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ORIGINAL RESEARCH

Antimicrobial Efficacy of Octenidine Hydrochloride, MTAD and Chlorhexidine Gluconate Mixed with Calcium Hydroxide

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ABSTRACT

Objective: The aim of this *in vitro* study was to investigate whether mixing with calcium hydroxide [$Ca(OH)_2$] affects the antimicrobial action of Octenidine hydrochloride (Octenisept), MTAD and chlorhexidine against *Enterococcus faecalis* and *Candida albicans.*

Materials and methods: Freshly grown cultures of *Enterococcus faecalis*, *Candida albicans* and a mixture of both strains were incubated in agar plates containing brain-heart infusion broth (BHIB). Zones of inhibition were measured at 24 and 48 hours. Statistical analysis was performed using Mann-Whitney U test and Kruskal-Wallis one-way analysis of variance (ANOVA, both $p = 0.05$).

Results: Mixing with $Ca(OH)_2$ significantly increased the antibacterial effect of Octenisept ($p < 0.05$), but did not alter its antifungal activity. Only chlorhexidine showed more antibacterial and antifungal efficiency compared to its $Ca(OH)_2$-mixed version (both $p < 0.05$). Mixing with $Ca(OH)_2$ decreased the antibacterial efficacy of MTAD, but increased its antifungal effect (both $p < 0.05$).

Conclusion: These results demonstrate the differential effects of $Ca(OH)_2$ addition on the antimicrobial action of the tested endodontic medicaments *in vitro*. $Ca(OH)_2$ was as effective as its combination with all of the tested medicaments.

Keywords: *C. albicans*, Chlorhexidine, *E. faecalis*, MTAD, Octenisept.

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INTRODUCTION

The primary goal of endodontic therapy is to eliminate microorganisms and their byproducts from the root canal system. Root canal asepsis is attempted by means of mechanical instrumentation and chemical irrigation,[1] but these procedures do not completely eliminate bacteria in the lateral and accessory root canals and apical deltas.[2] Particularly resistant species such as *Enterococcus faecalis* and *Candida albicans* may persist within the root canal system, and sustain the presence of apical periodontitis.[3,4] Thus, placement of an interappointment intracanal medicament has been recommended to further reduce bacteria in the root canal system.[5,6]

Chlorhexidine (CHX) gluconate has been suggested as an endodontic irrigant and intracanal medication by virtue of its wide antimicrobial spectrum, ability to maintain its antibacterial action for a prolonged duration when adhered to anionic substrates, slow release at low concentrations, lower cytotoxicity than sodium hypochlorite, and its efficient clinical performance.[7,8]

Octenidine hydrochloride, (N,N'-(1,10-decanediyldi-1(4H)-pyridinyl-4 ylidine) bis-[1-octanamine] dihydrochloride), a new bipyridine antimicrobial compound, has been developed as a potential antimicrobial/antiplaque agent for use in mouthwash formulations.[9,10] Octenidine hydrochloride appears to be more effective than CHX by means of prolonged antiadhesive activity on bacteria.[11] Octenidine hydrochloride has been suggested as an alternative endodontic irrigant based on its antimicrobial effects and low cytotoxicity.[4]

BioPure MTAD (Tulsa Dentsply, Tulsa, OK), is a mixture of doxycycline, Tween-80, and citric acid. Doxycycline and citric acid exhibit antimicrobial and acid etching properties, rendering MTAD as a promising antimicrobial against *Enterococcus faecalis*, and as a smear layer removal agent.[12] Calcium hydroxide [$Ca(OH)_2$] is one of the most versatile medications in dentistry, especially

for its use as an intracanal disinfectant in endodontic treatment.[13] Several researchers have studied the associations of $Ca(OH)_2$ with different vehicles and antimicrobial substances, such as antibiotics,[14] CHX,[15] camphorated paramonochorophenol[16] and glycerin.[17]

In light of these observations, the purpose of this *in vitro* study was to investigate and compare the antibacterial and antifungal activity of Octenisept, MTAD and CHX, alone or when mixed with $Ca(OH)_2$. The null hypothesis tested was that, addition of $Ca(OH)_2$ would significantly increase the antimicrobial effect of the tested endodontic medicaments.

MATERIALS AND METHODS

The medicaments tested in this study were: 0.1% octenidine hydrochloride (Octenisept, Schülke and Mayr GmBH, Norderstedt, Germany), MTAD (BioPure MTAD, Dentsply, Tulsa Dental, Tulsa, OK) and 2% CHX gluconate (Sigma, St. Louis, MO). Sterile saline solution served as a negative control. In approximation to common clinical practice, combinations of the medicaments with $Ca(OH)_2$ were prepared by mixing each solution with $Ca(OH)_2$ powder (Sultan Healthcare Inc, Englewood, NJ) to form a slurry at a ratio of 1.5:1 (vol/wt).[17] Freshly prepared pastes were used for each test.

Radial Diffusion Test

The test was performed as described by Lee et al[18] with some modifications. Briefly, *Enterococcus faecalis* (ATCC 29212) and *Candida albicans* (ATCC 10231) strains were cultured in brain-heart infusion broth (BHIB) for 24 hours. Agar plates were prepared by adding each organism to sterilized brain-heart infusion agars (BHIA) at 40°C, so that each agar would contain 10^6 CFU/ml of *Enterococcus faecalis* or 10^6 CFU/ml of *Candida albicans*, or a mixture of *Enterococcus faecalis* and *Candida albicans* (each 10^3 CFU/ml). The agars containing the microorganisms were poured into 8 × 8 cm^2 petri dishes to obtain a thickness of 3 mm. Following solidification of the agars, 5 to 6 wells with 3 mm diameter were opened. These wells were filled with the test medicaments or sterile distilled water (as negative control), and incubated at 37°C for 3 hours. Thereafter, the plates were overlaid with sterile BHIA, and incubated at 37°C for 48 hours. Zone diameters were measured at 24th and 48th hours. The zone diameters observed at 48 hours were the same with those observed at 24 hours, but were more distinctive.

Statistical Analysis

Comparison of inhibition zones among the microorganisms were assessed by the Mann-Whitney U test. Statistical differences among the test medicaments and their combination with $Ca(OH)_2$ were assessed by Kruskal-Wallis one-way analysis of variance (ANOVA). When the p-value from the Kruskal-Wallis test was statistically significant ($p < 0.05$), the multiple comparison test was used to determine the test groups that differed from others.

RESULTS

As expected, the negative control (saline) was ineffective against all tested microorganisms. Mixing with $Ca(OH)_2$ significantly increased the antimicrobial efficiency of Octenisept on *Enterococcus faecalis* and *Enterococcus faecalis* + *Candida albicans* (Mann-Whitney U test, $p < 0.05$), but its effect on *Candida albicans* was not statistically significant ($p > 0.05$, Table 1). Mixing with $Ca(OH)_2$ significantly decreased the efficiency of MTAD against *Enterococcus faecalis* and *Enterococcus faecalis* + *Candida albicans* (Mann-Whitney U test, $p < 0.05$, Table 2). When used alone, MTAD did not show any antifungal effect, while its combination with $Ca(OH)_2$ significantly increased the inhibition zones on *Candida albicans* ($p < 0.05$, Table 2). In contrast to other test medicaments, the use of CHX alone yielded a higher antibacterial and antifungal efficiency than its combined version with $Ca(OH)_2$ ($p < 0.05$, Table 3). Finally, the antibacterial and antifungal effect of $Ca(OH)_2$ was similar to that achieved by its mixture with the tested antimicrobial agents ($p > 0.05$, Table 3).

DISCUSSION

Chemomechanical cleaning and shaping of the root canal effectively reduces microbial numbers, but alone cannot accomplish disinfection of the entire root canal system.[19] Residual root canal infection may then sustain persistent or

Table 1: Inhibition zones (in mm) of octenisept and octenisept + $Ca(OH)_2$ on *E. faecalis*, *C. albicans* and *E. faecalis* + *C. albicans*

Microorganism	*OCT*		*OCT + $Ca(OH)_2$*		*p-value (Mann-Whitney U test)*
	X ± SD	*Median (min-max)*	*X ± SD*	*Median (min-max)*	
E. faecalis	8.67 ± 0.52	9 (8-9)	15.67 ± 0.52	16 (15-16)	0.002
C. albicans	14.67 ± 1.86	14 (13-17)	15.67 ± 0.52	16 (15-16)	0.39
E. faecalis + C. albicans	9.67 ± 0.52	10 (9-10)	15.67 ± 1.37	16 (14-17)	0.002

SD: Standard deviation; OCT: Octenisept

Table 2: Inhibition zones (in mm) of MTAD and MTAD + $Ca(OH)_2$ on *E. faecalis*, *C. albicans* and *E. faecalis* + *C. albicans*

Microorganism	*MTAD*		*MTAD+Ca(OH)$_2$*		*p-value (Mann-Whitney U test)*
	X ± SD	*Median (min-max)*	*X ± SD*	*Median (min-max)*	
E. faecalis	30.00 ± 0.89	30 (29-31)	16.33 ± 1.37	16 (15-18)	0.002
C. albicans	0.00 ± 0.00	0 (0-0)	16.33 ± 5.16	16 (16-17)	0.002
E. faecalis + C. albicans	0.00 ± 0.00	0 (0-0)	15.67 ± 5.16	(16 (15-16)	0.002

SD: Standard deviation

Table 3: Inhibition zones (in mm) of chlorhexidine and chlorhexidine + $Ca(OH)_2$ on *E. faecalis*, *C. albicans* and *E. faecalis* + *C. albicans*

Microorganism	*CHX*		*CHX + Ca(OH)$_2$*		*p-value (Mann-Whitney U test)*
	X ± SD	*Median (min-max)*	*X ± SD*	*Median (min-max)*	
E. faecalis	19.00 ± 0.89	19 (18-20)	16.33 ± 0.52	16 (16-17)	0.002
C. albicans	21.67 ± 0.51	22 (21-22)	15.67 ± 0.52	16 (15-16)	0.002
E. faecalis + C. albicans	20.33 ± 1.30	20 (19-22)	17.33 ± 1.37	17 (16-19)	0.009

SD: Standard deviation; CHX: Chlorhexidine

recurrent periapical disease.[5,6,19,20] Within an infected root canal, bacteria grow by forming biofilm colonies in conditions where the availability of carbohydrates is limited.[21] Microorganisms that are members of a biofilm community can be up to 1,000 times more resistant to antimicrobial agents than their planktonic counterparts,[22] which may jeopardize the success of endodontic irrigants and medicaments.[23] These observations justify the present attempt to evaluate the possible contributory effect of [$Ca(OH)_2$] on the antimicrobial efficacy of the tested endodontic medicaments.

Enterococcus faecalis and *Candida albicans* are considered to be the most resistant species in the infected root canals, and are often associated with endodontic treatment failures.[20] Both microorganisms have been used in many studies to test the efficacy of endodontic medicaments and irrigants, owing to their high resistance to antibacterial substances.[24,25] Consequently, this investigation utilized *Enterococcus faecalis* and *Candida albicans* as test microorganisms against which antibacterial action of different endodontic medicaments was investigated using a radial diffusion test.

Results of the present study showed that mixing with $Ca(OH)_2$ significantly increased the antibacterial effect of Octenisept, but did not alter its antifungal activity. Comparisons cannot be made due to the lack of previously published data. However, based on these initial findings, it might seem reasonable to mix Octenisept with $Ca(OH)_2$ in terms of potentiating its antibacterial efficacy.

Although, MTAD appears to be an effective solution against *Enterococcus faecalis*, the combination of MTAD with other medicaments may reduce its antimicrobial effect on this microorganism.[26-29] Our results confirm this finding, since mixing $Ca(OH)_2$ with MTAD resulted in a significant decrease in the inhibition zones of *Enterococcus faecalis* and *Enterococcus faecalis* + *Candida albicans*, compared with those achieved by using MTAD alone. However, our findings also demonstrate that addition of $Ca(OH)_2$ into MTAD may significantly increase the potential of inhibition against *Candida albicans*, as evidenced by the lack of antifungal effect when MTAD was used alone. Ruff et al[30] have also demonstrated that the use of MTAD alone showed no antifungal effect.

The present results showed that 2% CHX was highly effective against *Enterococcus faecalis* and *Candida albicans* (Table 3). In addition to its short-term antimicrobial effects, CHX is capable of adsorbing onto dental tissues and mucous membranes, resulting in a prolonged gradual release at therapeutic levels.[31] Previous studies have demonstrated that the antimicrobial activity of CHX may decrease, when mixed with a number of substances comprising $Ca(OH)_2$, urea and sulphate lauryl sodium.[13,32,33] As expected, both the antimicrobial and antifungal activity of CHX decreased upon combination with $Ca(OH)_2$. Other studies have observed similar antimicrobial activity of both CHX and CHX +$Ca(OH)_2$ against *Enterococcus faecalis*.[6,34] Such differences could be attributed to many factors including differences in study design or the type of microbial strain used. It should also be cautioned that although the widely used agar diffusion method provides a practical approach in determining the antibacterial potential of endodontic irrigants, it only indicates the potential of a medication to eliminate the microorganism, and at a technical standpoint, is directly dependent on the test substance's solubility and ability to diffuse through agar. Thus results of an agar diffusion test may not express

the actual efficacy of a medicament against tested microorganisms.[35]

$Ca(OH)_2$ is one of the most commonly used substances in endodontics, and its antibacterial property stems from its ability to increase the pH of a solution.[6,16] In the present study, the antibacterial and antifungal effect of $Ca(OH)_2$ was similar to that achieved by its mixture with the tested antimicrobial agents. Likewise, Delgado et al[6] showed that $Ca(OH)_2$ and CHX + $Ca(OH)_2$ had similar antimicrobial activity. However, it has also been demonstrated that CHX + $Ca(OH)_2$ exerted more antimicrobial activity against *Enterococcus faecalis* and *Candida albicans* when compared with $Ca(OH)_2$ alone.[17]

Based on the results obtained within the experimental conditions of this study, the null hypothesis should be rejected in part, owing to the differential effects of $Ca(OH)_2$ addition on antimicrobial action of the tested endodontic medicaments. The present results also support the use of Octenisept as an alternative endodontic irrigant, especially in combination with $Ca(OH)_2$. Likewise, mixing with $Ca(OH)_2$ increased the antimicrobial spectrum of MTAD. Finally, $Ca(OH)_2$ was as effective as its combination with all of the tested medicaments.

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