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Effect of N-Hexyl Cyanoacrylate, Calcium Hydroxide Plus Iodoform and Two Calcium-Silicate-Based Restorative Cements on Pulp Repair

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Abstract

Objective: The objective of this study was to evaluate the histological response of rat molars to four materials placed on the dental pulp.

Methods: We used 24 female Wistar rats (6 per group). The pulp chamber roof of the first and second upper molars was perforated. After control of bleeding (<5 minutes), a pulp coating material (ProRoot®MTA, Ifabond™, Calcipast+I or Biodentine™) was placed. After 30 days of treatment, the molars were observed under an optical microscope.

Results: ProRoot®MTA produced no inflammation of any pulp ($p<0.0005$). With Calcipast+I, inflammation was absent only in 18.7% of the pulps. All samples in all groups presented pulpal vitality. The ProRoot®MTA, Ifabond™ and Biodentine™ groups showed a dentin bridge and reparative dentin in all pulps. Only ProRoot®MTA showed a completely regular odontoblastic layer ($p<0.0005$). Ifabond™ and Biodentine™ generated an irregular odontoblastic layer in all pulps ($p<0.00l$) and Calcipast+I showed 50% regular and 50% irregular layers. All samples treated with ProRoot®MTA ($p<0.0005$), 81.25% with Calcipast+I and only 37.5% of those treated with Ifabond™ and Biodentine™ ($p<0.0005$) showed fibrosis. In all groups, calcifications appeared in areas outside the dentin bridge ($p=0.027$).

Conclusion: Although ProRoot®MTA had the best performance, the response of the pulp of rat molars to the materials used was similar according to the histological criteria of degree of inflammation and pulpal vitality. The regularity or irregularity of the odontoblastic layer did not influence the presence of a dentin bridge.

Keywords: Biodentine; Mineral aggregate trioxide; N-hexyl cyanoacrylate; Calcium hydroxide plus iodoform; Vital pulp therapy; Pulp capping; Pulpotomy

Clinical Significance

ProRoot®MTA, Ifabond™, Calcipast+I or Biodentine™ can be used on dental pulp in vital pulp therapy.

Introduction

The need to preserve the deciduous and permanent vital teeth to avoid orthodontic problems justified the appearance of Vital Pulp Therapy (VPT). The techniques it encompasses are direct pulpal protection in permanent teeth, and pulpotomy in deciduous and young permanent teeth. In all these techniques, the pulp must be healthy or able to heal and react to the placement of a material [1]. The success or failure of VPT depends on an exact diagnosis of the absence of infection and chronic inflammation, the absence of toxicity of the material used and the absence of filtration of bacteria or toxic products from the exterior to the pulp cavity and radicular pulp [2].

Calcium hydroxide [$Ca(OH)_2$] has traditionally been considered by many clinicians and researchers as the gold standard material for the management of exposed dental pulp. However, Mineral Trioxide Aggregate (MTA) is widely used today, with long-term clinical and radiographic controls, histological studies and, more recently, systematic reviews, showing better success rates [3-5].

N-hexyl cyanoacrylate is an adhesive widely used in medicine due to its antibacterial and hemostatic properties and ability to induce rapid tissue repair. There are studies on the use of isobutyl cyanoacrylate [6] and ethyl cyanoacrylate [7] as material for direct pulpal protection and pulpotomies.

Biodentine™ is a new material based on calcium silicates that has properties similar to $Ca(OH)_2$ and MTA. It has a stimulating effect on pulpal cells and promotes the formation of reparative dentin [8]. The fundamental difference is that it is simpler to handle than MTA [9].

Clinically, successful VPT is considered as the permanence of the tooth in the arch without mobility or the formation of fistulas or abscesses. Radiologically, the absence of periapical or furcation lesions, inter radicular resorption, or internal or external root resorption, and

the presence of a newly-formed dentin barrier with or without other pulpal calcifications is considered successful [10].

Not all authors consider the formation of a hard tissue barrier a success [11], but the formation of new dentin tissue indicates that the material used is biocompatible, without significant irritation and can stimulate odontoblasts or other pulpal pluripotent cells for the formation of hard tissue [12].

The aim of this study was to investigate the pulpal response of rat molars to four biomaterials: Biodentine™, Ifabond™, Calcipast+I and ProRoot®MTA.

Materials and Methods

Animals

The study followed the Directive 2010/63/EU on the protection of animals used for scientific purposes and was approved by the Experimental Animal Ethics Committee of the University of Murcia (CEEA Code: 292/2017). We used 24 female Wistar albino rats raised and fed in the animal care facilities of the University of Murcia (No. REGA ES300305440012, Murcia, Spain), with a mean weight of 230 ± 15 gm. Rats were divided into four groups of six. The animals were anesthetized by intramuscular injection with a mixture of Rompun® (2% xylazine hydrochloride, Bayer. Kiel, Germany) and Imalgene® 1000 (Ketamine hydrochloride 100 mg+chlorobutanol 5 mg; Merial, Barcelona, Spain) at 50%. The anesthetic dose was 0.2 ml/100 g of weight.

Experimental groups

MTA group (n=6): We used ProRoot®MTA (Dentsplay Maillefer, Ballaigues, Switzerland) as a positive control. Composition: Tricalcium silicate ($3CaO\text{-}SiO_2$); bismuth oxide (Bi_2O_3); dicalcium silicate ($2CaO\text{-}SiO_2$); tricalcium aluminate ($3CaO\text{-}Al_2O_3$); calcium sulfate dihydrate ($CaSO_4\text{-}2H_2O$). Mode of employment: The powder and distilled water ratio (3: 1) is placed on a mixing block and mixed until a creamy consistency is obtained. The material was applied directly on the pulp with a ball obturator.

N-hexyl cyanoacrylate group (n=6): We used Ifabond™ (FIMED, Quincié-en-Beaujolais, France). Composition: n-hexyl cyanoacrylate monomers. Mode of employment: the adhesive was applied directly from the ampoule onto the dental pulp. After 30 seconds, Ifabond™ offers good adhesion and polymerization.

Calcium hydroxide group with iodoform (n=6): We used Calcipast +I (CERKAMED Medical company Stalowa Wola, Poland). Composition: 10% calcium hydroxide; barium sulfate >5%; iodoform>30%; 1-2 propoanediol>10%; silicone oil. Mode of employment: Direct placement from the dispensing syringe onto the dental pulp.

Biodentine™ group (n=6): We use Biodentine™ (Septodont, Saint Maur des Fosses, France). Composition: The product consists of a powder and a liquid. The powder consists of tricalcium silicate (>80%), dicalcium silicate, calcium carbonate (10-25%), zirconium dioxide, calcium oxide and iron oxide. The liquid is composed of modified polycarboxylate and calcium chloride dihydrate (10-25%). Mode of employment: Five drops of liquid are added to the powder capsule which is then vibrated at 4000 rpm for 30 seconds on a Rotomix mechanical mixing machine. The mix is placed on the cavity with an amalgam applicator.

Experimental procedure

The surfaces of the healthy, caries-free first and second upper molars were cleaned with 0.12% chlorhexidine (PerioAid®; Dentaid, Barcelona, Spain). The pulp chamber of the molars was perforated with a 0.8 mm tungsten carbide bur (KOMET, Lemgo, Germany) mounted on a turbine (KaVo SMART torque LUX S615 L, Biberach an der Riss, Germany) with abundant cooling water to prevent overheating of the tooth. Bleeding was controlled with a cotton ball and sterile paper tips for not more than 5 minutes. The study material was placed on the exposed pulp. In the groups treated with MTA, Biodentine™ and Ifabond™ the molars were filled with the material. In the group treated with Calcipast+I, the molars were sealed with glass ionomer (Vitrebond™ Plus; 3M ESPE, St. Paul, MN, USA) which was polymerized for 40 seconds with a SmartLite Focus®Pen Style LED Curing Light (Dentsply, USA). All materials were allowed to act for 30 days, after which the rats were sacrificed by CO_2 inhalation.

The maxillary fragments containing the study molars were separated and remaining organic material was cleaned off, and the material immediately introduced (<5 min postmortem) in 10% formaldehyde for 15 days to fix the tissues. After fixation, the segments were immersed in a Shandon TBD-2 Decalcifier (77-80% water, 21-23% formic acid, >1% fluoride, >1% sodium citrate, >1% polyvinyl pyrrolidone; Thermo Fisher Scientific, Waltham, Massachusetts, USA) which was changed daily for two weeks, until decalcified. They were then dehydrated and embedded in paraffin. For the histological study, the tissues were cut into 8 µm microsections with a RM 2155 LEICA motorized microtome (Leica Microsystems, Wetzlar, Germany). Histological sections were stained with hematoxylin-eosin and observed under a Leica DM 5000 B optical microscope (Leica Microsystems, Wetzlar, Germany).

Histological evaluation

The histological evaluation used the following evaluation criteria: degree of inflammation, pulp vitality, existence of dentin bridge and reparative dentin, presence of odontoblastic layer, fibrosis and calcifications in the pulp not related to the bridge. In addition, scores were attributed to the various parameters to assess the best histologic response by group: Degree of inflammation (0. none, 1. slight, 2. moderate, 3. severe, 4. necrosis), vital pulp (0. absence; 1. presence), dentin bridge and reparative dentin (0. absence; 1. presence), odontoblastic layer (0. absence; 1. regular; 2. irregular), fibrosis (0. absence; 1. presence), calcifications in the pulp not related to the bridge. 0. absence; 1. presence).

Statistical analysis

Descriptive statistics were used to obtain the frequency distribution of each variable. Between-group comparisons were made using contingency tables with Pearson's X^2 test. We carried out residual analysis to determine significant associations. Statistical significance was set at $p<0.05$. The statistical analysis was performed using the SPSS 19.0 statistical software package (IBM SPSS Inc., New York, USA).

Results

In molars treated with MTA, no pulp showed inflammation ($p<0.005$). However, pulp treated with Calcipast+I presented mild (50%) or moderate (31.25%) inflammation ($p<0.005$). No material

used caused pulpal necrosis and all pulps were vital (Table 1 and Figures 1–4).

In the Calcipast+I group, there was no dentin bridge or reparative dentin in 18.75% of samples (p=0.024); dentine bridges were observed in all samples of the other three materials. Only pulps treated with MTA had a regular odontoblastic layer in all cases (p<0.005) (Table 1 and Figures 1–4).

Calcipast+I produced a regular odontoblastic layer in 50% of cases and irregular in 50%. The odontoblastic layer of the pulps treated with Ifabond™ and Biodentine™ generated an irregular odontoblastic layer in all cases (p<0.001) (Table 1 and Figures 1–4).

All pulps treated with MTA presented fibrosis (p<0.05) compared with 37.5% of pulps treated with Ifabond™ and Biodentine™ (p<0.005) (Table 1 and Figures 1–4).

Calcifications appeared in all groups in locations other than the bridge (p=0.027). Calcifications were present in all MTA and Calcipast +I sample and 75% of Ifabond™ and Biodentine™ samples (Table 1 and Figures 1–4).

Histological criteria	Degrees	ProRoot®MTA	Ifabond ™	Calcipast +I	Biodentine ™
Degree of inflammation	0. None	100	87.5	18.75	87.5
	1. Slight	–	12.5	5	12.5
	2. Moderate	–	–	31.25	–
	3. Severe	–	–	–	–
	4. Necrosis	–	–	–	–
Vital pulp	0. No	–	–	–	–
	1. Yes	100	100	100	100
Dentin bridge and reparative dentin	0. No	–	–	18.75	–
	1. Yes	100	100	81.25	100
Odontoblastic layer	0. No	–	–	–	–
	1. Regular	100	–	50	–
	2. Irregular	–	100	50	100
Fibrosis	0. No	–	62.5	18.75	62.5
	1. Yes	100	37.5	81.25	37.5
Calcifications in the pulp not related to the bridge	0. No	–	25	–	25
	1. Yes	100	75	100	75

Table 1: Histological evaluation of the pulpal response.

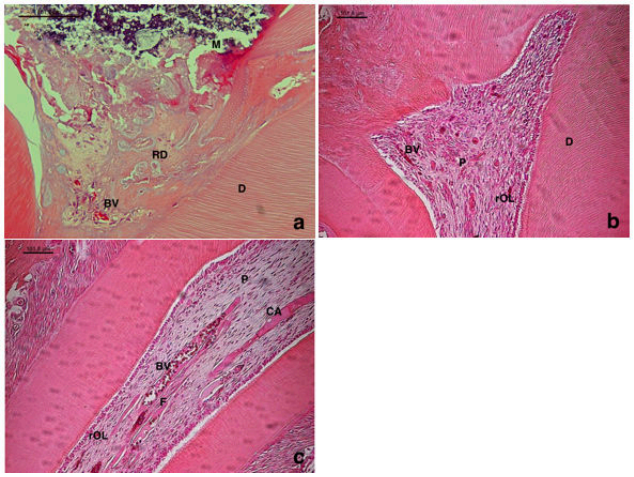

Figure 1: Histological observation of MTA group. Vital pulps with absence of inflammatory cells, presence of fibrosis and atubular reparative dentin in contact with the MTA. In this group we observed an odontoblastic layer that remained regular and calcifications in the dental pulp. Note: MTA. M: Material, RD: Reparative Dentin, rOL: regular Odontoblastic Layer, irOL: irregular Odontoblastic Layer. CA: Calcifications, F: Fibrosis; P: Dental Pulp; BV: Blood Vessels; D: Dentin.

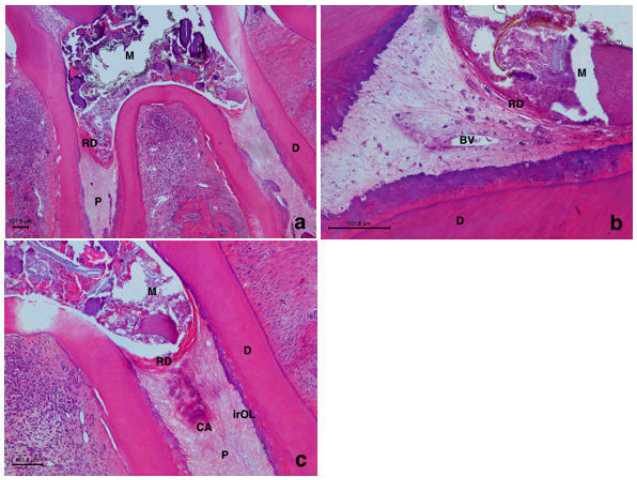

Figure 2: Histological observation of the n-hexyl cyanoacrylate group. The most common pattern was of a vital pulp with a regular odontoblast layer and reparative dentin or a dentin bridge covering the perforation. 87.5% of the samples showed no inflammation; 37.5% presented fibrosis and 75% calcifications in the dental pulp. Note: MTA. M: Material, RD: Reparative Dentin, rOL: regular Odontoblastic Layer, irOL: irregular Odontoblastic Layer. CA: Calcifications, F: Fibrosis; P: Dental Pulp; BV: Blood Vessels; D: Dentin.

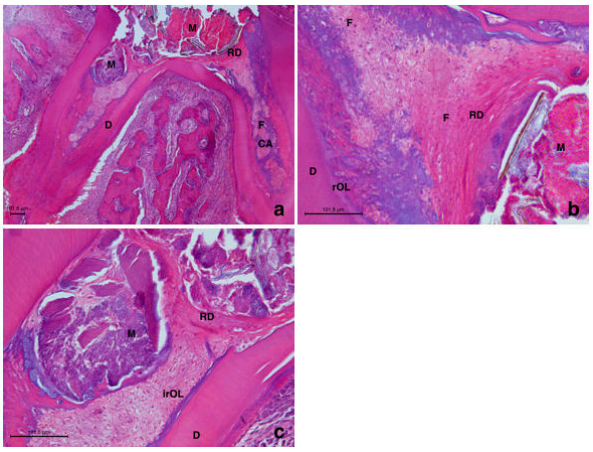

Figure 3: Histological observations of the calcium hydroxide with iodoform group. Vital pulp with many blood vessels was observed. The odontoblast layer remained regular in only 50% of samples. In 81.25% of samples there was fibrosis and a dentin bridge or reparative dentin. All samples presented calcifications. Note: MTA. M: Material, RD: Reparative Dentin, rOL: regular Odontoblastic Layer, irOL: irregular Odontoblastic Layer. CA: Calcifications, F: Fibrosis; P: Dental Pulp; BV: Blood Vessels; D: Dentin.

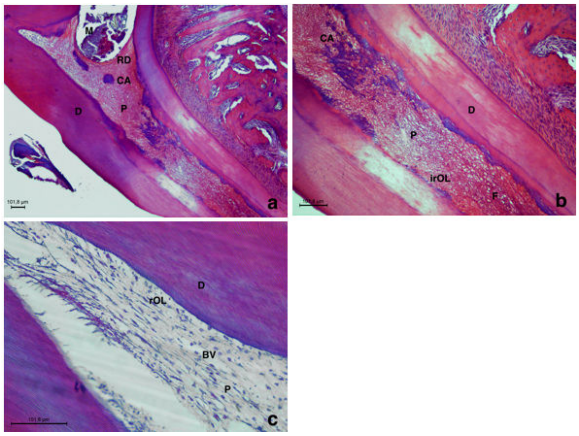

Figure 4: Histological observations of the Biodentine™ group. Vital pulp blood vessels and a regular odontoblastic layer and dentin bridge was observed in all samples: 87.5% of samples did not present inflammation. There was no fibrosis in 62.5%. Only 75% presented calcifications. Note: MTA. M: Material, RD: Reparative Dentin, rOL: regular Odontoblastic Layer, irOL: irregular Odontoblastic Layer. CA: Calcifications, F: Fibrosis; P: Dental Pulp; BV: Blood Vessels; D: Dentin.

Discussion

This study investigated the histological response of pulp to four materials after direct pulp protection on Wistar rat molars. We used this animal model despite criticisms of the difficulty of extrapolating the results obtained in animals to humans, since international codes of ethics for biomedical research consider that any experiment conducted on humans should be designed and based on the results of animal research (Nuremberg Code: 1947, Shuster E in 1997, Declaration of Helsinki). During recent years, many studies have been made in rodent models to evaluate the reaction of pulp after exposure to various materials. In fact, the rat molar is anatomically, histologically, biologically and physiologically similar to a miniature of the human molar, and the tissue reaction of the rodent pulp and the different stages of its healing are identical to those of other mammals, including humans [13].

We used MTA as a positive control since it has been shown to possess all the qualities required of a good material for VPT. It is biocompatible, bactericidal, maintains the pulp without inflammation, promotes its healing and the formation of reparative dentin, does not interfere with the normal process of root resorption, has low solubility and produces a hermetic seal of the pulp chamber [14].

We found that all pulps treated with MTA achieved histological success. That is, they were vital at 30 days, without signs of inflammation, with a regular odontoblastic layer, with reparative dentin and with fibrosis and intra-pulpal calcifications.

Although we made no radiographic study of the molars studied, both MTA and the rest of the materials used were clinically successful because no signs of pain, swelling, fistula, periapical or inter radicular lesion, or internal or external root resorption appeared.

The good performance of MTA has led dental professionals to consider it as a substitute for calcium hydroxide for procedures intended to preserve dental pulp vitality. However, disadvantages such as the difficulties in handling and application, the long time it takes to bond to the dental structure and its high cost has led researchers to look for alternative materials without these disadvantages. Nowicka et al., [3] compared the capacity for hard tissue production of $Ca(OH)_2$, MTA and Biodentine™ and found larger and more homogeneous dentin bridge formation with MTA and Biodentine™ and a smaller and more porous bridge with $Ca(OH)_2$, suggesting a better inductive and reparative process using calcium silicate, the main component of MTA and Biodentine™ compared with $Ca(OH)_2$, probably due to greater recruitment of pulpal stem cells.

In our study, dentin bridge formation occurred in all samples except for the Calcipast+I group, whose main component is $Ca(OH)_2$, in which 18.75% of samples did not show a dentin bridge.

The regenerative capacity of Biodentine™ is better than that of MTA due to the ability of the material to maintain good marginal integrity due to the production of hydroxyapatite at the interface with the tooth [15], which also allows it to be used as a sealing material without the need for final restoration [16].

The dentin bridge induced by $Ca(OH)_2$ is different from that induced by MTA and Biodentine™. While calcium silicates induce the formation of dental tissue with a morphology similar to dentin, $Ca(OH)_2$ induces the formation of a porous dental tissue, which looks more like a defense response to an irritant [3]. In fact, the Calcipast+I group was the only one that presented significant mild or moderate inflammation, which may be explained by the effect of the local irritant of iodoform added to the necrotizing effect of $Ca(OH)_2$ [15].

We used n-hexyl cyanoacrylate, a material belonging to the cyanoacrylate group of adhesives, which are widely used in medicine and surgery due to their hemostatic and bacteriostatic properties, since they induce rapid tissue repair, reducing the degree and duration of the inflammatory response [7].

Cvek et al. [6] used isobutyl cyanoacrylate to make pulp coatings in monkeys and found the formation of a dentin bridge in seven out of nine treated teeth. De Albuquerque, Gominho and Dos Santos [7] used ethyl cyanoacrylate and observed the formation of hard dental tissue in all pulpotomies performed in dogs, with a complete dentin bridge in 83.3% of cases. We have obtained results similar to those obtained with the other cyanoacrylate esters using the "n-hexyl" ester. Molars subjected to VPT (direct pulpal protection) presented hard tissue dentin formation in all cases, with no necrosis or inflammation. Cvek et al. [6] argue that the presence of a certain degree of irritation and inflammation is important to ensure a good pulpal response.

The absence of inflammation may be due to the fact that 30 days passed from application to sacrifice and that entire inflammatory process was resolved positively as the formation of dental hard tissue. In fact, Moretti Neto et al. [17], having demonstrated the good compatibility of these types of adhesives, observed an acute inflammatory component that was resolved over time, and whose intensity and duration depended on the type of cyanoacrylate monomer used.

Fibrosis and calcifications in other locations may be considered a normal part of the healing process of the dental pulp by the coating materials. In fact, the absence of any pulpal coating agent produces the spontaneous formation of calcospherites (spheres of calcium salts) that induce the deposition of a matrix similar to osteodentin interspersed with pieces of demineralized pulp [13].

A dentin barrier is an indication of the presence of the odontoblastic layer, which is located just below the barrier, and thus analyzed the histological presence of the odontoblastic layer. Not all groups presented a regular odontoblastic layer, but there was hard tissue formation in all groups. The mechanism of action of these materials in terms of the arrangement of mineralized tissue seems to be similar: they can regulate the differentiation of odontoblast-like cells and induce the deposition of a mineralized matrix. This might be a normal response of healthy pulp or perhaps a pulpal reaction to irritation. The morphology of this hard tissue probably differs depending on the regularity or irregularity of the odontoblastic layer [18,19].

Conclusion

Although MTA performed best, the response of rat molar pulp to the materials used was similar in terms of the histological criteria of the degree of inflammation and pulp vitality. The regularity or irregularity of the odontoblastic layer did not influence the presence of a dentin bridge. Ifabond™ and Biodentine™ did not present fibrosis in all molars, a necessary response for the production of hard dental tissue, and, therefore, there were fewer calcifications in locations other than the site of pulp chamber perforation.

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