Original Article

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Dentistry Section

Alexidine: A Safer and an Effective Root Canal Irrigant than Chlorhexidine

L.R SURENDER[1], A. SHIKHA[2], A SWATHI[3], C MANASWINI[4], AISHA HABEEB[5], S SHASHI PRABHA[6]

ABSTRACT

Introduction: Chlorhexidine (CHX) is generally used as the final irrigating solution in root canal therapy. Recent studies have reported that, toxic precipitates containing Parachloroaniline (PCA) are formed when CHX reacts with Sodium Hypochlorite (NaOCl). Whereas, Alexidine (ALX), a bisbiguanide disinfectant similar to CHX, has proven to form no precipitates with NaOCl.

Aim: To compare antimicrobial activity of different concentrations of ALX with CHX individually and when combined with NaOCl against *E. faecalis* strains.

Materials and Methods: Different concentrations of ALX and CHX (0.5%, 1%, and 2%) were tested individually and when mixed with 2.5% NaOCl (1:1 ratio) using disc diffusion method against *E. faecalis*. After 24 hours incubation at 37°C, zones of inhibition were measured for each solution. The results obtained were statistically analysed using one way ANOVA and Scheffe's post-hoc tests. The p-value <0.001 was considered as highly significant.

Results: Regardless of the concentrations, ALX obtained the best results in comparison to CHX. There was no statistically significant difference between ALX + NaOCl and CHX + NaOCl mixtures.

Conclusion: The present study showed that, the antimicrobial property of ALX against *E. faecalis* was found to be superior to CHX at same concentrations.

Keywords: Antimicrobial property, Disc diffusion method, Parachloroaniline, Sodium hypochlorite

INTRODUCTION

Elimination of microbial contamination from the root canal is essential for a successful outcome in endodontic therapy. Several studies have recommended the use of antimicrobial irrigants in order to ensure complete disinfection of the root canal [1,2].

Among the various irrigating solutions, NaOCl is most commonly used due to its excellent organic tissue dissolving property [3] and its effectiveness as an antimicrobial agent [4]. The antimicrobial activity of NaOCl is due to the irreversible inactivation of bacterial essential enzymatic sites. NaOCl results in dissolution of organic tissue through saponification. It destroys fatty acids and lipids forming soap and glycerol [5].

CHX, a bisbiguanide with antimicrobial activity against both Gram-positive and Gram-negative bacteria, is generally used as the final irrigating solution in endodontic therapy. It is an effective antifungal agent, especially against *C. albicans*, and has the unique property of antimicrobial substantivity owing to its cationic structure [6]. It interacts with the anionic compounds located on the surface of the bacteria i.e., phosphate groups from Lipoteichoic Acid (LTA) in the Gram-positive bacteria and Lipopolysaccharide (LPS) in the Gram-negative bacteria, and alters the integrity of the cell membranes [7].

As both NaOCl and CHX are frequently used as root canal irrigants, possible chemical interactions between the two solutions may be expected in a clinical scenario. Colour change and the formation of precipitates containing PCA have been reported when NaOCl and CHX were used to irrigate the canal [8]. PCA is an aromatic amine and is known to be toxic in nature; short term exposure to these chemical results in cyanosis, a manifestation of methemoglobin formation [9].

Hence, there exists the need for an irrigating solution that possesses antimicrobial and substantivity properties similar or superior to those of CHX. In addition, the solution must not have any potential interactions with NaOCl. In fact, a synergistic action between the solution and NaOCl would be advantageous.

ALX, another bisbiguanide that chemically differs from CHX, has antimicrobial activities towards both Gram-positive and Gram-negative bacteria, and provides faster bactericidal activity as well as bacterial permeabilization when compared with CHX [10]. It helps to inhibit the immune response of major bacterial virulence factors including LPS and LTA [11]. Interactions between ALX and NaOCl do not produce PCA or other precipitates [12]. Furthermore, it has been shown that the antimicrobial substantivity of ALX is longer than that of CHX [13].

Enterococcus faecalis (E. faecalis), a Gram-positive, facultative anaerobic bacterium, is more likely to be found in persistent infections than in primary infections [14]. The inherent ability of *E. faecalis* to adhere to and invade the dentinal tubules [15], and form communities in an organized biofilm may contribute to both bacterial resistance as well as persistence of infection after root canal treatment [16].

In the present study, the antimicrobial activities of different concentrations of ALX and CHX were tested individually and in combination with NaOCl to evaluate the presence; or absence; of synergistic or antagonist actions between the solutions.

As the interactions between ALX and NaOCl do not produce PCA or other precipitates [12], and have a proven antimicrobial property, it was hypothesized that the combination of ALX with NaOCl would produce a synergistic antimicrobial action.

MATERIALS AND METHODS

This in vitro study was conducted for a period of 24 hours in November 2014 to check the zone of inhibition in Department of Microbiology, SVS Institute of Medical Sciences, Mahabubnagar, Telangana, India.

Bacterial strain:

Pure strains of *E. faecalis* (ATCC® 29212™) obtained from the Department of Microbiology, SVS Institute of Medical Sciences were subcultured on blood agar plate and incubated at 37°C for 24 hours.

Groups	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for mean		Minimum	Maximum
					Lower bound	Upper bound		
2% ALX	8	13.1488	0.12699	0.04490	13.0426	13.2549	12.98	13.34
2% CHX	8	12.6387	0.27430	0.09698	12.4094	12.8681	12.29	13.02
2% ALX+NaOCl	8	8.5513	0.28578	0.10104	8.3123	8.7902	8.18	9.00
2% CHX + NaOCl	8	6.3550	0.33179	0.11731	6.0776	6.6324	5.63	6.63
1% ALX	8	12.4950	0.19383	0.06853	12.3330	12.6570	12.32	12.90
1% CHX	8	11.3575	0.14830	0.05243	11.2335	11.4815	11.15	11.63
1% ALX + NaOCl	8	8.5050	0.06740	0.02383	8.4487	8.5613	8.41	8.62
1% CHX + NaOCl	8	8.0825	0.08515	0.03010	8.0113	8.1537	8.00	8.23
0.5% ALX	8	11.3788	0.12766	0.04514	11.2720	11.4855	11.19	11.64
0.5% CHX	8	10.6313	0.36053	0.12747	10.3298	10.9327	10.17	11.08
0.5% ALX + NaOCl	8	9.2625	0.22102	0.07814	9.0777	9.4473	9.04	9.71
0.5% CHX + NaOCl	8	8.1950	0.22149	0.07831	8.0098	8.3802	8.02	8.71
Total	96	10.0501	2.10937	0.21529	9.6227	10.4775	5.63	13.34

[Table/Fig-1]: Descriptive analysis for zone of Inhibition for the 2%, 1% and 0.5% test groups.

Variables	Sum of squares	df	Mean Square	F value	p-value
Between Groups	418.507	11	38.046	762.613	<0.001 Highly significant
Within Groups	4.191	84	0.050		
Total	422.698	95			

[Table/Fig-2]: ANOVA test for comparison in between and within the groups.

Comparison	Mean Difference	p-value
2% ALX vs 2% CHX	0.510	0.051
2% ALX+NaOCl vs 2% CHX +NaOCl	2.196	<0.001**
1% ALX vs 1% CHX	1.138	<0.001**
1% ALX + NaOCl vs 1% CHX + NaOCl	0.423	0.238
0.5% ALX vs 0.5% CHX	0.748	<0.001**
0.5% ALX + NaOCl vs 0.5% CHX + NaOCl	1.068	<0.001**

[Table/Fig-3]: Multiple comparison between different concentrations using Scheffe's post-hoc test. **, p<0.001 is highly significant.

A pure, single *E. faecalis* colony was isolated from the same cultured plate and inoculated in Brain Heart Infusion (BHI) broth to prepare a bacterial suspension that can be swabbed on the blood agar plates. The broth culture was incubated until it achieved turbidity of the 0.5 McFarland standard with the suspension containing approximately 1 x10^{11} bacteria. These agar plates were inoculated with prepared *E. faecalis* suspension by evenly swabbing the plates to obtain a lawn culture.

The study had following groups which are as follows with eight blood agar plates for each concentration:

Group 1: ALX

Group 2: CHX

Group 3: ALX+ NaOCl

Group 4: CHX + NaOCl

Sterile distilled water was used as a negative control.

ALX was mixed with NaOCl to check if there was any synergistic action i.e., a larger zone of inhibition than ALX/CHX individually.

Preparation of solutions

Commercially available 2% CHX (Amrit Chem. & Min.Ag.Mohali) was used which was further serially diluted with distilled water to obtain 1% and 0.5% solutions. Commercially available NaOCl (Vishal dentocare pvt., ltd., Ahmedabad, Gujarat, India) was used whereas ALX was prepared by dissolving 2 gram of alexidine dihydrochloride powder (Toronto Research Chemicals Inc, Canada) in 100 ml of dimethyl sulfoxide (DMSO, Bhavani chemicals, Hyderabad) which is an organic solvent to obtain a concentration of 2%, which was further serially diluted with distilled water in a 1:2 ratio to obtain 1% and 0.5%. ALX and CHX solutions were mixed with NaOCl in 1:1 ratio.

Eight cultured plates were assigned to each of the freshly prepared 12 solutions which are as follows

- 0.5, 1, 2% ALX
- 0.5, 1, 2% CHX
- 0.5, 1, 2% ALX +2.5% NaOCl
- 0.5, 1, 2% CHX +2.5% NaOCl
- distilled water (Negative control)

The filter paper disks were standardized to 3 mm in diameter and were applied with the help of sterile forceps on the agar plates and pressed gently to ensure even contact with the medium. Around 100 µl (0.1 ml) of each solution was placed on the paper disc with the help of micropipettes. The plates were kept for incubation at 37°C for 24 hours. Zones of inhibition were measured at the end of 24 hours for each solution.

The diameter of the zone of inhibition was measured in millimetres with the help of a digital vernier calliper {Precision Scientific Instruments Corporation, Delhi, India}. Measurement accuracy was taken as +/- 0.03 mm and the values recorded. The zone edge was taken at the point of abrupt disappearance of growth, which corresponds to the point of complete inhibition of growth. The cut off taken for the determination of zone of inhibition was 6 mm (since this is the least value which was obtained with the combination of CHX and NaOCl).

STATISTICAL ANALYSIS

SPSS Statistics version 17.0. (SPSS Inc.Chicago) was used for statistical analysis. One-way analysis of variance (ANOVA) and Scheffe's post-hoc test were performed to compare antimicrobial efficacy between the groups. The p<0.001 was considered as statistically highly significant.

RESULTS

Among the 2% test solutions, the largest zone of inhibition was observed for ALX with a mean value of 13.148 mm followed by CHX with a mean value of 12.638 mm [Table/Fig-1]. The mean difference observed between the two agents was 0.51. The smallest zone of inhibition was observed in the CHX + NaOCl mixture with a mean value of 6.355 mm. The mean value for the ALX+NaOCl solution was 8.551 mm with a mean difference of 2.196 between the two combined test solutions [Table/Fig-1].

As seen in [Table/Fig-1], the largest zone of inhibition among the 1% test solutions was observed with ALX (mean=12.495 mm) followed by CHX with a mean value of 11.357 mm. The mean difference between the two individual test solutions was 1.138. The zones of inhibition for the ALX + NaOCl and CHX + NaOCl mixtures were almost equal with mean values of 8.505 mm and 8.0825 mm respectively. The mean difference between the two groups was 0.423 [Table/Fig-1].

In the case of the 0.5% test solutions, the mean values of the zones of inhibition for ALX and CHX were 11.378 mm and 10.631 mm, respectively [Table/Fig-1]. The mean difference observed between the two individual solutions was 0.748 mm. Mean values of the zones of inhibition for ALX + NaOCl and CHX + NaOCl were 9.262 mm and 8.195 mm, respectively with a mean difference of 1.068 mm [Table/Fig-1].

[Tables/Fig-2,3] illustrate the results of the One-way ANOVA and Scheffe's post-hoc tests, respectively.

As seen in [Table/Fig-3], the Scheffe's post-hoc test revealed statistically significant differences between the following groups:

- 2% ALX + N_aOCl and 2% CHX + N_aOCl;
- 0.5% ALX + N_aOCl and 0.5% CHX + N_aOCl;
- 1% ALX and 1% CHX; and
- 0.5% ALX and 0.5% CHX.

The combination of ALX and NaOCl did not show any synergistic effects on the antimicrobial properties. Furthermore, the zone of inhibition was smaller in the combined solutions (ALX + NaOCl and CHX + NaOCl) when compared with those of the individual test solutions.

DISCUSSION

The present study aimed at evaluating the antimicrobial efficacy of ALX and CHX against *E. faecalis*. The *E. faecalis* ATCC® 29212™ strain was chosen in this study because these microorganisms are commonly found in retreatment cases. They are capable of surviving in environments where the availability of nutrients is scarce, and commensality with other bacteria is minimal [17].

Both ALX and CHX have the same bisbiguanide backbone; however, the p-chloro-aniline end groups of CHX are replaced by ethyl-hexyl substituents in ALX [5]. Moreover, both ALX and CHX are cationic molecules that disrupt the integrity of the bacterial cytoplasmic membrane, which results in leakage of the intracellular contents. ALX has a greater affinity for the major bacterial virulence factors than CHX. As ALX contains two hydrophobic ethylhexyl groups in its structure whereas CHX has p chlorophenyl end groups, hydrophobic interaction between ALX and the hydrophobic acyl chains in lipid A may be stronger due to the more favourable packing of alkyl chains of ALX than that of the p-chlorophenyl group of CHX [11].

It has been reported that on combining NaOCl and CHX as root canal irrigants colour change and the formation of precipitates occurs [18,19]. Change in colour might have some clinical relevance because of staining, whereas the sealing of root fillings on to the root canal wall may be interfered by the formed precipitates [18].

In previous studies, it has been observed that the chemical interactions between ALX and NaOCl do not produce any precipitate [12]. However, to the best of our knowledge, there are no reports on the antimicrobial efficacy of these two agents when used together. Hence, this study focused on assessing the antimicrobial efficacy (presence of synergistic or antagonistic activities) of ALX or CHX alone, and in combination with NaOCl by using the agar disc diffusion method.

Initially, when the test solutions were prepared to check for the zones of inhibition, a peach brown discoloration was observed with the NaOCl and CHX mixture, whereas, the mixture of ALX and NaOCl resulted in a light yellow colour solution. These findings are in agreement with those reported in previous studies [8,12].

The microbiological agar disc diffusion test results in the present study showed a decrease in inhibition zone formation when higher concentrations of CHX were combined with NaOCl.

This may be attributed to the large quantities of precipitates formed, which may restrict diffusion through the agar plates, thereby creating smaller inhibition zones.

Even though studies have shown that precipitate PCA is not formed on combining ALX and NaOCl [9], no increase in zone of inhibition was observed when these two test solutions were combined. Thus, suggesting that there was no synergistic effect following the combination of ALX and NaOCl. However, the zone of inhibition for the combined solution of these two agents was larger than that obtained with the CHX and NaOCl mixture (for all three concentrations used in the study).

The findings of this study demonstrate that the antimicrobial property of ALX was superior to that of CHX as the zone of inhibition was greater with the various concentrations of ALX when compared with CHX. This is in agreement with previous study where it was demonstrated that ALX has greater antimicrobial activity than CHX using *Enterococcus faecalis*-infected dentin blocks [20]. In another study, it was shown that ALX and cetrimide has a superior antimicrobial property than CHX against *Streptococcus mutans* biofilm [21]. It has also been proved that ALX has superior antimicrobial substantivity when compared to CHX on dentin blocks [13].

LIMITATION

As the methodology opted was agar diffusion test, further studies should be done to evaluate and substantiate the results by using equipments like scanning electron microscopy and confocal laser microscopy using dentin bocks. Also, effects on antimicrobial substantivity should be measured which is not measured in current study.

CONCLUSION

Within the limitation of the study, it can be concluded that the antimicrobial property of ALX individually is more than CHX. Further, other properties of ALX should also be explored on various other microorganisms and if proved to be better than CHX, it can be used as an alternative to CHX.

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PARTICULARS OF CONTRIBUTORS:
1. Professor and Head, Department of Conservative Dentistry and Endodontics, SVS Institute of Dental Sciences, Mahabub Nagar, Telangana, India
2. Postgraduate Student, Department of Conservative Dentistry and Endodontics, SVS Institute of Dental Sciences, Mahabub Nagar, Telangana, India.
3. Senior lecturer, Department of Conservative Dentistry and Endodontics, SVS Institute of Dental Sciences, Mahabub Nagar, Telangana, India.
4. Postgraduate Student, Department of Conservative Dentistry and Endodontics, SVS Institute of Dental Sciences, Mahabub Nagar, Telangana, India.
5. Postgraduate Student, Department of Conservative Dentistry and Endodontics, SVS Institute of Dental Sciences, Mahabub Nagar, Telangana, India.
6. Postgraduate Student, Department of Conservative Dentistry and Endodontics, SVS Institute of Dental Sciences, Mahabub Nagar, Telangana, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. L.R Surender,
Professor and Head, Department of Conservative Dentistry and Endodontics,
SVS Institute of Dental Sciences, Appannapally Village Yedira Post, Mahabub Nagar-509002, Telangana, India.
E-mail: aishahabeeb9@gmail.com

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