Antimicrobial Efficacy of Octenidine Hydrochloride and Calcium Hydroxide with and Without a Carrier: A Broth Dilution Analysis

Abstract

Background: An efficient antimicrobial agent action is required for a predetermined time period for absolute elimination of root canal microbes. Till date, there is limited or no data on the antimicrobial effect of octenidine as an intracanal medicament with chitosan (CTS) as a carrier against Candida albicans and Enterococcus faecalis. Aim: The aim of this microbiological study was to compare the antimicrobial efficacy of octenidine hydrochloride (OHC) and calcium hydroxide (Ca(OH)₂) as intracanal medicaments, both independently and along with CTS as a carrier molecule against the common resistant endodontic pathogens. Materials and Methods: A total of 160 single-rooted anterior teeth were selected, root canal preparation was done, and teeth were divided into two groups and contaminated with C. albicans and E. faecalis, which were further divided into four test groups each according to intracanal medicaments used. CTS was used as a vehicle for OHC and Ca(OH)₂ and antimicrobial assessment was performed on day 2 and day 7 following broth dilution method. Dentine samples were collected after each time interval, and the number of colony-forming units was determined. Results: All four medicaments used in this study showed antifungal and antibacterial activity that diminished from day 2 to day 7. Group I (OHC alone) and Group IV (Ca(OH)₂ alone) showed significant antimicrobial activity against C. albicans and E. faecalis, respectively, than the other groups. Conclusion: A combination of OHC + CTS and Ca(OH)₂ + CTS produced inferior results than that of the medicaments used alone.

Keywords: Antimicrobial efficacy, broth dilution analysis, calcium hydroxide, chitosan, octenidine hydrochloride

Introduction

The absolute elimination of vital or necrotic pulp tissue and microorganisms from the root canal system, ensuring total canal disinfection, is the unconditional goal of endodontic therapy.[1] However, the routine root canal procedures do not completely eliminate microorganisms owing to accessory root canals, apical deltas, dentinal tubules, grooves, and other root canal irregularities.[2]

The proportional decrease of the aerobic bacteria and the concomitant increase of strict anaerobic bacteria with time, which are more persistent and resistant forms, make its complete elimination an absolute necessity.[3]

Candida albicans due to its collagenolytic activity uses dentin as a nutrient source to promote its intracanal colonization. Enterococcus faecalis is small enough to competently invade and survive within the dentinal tubules and is resistant to low concentrations of sodium hypochlorite (NaOCl) irrigant.[1,4] Due to these features of the microorganisms, the use of intracanal medicament is vital to eliminate any residual bacteria in a root canal, after instrumentation and irrigation.[1]

The most routinely used intracanal medicament, calcium hydroxide (Ca(OH)₂), is believed to possess many of the ideal properties, mainly due to its alkaline pH. It has been demonstrated that C. albicans and E. faecalis are resistant to the antimicrobial effect of Ca(OH)₂.[1] E. faecalis remains alive in dentinal tubules even with Ca(OH)₂ as an intracanal medicament.[5]

However, octenidine hydrochloride (OHC), a new bipyridine antimicrobial compound, a potential antimicrobial/antiplaque agent, appears to be more effective than chlorhexidine by means of its prolonged antiadhesive activity on bacteria.[2] Its bactericidal/fungicidal action is by interfering with cell walls and cell membranes.[6]

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Within the dentinal tubules, these microorganisms may be guarded from the intracanal medicaments by the inhibiting effects of dentin and progressive resistance to intracanal medicaments after direct contact with them. Hence, to ensure complete elimination of root canal microbes, an effective antimicrobial agent action is required for a predetermined time period. Furthermore, to increase the intracanal medicament stability, insolubility, and controlled release, a drug carrier or vehicle such as chitosan (CTS) can be used, which is a natural copolymer of glucosamine and N-acetylglucosamine, present in some crustaceans, insects, and fungi. It has a myriad of biological properties such as hypocholesterolemic, antimicrobial, antifungal, mucoadhesive, wound healing, and sustained-release effect.

Dental literatures have elaborated various properties of newer intracanal medicaments and carrier molecules such as CTS. However, till date, there is limited or no data on the antimicrobial effect of octenidine as an intracanal medicament with CTS as a carrier against C. albicans and E. Faecalis.

Therefore, this study was designed to compare the antimicrobial efficacy of OHC and Ca(OH)$_2$, as intracanal medicaments, both independently and along with CTS as a carrier against C. albicans and E. Faecalis.

Materials and Methods

Sample preparation

A total of 160 intact, noncarious, extracted human permanent single-rooted teeth, with fully formed apices collected, were decoronated at 16 mm length from the apex using a rotary diamond disk. The internal diameter of the root canals was standardized using Gates Glidden (GG) drill No. 3 and the canals were cleaned with 17% ethylenediaminetetraacetic acid for 5 min, followed by 5% NaOCl for 5 min.

Then, the teeth were dipped in distilled water to remove any remaining irrigants and then twice sterilized in an autoclave. For the second sterilization, the teeth were divided into two groups ($n = 80$ each). The first group was immersed in brain–heart infusion (BHI) broth and the second group in Sabouraud Dextrose Agar (SDA) broth.

Contamination of the teeth

24-h colonies of pure culture of C. albicans and E. faecalis were grown on SDA and BHI agar, respectively, and were suspended in 5 ml of their respective broth and then incubated at 37°C for 24 h.

Then, a total of 50 µl of the inocula was transferred to the individual microcentrifuge tubes with 1 ml of the respective broth and teeth and the contamination of the teeth was carried out for 24 h [Figure 1a and b].

Antimicrobial assessment

Then, all the teeth were irrigated with 5 ml of sterile saline to remove the incubation broth. The intracanal medicaments were prepared in four groups and placed within the canals of the respective sample teeth:

- Group 1: Octenidine + saline (OHC)
- Group 2: Octenidine + CTS (OHC + CTS)
- Group 3: Ca(OH)$_2$ + CTS (CH + CTS)
- Group 4: Ca(OH)$_2$ + saline (CH).

All the teeth with the intracanal medicament were then sealed with a temporary restorative material (Cavit) and incubated at 37°C, till the antimicrobial assessment was carried out at day 2 and day 7 with 10 teeth in each group for each time interval [Figure 1c].

The teeth were then washed with sterile saline to remove the medicament. The dentinal debris was then harvested at a depth of 200 µm with GG drill No. 4 and collected in 1 ml of phosphate-buffered saline solution [Figure 1d].

The diluted solutions were then transferred into their respective culture media and incubated at 37°C for 24 h after which the number of colony-forming units (CFUs) was counted at the end of days 2 and 7 and the values were compared for all the four groups [Figure 2a and b].

On completion of the study, the data were analyzed by one-way ANOVA and Tukey’s multiple post hoc tests.

Results

Based on the results obtained from the comparison of the four groups with respect to the CFU of C. albicans, there was a progressive increase in the growth of the microorganisms in all the groups from day 2 to day 7 except for the OHC alone group which showed a statistically significant decrease of 29.44% in the CFU.
counts of *C. albicans* \((P = 0.0430)\). However, the maximum significant growth% of *C. albicans* was seen in the OHC + CTS group \((P = 0.0001)\). Moreover, when compared between the groups, OHC group showed significantly better antimicrobial efficacy against *C. albicans* than all the other three groups \((P = 0.0002)\) [Table 1 and Figure 3].

However, when comparison of the groups was made for *E. faecalis*, all the groups showed a progressive increase in the CFU counts from day 2 to day 7. Although the group with CH alone showed least amount of CFU at day 2 \((51.10 \pm 44.02)\), the antimicrobial efficacy of the molecule reduced significantly in CH + CTS group. However, the addition of CTS to octenidine did not significantly alter the antimicrobial efficacy of octenidine \((P = 0.9848)\). Furthermore, it can be noted that none of the medicament groups were able to completely eradicate both the test microorganisms [Table 2 and Figure 4].

**Discussion**

Favorable outcome of root canal treatment is significantly higher only if infection is eradicated effectively before obturation. Failure to do so will result in a higher risk of treatment failure owing to persistent microorganisms. This importunate infection is usually composed of Gram-positive bacterial species such as *E. faecalis* and fungi such as Candida which are extremely resistant to several medicaments, including Ca(OH)\(_2\)\(^{[8-10]}\). Therefore, *E. faecalis*.

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**Table 1: Comparison of four groups with respect to colony-forming units counts of *Candida albicans* at day 2 and day 7 by one-way ANOVA**

| Groups       | Day 2          | Day 7          | Changes from day 2 to day 7 |
|--------------|----------------|----------------|-----------------------------|
|              | Mean±SD        | SE             | Mean±SD        | SE             | Mean±SD        | SE             |
| OHC          | 311.50±60.89   | 19.25          | 219.80±93.67   | 29.62          | −91.70±123.20  | 38.96          |
| OHC + CTS    | 15.00±17.22    | 5.44           | 275.70±58.05   | 18.36          | 260.70±54.38   | 17.20          |
| CH + CTS     | 99.30±87.61    | 27.70          | 198.10±68.53   | 21.67          | 98.80±87.67    | 27.72          |
| CH           | 40.00±55.55    | 17.57          | 203.50±57.50   | 18.18          | 163.50±57.62   | 18.22          |
| Percentage of change in OHC | −29.44, \(P=0.0430^*\) |                      |                |                | 1738.00, \(P=0.0001^*\) |                      |
| Percentage of change in OHC + CTS | 99.50, \(P=0.0061^*\) |                      |                |                | 408.75, \(P=0.0001^*\) |                      |
| Percentage of change in CH + CTS | −29.44, \(P=0.0430^*\) |                      |                |                | 1738.00, \(P=0.0001^*\) |                      |
| Percentage of change in CH | 40.00±55.55 | 17.57          | 203.50±57.50   | 18.18          | 163.50±57.62   | 18.22          |

**Pairwise comparisons by Tukey’s multiple post hoc procedures**

| Groups       | Day 2          | Day 7          | Changes from day 2 to day 7 |
|--------------|----------------|----------------|-----------------------------|
| OHC versus OHC + CTS | 0.0002*          | 0.3083         | 0.0002*                     |
| OHC versus CH + CTS | 0.0002*          | 0.9027         | 0.0002*                     |
| OHC versus CH | 0.0002*          | 0.9554         | 0.0002*                     |
| OHC + CTS versus CH + CTS | 0.0187          | 0.0867         | 0.0009*                     |
| OHC + CTS versus CH | 0.7944          | 0.1231         | 0.0696                      |
| CH + CTS versus CH | 0.1476          | 0.9983         | 0.3412                      |

\(^*P<0.05, ^*Applied paired t-test. SD: Standard deviation; OHC: Octenidine hydrochloride; CTS: Chitosan; CH: Calcium hydroxide; SE: Standard error\)
Table 2: Comparison of four groups with respect to colony-forming units counts of Enterococcus faecalis at day 2 and day 7 by one-way ANOVA

| Groups       | Mean±SD | SE | Mean±SD | SE | Mean±SD | SE |
|--------------|---------|----|---------|----|---------|----|
| OHC          | 169.60±63.10 | 19.95 | 344.10±61.91 | 19.58 | 174.50±95.91 | 30.33 |
| OHC + CTS    | 160.00±55.50 | 17.55 | 388.90±43.36 | 13.71 | 228.90±76.51 | 24.19 |
| CH + CTS     | 245.00±76.19 | 24.09 | 373.80±85.51 | 27.04 | 128.80±89.68 | 28.36 |
| CH           | 51.10±44.02 | 13.92 | 368.60±67.01 | 21.19 | 317.50±73.50 | 23.24 |

Percentage of change in OHC: 102.89%, \( P=0.0003^* \)
Percentage of change in OHC + CTS: 143.06%, \( P=0.0001^* \)
Percentage of change in CH + CTS: 52.57%, \( P=0.0014^* \)
Percentage of change in CH: 621.33%, \( P=0.0001^* \)

F: 17.2249
P: 0.0001*

Pairwise comparisons by Tukey’s multiple post hoc procedures

| Groups                  | Day 2 | Day 7 | Changes from day 2 to day 7 |
|-------------------------|-------|-------|----------------------------|
| OHC versus OHC + CTS    | 0.9848 | 0.4401 | 0.4828                      |
| OHC versus CH + CTS     | 0.0418* | 0.7484 | 0.6243                      |
| OHC versus CH           | 0.0007* | 0.8409 | 0.0031*                     |
| OHC + CTS versus CH + CTS | 0.0177* | 0.9562 | 0.0551                      |
| OHC + CTS versus CH     | 0.0017* | 0.9018 | 0.1063                      |
| CH + CTS versus CH      | 0.0002* | 0.9981 | 0.0002*                     |

\( *P<0.05, ^* \text{Applied paired } t\text{-test. SD: Standard deviation; OHC: Octenidine hydrochloride; CTS: Chitosan; CH: Calcium hydroxide; SE: Standard error} \)

Figure 4: Comparison of four groups with respect to colony-forming units counts of Enterococcus faecalis at day 2 and day 7

and C. albicans, which are considered the most common and defiant species in the infected root canals, were used as test microorganisms against which the antimicrobial action of different endodontic medicaments was investigated.

The broth dilution test was done with methodology similar to the study performed by Jaheer Shaikh et al. where extracted human root dentin specimens were used to check the antimicrobial efficacy of the four medicament groups against E. faecalis and C. albicans.[2] The inherent factors in the root dentine that protect the bacteria against the antibacterial effect of the medicaments made the presence of actual dentin scrapings per se a vital element in measuring the efficacy of medicaments.[11] Broth dilution test which in this case uses actual dentin samples to mimic clinical scenario rated superior to an agar diffusion test, thus authenticating itself as a superior method in evaluating antimicrobial efficacy.

From the results obtained, it was evident that octenidine and Ca(OH)\(_2\) showed the highest antimicrobial efficacy against C. albicans and E. faecalis, respectively. Furthermore, the addition of CTS to octenidine, though reduced the antimicrobial efficacy of the medicament, showed comparable results and there was no significant difference between the two groups. However, the addition of CTS to Ca(OH)\(_2\) drastically reduced the antimicrobial efficacy of Ca(OH)\(_2\) against E. faecalis and C. albicans (\( P=0.0002 \) and \( P=0.0369 \), respectively).

However, octenidine with CTS group showed greater antimicrobial efficacy against C. albicans than Ca(OH)\(_2\) with CTS group which was statistically significant (\( P = 0.0339 \) and \( P = 0.0187 \)). These findings suggest that the addition of CTS to octenidine did not significantly reduce the antifungal property of octenidine and was able to maintain it, while the addition of CTS to Ca(OH)\(_2\) drastically reduced the antifungal property of Ca(OH)\(_2\). Whereas, against E. faecalis, the reverse was true, though the Groups II and III had comparable yet insignificant differences in their antimicrobial efficacy.

The broth dilution results showed that the addition of CTS to the medicaments was not able to produce any additive or synergistic antimicrobial effect, but instead, its addition reduced the efficacy of the pure compound against the test microorganisms. However, when compared with Ca(OH)\(_2\),
CTS group, the CTS combination with octenidine was able to show better antifungal activity. This was in accordance with the study conducted by Balicka-Ramisz et al., which showed that CTS has good antifungal activity against C. albicans.[12]

However, the results in the present study are in contrast to that from a study conducted by Jaheer sheikh et al. in 2014, where they compared the antimicrobial efficacy of Triple antibiotic paste (TAP) and Ca(OH)₂ with and without CTS as carrier against E. faecalis and C. albicans. Their study results showed that the combination of the medicaments with CTS produced better results compared with the combination of medicaments with saline.[13]

Moreover, several studies have shown variable inhibition of different root canal medicaments including Ca(OH)₂ by the root dentine powder, bovine serum albumin, hydroxylapatite, and dentine matrix (collagen).[11,13] In this study, the broth dilution results showed that none of the medicament groups were able to completely inhibit the growth of microorganisms, and over a period of time from day 2 to day 7, they lost their antimicrobial efficacy except the octenidine group (Group I) that showed a significant 30% reduction in the growth of C. albicans from day 2 to day 7 (P = 0.0430), suggesting that octenidine is a comparatively stronger antifungal agent and that its combination with a carrier molecule such as CTS is not necessary for its prolonged duration of action.

Similar results to the broth dilution test of this study was obtained by Gomes et al. in 2003, when they studied the effect of prolonged incubation with the medicaments on the antibacterial effect of Ca(OH)₂ and 2% chlorhexidine, alone and in combination, and found that all the medicament groups started to lose their antibacterial effect from day 2 to day 15.[14]

However, further studies are required to test these medicament combinations with CTS as a drug carrier in in vivo studies. The depth of penetration of the medicament combinations into dentinal tubules, their duration of action, concentration of the medicaments, and volume of the medicament to be given are to be investigated and compared.

Conclusion
The broth dilution analysis showed that the combination of OHC + CTS and Ca(OH)₂+ CTS produced inferior results than that of the medicaments used alone suggesting that the addition of chitosan to the medicaments was not able to produce any additive or synergistic antimicrobial effect, but instead its addition reduced the efficacy of the pure compound against the test microorganisms.

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Conflicts of interest
There are no conflicts of interest.

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