

Full Length Research Paper

Antibacterial effect of gaseous and aqueous ozone in root canals contaminated with *Pseudomonas aeruginosa*

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This present study attempted to identify the antibacterial effects of aqueous and gaseous ozone in human root canals that are contaminated with *Pseudomonas aeruginosa*. The differences and antibacterial effects of gaseous and aqueous ozone (manual and ultrasonic) have not been compared to each other yet in any studies. Eighty single-root premolar teeth were prepared and then disinfected and sterilized. *P. aeruginosa* were incubated in root canals and kept at 37°C for 24 h. The root canals were contaminated with *P. aeruginosa* divided into 1 positive control, sodium hypochlorite (NaOCl) and three experimental groups: aqueous ozone with manual technique, aqueous ozone with ultrasonic technique and gaseous ozone (n=10). Disinfection procedures were performed for 5 min in order to ensure standardization among all working groups. Remaining microorganism colonies were counted on blood agar plates. Then, data were evaluated and statistically analyzed by one-way ANOVA and Tukey's test. As a result, although there were no statistically significant differences between the three groups (NaOCl, aqueous ozone with manual, and ultrasonic technique) ($P>0.05$), there were statistically significant differences between the gaseous ozone and all other groups ($P<0.05$). Both the manual and ultrasonic techniques of aqueous ozone achieved complete elimination of *P. aeruginosa* in root canals.

Key words: Gaseous ozone, aqueous ozone, microorganisms.

INTRODUCTION

The fundamentals for root canal treatments are the removal of the remaining pulp tissue, dentinal debris, and the elimination of microorganisms with chemomechanical preparation from the root canal system. Essentially, pulpal and periapical inflammation occurred because of pathogen microorganisms (Siqueira, 2002). One of the mostly eliminated microorganisms from root canals is *Pseudomonas aeruginosa*. Some studies reported that *P. aeruginosa* was isolated as an endodontic infectious

agent. Its morphology is highly similar to other gram-negative rods commonly found in endodontic infections (Ranta et al., 1988; Haapasalo et al., 1983).

Various irrigation solutions have been used during chemomechanical preparation to eliminate or reduce the number of microorganisms in endodontics. Moreover, an ideal endodontic irrigant should be antimicrobial, nontoxic to periapical tissues, capable of dissolving tissue or debris, able to lubricate the canal, and improve the

removal of the smear layer (Harrison and Hand, 1981). The most commonly preferred irrigation solution is 5.25% sodium hypochlorite (NaOCl). Recent researches demonstrated its strong antibacterial efficacy in infected root canals (Baumgartner and Cuenin, 1992; Huth et al., 2009; Kustarcı et al., 2009; Zan et al., 2013). Furthermore, the bactericidal ability of NaOCl occurred because of the formation of hypochlorous acid (HOCl), when it comes in contact with organic debris. HOCl shows its effects by oxidizing sulfhydryl groups within bacterial enzyme systems, thereby disrupting the metabolism of the microorganism (Siqueira et al., 1997), resulting in the death of the bacterial cells (Baumgartner and Cuenin, 1992). However, unfortunately, NaOCl causes undesirable side effects. For instance, several case reports have described the symptomatology of sodium hypochlorite when injected into the periapical tissues (Becking et al., 1991). When 5.25% sodium hypochlorite was forced beyond the apex, it led to immediate strong reactions and extreme pain. Within a few seconds the patient's cheek and upper lip showed signs of hematoma, ecchymosis and profuse hemorrhage from the root canal (Becker et al., 1974).

Ozone, which is one of the different approaches to eliminating microorganisms from root canal systems, has been proposed for the disinfection of root canals (Lussi et al., 1995). In dentistry, ozone has been used either in gaseous or aqueous form for elimination in the disinfection of root canals (Huth et al., 2005; Stübinger et al., 2006). Gaseous ozone shows its disinfection process with micro-organisms breaking down by neutralizing or preventing their growth (Nagayoshi et al., 2004a). Aqueous ozone is highly effective against bacteria, fungi and viruses, and is more economical compared to other disinfectants (Nagayoshi et al., 2004b). Therefore, aqueous ozone was useful in reducing the number of oral microorganisms for the treatment of endodontic infections (Hems et al., 2005). Some studies reported the antibacterial effect of aqueous ozone against a number of *P. aeruginosa* (gram-positive bacteria) bacteria in infected root canals (Huth et al., 2005; Białoszewski et al., 2011).

We have established the hypothesis of the present study and the need to seek new alternative irrigation agents instead of NaOCl. The null hypothesis of the present study is that gaseous and aqueous ozone with manual and ultrasonic techniques show antibacterial effects for the disinfection of the root canals contaminated with *P. aeruginosa*.

MATERIALS AND METHODS

In the present study, 80 single-rooted single canal human mandibular permanent premolar teeth that were extracted for orthodontic or periodontal reasons, were used. Digital radiographs of teeth were taken from buccal and approximal directions to determine the number and morphology of canals. Informed consent was obtained from the patients before the study, and the study was

approved by the Local Ethics Committee on Human Research of Cumhuriyet University (2010-02/08).

After been cleaned, the residues of the fresh-extracted human teeth, were kept at +4°C and 0.9% saline solution until the study was applied. Below the level of the enamel-cementum junction, the coronal portions of teeth were removed using sterile diamond discs under cooling water to obtain the 14 to 16 mm length of each root. Then, the root canals were entered with # 15 K-File (Mani Inc., Tochigi, Japan) hand tools and the path of the canal was determined. The tip of the file was transmitted to measure the length of each canal until it became visible in the apical foramen. Then it was withdrawn 1 mm from measured length. The root canals were started to be shaped with ProTaper (Dentsply, Tulsa Endodontics, OK, USA) rotary Ni-Ti instruments using crown-down method by the electric motor (Denta Ports DP-ZX, J. Morita MFG, CORP, Kyoto, Japan). Firstly, the coronal third of roots were expanded with SX files. Then, the median third of roots were reached with S1 and S2 files. The F1, F2 and F3 files were applied respectively to shape the apical third of canals. Canals were irrigated with 1 ml of 5.25% solution of NaOCl after the variation of the each file.

The roots were irrigated respectively with 17% EDTA, 5.25% NaOCl, and distilled water for 5 min to remove the smear layer that formed during the root canals' preparation. Then they were dried with paper point. Bottles were placed in autoclave to ensure the sterilization for 20 min at 121°C (Melag, Euroklav 23V-S, Germany). Then 3-fold nail polish (L'Oreal Jet-Set Diamond, Paris, France) was applied to the whole root surfaces of the teeth including root tips. After rubber caps were embedded, the teeth were sterilized with ethylene oxide (EtO). Then these caps were placed in bottles.

Microbiologic procedures

P. aeruginosa (ATCC 27853) strains were revived in the liquid nutrient media [brain heart infusion broth, (Acumedia Manufactures, Inc. Lansing, Michigan, USA)] and were incubated at 37°C for 24 h. Prior to each experiment, 0.5 McFarland turbidity was set with the Crystalspec™ device and McFarland standard number 0.5 was used to improve blood agar plates to obtain the bacterial growth in the amount of 1.5×10^8 colony-forming units (CFU/ml). The value of 10 µl of bacterial culture was transferred to the mechanically expanded lumen of the root canal using a sterile micropipette and then kept at 37°C for 24 h. In order to control bacterial growth, the sterile paper points (Dentsply, Maillefer) were placed in the root canals inoculated with bacteria. Paper points were waited along 5 min within the root canal, soaked with the broth. Then paper points were taken in sterile Eppendorf tubes containing 0.5 ml brain heart infusion broth (Merck 1.13825). After waiting 15 min, 50 ml of liquid medium was taken with a sterile micropipette from Eppendorf with mixed vortex and were smear-planted to a solid medium (blood agar plates) which split before and after the applications of disinfection.

Experimental groups

NaOCl (positive control) group

Infected root canals were irrigated for a duration of 5 min with 5.25% NaOCl.

Aqueous ozone with manual technique group

Aqueous ozone was obtained with the custom-made ozone generator (TeknO₃zone, Izmir/Turkey). The amount of aqueous ozone was measured with the help of the probe that is in the

reaction tank connected to the generator. The ozone density of the distilled water in the reactor tank was shown by the digital indicator, which is on the generator. Infected root canals were irrigated for a duration of 5 min with 4 ppm aqueous ozone.

Aqueous ozone with the ultrasonic technique group

Aqueous ozone was obtained with the custom-made ozone generator by the TeknO₃zone Company. Infected root canals were irrigated for a duration of 5 min with the intensity of a 4 ppm aqueous ozone with using an ultrasonic technique. Power control was done automatically by means of the automatic balancing system.

Gaseous ozone group

Gaseous ozone was obtained from an ozone generator (HealOzone, KaVo, Germany) by vacuum to the root canals for 5 min with the help of a handpiece on which were fixed 5 mm diameter silicone caps and endodontic cannulas.

Bacterial evaluation

Root canals were contaminated with *P.aeruginosa* and then waited for 24 h. Paper points were placed and waited for 5 min to counting the existence of bacteria both before and after root canal disinfection in the root canals. The bacteria were counted before irrigation to ensure standardization and then the examples whose CFU values were under 1.5×10^8 CFU/mL were excluded. After the application of irrigation, CFU counts of the breeding colonies of microorganisms were performed in blood agar plates. Then the logs of the CFU counts were calculated.

Statistical analysis

Variation data of the irrigation solutions were analyzed using a SPSS statistical software program (14.0 version, SPSS Inc., Chicago, USA). The data were subjected to statistical analysis among five different irrigation solutions, using one-way ANOVA and Tukey's test was applied to examine pairwise differences at a significance level of 0.05.

RESULTS

Mean \pm standard deviation and bacterial reduction (%) values obtained from the experimental groups applied for the disinfection, are shown in Table 1. A statistical comparison of the log CFU mean numbers of remaining *P. aeruginosa* is shown in Table 1. Cross-sectional images of bacteria on blood agar plates of samples infected by *P. aeruginosa* which was isolated from root canals before and after irrigation, are shown in Figure 1.

As a result, although there were not statistically significant differences between the three groups (NaOCl, aqueous ozone with manual technique, and aqueous ozone with ultrasonic technique) ($P>0.05$), there were statistically significant differences between gaseous ozone and all other groups ($P<0.05$). On the other hand, there was no statistically significant difference between

the manual and ultrasonic techniques of aqueous ozone groups ($P>0.05$).

DISCUSSION

In endodontics, many alternative disinfectants have been researched because the resistance of various microorganisms has gradually increased. For instance, some researchers investigated the antibacterial efficacy of NaOCl against *P. aeruginosa*. These investigations found that a high concentration of NaOCl (5.25%) became sufficient for complete sterilization in root canals contaminated with *P. aeruginosa* (Huth et al., 2009; Baumgartner and Cuenin, 1992; Piccolomini et al., 2002). In the present study, 5.25% NaOCl was applied as a negative control group to compare the antibacterial efficacy of experimental groups against *P. aeruginosa* in root canals. Consequently, the application of 5.25% NaOCl achieved exact sterilization in root canals like in the above mentioned studies (Baumgartner and Cuenin 1992; Huth et al., 2009; Kustarcı et al., 2009, Zan et al., 2013).

Recently, ozone has been one of the most researched alternative disinfection agents. Moreover, only a few studies have investigated the antibacterial effects of ozone against *P. aeruginosa* in endodontics. Particularly, aqueous (Bialoszewski et al., 2011) and gaseous (Bialoszewski et al., 2011; Huth et al., 2009; Case et al., 2012) ozone were emphasized against *P. aeruginosa* in root canals. Furthermore, this subject has attracted the attention of researchers. For instance, Bialoszewski et al. (2011) investigated the bactericidal efficiency of aqueous ozone in root canals infected by *P. aeruginosa*. Aqueous ozone was found to be effective after at least 30 s of application. In an other study, Huth et al. (2009) also assessed the antibacterial efficacy of aqueous and gaseous ozone against *P. aeruginosa* for 1 min. As a result, *P. aeruginosa* was almost eliminated following the application of two forms of ozone. In another research, Case et al. (2012) examined the effects of gaseous ozone against *E. faecallis*. Gaseous ozone was found useful as an additional disinfection agent in root canals. In the present study, we demonstrated the antibacterial effect of aqueous and gaseous ozone on elimination of *P. aeruginosa*. Consequently, although aqueous ozone achieved the complete elimination of *P. aeruginosa*, a few bacteria were detected in the gaseous ozone application in root canals. This outcome of the present study showed better results than the previous studies' (Bialoszewski et al., 2011; Huth et al., 2009; Case et al., 2012) results because of the higher concentration and longer application time for the disinfection of root canals.

The new root canal irrigation techniques have been investigated to reach the maximum antibacterial effect in endodontics. For this purpose, very few previous investigations determined the antimicrobial efficacy of

Table 1. The data values of log CFU enumeration that were obtained after the application belonging to the group of *P. aeruginosa* and statistical comparisons among groups.

Groups	Before Irrigation (CFU mL ⁻¹)	Mean ± Standard Deviation (Log CFU mL ⁻¹)	Bacterial reduction (%)
Group 1			
NaOCl (positive control)	1.5 X 10 ⁸	0.000 ± 0.00 ^a	100
Group 2			
Aqueous Ozone with manual technique	1.5 X 10 ⁸	0.000 ± 0.00 ^b	100
Group 3			
Aqueous Ozone with ultrasonic technique	1.5 X 10 ⁸	0.000 ± 0.00 ^c	100
Group 4			
Gaseous Ozone	1.5 X 10 ⁸	0.300 ± 0.16 ^{a,b,c}	99.97

n=20 specimens per experimental condition. CFU, Colony-Forming Unit; By the one-way ANOVA, F= 36.000 P=0.000, P<0.05; ^{a,b,c}Values with same superscript letters are statistically significant (P<0.05, Tukey's test).

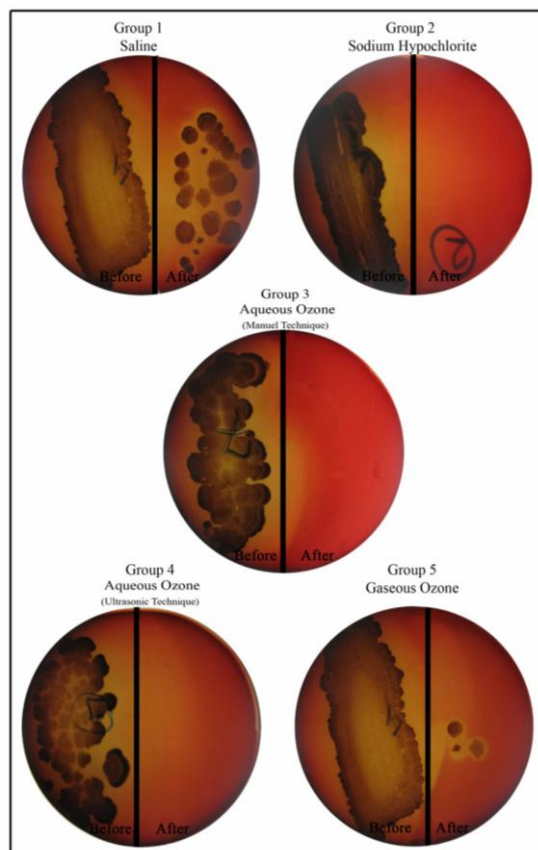


Figure 1. Cross sectional images of bacteria on blood agar plates of samples infected by *P. aeruginosa* which was isolated from root canals before and after irrigation.

aqueous ozone combined with the ultrasonic technique against microorganisms in root canals (Estrela et al., 2007; Arita et al., 2005). Moreover, another study examined the effect of aqueous ozone with the ultrasonic technique against *E. faecalis* and *Streptococcus mutans*

in bovine dentin. In conclusion, the viability of microorganisms was significantly decreased. Additionally, the ultrasonic technique showed a stronger antibacterial effect than the manual technique (Nagayoshi et al., 2004). In another similar study, the effect of aqueous ozone was examined against *C. albicans* inoculated on an acrylic denture plate. The application of aqueous ozone could reduce the number of fungi and especially the ultrasonic technique exhibited a higher degree of antifungal efficacy than the manual technique (Cardoso et al., 2008). In the present study, aqueous ozone with the ultrasonic and manual techniques achieved complete elimination of *P. aeruginosa* in human root canals. Unlike the results of the above-mentioned studies, we found the same antibacterial efficacy between aqueous ozone with the ultrasonic and manual technique (Estrela et al., 2007; Arita et al., 2005). In our opinion, this result grew out of differences in the use of the human root canal and different types of microorganisms.

In conclusion, the results of the present study indicate that NaOCl, aqueous ozone with the manual technique, and aqueous ozone with the ultrasonic technique groups showed the highest and same antibacterial efficacy against *P. aeruginosa*. Furthermore, the aqueous ozone with manual and ultrasonic technique groups showed an equal potential to kill *P. aeruginosa*. Aqueous ozone may be used with both technique for disinfection of root canals inoculated with *P. aeruginosa*. Moreover, the aqueous ozone with manual and ultrasonic technique groups should be preferred despite NaOCl for the ideal sterilization of root canals infected with *P. aeruginosa* and gaseous ozone was not suitable for complete sterilization of root canals because gaseous ozone could not achieve the total elimination of bacteria from root canals.

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