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Estimating The Antimicrobial Efficacy Of Calcium Hydroxide With Different Carrier Vehicles

Research Article

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Abstract

Introduction: Since its introduction in 1920 (Hermann 1920), calcium hydroxide has been widely used in endodontics. It is a strong alkaline substance, which has a pH of approximately 12.5. In an aqueous solution, calcium hydroxide dissociates into calcium and hydroxyl ions. Bacterial micro flora of primary endodontic infections differs vastly from secondary or persistent peri radicular lesions. The most frequent survivors present in a very high proportion of root canal failure cases are Enterococcus faecalis with the prevalence of 24%–77% and Candida albicanswith the prevalence of 6%–18%..
Materials & Methods: The study was done by assessing zone of inhibition forEnterococcusfaecalis and Candida albicanswith 4 different carriers for calcium hydroxide.The statistical package SPSS (Statistical package for social science) and Microsoft Excel was used for statistical analysis.
Results: The obtained results state that calcium hydroxide mixed with saline showed an increased antimicrobial effect on facultative anaerobes such aE. faecalis and C. albicans, with calcium hydroxide mixed with distilled water as standard.
Conclusion: The obtained results state that Saline was promoting the antibiotic effect whereas Local anaesthesia and MTA were inhibiting the antibiotic activity of calcium hydroxide.

Keywords: Calcium Hydroxide; Antimicrobial Effect; MTA; Local Anaesthesia; Saline.

Introduction

The prevention and control of pulpal and peri-radicular infections is essentially projected toward the success of endodontic treatment [1]. It is clear that the outcome of the endodontic therapy depends on their reduction or elimination of the microorganisms involving the peri-radicular lesions. Complete chemo-mechanical preparation may be considered an essential step in root canal disinfection.[2] However, it is totally difficult to accomplish total elimination of bacteria.Since its introduction in 1920 (Hermann 1920), calcium hydroxide has been widely used in endodontics. [3] It is a strong alkaline substance, which has a pH of approximately 12.5. In an aqueous solution, calcium hydroxide dissociates into calcium and hydroxyl ions. Numerous biological properties have been attributed to calcium hydroxide, such as antimicrobial activity, inhibition of tooth resorption, induction of repair, tissue-dissolving ability and by hard tissue formation [4]. Because of all the beneficiary effects calcium hydroxide is been recommended for use in several clinical situations [5].

Recent results show that calcium hydroxide paste used as a dressing in carefully instrumented and irrigated root canals kills the bacteria so effectively that the treatment of initially infected root canals can be completed at the second visit [6]. Calcium hydroxide was originally introduced to the field of endodontics by Herman in 1920 as a pulp-capping agent. It is a white odourless powder with the formula $Ca(OH)_2$ and has a molecular weight of 74.08. $Ca(OH)_2$ has low solubility in water (about 1.2 gm-L^{-1} at 25),

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which decreases as the temperature rises.

The dissociation coefficient of $Ca(OH)_2$ (0.17) permits a slow, controlled release of both calcium and hydroxyl ions [6, 7]. The low solubility is a good clinical characteristic because a long period is necessary for $Ca(OH)_2$ to become soluble in tissue fluids when in direct contact with vital tissues. $Ca(OH)_2$ has a high pH (about 12.5-12.8), is insoluble in alcohol, and is chemically classified as a strong base.(8) Its main actions result from the ionic dissociation of th $Ca2^+$ and OH^- ions and their effect on vital tissues, such as inducing hard tissue deposition and being antibacterial. $Ca(OH)_2$ dissociates into calcium and hydroxyl ions on contact with aqueous fluids. $Ca(OH)_2$ in water has a thixotropic behaviour, which means that it will be very fluid when agitated. When $Ca(OH)_2$ is exposed to carbon dioxide (CO_2) or carbonate ions (CO_3) in biological tissue, the dissociation of the chemical leads to the formation of calcium carbonate ($CaCO_3$) and an overall consumption of $Ca2^+$ ions [9].

Currently, this chemical substance is acknowledged as one of the most effective antimicrobial dressings during endodontic therapy [9, 10]. However, one study showed that after 30 days of exposure to carbon dioxide, six preparations of $Ca(OH)_2$ still maintained a purportedly bactericidal pH within the root canal. In studies that have demonstrated the antimicrobial efficacy of calcium hydroxide, the root canals had been dressed for at least 1 month. It is not clear, however, what minimum time might be needed for a calcium hydroxide dressing to achieve an optimal antibacterial effect [10].

The main etiological agents of pulp necrosis and peri-radicular lesions are the microorganisms, bacteria, and their products.Candidaalbicans is the most common species of fungi cultured from root canals of teeth with failed endodontic treatment. Because of collagenolytic activity, it may be possible for the yeast to use dentin as a nutrient source and promote colonization in the root canal. Another organism commonly found in cases of failed endodontic infections and endodontic flare-ups is Enterococcus faecalis. It has the ability to survive in root canal system as a single organism without the support of other bacteria and is small enough to proficiently invade and live within the dentinal tubules [11]. Many studies have reported that C. albicans and E. faecalis are able to invade dentinal tubules to variable depth. [12] So, to ensure complete elimination of root canal bacteria, an effective antimicrobial agent in the root canal is required for a predetermined time for complete eradication of any remaining bacteria [12, 13].

Calcium hydroxide has been widely used as an intracanal medicament in endodontics, and it has been demonstrated that it is very effective against obligate anaerobes but facultative anaerobes like C. albicans and E. faecalis have been reported to be resistant to the antimicrobial effect of calcium hydroxide [12-14]. Therefore, the purpose of the present study was to assess in vitro antimicrobial activity of Calcium hydroxide mixed with different vehicles against E. faecalis and C. albicans by agar diffusion method.Our research experience has prompted us in pursuing this study [15-24].

Materials And Methods

The test organisms chosen to use for this study were C. albicans and E. faecalis as they are the most frequently isolated bacterial species from the root canals of endodontically failed teeth. Culture of C. albicans and E. faecalis were grown on Nutrient agar and incubated for 24 h at 37°C. All solutions were at a concentration of 100µl. [25, 26].

Intracanal medicaments were prepared for four solutions:
Solution I: calcium hydroxide [$Ca(OH)_2$] + saline
Solution II: calcium hydroxide [$Ca(OH)_2$] + distilled water
Solution III: calcium hydroxide [$Ca(OH)_2$] + Local anaesthesia
Solution IV: calcium hydroxide [$Ca(OH)_2$] +MTA (Mineral trioxide aggregate)

Diluted solution was transferred to their respective medium for culture. Plates were incubated for 24 h at 37°C. Zone of inhibition was measured after adding the four different solutions to the culture plates.

The statistical package SPSS (Statistical package for social science) and Microsoft Excel was used for statistical analysis.

Results

The obtained results state that calcium hydroxide mixed with saline showed an increased antimicrobial effect on facultative anaerobes such a E. faecalis and C. albicans,with calcium hydroxide mixed with distilled water as standard. Calcium hydroxide combined with MTA reduces the anti-microbial efficacy of calcium hydroxide.

Discussion

Root canal irrigating solutions and intracanal medicaments are used to eliminate the bacteria from the root canals [27]. Antibiotics are used in dentistry both systemically and topically. During systemic administration of antibiotics, negligible concentrations reach the root canal, whereas during the local administration of antibiotics, greater concentrations can be used as intracanal medicaments, to decrease systemic consequences and complications. [11, 28]. Dealing with the complexity of root canal infection, a single irrigant or a medicament could not result in effective sterilization of the root canal [27-29]. Combination of irrigants or

Table 1. Zone Of Inhibition In Millimeters.

Solutions(100µl)	E. faecalis	C. albicans
calcium hydroxide [Ca (OH)2] + saline	18mm	21mm
calcium hydroxide [Ca (OH)2] + distilled water	17mm	20mm
calcium hydroxide [Ca (OH)2] + Local anaesthesia	16mm	19mm
calcium hydroxide [Ca (OH)2] +MTA (Mineral trioxide aggregate)	15mm	15mm

Figure 1. Efficacy of each solution.

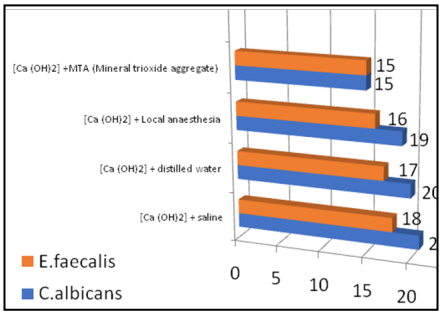


Figure 2. *E.faecalis* - Zone Of Inhibition.

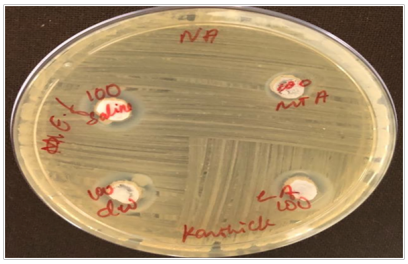
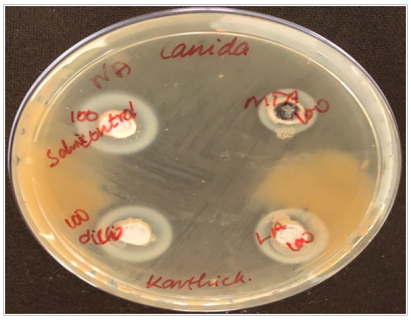


Figure 3. *C. albicans* - Zone Of Inhibition.



medicaments decreases the development of resistant bacterial strains and produces synergistic effect, whose antimicrobial action lasts longer and also sustains release of medicaments occurs. In the present study, $Ca(OH)_2$+ saline has shown better antifungal efficacy against C. albicans and against E. faecalis compared with the remaining groups of solutions, with $Ca(OH)_2$+ distilled water as standard, which is significant. $Ca(OH)_2$ + MTA showed the least antimicrobial efficacy among the chosen vehicles [30].

Various methods have been describedfor the antimicrobial activity of endodontic disinfectants and medicaments. The generally accepted procedure worldwide for determining in vitro sensitivity under routine laboratory conditions is Agar diffusion test. The majority of research on Calcium hydroxide pastes has used the agar diffusion method. This method is simple, standard, and reproducible. Even though the agar method is one of the popular tests, it may have some limitations.Some factors (such as pH of the substrate, incubation period, toxicity, sensitivity, and diffusion capacity of the drug) may have an effect on the antimicrobial activity of the test materials in the plates. However, there is also evidence that agar diffusion tests show good correlation with other antimicrobial susceptibility tests [25]. Results of agar diffusion tests should be carefully examined when materials such as Calcium hydroxide are used, because its antibacterial effect is mainly obtained from its high alkalinity.With the presence of buffering agents in the culture medium, regardless of whether Calcium hydroxide can diffuse through agar plates very well, its pH may not reach sufficient level to give enough antimicrobial activity. Buffering ability of the agar media may alter its antimicrobial activity. On the other hand, similar buffering effect may be generated by tissue fluids and dentin in ex vivo or in vivo conditions [30, 31].

The effect of Calcium hydroxide on C. albicans is very controversial in the literature. In the present study, all Calcium hydroxide preparations were more effective on C. albicans than on E. faecalis.[32] In contrast, it has been previously found that Calcium hydroxide is not an effective agent on C. albicans. However, there are some methodologic differences among these studies.[33] Waltimo et al. used saturated aqueous Calcium hydroxide, which has a high pH. Because C. albicans can survive in a wide range of pH values, the pH may not be a critical factor in this situation. In addition, Calcium hydroxide solution may act as a source of free cationswhich have a critical role in morphogenesis and pathogenesis of C. albicans.[34] Furthermore, saturated Calcium hydroxide solutions are sensitive to inhibition, particularly by dentin, albumin, and hydroxyapatite. If the Calcium hydroxide solution does not have a backup reservoir of a paste, it may lose its high pH easily after interaction with organic and inorganic substances and may become ineffective. Ferguson et al. investigated antifungal effect of different irrigants and medications and concluded that the paste form of Calcium hydroxide was very effective in killing C. albicans although aqueous Calcium hydroxide had no antifungal activity [35]. Therefore, they stated that Calcium hydroxide must be in direct contact with microorganisms to show its potent an-

timicrobial properties. In addition to different forms of Calcium hydroxide preparation, the microbiologic methods were also different in other studies.Ercan et al. used direct contact test and observed that C. albicans was more resistant than E. faecalis to Calcium hydroxide. Moreover, Gomes et al. investigated antimicrobial activity of Calcium hydroxide pastes by 2 methods: agar diffusion and direct contact tests.[36] They found that Calcium hydroxide pastes showed antimicrobial action only by direct contact test; they did not produce any inhibitory zone against any of the tested microorganisms. However, both studies used brain heart infusion broth or agar for growing C. albicans. Brain heart infusion is not a specific medium for fungi and may affect growth conditions of C. albicans [36, 37]. These discrepancies regarding antimicrobial effects of disinfectants may also be related to the strain type of C. albicans used in different studies.Yeast cells have a high capacity of phenotypic switching and may rapidly gain resistance to antimicrobial agents [36-38].

Conclusion

Under the limits of the study, the above obtained results state that Saline was promoting the antibiotic effect whereas local anaesthesia and MTA were inhibiting the antibiotic activity of calcium hydroxide. On the basis of the results and of the experimental conditions used in the present study, antimicrobial activity of Calcium hydroxide against different microorganisms may change with the type of the vehicle. Additive antibacterial and antifungal action can be achieved by combining Calcium hydroxide with saline or distilled water. However, in vitro results should be carefully analysed before their adaptation to clinical use.

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