Vol 9, Special Issue 2, 2017

INTERNATIONAL JOURNAL OF APPLIED PHARMACEUTICS

ISSN - 0975-7058

Research Article

EFFECTS OF A 980-NM DIODE LASER'S ACTIVATION OF 2.5% NAOCL AND 2% CHLORHEXIDINE ANTIFUNGAL IRRIGATION SOLUTIONS ON *CANDIDA ALBICANS* BIOFILMS

CHITRA ISELINNI, RATNA MEIDYAWATI*, NILAKUSUMA DJAUHARIE

Department of Conservative Dentistry, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia. Email: meidyawati58@gmail.com

Received: 16 September 2017, Received and Accepted: 05 October 2017

ABSTRACT

Objective: *Candida albicans* is the most frequently found fungi in persistent root canal infections; it can form a biofilm and penetrate into dentinal tubules. Endodontic irrigants, such as 2.5% sodium hypochlorite (NaOCl) and 2% chlorhexidine (CHX), have antifungal properties, but limited penetration into dentinal tubules, which somewhat protects the *C. albicans* fungi. The use of a diode laser is an innovative approach in root canal treatments because it is able to penetrate deeper into the dentinal tubules. This study examined the effect of a 980-nm diode laser on the antifungal properties of 2.5% NaOCl and 2% CHX on *C. albicans* biofilms.

Methods: The number of *C. albicans* colonies in the biofilms was recorded after irrigation using 2.5% NaOCl and 2% CHX. Then, the biofilms were radiated using a 980-nm diode laser.

Results: Showed statistically significant differences between the use of the irrigants only and the use of the irrigants plus the diode laser treatment in the reduction of the *C. albicans* colonies in the biofilm.

Conclusion: Diode laser is able to activate the antifungal properties of the 2.5% NaOCl and 2% CHX endodontic irrigants.

Keywords: *Candida albicans*, Diode laser, Endodontic irrigants.

 DOI: http://dx.doi.org/10.22159/ijap.2017.v9s2.04

INTRODUCTION

Endodontic is a branch of dentistry that focuses on the prevention, diagnosis, and treatment of dental pulp pathosis [1]. Pulp and periapical tissue infections occur when microorganisms invade the hard tissues in teeth and progress into the root canal [2]. The main objective of a root canal treatment is to eliminate microorganisms, toxins, and pathological debris from the root canal, preventing further infection to the surrounding bone [3,4]. At least 300 different microorganisms species found in root canal infections, including fungal organisms, are found in pure culture or with bacteria in primary, secondary, and persistent root canal infections. *Candida albicans* is the most common fungi in the oral cavity and the root canal [5]. The incidence of *C. albicans* in a healthy oral cavity is approximately 30–45%, but its incidence can be as high as 95% in immune compromised individuals [6]. The prevalence of *C. albicans* in root canal infections is approximately 1–17% [7]. *C. albicans* is more frequently found in teeth with failed root canal treatments [8].

C. albicans dentin colonization plays an important role in persistent root canal infection, and the invasion of the *C. albicans* into the dentinal tubules can protect it from intracanal procedures. *C. albicans* is a dimorphic fungus that can alter its morphology in response to a host's attempts to fend off infection. *C. albicans* has hyphae morphology with a diameter around 1.9–2.6 μm, which suggests that it has the ability to penetrate dentinal tubules [8].

Endodontic treatment procedures rely on mechanical instrumentation, irrigants, and medicaments to disinfect the root canal [5]. Irrigants help remove microorganisms that are untouched by mechanical instrumentation [9]. The most commonly used endodontic irrigant is sodium hypochlorite (NaOCl), which works by turning the fatty acid in microbes' cell membranes into fatty salt and glycerol, damaging the cell membranes [10]. Another endodontic irrigant is CHX. Cationic molecules from CHX bind to microbes' cell membranes, which are negatively charged, causing cell lysis. These irrigants must be in direct contact with the microorganisms to be effective, but they have limited penetration into dentinal tubules [11].

Root canal disinfection is one of the basic principles to retain tooth treated root canals in the long term. Root canal disinfection is the primary challenge in endodontic procedures, and although the use of irrigants may decrease the number of microbes in infected root canals, they do not achieve the total disinfection of entire root canal systems. Lasers are one method used to overcome this challenge because lasers can access tubular systems that irrigants cannot [5].

The antimicrobial effect of lasers depends on the dosage of the heat delivered to the target [12]. Various types of lasers, such as diode lasers, are used in dentistry. The use of diode lasers in endodontics is an innovative approach to root canal disinfection because these lasers have the ability to penetrate deeper into dentinal tubules [3]. Diode lasers have become the method of choice due to their advantages including their ease of use and relatively small size compared to other types of lasers [13].

The antimicrobial effect of lasers has been shown in previous studies. According to some studies, laser alone is not more effective than irrigants [12]. Baz *et al.* conducted an *in vitro* study on the disinfection of 60 single root canals and found that disinfection by NaOCl irrigants is significantly better than disinfection using diode lasers alone. However, disinfection with a combination of irrigants and diode lasers resulted in the greatest bactericidal effect. Therefore, the diode laser is considered as an adjunct to enhance the bactericidal effect of endodontic irrigants [10].

Kaiwar *et al.* found similar results when they studied the use of diode lasers combined with irrigants. This combination resulted in the highest root canal disinfection rate when compared with disinfection by irrigants or lasers alone. Differences in the disinfection rates were statistically significant [13].

Gerek *et al*. conducted an *ex vivo* study on 176 single-rooted teeth contaminated by bacteria and *C. albicans.* These teeth were treated using endodontic irrigants and an 810-nm diode laser. The method did not achieve complete sterilization of the root canal system, but it achieved a statistically significant decrease in the bacterial and *C. albicans* counts [14].

Many studies on the effect of diode lasers in root canal bacterial disinfection treatments have been conducted, but studies on the effect of 980-nm diode lasers on fungal organisms are rare. The objective of this study is to evaluate a 980-nm diode laser's enhancement of the antifungal properties of 2.5% NaOCl and 2% CHX against a *C. albicans* biofilm.

METHODS

This is a laboratory experimental study. *C. albicans* strain ATCC 10231 — provided by the Oral Biology Laboratorium in the Faculty of Dentistry, University of Indonesia — was the sample used in this study. The *C. albicans* biofilms were created on a well plate. After the biofilms were created, they were subjected to 2.5% NaOCl or 2% CHX solutions followed by 980-nm diode laser treatments. A swab of biofilm that has been treated was taken and put inside an Eppendorf tube filled with phosphate-buffered saline (PBS) solution and diluted to −6. Each sample was cultured in a Sabouraud dextrose agar medium (SDA). The culture was incubated for 24 h at 37°C.

An assessment of the diode lasers' ability to enhance the antifungal properties of 2.5% NaOCl and 2% CHX against the *C. albicans* biofilms was completed by visually counting the colony-forming units (CFU) of *C. albicans*. The living and colonized *C. albicans* on the agar were manually counter after it was exposed to the irrigants and the 980-nm diode laser. If more colonies were formed, the CFU/ml score increased, and the antifungal effect of the irrigant and diode laser was lower on the testing materials.

Statistical analysis was conducted using SPSS 22.0 (IBM, United States). Normality tests were conducted using the Sapiro-Wilk test, which found that the data had a normal distribution (p>0.05). Homogeneity test discovered that the data were homogenous (p≥0.05). Therefore, data qualify for the parametric test (one-way ANOVA), which was calculated at p<0.05 (p=0.000). Finally, a *post hoc* Bonferroni test was conducted.

RESULTS

The mean values of the *C. albicans* count from the five experimental groups can be seen in Table 1. The 2.5% NaOCl only and 2% CHX only groups showed lower *C. albicans* counts than the biofilm *C. albicans* group. Therefore, both 2.5% NaOCl and the 2% CHX have antifungal properties. The 2.5% NaOCl plus diode laser group had the lowest *C. albicans* count (5.67 CFU/mL), which suggests that the use of 2.5% NaOCl with subsequent diode laser radiation has stronger antifungal effects than the use of 2.5% NaOCl alone (154.00 CFU/mL).

The *post hoc* Bonferroni test results (Table 2) found a statistically significant difference in *C. albicans* count between the biofilm *C. albicans* group, 2.5% NaOCl group, and the 2% CHX group (p=0.001). No significant difference between the 2.5% NaOCl group and the 2% CHX group was found (p=1.000), which suggests that both the 2.5% NaOCl and 2% CHX have similar antifungal effects on the *C. albicans* biofilm.

A statistically significant difference in *C. albicans* count was found between the 2.5% NaOCl only group and 2.5% NaOCl plus diode laser group (p=0.001). Similar results were found between the 2% CHX only group and 2% CHX plus diode laser group (p=0.001). This suggests that the diode laser enhances the antifungal effects of both the 2.5% NaOCl and the 2% CHX.

Table 1: The mean values of the *C. albicans* count before and after the application of the 2.5% NaOCl and the 2% CHX with and without diode laser radiation (CFU/ml)

Experimental group	n	Mean±SD	95% CI	
			Lowest value	Highest value
C. albicans biofilm	3	228.00±9.00	219	237
NaOCl 2.5%	3	154.00±4.00	150	158
CHX 2%	3	151.67±22.50	129	174
NaOCl 2.5%+diode laser	3	5.67±3.22	2	8
CHX 2%+diode laser	3	6.00±1.00	5	7

C. albicans: *Candida albicans*, CHX: Chlorhexidine, CFU: Colony-forming units, SD: Standard deviation, CI: Confidence interval

Table 2: The p value of the *C. albicans* count before and after the application of the 2.5% NaOCl and 2% CHX with and without the application of the diode laser at 980-nm of radiation

Experimental group	p
C. albicans biofilm versus NaOCl 2.5%	0.001
C. albicans biofilm versus CHX 2%	0.001
C. albicans biofilm versus NaOCl 2.5%+diode laser	0.001
C. albicans biofilm versus CHX 2%+diode laser	0.001
NaOCl 2.5% versus CHX 2%	1.000
NaOCl 2.5% versus NaOCl 2.5%+diode laser	0.001
NaOCl 2.5% versus CHX 2%+diode laser	0.001
CHX 2% versus NaOCl 2.5%+diode laser	0.001
CHX 2% versus CHX 2%+diode laser	0.001
NaOCl 2.5%+diode laser versus CHX 2%+diode laser	1.000

Post hoc Bonferroni statistic test with p<0.05. *C. albicans*: *Candida albicans*, CHX: Chlorhexidine

DISCUSSION

The CFU counts in the 2.5% NaOCl and 2% CHX groups were lower than the control group, which suggests that both 2.5% NaOCl and 2% CHX have antifungal properties with regard to *C. albicans*. Siquiraand Sen*.* also found that these irrigants had antifungal effects on *C. albicans* [8]. The 2.5% NaOCl group and 2% CHX group's CFU counts had no statistically significant difference, which suggests that 2% CHXs antifungal properties are comparable to 2.5% NaOCl's antifungal properties against the *C. albicans* biofilm. Sena *et al*. found similar result that both NaOCl and CHX have antifungal properties but do not differ significantly, supporting the findings of this study [15].

Gopikrishna *et al.* found that a 60°C increase in the 1.25% NaOCl temperature can significantly reduce the viscosity of NaOCl and may influence the movement of NaOCl solution on root canal in clinical applications [16]. Gulsahi also found that a 37°C increase in the 2.5% NaOCl temperature can increase the effectiveness of NaOCl against *C. albicans* [17].

The use of a diode laser is an innovative approach to root canal disinfection. Diode lasers are able to create heat increasing the temperature of the irrigants [18]. An increase in the temperature of low concentrations of NaOCl solutions can enhance the solution's antimicrobial properties [16,17,19]. Table 1 summarizes a reduction in the CFU in the 2.5% NaOCl plus diode laser radiation group when compared to the 2.5% NaOCl, only group. Table 2 summarizes the statistically significant difference between the two groups.

Table 1 summarizes a reduction in the CFU in the 2% CHX plus diode laser radiation group when compared with the 2% CHX only group. Table 2 summarizes the statistically significant difference between the two groups. Therefore, the use of a diode laser enhances the effect of 2% CHX on *C. albicans*.

No studies or literature have been found regarding the use of a 980-nm diode laser combined with 2% CHX against *C. albicans* the effect of changing the 2% CHX temperature in root canal disinfection. Hmud *et al.* found that a 980-nm diode laser can induce cavitation on a water-based medium by the formation and implosion of water vapor. Bubble pressure from cavitation can increase the breakdown of the irrigation solution, which enhances disinfection. In the root canal, the bubble pressure can potentially destroy microorganisms' biofilms, rupture cell membranes, and to clear the smear layer and debris [20]. The 2% CHX solution is thought to create cavitation after subsequent diode laser radiation, supporting the findings of this study, which showed an increase in the antifungal properties of 2% CHX after diode laser radiation.

An explanation of the effect of a 980-nm diode laser on the *C. albicans* biofilm has not been discussed in previous literature. However, many studies researched the effect of diode lasers on bacteria are assumed to be applicable to *C. albicans*. Bago *et al.* and Baz *et al*. studied *E. faecalis* and concluded that diode lasers have a better effect if they are used in conjunction with endodontic irrigants [10,12]. Similar results were found in this study. The CFU of *C. albicans* was significantly lower in the irrigants plus diode laser groups than the irrigants only groups. Therefore, we concluded that the diode laser activates the antifungal properties of the 2.5% NaOCl and the 2% CHX against *C. albicans*.

CONCLUSION

This study found that the 2.5% NaOCl and 2% CHX endodontic irrigants have antifungal properties. The use of a diode laser in addition to the irrigants can activate the antifungal properties of the 2.5% NaOCl and 2% CHX.

ACKNOWLEDGMENT

The publication of this manuscript is supported by Universitas Indonesia.

REFERENCES

1. Garg N, Garg A, editors. Introduction and scope of endodontics. In: Textbook of Endodontics. 3rd ed. New Delhi: Jaypee Brothers Medical Publishers; 2014.
2. Mathew J, Emil J, Paulaian B, John B, Raja J, Mathew J. Viability and antibacterial efficacy of four root canal disinfection techniques evaluated using confocal laser scanning microscopy. J Conserv Dent 2014;17:444-8.
3. Jonathan R, Mathew J, Suganthan P, Samuel A, John B. Comparative evaluation of the antibacterial efficacy of four different disinfection techniques in minimally instrumented experimentally infected root canals: An *in vitro* study. Int J Laser Dent 2013;3:49-54.
4. Shenoy A, Mandava P, Bolla N, Raj S, Kurien J, Prathap MS. Antibacterial efficacy of sodium hypochlorite with a novel sonic agitation device. Indian J Dent Res 2013;24:537-41.
5. Onay EO, Alikaya C, Seker E. Evaluation of antifungal efficacy of erbium, chromium: Yttrium-scandium-gallium-garnet laser against *Candida albicans*. Photomed Laser Surg 2010;28 Suppl 1:S73-8.
6. Mohammadi Z, Asgary S. Antifungal activity of endodontic irrigants. Iran Endod J 2015;10:144-7.
7. Shenoy A, Nayak S, Bella N. An *in vitro* comparison of the antimicrobial effects of sodium dichloroisocyanurate, CHX and sodium hypochlorite against *Enterococcus faecalis* and *Candida albicans*. Guident 2013;6:69-74.
8. Siqueira JF Jr, Sen BH. Fungi in endodontic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:632-41.
9. Garg N, Garg A. Irrigation and intracanal medicaments. In: Garg N, Garg A, editors. Textbook of Endodontics. Malaysia: Unipress Publishing; 2008.
10. Baz P, Biedma B, Pinon M, Mundina B, Bahillo J, Prado R, *et al.* Combined sodium hypochlorite and 940 nm diode laser treatment against mature *e.Faecalis biofilms in vitro*. J Lasers Med Sci 2012;3:116-21.
11. Mehrvarzfar P, Saghiri MA, Asatourian A, Fekrazad R, Karamifar K, Eslami G, *et al.* Additive effect of a diode laser on the antibacterial activity of 2.5% NaOCl, 2% CHX and MTAD against *Enterococcus faecalis* contaminating root canals: An *in vitro* study. J Oral Sci 2011;53:355-60.
12. Bago I, Plecko V, Gabric Panduric D, Schauperl Z, Baraba A, Anic I. Antimicrobial efficacy of a high-power diode laser, photo-activated disinfection, conventional and sonic activated irrigation during root canal treatment. Int Endod J 2013;46:339-47.
13. Kaiwar A, Usha HL, Meena N, Ashwini P, Murthy CS. The efficiency of root canal disinfection using a diode laser: *In vitro* study. Indian J Dent Res 2013;24:14-8.
14. Gerek M, Asci S, Yaylali D. *Ex vivo* evaluation of antibacterial effects of ND: YAG and diode lasers in root canals. Biotechnol Biotechnol Equip 2010;23:2031-4.
15. Sena NT, Gomes BP, Vianna ME, Berber VB, Zaia AA, Ferraz CC, *et al. In vitro* antimicrobial activity of sodium hypochlorite and CHX against selected single-species biofilms. Int Endod J 2006;39:878-85.
16. Gopikrishna V, Ashok P, Kumar AP, Narayanan LL. Influence of temperature and concentration on the dynamic viscosity of sodium hypochlorite in comparison with 17% EDTA and 2% CHX gluconate: An *in vitro* study. J Conserv Dent 2014;17:57-60.
17. Gulsahi K, Tirali RE, Cehreli SB, Karahan ZC, Uzunoglu E, Sabuncuoglu B. The effect of temperature and contact time of sodium hypochlorite on human roots infected with *Enterococcus faecalis* and *Candida albicans*. Odontology 2014;102:36-41.
18. Pradhan S, Kamik R. Temperature rise on external root surface during laser endodontic therapy using 940 nm diode laser: An *in vitro* study. Int J Laser Dent 2011;1:29-35.
19. Sirtes G, Waltimo T, Schaetzle M, Zehnder M. The effects of temperature on sodium hypochlorite short-term stability, pulp dissolution capacity, and antimicrobial efficacy. J Endod 2005;31:669-71.
20. Hmud R, Kahler WA, George R, Walsh LJ. Cavitational effects in aqueous endodontic irrigants generated by near-infrared lasers. J Endod 2010;36:275-8.