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In vitro comparison of the antibacterial effect of three intracanal irrigants and diode laser on root canals infected with *Enterococcus faecalis*

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ABSTRACT

Introduction: Bacteria are the primary etiology of pulpal and periradicular pathosis. In endodontically treated teeth with persistent infections only one or a few bacterial species are present of which the most important is *Enterococcus faecalis*. The aim of this study was to compare antibacterial efficacy of canal disinfectants including 5.25% sodium hypochlorite, 2% chlorhexidine, MTAD (a mixture of doxycycline, citric acid and a detergent (Tween 80) and 830 nm diode laser.

Methods and Materials: The canals of 135 extracted single rooted human teeth were prepared using rotary instruments. The canals were contaminated with *Enterococcus faecalis* for 4 weeks and then were divided into 4 groups of 30 teeth in each, a positive control group containing 10 teeth and a negative control group of 5 teeth. After using the disinfectants, samples obtained from canals by paper points and also shaving the canal walls were cultured. The Kruskal-Wallis test was used to analyze the results.

Results: The results showed the bacterial reduction as follows: 99.97 ± 0.14 for sodium hypochlorite, 99.65 ± 1.13 for chlorhexidine, 97.56 ± 6.36 for laser and 96.91 ± 5.60 for MTAD. The count of CFU obtained from dentin shavings was: 16/96 ± 91/23 for sodium hypochlorite, 82/73 ± 186/63 for chlorhexidine, 47/26 ± 112/21 for laser and 341/34 ± 1139/83 for MTAD.

Conclusion: According to the results, sodium hypochlorite was the most effective agent against *Enterococcus faecalis*.

Keywords: *Enterococcus faecalis*, sodium hypochlorite, chlorhexidine, laser

INTRODUCTION

Bacteria are the main causes of pulpal and periapical lesions, directly or by toxins which they produce (1-3). Inability of immune system to reach the root canal system leads to inefficiency of this system in fully eradicating the endodontic infections (4).

One of the primary goals of the root canal treatment is to reduce the number of microorganisms and disinfecting the root canal system (5-8). To achieve this goal, mechanical preparation, irrigation, disinfection and obturation of the root canal system is necessary (9-11).

Although mechanical cleaning is an inseparable part of conventional root canal treatments, according to studies, deep penetration of microorganisms into anatomic regions such as lateral canals, apical ramifications, isthmuses dentinal tubules and also presence of smear layer, reduces its efficacy (10-13). Simultaneous use of antimicrobial agents and mechanical debridement reduces the number of bacteria in the root canal system (6, 10). Long term success of treatments depends on different factors such as anatomy of root canal system, resistance of bacteria and canal micro flora when achieving the root canal treatment (7).

Commonly, remaining of microorganisms in the canal, especially in the apical region, is known as

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one of the main causes of post treatment diseases and studies show that even after the best possible treatments, there is a probability of failure because of presence of resistant microorganisms in the canal (14-17).

Despite the primary endodontic infections which are polymicrobial and the dominant bacteria are the Gram negative anaerobic rods, the microorganisms related to secondary or refractory infections are restricted to one or few species of bacteria (11, 18). *Enterococcus faecalis* is one of the most resistant bacteria in the endodontic infections and its presence is related to higher probability of failures of endodontic treatments (10, 16). Considering the ability of this microorganism to penetrate into dentinal tubules and resistance against intra canal medicaments such as $Ca(OH)_2$, it is necessary to use an irrigant which is able to eliminate the bacteria (17, 19).

Sodium hypochlorite (NaOCl) is widely used as an antimicrobial irrigant. NaOCl is able to dissolve vital and necrotic tissues and has good antimicrobial activity (4). However it is severely toxic, has bad smell and taste, makes corrosion in instruments, has low wetting ability and is unable to remove smear layer (20, 21).

Chlorhexidine digluconate (CHX) has a broad antimicrobial spectrum and is effective against Gram negative and Gram positive bacteria, yeasts, fungi and viruses with lipid membrane (14, 16, 20). It also has the advantage of substantivity; however it lacks the ability of tissue solving (4, 12, 14). BioPure MTAD (a mixture of doxycycline, citric acid and detergent) is suggested as final rinse because of its antimicrobial properties and its ability to remove the smear layer (18, 21).

Laser has been shown to possess good antimicrobial properties. Different studies evaluated various types of wavelength for disinfection of root canals. Gutknecht *et al*. reported that Nd:YAG laser had the ability to kill 99.91% of *Enterococcus faecalis* under *in vitro* condition (22). In another study in assessing bacterial effect of diode laser (810 nm) in deep layers of infected root canal wall achieved to 74% bacterial reduction (23). Also, when diode laser with wavelength of 980 nm in continuous mode was used could increase the success rate of treatment by elimination of bacteria in deep dentin (24). Besides, Nd:YAG laser is effective in deep dentin even though the intensity of laser irradiation decreased in deeper layer (25). Moreover diode laser showed promising results in invivo condition (26). It can deeply penetrate into dentinal tubules and eliminate the microorganisms (6-15).

The aim of this study was to compare antibacterial efficacy of canal disinfectants including 5.25% sodium hypochlorite, 2% chlorhexidine, MTAD (a mixture of doxycycline, citric acid and a detergent (Tween 80)) and 830 nm diode laser.

MATERIALS AND METHODS

One hundred and forty extracted single rooted teeth with one canal were used in the study. The external surfaces of the roots were debrided using a curette. Then all teeth were placed in 0.5% NaOCl for 24 hours and then in saline until used. The crowns of the teeth were cut at CEJ by a disk under copious water spray. The apical foramina were determined using #10 K-file (Mani, Japan). One millimeter was subtracted from the length and the roots were cut again, so that a working length of 14 millimeters was obtained.

All canals were prepared using Profile rotary files (Maillefer, Ballaigue, Switzerland) up to size 35 with 0.06 tapering. Between filings, 1.3% NaOCl and 17% EDTA were used alternatively as irrigant and then EDTA was placed in canals for 1 minute and finally all canals were rinsed with 5 ml of saline.

After preparation, the apical foramina of all canals were sealed with glass ionomer cement (Fuji, Japan). The specimens were placed in acryl and were sterilized using an autoclave (20 min, 121 , 20 psi). To get sure of sterilization, specimens were incubated at 37°C for 24 hours and samples from canals of 5 specimens were obtained using a #50 Hedström file (Mani, Japan) and cultured.

No bacterial growth was observed. To induce infection, pure *E. faecalis* (ATCC 29212) suspension in BHI broth with a concentration of 1 Mc Farland (3x108 bacteria per ml) was injected into canals using insulin syringes. Five specimens received sterile BHI broth and served as negative control group. All specimens were placed in an incubator at 37°C for 4 weeks. During this period, canals were replenished with fresh bacterial suspension every 48 hours.

After incubation, the canals were divided into four groups of 30 canals each and a group of 10 canals as positive control group. They were filled with sterile saline and then each canal was dried by 3 sterile #35 paper points with intervals of 30 seconds. The paper points were transferred into test tubes containing 1 ml sterile saline. To obtain a suspension of bacteria,

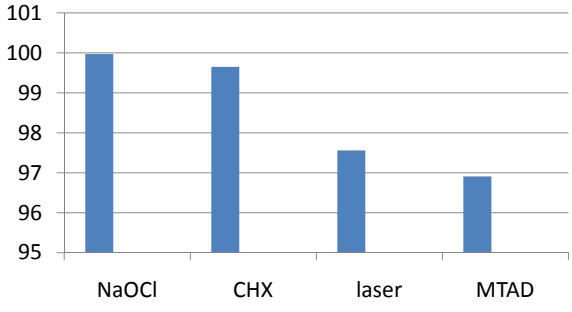

Fig. 1. Bacterial reduction in the canals in different groups.

the test tubes were placed in Vortex Mixer shaking machine for 20 seconds. After preparing 10^{-1}, 10^{-2} and 10^{-3} dilutions, 0.1 ml of the suspensions were cultured in BHI agar and colonies were counted (CFU-1).

Subsequently test irrigants were used as follows: in group 1, canals were rinsed with 5 ml of 5.25% NaOCl (Merk, Germany) and in group 2 with 2% CHX (Consepsis, Ultradent, USA) after 5 minutes canals were dried with paper points. In group 3, canals were rinsed with 1 ml of MTAD at first and then after 5 minutes with 4 ml of MTAD (according to the manufacturer) and dried with paper points.

In group 4 canals were rinsed with 5 ml sterile saline and then dried by paper points. In all canals 28 gauge needles (Dentsply, Tulsa, USA) were used for irrigation.

In group 5, diode laser with wavelength of 830 nm, output power of 1.5 Watt which was checked by power meter and frequency of 20 Hz was used (Medizinische Laser Technologie, Germany). A fiber optic of 200 µm diameter was inserted into the canal at the working length and after activation of the apparatus, the fiber was guided outward with a circumferential motion with a speed of 2 mm/s. The pulse duration was 50 ms. This process was repeated 4 times. There was a 15 seconds interval between the cycles.

After disinfecting the canals, all of them were rinsed with 5 ml sterile saline. To determine the amount of reduction of intra canal bacteria, all canals were filled with sterile saline and the same procedures explained in relation to determination of CFU-1 were repeated.

In the next step, to determine the amount of bacteria inside the dentinal tubules, 1 mm around the lumen of the canal was marked with a marker and dentin chips were shaved from the canal walls using #50 Hedström files (Mani, Japan) into sterile plates. Dentin chips were weighted and transferred into test tubes containing sterile saline. After preparation of 10^{-1}, 10^{-2} and 10^{-3} dilutions, the samples were cultured in BHI agar at 37ºC .

The colonies of bacteria were counted and reported as CFU obtained from dentin chips (CFU/mg). Kruskal-Wallis test and SPSS software were used for the statistical analysis of the data.

RESULTS

The results showed the bacterial reduction in the canals as below: %99.97 ± 0.14 for sodium hypochlorite, %99.65 ± 1.13 for chlorhexidine, %97.56 ± 6.36 for laser and %96.91 ± 5.60 for MTAD (Fig. 1). There was a significant difference between NaOCl and other methods ($p < 0.004$). CHX was more effective than laser and MTAD ($P < 0.004$). There was no significant difference between laser and MTAD group ($P > 0.004$).

The count of CFU obtained from dentin shavings was reported as CFU/mg: 16.96 91.23 for sodium hypochlorite, 82.73 186.63 for chlorhexidine, 47.26 112.21 for laser and 341.34 1139.83 for MTAD. There was a significant difference between NaOCl and other methods ($p < 0.004$). There was no significant difference between laser and CHX ($P > 0.004$). MTAD was less effective than the other disinfectants ($p < 0.004$).

DISCUSSION

Success of endodontic treatments is related to disinfection of root canal system. *E. faecalis* is a Gram positive coccus which is very frequently found in canals needing retreatment. Especial attention to this microorganism is because of its resistance against intra canal medicaments such as calcium hydroxide (27). Baumgartner *et al.* showed that 3 weeks of incubation of this microorganism in root canals, lead to a dense infection in dentinal tubules (28). To get sure of adequate infection, we inoculated the bacteria for 4 weeks.

The laser used in the study was diode laser. Because of possessing favorable antibacterial properties and relative safety and also more suitable price, this kind of laser has gained popularity. Radaelli showed that diode laser (830 nm) at a power of 2.5 and 3 W in continuous mode can be safely used in endodontics (29).

Group of sodium hypochlorite showed the greatest number of specimens with no bacterial growth (CFU = 0). This agrees with the results of Baumgartner's

study (28). This group also showed the least bacterial group among experimental groups. This agrees with the findings of Krause (30).

According to results, NaOCl seems to be more effective than chlorhexidine. Dunavant *et al*. and Abdullah *et al*. reached the same results (10, 31).

Although some in vitro studies have shown higher antibacterial efficiency of CHX than NaOCl, in in vitro environment, dentinal components can reduce the efficiency of CHX (4, 21, 32).

In present study NaOCl was more effective than MTAD. Giordino showed that %5.25 NaOCl was the only irrigant that could disrupt the biofilm of *E. faecalis* within 5 minutes. MTAD was unable of doing that at any time (33).

In another study conducted by Kho *et al*., there was no significant difference between %5.25 NaOCl/%15 EDTA and %1.3 NaOCl/MTAD (34). The study was achieved on apical 5 millimeters of roots. Considering the fact that most of the ramifications and lateral canals are at this region, probably many portions of the canals have been remained out of reach of both irrigants.

Antibacterial effect of MTAD is attributed to its doxycycline. It should be noted that doxycycline is a bacteriostatic agent. However enterococci are resistant against a broad spectrum of antibiotics including doxycycline (31, 35, 36).

Results of present study are in contrast to the results obtained from Torabinejad *et al*. and Shabahang *et al*., who found MTAD to be more effective than NaOCl (37, 38). In these studies %1.3 NaOCl has been used before irrigation with MTAD and this may overshadow the results in mentioned studies. Mechanism of MTAD is not clearly explained yet. It seems that Tween 80 has little antimicrobial activity, but enhances the antimicrobial effect of other ingredients (39).

The present study is in agreement with Eldeniz *et al*., who compared the efficacy of Er, Cr:YSGG and NaOCl against *E. faecalis*. Results showed %100 elimination of bacteria by %3 NaOCl; whereas efficacy of laser was significantly lower (6).

Kimura *et al*. suggested that although removal of debris and smear layer is possible by laser, it is difficult to clean all of root canal walls because the laser energy is emitted straight ahead, making it almost impossible to irradiate the lateral canal walls (40). Using side firing tips may enable the lasers to irradiate all areas of root canal walls and increase their antibacterial efficacies.

Despite the CFU obtained from canals, there was a significant difference between CFU obtained from dentin chips between groups of MTAD and laser. This issue may be explained by deeper penetration of laser compared to MTAD and thus higher antibacterial effect.

In agreement with current study, de souza *et al.* in assessing the same wavelength of diode laser (830 nm) in disinfection of root canal used 3 W output power which provided increased disinfection in deep dentin (41).

In conclusion, all techniques were effective against *E. faecalis,* with the NaOCl being more effective than others. Diode laser can be considered as an alternative technique for root canal disinfection.

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