide preparation (HYDRO C, Dentsply Ind. e Com. Ltda., Petrópolis, RJ, Brazil).

In both groups, the remaining part of the cavity in the crown of every tooth was filled with amalgam.

Thirty days later, the animals were sacrificed with an overdose of anesthetic and the maxillas were removed. The specimens were fixed in a 10% neutral buffered formalin solution and stored in these individual recipients for 24 hours.

The specimens then underwent routine histologic preparation. After these procedures, 6 micrometer (6 μm) sections were cut and stained with Hematoxylin and Eosin and Golmori Trichrome. The sections were then blindly evaluated by a histologist using the following criteria for histological assessment:

Hard tissue barrier

The hard tissue barrier was classified as continuous, discontinuous or absent. The criterion used to assess barrier formation was the typical appearance of a barrier induced through the action of calcium hydroxide. Characteristically, such a barrier consists of a layer of bone-like tissue and a subsequent layer of dentin-like tissue located at a certain distance (about 1 mm) from the original level of the wound surface[10]. When the barrier was continuous through all sections and no vital tissue was seen above, it was classified as continuous; the barrier was termed discontinuous when it presented fissures and pulpal tissue was found above it.

Condition of the pulpal tissue

Pathologic changes in the pulp were classified as without inflammation, slight inflammation (scattered inflammatory cells), moderate inflammation (accumulation of inflammatory cells) or severe inflammation (accumulation of inflammatory cells and abscess) or necrosis. The condition recorded was the one with the most advanced degree of inflammation.

Data were analyzed by the Fisher Exact test to compare the two groups. The critical level of statistical significance was set at p = 5%.

RESULTS

Histological assessment

The results of the findings are summarized in Table 1.

In the present study 3 teeth from each group were excluded because they could not be suitably processed, leaving 12 teeth to be analyzed from each group.

Six teeth capped with ethyl-cyanoacrylate showed formation of a continuous hard tissue barrier at the level of pulpal amputation, which seemed, in most instances, to be a combination of hard tissue induced by the medicament and the apposition of hard tissue on the dentinal walls. These hard tissue barriers consisted of a bone-like layer at the surface and an underlying layer of dentin-like tissue. None of these teeth contained necrotic tissue between the barrier and the ethyl-cyanoacrylate. The pulpal aspect, underneath the barrier, showed signs of vitality with the presence of a chronic inflammatory process, where congested blood vessels, lymphocytes and plasmocytes, and a few macrophages could be observed (Figure 1). The inflammatory response was considered to be moderate in this area, since the other two-thirds of the pulpal tissue were free of inflammation.

Four teeth from the experimental group presented discontinuous hard tissue barriers. The presence of pulpal tissue with numerous fibroblast-like cells located between the space of the lateral protuberances from the bone-like tissue and the dentin-like tissue could also be observed extending into the coronary cavity. The pulpal appearance, in this case, was also that of a moderate inflammatory infiltrate, characterized by the presence of plasmocytes and lymphocytes.

With regard to the experimental group, the pulpal surface in the teeth that did not have a hard tissue barrier consisted of a dense, collagen-rich tissue in contact with ethyl-cyanoacrylate and a moderate inflammatory infiltrate with the presence of congested blood vessels, lymphocytes, plasmocytes and a few macrophages. Areas of pulpal fibrosis were also observed (Figure 2).

The continuous hard tissue barriers seen on nine of the twelve teeth from Group 2 (control group) consisted of superficial dentin-like layers of tissue that were thicker than the ones found in Group 1 and they were also covered with an amorphous tissue, similar to necrotic tissue, which was in contact with the capping agent. The pulpal tissue underneath the barrier presented a moderate inflammatory infiltrate with plasmocytes, lymphocytes, macrophages and congested blood vessels (Figure 3). The pulpal tissue under the wound appeared to be vital.

In the remaining teeth of the control group, a discontinuous hard tissue barrier with a tunnel-

TABLE 1 - Hard tissue barrier results.

Group	Discontinuous		Continuous		Absence		Total	
	N	%	N	%	N	%	N	%
1	4	33.33	6	50.0	2	16.6	12	100.0
2	3	25.0	9	75.0	0	0	12	100.0
Total	7	29.16	15	62.5	2	8.33	24	100.0

N = number of teeth.

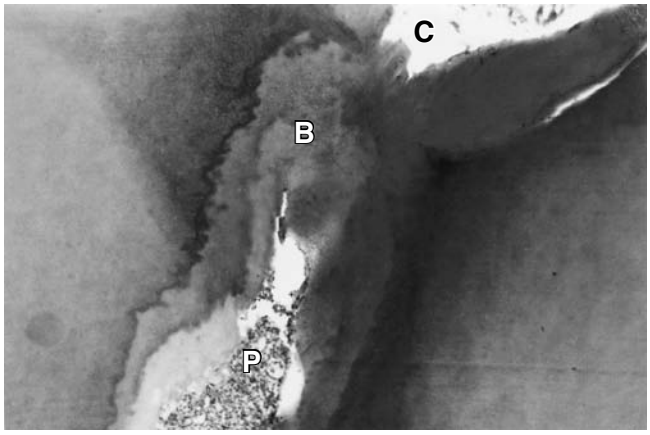

FIGURE 1 - Continuous hard tissue barrier (B) formed underneath the coronary cavity (C). Pulpal tissue (P) presenting a chronic inflammatory infiltrate. Cyanoacrylate (H & E stain). 200 X.

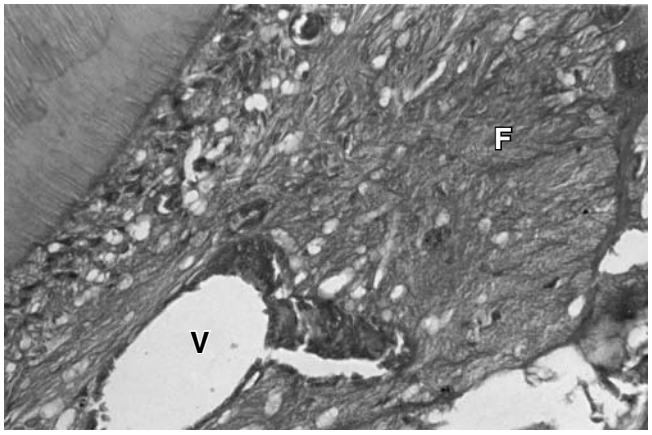

FIGURE 2 - Pulpal tissue presenting blood vessels (V) and the predominance of collagenous fiber (F). Cyanoacrylate. (H & E stain) 400 X.

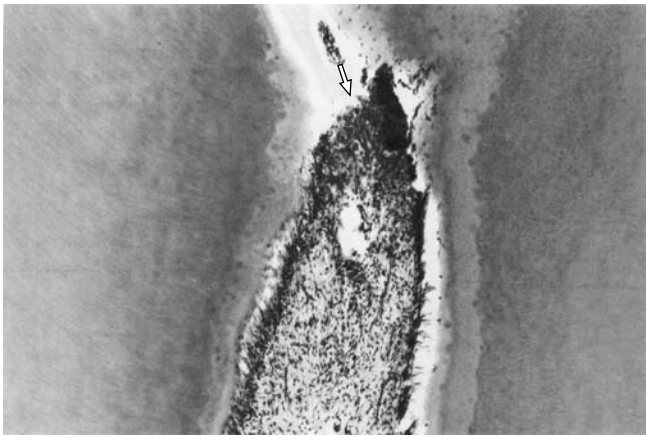

FIGURE 3 - Inflammatory infiltrate (arrow) in pulpal tissue underneath the continuous hard tissue barrier. Calcium Hydroxide. (H & E stain) 200 X.

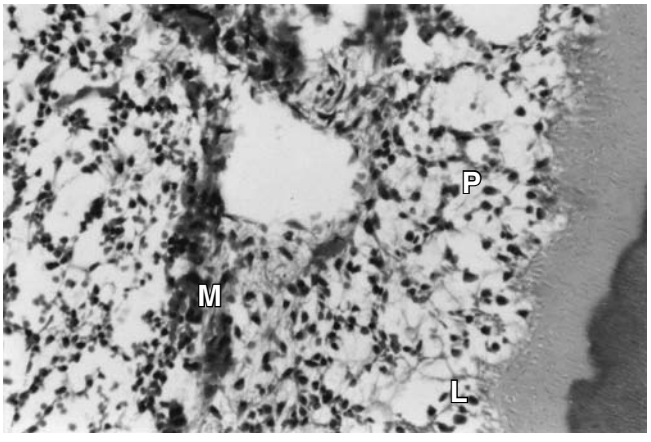

FIGURE 4 - Inflammatory infiltrate in pulpal tissue underneath the discontinuous hard tissue barrier with presence of lymphocyte (L), plasmocyte (P) and macrophage (M) cells. Calcium Hydroxide. (H & E stain) 400 X.

like appearance could be observed. These tunnels contained pulp tissue with fibroblast-like cells that had proliferated towards the coronal surface. In addition, a moderate infiltrate of lymphocytes and plasmocytes next to the barrier was also found (Figure 4). Viable pulpal tissue was observed within the other two-thirds of the root canal. The appearance of the barrier was similar to that of the barriers described above.

It is important to emphasize that pulpal necrosis was not found in any of the twenty-four teeth analyzed in this study.

Data were statistically analyzed by the Fisher exact test comparing the two groups, with critical level of statistical significance set at p = 5%. With regard to the presence or absence of hard tissue barrier formation between groups, the significance level was p = 0.48, which did not represent a statistically significant difference. As to the type of the hard tissue barrier between the groups, the differences were likewise not statistically significant (p = 0.652).

DISCUSSION

In this study, 83.3% of the pulps in Group 1 and 100% in Group 2 reacted to the experimental procedures with the formation of a hard tissue barrier.

With regard to the ethyl-cyanoacrylate group only two teeth did not present barrier formation.

However, in this group, the formation of a continuous hard tissue barrier was found in 50% of the teeth; in 33.33% of the teeth the barriers were found to be discontinuous. The barriers formed in this group were thin and located at the level of the wound, although the histological appearance was similar to that induced by calcium hydroxide. These findings only confirm the theory of Cvek *et al.*[3] (1987) that a low degree of degeneration is responsible for the formation of a hard tissue barrier.

Also, along the experiment, fragments of dentin were found in two teeth with discontinuous barriers and in one that did not form a barrier. According to Holland *et al.*[6] (1978), the presence of these dentinal fragments on pulpal surfaces can impair a good contact between the material and the tissue, resulting in a partial or absent barrier.

There were therefore no significant differences between the materials regarding the appearance of the pulp: both calcium hydroxide and ethyl-cyanoacrylate induced the formation of hard tissue barriers in dog dental pulps; the barriers produced in the cyanoacrylate group, however, were thinner, which may be an advantage, but only if pulpectomy is needed.

On the basis of the characteristics presented by the discontinuous barrier (dentin-like and bone-like tissue), the findings of the present study suggest that an analysis should be performed at 60 and 90 days to evaluate the appearance of the pulp over a longer period of time. This study was conducted only for 30 days in order to have a previous view on the behavior of ethyl-cyanoacrylate when used as a pulp capping material.

However, the most important finding, bearing in mind the conditions under which this study was conducted, is that both groups (calcium hydroxide and ethyl-cyanoacrylate) induced the formation of a hard tissue barrier on dog pulps in thirty days.

CONCLUSIONS

It can be concluded that:

- Both calcium hydroxide and ethyl-cyanoacrylate induced the formation of a hard tissue barrier in dog dental pulps.
- Although the presence of a hard tissue barrier was found in a higher percentage in the calcium hydroxide group than in the cyanoacrylate group, the statistical difference was not significant.
- Within each group, the differences observed between continuous and discontinuous barriers were also not significant.
- There was no pulpal necrosis in the studied groups.

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