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Antimicrobial effect of Microdacyn 60, OxOral, and sodium hypochlorite 5.25% in anaerobic bacteria

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Irrigants have traditionally been used in the root canal. Sodium hypochlorite (NaOCl) is the most commonly used solution, because of its advantages; however, it is known to be toxic to periradicular tissues and can cause necrosis of support tissues and edema. The objective of this study is to compare the antimicrobial effect of Microdacyn 60®, OxOral®, and NaOCl 5.25% against *Streptococcus sobrinus, Porphyromona gingivalis, Streptococcus intermedius, Tanerella forsytensis*, and *Enterococcus faecalis*. Thirty-three extracted uniradicular teeth were inoculated with 10 µl of a mixture of anaerobic bacteria. After seven days, the samples were irrigated with the solutions and samples were taken. These were placed in an Eppendorf tube and incubated at 37°C. After seven days, a sample was taken for a bacterial count. Polymerase chain reaction (PCR) was performed to identify the bacteria present in the samples. Student´s *t* test and analysis of variance were used with a 95% confidence interval. NaOCl showed complete bacteria elimination with no statistically significant difference with OxOral. The saline and Microdacyn 60 groups showed greater bacterial growth than OxOral. In the Microdacyn 60 group, *E. faecalis* was the most resistant microorganism. NaOCl 5.25% had a greater antibacterial effect, followed by OxOral, and lastly by Microdacyn 60.

Key words: Irrigants, sodium hypochlorite (NaOCl), *Enterococcus faecalis*, Microdacyn 60, OxOral, irrigation.

INTRODUCTION

Successful treatment of the root canal system depends on the method and quality of instrumentation, irrigation, disinfection, and three-dimensional obturation of the root canal space (Himel and DiFore, 2009; Canalda-Sahli, 2006). For this, different types of handpieces, machining, and irrigation solutions have been used to obtain a clean space, shaped to receive a filling (Sen et al., 1995). Basrani and Cañete (1988) define endodontic irrigation as the introduction of one or more solutions in the pulp chamber and the root canals with subsequent aspiration. Furthermore, it is an essential complement to instrumentation; therefore, it must be used before, during, and after it. Sodium hypochlorite (NaOCl) is the most commonly used irrigating solution in current practice because of its effectiveness in eliminating vital and non-vital tissue and its broad effect against bacteria, spores, fungi and viruses (including HIV, rotavirus, HSV-1 and Hepatitis A and B) (Siqueira et al., 2000). However, NaOCl has toxic effects on vital tissues, which can result in haemolysis, skin ulceration, and necrosis (Hülsmann and Hahn, 2000).

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It does not remove the smear layer since it only acts on the organic matter in the pulp and predentine (Di Lenarda et al., 2000). In the past 20 years, super oxidized solutions (SOSS) have shown to be potent antimicrobial agents and disinfectants through oxidative damage (Huang et al., 2008). Electrolyzed water (EW) contains a mixture of inorganic oxidants such as hypochlorous acid (HClO), hypochlorous acidic ion (ClO^-), chlorine (Cl_2), hydroxide (OH), and ozone (O_3), which are effective for inactivating a variety of microorganisms (Chittoria, 2007). This product is prepared with purified water plus a saturated solution of sodium chloride (NaCl). SOSS have been used mainly as disinfectants in endoscopic equipment and minimally invasive instruments as well as for washing and disinfecting hemodialysis equipment. Different types of electrolyzed solutions have been the subject of many studies and reviews as a method of decontaminating vegetables, killing *Escherichia coli, Listeria, Salmonella, Bacillus anthracis*, and many other infectious strains (Horiba et al., 1999; Guentzel et al., 2008). Their mechanism of action is based on the effect that Na^+, Cl^-, and O_2 ions have on the bacterial wall, where they cause protein denaturation, and fragmentation of carbohydrates and lipids, in addition to viral alteration of capsids, DNAses, and RNases (Tang et al., 2011). They have been approved by the Food and Drug Administration (FDA) to debride traumatic wounds and burns, to lubricate and moisten wounds and burns, and to disinfect medical equipment (Gutierrez, 2006). OxOral® is a pH neutral SOSS 0.005% (50 ppm) of active Cl, formulated for dilution at 1:5. It is effective against viruses, fungi, and Gram-positive and Gram-negative bacteria (PLM México, 2012). Microcyn 60® (60 ppm) is an electrolyzed solution manufactured in Mexico for the care of skin wounds. It does not cause irritation, or sensitize, and requires no rinsing. It is an antiseptic and disinfectant with a neutral pH and a broad spectrum against microorganisms. Its bactericidal effect takes place in 30 s and disinfection occurs in 15 min with a long shelf life (>12 months). It is a hypotonic solution with an osmolarity of 13 mOsm/kg (Chittoria et al., 2007; Landa-Solis et al., 2005; Yahagi et al., 2000).

The aim of this study is to compare the antimicrobial effect of two electrolyzed water solutions, Microdacyn 60 and OxOral, and NaOCl 5.25% against *Streptococcus sobrinus*, *Porphyromona gingivalis*, *Streptococcus intermedius*, *Tanerella forsytensis*, and *Enterococcus faecalis*.

MATERIALS AND METHODS

Five anaerobic bacteria strains from the laboratory of the School of Dentistry of the Universidad Autonoma de Nuevo Leon that were present in necrotic tissue were selected: *S. sobrinus* (ATCC 27607), *P. gingivalis* (ATCC 33277), *S. intermedius* (ATCC 27335), *T. forsytensis* (ATTC43037) and *E. faecalis* (ATCC 11420). Uniradicular teeth with a single straight canal, complete root formation, free of fractures, and free of caries in their root portion with a closed apex were also selected.

Preparation of teeth

A total of 33 extracted human single root teeth were selected. The crowns were sectioned at the cementoenamel junction and the working length was determined with #15 K-type Maillefer file subtracting a millimeter from its outlet, flush with the apical foramen. The teeth were instrumented to a #40 apical diameter with ProTaper Universal files (Dentsply International, York, PA) and using irrigation with NaOCl 5.25%. After instrumentation to maintain the canal permeable, root canals were dried with Hygenic #40 Sterile Absorbent Paper Points (Hygenic Corporation, Akron, Ohio) and canals were filled with 17% ethylenediaminetetraacetic acid (EDTA) for 5 min after which the solution was removed with a NaOCl 5.25% wash and again dried with paper points. The outer surface of the roots was covered with a layer of clear lacquer, which also sealed the apical foramen to avoid external contamination.

At the end of this process, teeth were placed in a rack made of heavy silicone (Speedex Trial™, Coltene whaledente, Altstaetten, Switzerland). The teeth were autoclaved for 30 min at 121°C and under 15 pounds of pressure. After this, a sample of the specimens was taken with a #40 Hygenic sterile paper point (Coltene) and placed in an Eppendorf tube with trypticase soy broth for incubation for 24 h. At the end of this time, if there was no turbidity, this showed that there were no bacteria. If turbidity was present, the presence of bacteria needed to be confirmed by seeding this broth in a Petri dish with 5% sheep blood agar and incubating it for 24 h. Subsequently, the test specimen was resterilized and the procedure repeated.

Preparation of strains

Inside an anaerobic chamber, 100 µl of each bacterium was obtained with an Eppendorf pipette and inoculated individually in Eppendorf tubes with trypticase soy broth. The tubes were placed in an incubator at 37°C for 7 days to activate them. At the end of this time, 100 µl from each tube was inoculated and plated on 5% sheep blood agar plates individually, sealing them with tape and placing them in airtight bags. The dishes were taken to the incubator for 7 more days. After this time, Gram staining of the bacterial colonies was performed to observe their morphology with an optical microscope at 100x, and the viability of each bacterial strain was confirmed.

To confirm optimal growth of the strains, 1000 µl were taken from each tube containing the reactive bacteria and this was placed in a single test tube containing 5000 µl of trypticase soy broth to mix the five bacterial strains, obtaining a total volume of 10,000 µl, simulating the clinical conditions of the oral environment, living as an ecological community and not isolated.

Inoculation of specimens and placement of solutions

The teeth were divided into three experimental and one control groups (10 teeth in each experimental group and 3 teeth in the control group). These were placed in individual silicone racks for easy handling inside the anaerobic chamber.

After sterilization of dental specimens was proven and the bacteria mixture cultured, starting with a bacterial concentration of 0.5×10^8 CFU/ml, 10 µl of this mixture was used to fill the root canal using an Eppendorf micropipette, slowly withdrawing it from the canal and later sealing it with sterile tape. Each group was placed in airtight sealed bags and subsequently incubated for 7 days. After bacterial incubation, we proceeded to irrigate the samples with different solutions under aseptic conditions and in strict

anaerobiosis: Group 1, sterile saline/NaCl (n = 3); Group 2, Microdacyn 60 (n = 10); Group 3, OxOral (n = 10); and Group 4, Sodium Hypochlorite/NaOCl 5.25% (n = 10). The samples were irrigated with 5 ml of each solution for 5 min with a 5 ml syringe and a NaviTip ISO 30 (Ultradent) hypodermic needle, and afterwards rinsed with sterile saline solution. The same procedure was performed for each group.

Sampling

Five minutes after irrigating each specimen, they were dried with sterile paper points and a sample was collected from each with a paper point previously dampened with sterile saline. Each sample was introduced into an Eppendorf tube with 1,000 µl of trypticase soy broth. Subsequently, the tubes were placed in a Vortex Maxi-Mix Thermolyne M16700 (Thermo, Fisher Scientific Inc., Asheville, NC) for homogenization, and again in the anaerobic chamber; 100 µl was drawn from each tube and its contents were seeded in Petri dishes with 5% sheep blood agar and incubated at 37°C for seven days.

Bacterial count

After seven days, a sample was taken from each of the dishes to make a 10^{-3} dilution in sterile bidistilled water, since this dilution proved to be the best to carry out a viable cell count. From the dilution, 10 µl was obtained and placed in a Neubauer chamber and a bacterial cell count was performed under an optical microscope to determine the number of cells per milliliter. Mean colony-forming unit (CFU) counts were log transformed for performing calculations and statistical analyses.

Statistical analysis

To compare results between irrigation solutions and each irrigation solution, analysis of variance (ANOVA) was used. Tukey honestly significant difference (HSD) test was also applied to specifically determine significant differences between irrigants. The tests were performed considering an alpha of 0.05 and a confidence interval (CI) of 95%.

RESULTS

The mean number of bacteria present in the different solutions was the following: the three elements irrigated with NaCl (control group) had an average of 3.52 ± 0.16 CFU/ml (95% CI: 3.12 to 3.92). The 10 elements irrigated with Microdacyn 60 had a mean of 2.57 ± 1.58 CFU/ml (95% CI: 1.44 to 3.69), while the 10 elements irrigated with OxOral had a mean of 0.26 ± 0.46 CFU/ml (95% CI: 0.08 to 0.59). The 10 elements irrigated with NaOCl showed no bacterial growth (Figure 1). Values for NaCl were in the range of 3.40 to 3.70 CFU/ml. The sample irrigated with Microdacyn 60 had values between 0.90 and 5.85 CFU/ml, while the sample irrigated with OxOral was 0.00 to 1.40 CFU/ml. The best antimicrobial effect was observed with NaOCl, followed by OxOral. There was no statistically significant difference between OxOral and NaOCl ($P = .924$) or between NaCl and Microdacyn 60 ($P = .408$). A statistically significant difference was found between the groups NaCl and Microdacyn 60 versus OxOral and NaOCl ($P < .01$).

DISCUSSION

The results show a greater antibacterial effect with NaOCl, followed by OxOral, with a statistically significant difference between the control group (NaCl) plus Microdacyn 60 and OxOral and NaOCl. These results are similar to those by Prabhakar et al. (2010) who analyzed the antimicrobial efficacy of Triphala, green tea polyphenols, MTAD, and NaOCl 5% against *E. faecalis* biofilm formation on tooth substrate. NaOCl 5% showed complete bacterial inhibition against *E. faecalis* biofilm formation.

Retamozo et al. (2010) investigated the concentration and irrigation time of NaOCl for disinfecting dentin canals colonized by *E. faecalis.* Their findings were that irrigation with NaOCl 5.25% for 40 min was the most effective, while irrigation with lower concentrations and lower exposure times to NaOCl were not effective against *E. faecalis*. They concluded that high concentrations and long exposure to NaOCl are needed for removal of *E. faecalis* in contaminated dentin. These results agree with those of the present study, although the exposure time in our study was 5 min; however, one of the drawbacks of our study was that NaOCl was not neutralized with sodium thiosulfate. Prabhakar et al. (2010) used human single premolars and NaOCl 5% without neutralization by sodium thiosulfate and found maximum antibacterial activity against *E. faecalis* biofilm with an exposure time of 10 min.

Root canal irrigation is an important part of the preparation procedure. NaOCl and H_2O_2 are the irrigants most frequently used in the debridement and disinfection of the root canal. In the clinical setting, irrigant exposure time should be short to avoid patient discomfort and allow better endodontic conditions for the clinician. In a review of complications of NaOCl during root canal irrigation, Hulsmann and Hahn (2000) reported that NaOCl has toxic effects on vital tissues, resulting in haemolysis, skin ulceration and necrosis. It can also damage clothing and the eye, and produce tissue necrosis when NaOCl is injected beyond the apical foramen when wide apical foramina is present or the apical constriction has been destroyed during root canal preparation.

Electrolyzed water (superoxidized solution) has been used to disinfect produce because of its strong bactericidal effect (Guentzel et al., 2008; Huang et al., 2008). The active substances in SOSS are Cl_2, HClO, and ClO^- (Tang et al., 2011; Huang et al., 2008; Landa-Solis et al., 2005). It has been used in dentistry for disinfection of dental instruments, and root canal irrigation (Horiba et al., 1999). Horiba et al. (1999) also studied the bacteriostatic/bactericidal effect of electrolyzed neutral water against 15 bacteria strains obtained from infected root canals. They found that electrolyzed neutral water had a bactericidal or growth-inhibitory effect against

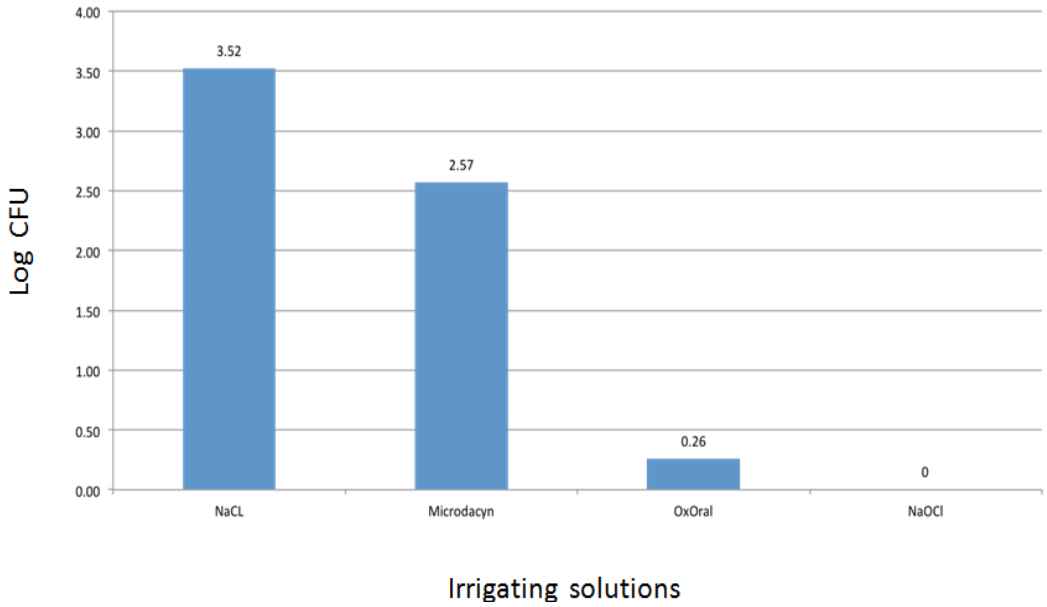

Figure 1. Effect of NaCl, superoxidized solutions (Microdacyn 60, OxOral), and NaOCl 5.25%, on mean bacterial growth.

the bacteria studied.

With regard to biofilm, Ozaki et al. (2012) studied biofilm removal and antimicrobial effects of microbubbled tap water and electrolyzed water with *Candida albicans* and *Streptococcus mutans*. They found that electrolyzed water in the microbubbled state was effective against both planktonic and biofilm microorganisms. This is evidence that superoxidized solutions could be effective in removing biofilm. Studies with microorganisms present in the oral cavity and dental materials need to be carried out.

Our results found a growth-inhibitory effect with the two electrolyzed solutions studied (Microdacyn 60 and OxOral). OxOral showed a greater effect than Microdacyn 60 and an effect similar to NaOCl, although this similarity was not statistically significant. The difference in effectiveness between Microdacyn 60 and OxOral could be because one comes in a closed container and the other in an open container, but this requires further study.

Superoxidized solutions represent a possible alternative to NaOCl for irrigating root canals; however, these formulas need to be tested clinically in order to determine their antibacterial effect in root canal procedures when confronted with the diversity of organisms that populate necrotic root canals.

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