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ORIGINAL ARTICLE

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Antimicrobial activity of calcium hydroxide associated with a new vehicle (Triethanolamine)

Atividade antimicrobiana do hidróxido de cálcio associado à um novo veículo (Trietanolamina)

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ABSTRACT

Objective: The aim of this study was to evaluate the antimicrobial effectiveness of calcium hydroxide associated with a two conventional (anesthetic liquid and olive oil) and a new vehicle (Triethanolamine). **Material and methods:** Initially, microorganisms were collected from four upper incisors presenting unsatisfactory root canal treatments and persistent apical periodontitis from the same patient using sterile absorbent paper cones placed inside the root canal following initial access and root filling removal. Next, they were grown in a BHI culture medium for 24 h. Afterwards, they were placed in 10 Petri dishes with 3 holes, where different formulations of the medications studied were placed. After 48 and 72 h, readings were taken of the growth inhibition halos. **Results:** After using Kruskal-Wallis test with significance level of 1% ($\alpha = 0.01$) it was observed that, calcium hydroxide associated with Triethanolamine, produced results similar to those produced by the anesthetic liquid. Both combinations were superior to the association with olive oil. **Conclusion:** Considering the results of this *in vitro* study, it is possible to consider that Triethanolamine can be a viable alternative to be used as a vehicle associated with the calcium hydroxide.

KEYWORDS

Pharmaceutical Vehicles; Calcium hydroxide; Endodontics; Triethanolamine.

RESUMO

Objetivo: O objetivo deste estudo foi avaliar a capacidade antimicrobiana do hidróxido de cálcio associado à dois veículos convencionais (anestésico líquido e azeite de oliva) e um novo veículo (Trietanolamina). **Material e Métodos:** Inicialmente, os micro-organismos foram coletados de quatro incisivos superiores de um mesmo paciente que apresentavam tratamentos endodônticos insatisfatórios e periodontites apicais persistentes utilizando cones de papel absorvente esterilizados inseridos nos canais radiculares após a remoção do material obturador. Em seguida, estes foram cultivados em BHI durante 24 h e colocados em 10 placas de Petri com três orifícios preenchidos com as medicações avaliadas. Após 48 e 72 h, os halos de inibição foram aferidos. **Resultados:** Após aplicação do teste de Kruskal-Wallis com nível de significância de 1% ($\alpha = 0,01$) foi observado que, hidróxido de cálcio associado à Trietanolamina, apresentou resultados semelhantes àqueles produzidos pelo líquido anestésico. Ambas as combinações foram superiores ao hidróxido de cálcio veiculado ao azeite de oliva. **Conclusão:** Considerando os resultados deste estudo *in vitro*, é possível concluir que Trietanolamina pode ser uma alternativa viável para ser utilizada como veículo associado ao hidróxido de cálcio.

PALAVRAS-CHAVE

Veículos farmacêuticos; Hidróxido de cálcio; Endodontia; Trietanolamina.

INTRODUCTION

Calcium hydroxide pastes are the most widely used intracanal medications for infectious processes involving primarily the pulp tissue and subsequently the periradicular tissues [1-4]. In regard to the mechanism of action of these pastes, hydroxyl ion diffusion alters the pH of the medium and compromises the enzymatic metabolism of bacteria, causing protein denaturation and irreversible damage to the cytoplasmic membrane [5,6].

With this in mind, many substances have been researched to act as vehicles associated with calcium hydroxide [7-9]. Studies *in vitro* have shown that the specific type of vehicle is directly related to the effectiveness of the ionic dissociation and the antimicrobial action of this medication [10,11].

Triethanolamine is clear, totally water soluble and miscible with most liquid oxygen organic solvents. It is used for different purposes in the pharmaceutical field, such as a surfactant and emulsifier in manufacturing soaps [12]. Therefore, it has shown an excellent alkalizing potential. These characteristics enable Triethanolamine to stabilize the pH of different solutions [13-16].

Considering the importance of pH for the antimicrobial effects of calcium hydroxide pastes associated with different vehicles, the aim of this study was to evaluate the antimicrobial effects of calcium hydroxide associated with Triethanolamine, anesthetic liquid and olive oil.

MATERIALS AND METHODS

Initially, microorganisms were collected from four upper incisors presenting unsatisfactory root canal treatments and persistent apical periodontitis from the same patient using sterile absorbent paper cones (Tanari, São Paulo, Brazil) placed inside the root canals following initial access and root filling removal by using 1016HL and Endo Z drills (KG Sorensen, Barueri, Brazil), Gattes Glidden and Hedstroen files (Dentsply Maillefer, Ballaigues, Switzerland), respectively [17]. Afterwards, fresh cultures of these microorganisms were cultivated in 5ml of brain heart infusion (BHI) broth culture medium, incubated at 37 °C for 72 h under aerobic conditions [18]. After the incubation period, the culture was standardized at a turbidity of 0.5 McFarland scale (approximately 1.5 x 108CFU / mL) in a new tube containing 5 ml of sterile BHI broth, for use as test inoculums [19].

Ten Petri dishes with Mueller-Hinton agar were inoculated with 0.1ml of culture using a Drigaski handle [20,21]. After absorption of the culture, three equidistant holes were made, standardized with an autoclaved copper ring 4 mm in diameter by 3 mm in height, and then filled with the test solutions.

The plates were left to stand at room temperature for 2 h so that the paste in the culture medium could spread, and then incubated in a bacteriological incubator (FANEM ®, São Paulo, Brazil) at 37 °C for 48 h. After incubation, a reading was taken of the plates to determine the presence or absence of the inhibition zones. These were measured by three observers using a transparent millimeter ruler. The values measured were then tabulated and submitted to statistical analysis by using Kruskal-Wallis test with significance level of 1% ($\alpha = 0.01$).

RESULTS

The mean measurements in mm of the inhibition zones, at the two time periods examined (48 and 72 h) are shown in Table 1.

Table 1 - Average inhibition zones considering the materials and the periods analyzed

MATERIALS	TIME	
	48 h	72 h
Triethanolamine*	9.5 mm	10.1 mm
Olive oil	0.0 mm	0.0 mm
Anesthetic liquid*	7.8 mm	8.4 mm

* Significant differences at a 1% level ($\alpha = 0.01$) by using Kruskal-Wallis test.

DISCUSSION

The use of calcium hydroxide pastes as an intracanal medication, especially in cases of infection, is a widespread clinical strategy accepted worldwide [22,23]. Not only do the pastes act effectively against microorganisms that remain and develop inside the root canal, but they also play an active role in repairing periradicular tissues, due to alkaline phosphatase action [23]. Since all of these mechanisms have a direct or indirect relation to the pH of this medication, vehicles associated with calcium hydroxide play a key role in its action [24-26].

Different substances reported in the literature, such as anesthetics [27], saline solution [28] and distilled water [29], have been associated with calcium hydroxide to evaluate the antimicrobial effects of this medication. Like the present study, these studies attempted to evaluate different formulations to determine its antimicrobial effects. Triethanolamine was chosen by the authors as a vehicle to be associated with calcium hydroxide, mainly based on a study that aimed to make a histological evaluation of the biocompatibility of calcium hydroxide associated with this substance, compared with polyethylene glycol, saline solution and olive oil. Fifty mice of the guinea pig species were randomly divided into 5 groups (n.10), according to each vehicle used: G1: calcium hydroxide, G2: triethanolamine, G3: polyethylene glycol, G4: saline solution, and G5: olive oil, and further divided into subgroups, according to the two analysis periods: (a) 30 and (b) 90 days. Teflon carriers filled with the evaluated substances were placed in standardized bone cavities in the anterior mandible region. The animals were euthanized to perform a histological analysis after the time periods analyzed. In 30 days, specimens from Groups 1, 3 and 5 showed a very pronounced inflammatory response. Specimens from Group 2 showed an inflammatory reaction ranging from mild to severe, with rapid resorption of the material and progressive advancement of osteoid tissue into the Teflon carriers. Specimens from Group 4 showed a moderate inflammatory reaction. In 90 days, specimens from Group 1 showed a very pronounced fibrous replacement. In regard to Group 2 specimens, the tested material was solubilized and replaced by newly formed bone tissue. As for Group 3 and 5 specimens, the inflammatory reaction went from acute to moderate. In relation to Group 4 specimens, an organized bone formation process was observed. Specimens from Group 2 showed higher biocompatibility, especially as compared with the specimens from Groups 3 and 5. [12].

The agar diffusion method has been widely used in studies to analyze the antimicrobial activity of different substances employed in endodontic therapy, including calcium hydroxide pastes associated with different vehicles [30]. Also based on previous studies, we chose Mueller-Hinton as a culture medium that would enable microorganisms to survive [20,21], and the McFarland scale to enable standardization of the number of microorganisms [19]. In relation to the periods of analysis, a minimum period of 48 h was chosen to promote microbial proliferation [26].

In this study, we have decided to use microorganisms collected from teeth with previous endodontic treatment and apical periodontitis without identification. It can be considered as a methodological deficiency from a microbiological standpoint. However, initial clinical and subsequent atmospheric conditions were responsible for the natural selection of these microorganisms. Only cultivable specimens under aerobic conditions could influence the results. This was an intentional strategy seeking naturally select the most difficult microrganisms to be eliminated from the root canal system, i.e, microrganisms associated with persistent infections and resistant to different atmospheric conditions [31-33].

Calcium hydroxide associated with Triethanolamine showed results similar to those for the same medication combined with the

anesthetic liquid, and statistically superior to that of the olive oil combination. Since the study periods were relatively short (48 and 72 h), the findings of the study could be attributed to the slower release of hydroxyl ions and obtaining a low pH when oily vehicles are used in associated with calcium hydroxide. These results are in agreement with Ferreira et al. [34] that showed after 72 h, calcium hydroxide associated with camphorated paramonochlorophenol and saline solution raise the pH from 9.6 and 8.2 to 11.4 and 11.3, respectively, while LC paste and calcium hydroxide containing gutta-percha points showed significantly lower results.

Triethanolamine appears to be a promising substance for use as a vehicle associated with calcium hydroxide. More research should be designed with more accurate methodologies to analyze the release of calcium ions and hydroxyl, pH and antimicrobial capacity of this association.

CONCLUSIONS

Considering the limitations of this *in vitro* study, the associations of calcium hydroxide with Triethanolamine and with the anesthetic liquid showed results superior to the olive oil association at both periods of the study (48 and 72 h). However, the two former associations showed no statistically significant differences between each other.

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