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Tissue Reaction to Different Types of Calcium Hydroxide Paste in Rat

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Abstract

The purpose of this study was to compare the biocompatibility of two types of calcium hydroxide paste in subcutaneous tissue in rat. Twenty-two Wistar rats were divided into 4 experimental (n = 5 each) and one control (n = 2) group. A polyethylene tube filled with either Dentsply or Sure-Paste was implanted in each rat in the experimental groups, while an empty polyethylene tube was used in the control group. After 15 or 60 days, the animals were sacrificed and histopathological examination carried out. Tissue reaction was assessed by inflammatory cell infiltration using a 4-point scoring system, ranging from 0 to 3. Data were analyzed with the Kruskal-Wallis, Wilcoxon, and McNemar tests. Both types of paste induced an inflammatory response at each time point, although the intensity varied. A significant reduction in the number of inflammatory cells was observed at 60 days. Dentsply appeared to induce a more marked inflammatory response at both time points, although the difference was not significant. These results suggest that both types of paste are biocompatible with subcutaneous tissue in rat.

Key words: Biocompatibility — Calcium hydroxide — Implantation — Subcutaneous — Tissue reaction

Introduction

Application of intracanal dressing is indicated in root canal treatment as mechanical preparation alone is insufficient to eliminate microorganisms. Ideally, such dressing should show characteristics such as antimicrobial activity and biocompatibility[2,3)]. The biocompatibility of an intracanal medicament is an important factor in its selection, as it will be coming into direct contact with pulp and periodontal ligament, both vital tissues[3,23)]. All such materials will cause some kind of tissue reaction, the severity and duration of which may influence the outcome of endodontic treatment[5)].

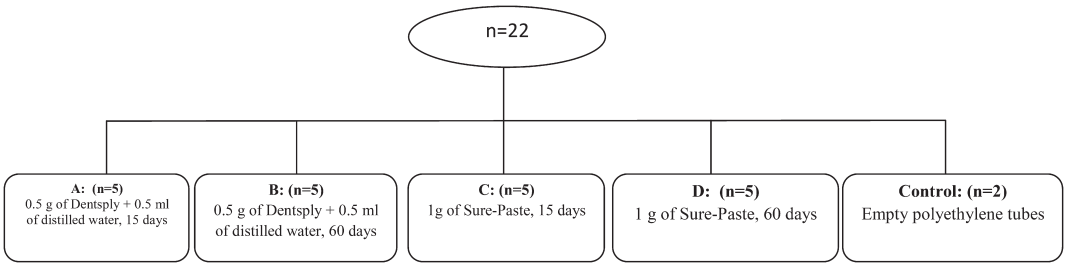

Fig. 1 Diagram of material applied in each group

Calcium hydroxide [$Ca(OH)_2$] is widely used in the treatment of necrotic root canals and open apex teeth due to its biological and antimicrobial properties, ability to dissolve organic tissues, stimulation of mineralization, and capacity to inactivate bacterial endotoxins[15].

Although $Ca(OH)_2$ shows excellent biocompatibility, the commercial addition of other substances to this compound can affect its biological properties[18,22,24]. In recent decades, a number of $Ca(OH)_2$-based medicaments have appeared on the market, including Sure-Paste (SureDent Corp., Seongnam-si, Korea) and Dentsply (Dentsply, Petropolis, RJ, Brazil).

Sure-Paste is basically composed of $Ca(OH)_2$, a radiopacifier, and propylene glycol as vehicle, yielding a high pH value[12]. On the other hand, Dentsply consists of $Ca(OH)_2$, distilled water, bismuth subcarbonate, and olive oil as the vehicle. Many studies have evaluated tissue reaction to $Ca(OH)_2$-based root canal sealers[4,8,26]. To our knowledge, however, no studies to date have compared tissue reaction to different types of $Ca(OH)_2$ paste used as an intracanal medicament.

Tissue response may differ depending on the variation in content of each type of commercially available $Ca(OH)_2$ paste. The purpose of this study, therefore, was to compare two types of commercially available $Ca(OH)_2$ paste in terms of severity of tissue inflammatory response and determine whether that response changed over time.

Materials and Methods

The current study was approved by the Ethics Committee of Mashhad University of Medical Sciences. A total of 22 male Wistar rats weighing 200 ± 15 g each were evaluated. By using the blocking technique, the rats were randomly divided into 4 experimental ($n = 5$ per group) and one control ($n = 2$) group.

The animals were anesthetized by intraperitoneal injection of ketamine hydrochloride 10% (47.5 mg/kg) and xylazine 2% (10 mg/kg). Polyethylene tubes to be used for application of the study materials (Mediflon, Eastern Medikit Ltd., Delhi, India) were first sterilized with ethylene oxide; each tube was 10.0 mm in length and 1.5 mm in diameter.

An area of dorsal skin was disinfected with 10% povidone-iodine and then shaved. A pocket was created to a depth of 20 mm by blunt dissection for implantation of each material. A sterilized polyethylene tube was then immediately implanted in the dorsal subcutaneous connective tissue. Each animal received one tube containing one material in the inter-scapular region. In groups A and B, the tubes contained 0.5 g of Dentsply (test paste #1) plus 0.5 ml of distilled water; in groups C and D, the tubes contained 1 g of Sure-Paste (test paste #2); empty polyethylene tubes were used in the control group (Fig. 1).

Finally, the wound was closed with surgical 4.0 silk sutures (Supa, Tehran, Iran). All the animals were kept at room temperature (24°C) with free access to water and food

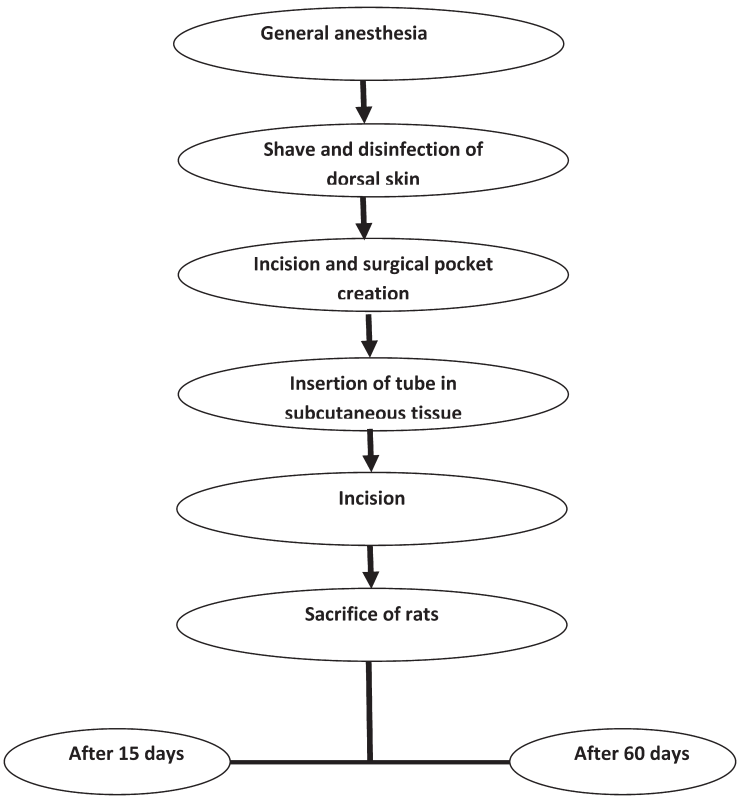

Fig. 2 Diagram of experimental procedure

at the Animal Research Center of Mashhad University of Medical Sciences. The animals in the experimental groups were sacrificed with an overdose of ethyl ether after 15 or 60 days, while one was sacrificed at each time point in the control group (Fig. 2).

The implanted polyethylene tubes and surrounding tissues (1×1 cm) were removed and immersed in 10% buffered formalin (Bayer, Germany). They were then fixed for 24 hr, after which they were processed for paraffin embedding. A series of sections 6 μm in thickness were cut parallel to the long axis of the tube and stained with Hematoxylin and Eosin (H&E).

The specimens were examined with a light microscope (Olympus, Tokyo, Japan) at 40× and 100× magnification by a trained pathologist. The mean count of inflammatory cells for 10 fields, presence or absence of necrotic zones, edema, collagen fiber deposits, and fibrosis were evaluated in each group.

The severity of inflammatory response was classified as follows[29]: grade 0: no inflammation (0–2 cells), grade 1: mild (2–5 cells); grade 2: moderate (5–10 cells); or grade 3: severe (more than 10 cells). Evaluation of necrosis was according to existence or absence of necrotic tissue. Edema was evaluated according to increase in myxoid tissue. Assessment of fibrosis was performed by comparisons of the experimental and control groups and 15- and 60-day groups. The results were recorded as mild, indicating a thin layer of collagen fiber around the material; moderate, a thick layer of collagen fiber around the material; or severe, a dense network of collagen fibers[8].

Statistical analysis of the data was performed using a non-parametric Kruskal-Wallis test

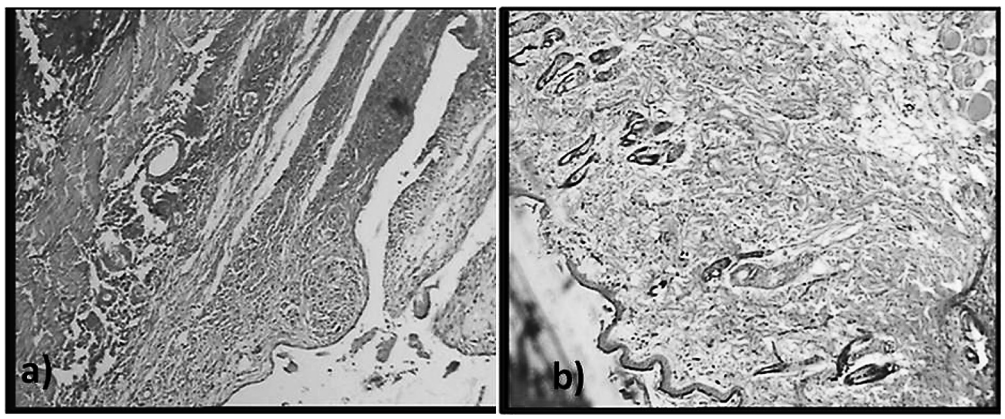

Fig. 3

a) Sure-Paste induced severe inflammatory infiltration at 15 days; Hematoxylin & Eosin, original magnification (×40).
b) Dentsply induced mild inflammatory infiltration at 60 days; Hematoxylin & Eosin, original magnification (×40).

Table 1 Severity of inflammation at each time point

Interval	Severity of inflammation			p-value
	Mild	Moderate	Severe	
15 days	20% (2)	30% (3)	50% (5)	0.096
60 days	30% (3)	60% (6)	10% (1)	

and Wilcoxon and McNemar tests at a 5% significance level.

Results

An inflammatory response was induced in all groups at each observation point (Figs. 3a and 3b). Inter-group evaluation revealed no significant difference between the two experimental time points ($p=0.096$) (Table 1). A mild inflammatory reaction, with mild fibrosis and scattered deposition of collagen bundles surrounded by apparently healthy tissue was observed in the control group. No necrotic reaction was seen in the control group.

Figure 4a shows the results at 15 days in each group. Test paste #1 induced a more severe response, although the difference was

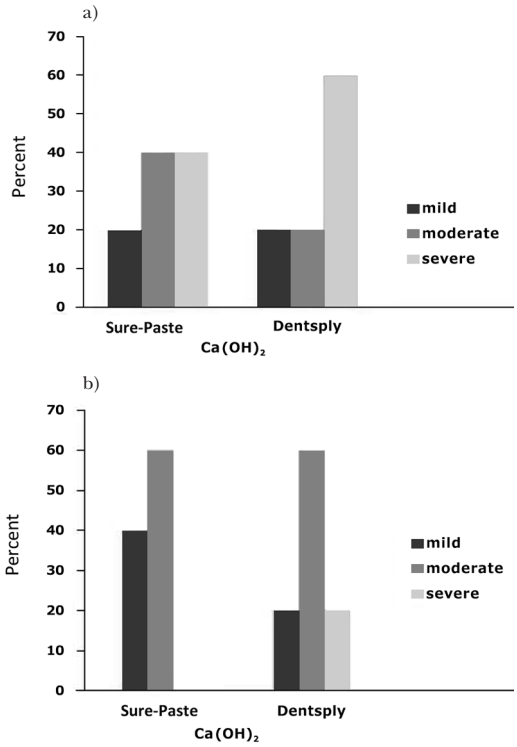

Fig. 4

a) Inflammatory response at 15 days.
b) Inflammatory response at 60 days.

Table 2 Tissue edema by contact with paste at each time point

Interval	Edema		p-value
	+	−	
15 days	70% (7)	30% (3)	0.5
60 days	50% (5)	50% (5)	

Table 3 Fibrosis by contact with paste at each time point

Interval	Fibrosis			p-value
	Mild	Moderate	Severe	
15 days	30% (3)	40% (4)	30% (3)	
60 days	—	30% (3)	70% (7)	0.014

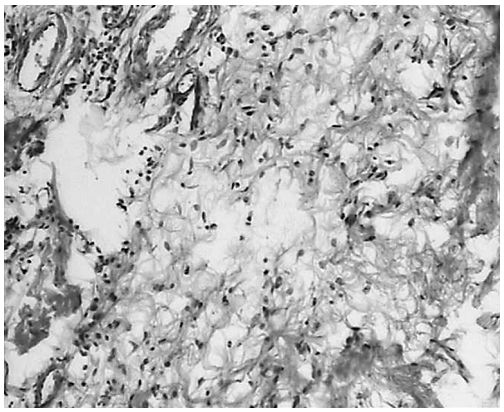

Fig. 5 Tissue edema at 15 days with Sure-Paste (×100)

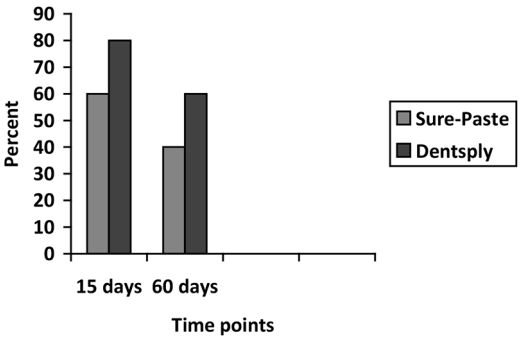

Fig. 6 Tissue edema at 15 and 60 days with Sure-Paste and Dentsply

insignificant. The inflammatory reaction was strongest at 15 days, regardless of the type of $Ca(OH)_2$ used. Figure 4b shows the results at 60 days in each group. Test paste #1 elicited a more severe response than #2, although the difference was not significant ($p>0.05$). Only a moderate inflammatory response was observed at this time point, regardless of the type of paste, showing a reduction compared to at 15 days. At 15 days, inflammatory cell infiltration mainly comprised polymorphonuclear neutrophils, macrophages, and eosinophils, while at 60 days, macrophages and eosinophils showed the strongest response.

In comparison with the control group, no necrotic reaction was seen in the test paste #2 group at 15 or 60 days, although some areas of coagulative necrotic reaction were observed with test paste #1 at each time point. At 60 days, tissue edema decreased in both $Ca(OH)_2$ groups in comparison with at 15 days or in the control group, although the difference was insignificant ($p=0.5$) (Table 2 and Figs. 5 and 6).

Also, at 15 days, moderate dissociation of collagen fibers and few fibroblasts were seen, unlike at 60 days, when there was a dense network of collagen fibers and fibroblasts. At 60 days, fibrosis was better organized, showing formation of capsules around each material, with moderate or intense density ($p=0.014$) (Table 3).

Discussion

The results of the present study revealed no significant difference in the density of inflammatory cells induced by the two types of $Ca(OH)_2$ paste.

The biocompatibility of endodontic materials such as cement medicaments and filling materials has been evaluated based on immune stimulating properties, cell viability, and anti-inflammatory properties[27,28]. The most highly recommended tests for biocompatibility include *in vitro* cytotoxicity and *in*

vivo implantation of materials in the bone or subcutaneous tissue[5,14,15]. In the present study, subcutaneous implantation was employed because of its high validity in screening for biocompatibility[5].

Wistar rats were used as their genetic similarity is suitable for assessing a homogeneous response pattern to the same stimulus[7]. Polyethylene tubes were also considered the most suitable option for bringing the targeted materials into contact with living tissue. As evidence of this, only a small inflammatory reaction was caused by these tubes in the control group, a result that is consistent with that of earlier studies[5,22,28].

Many studies have shown that the inflammatory response continues until the end of the second week[13,23]. Therefore, 15 days was selected as the first time point for observation in the present study. If the life span of a rat is estimated to be approximately 3 years (life span of humans <90 years), then 2 months in the life of a rat is equivalent to 60 months in the life of a human[17]. Therefore, the 60-day time point was selected as being suitable for assessing long-term response. Several studies have compared the biocompatibility of $Ca(OH)_2$ with other types of sealer[5,8,11,12,25,26]. However, to our knowledge, no other studies have compared the biocompatibility of different types of intracanal $Ca(OH)_2$ paste. The present results showed no significant difference in response to each type of paste, although that to test paste #1 was a little more severe than that to test paste #2.

Calcium hydroxide powder is mixed with various types of vehicle in the preparation of paste, which has a significant effect on the dissociation of $Ca(OH)_2$ into OH^- and Ca^{2+}, and consequently on its properties and biocompatibility. Such vehicle comprises 3 major types: water-soluble, viscous, and oil-based substances[16]. Pacios *et al.* concluded that the type of vehicle used had a significant effect on the pH of the resulting $Ca(OH)_2$ paste[21]. In the present study, however, the results showed similar results for each type of paste, which indicates that the vehicle in each exerted little effect on tissue reaction.

Distilled water and olive oil are used as vehicle in test paste #1, while propylene glycol, which is classified as a viscous vehicle, is used in test paste #2. This type of vehicle releases OH^- and Ca^{2+} more slowly and for longer periods than water-soluble or oil-based materials[19]. It has been reported that addition of propylene glycol does not interfere with the biocompatibility of mineral trioxide aggregates in rat subcutaneous tissues[10]. Moreover, the present results are consistent with those of an earlier study by Andolfatto, which showed that UltraCal XS and Hydropast $Ca(OH)_2$ elicited similar biological behaviors, despite the different type of vehicle used in each[2].

Various types of radiopacifier are also used in endodontic materials, such as bismuth oxide, barium sulfate, and zinc oxide[1,20]. The present results indicated that type of radiopacifying agent had no effect on tissue reaction. The bismuth carbonate of Dentsply did not affect the biological properties of $Ca(OH)_2$[19,25]. Barium sulphate, the radiopacifying agent of Sure-Paste, was also considered biocompatible as it caused no detrimental effect on subcutaneous tissue in rat[20].

In the present study, the severity of inflammation at 60 days was less than at 15 days. At 15 days, an intense inflammatory reaction (50%) and foci of coagulative necrosis were observed in the capsule adjacent to the implanted $Ca(OH)_2$. At 60 days, a reduction (10%) in inflammatory reaction was observed.

The severe inflammatory reaction observed at 15 days may be attributed to "superficial" necrosis promoted by $Ca(OH)_2$[6]. The alkaline pH of $Ca(OH)_2$ induces formation of a coagulative necrosis zone when it comes into contact with connective tissue[9]. When the integrity of necrotic cell membrane is lost, the cellular contents leak into the surrounding tissue, triggering an inflammatory response characterized by removal of cellular debris by phagocytosis and subsequent healing. The reduction in inflammatory reaction seen at 60 days in the present study may be explained by tissue tolerance and repair[6].

Finally, used as an intracanal medicament,

$Ca(OH)_2$ shows properties such as solubility and change in environmental pH. Induction of an immediate reaction, followed by healing, is the optimal result. In the present study, no significant difference was observed in tissue reaction between the two types of commercially available $Ca(OH)_2$ investigated. However, the inflammatory response was weaker with test paste #2 (Sure-Paste) than that with #1 (Dentsply).

Conclusion

The present findings indicate that Sure-Paste and Dentsply are biocompatible in subcutaneous tissue in rat.

Conflicts of Interest

The authors declare no conflict of interest related to this study.

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