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Pesquisa Científica

Is ozonated water a suitable alternative to NaOCl in root canal disinfection?

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Abstract

This study evaluated the disinfection ability of ozonated water in comparison to NaOCl solution in root canals infected with *Enterococcus faecalis*. Forty single-rooted bovine teeth were standardized and prepared using K-files. The root canals were contaminated for 21 days and the first microbial sample was collected from root canals (S1). The teeth were divided into 5 groups: Saline solution, 0.5% NaOCl, Ozonated water, and 2 control groups. The root canals were irrigated with 20 mL of the selected solution for an uninterrupted period of 20 minutes, the irrigant solution was maintained resting for 5 minutes and then, samples were collected from root canals (S2). The data was evaluated using Kruskal Wallis and Wilcoxon tests ($P<0.05$). The NaOCl group had lower microbial counts ($P<0.05$) and a higher percentage of microbiological reduction than the other groups ($P<0.05$). It was determined that ozonated water was not a suitable substitute for NaOCl.

KEYWORDS: Disinfection; Endodontics; Ozone; Sodium hypochlorite.

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Introduction

The presence of bacteria within the root canal system might result in the persistence of periapical lesions and, consequently, in the failure of endodontic treatment[1]. Its elimination takes place through the mechanical action of the instruments along the root canal walls and the flow of irrigating solutions[2]. Since the instruments leave around 10 to 50 percent of the canal walls unprepared, the use of irrigant solutions with adequate properties in endodontic therapy is critical[3].

Currently, no solution has all the desired characteristics for an ideal irrigant. The most popular irrigant solution used in endodontic treatment is sodium hypochlorite (NaOCl). NaOCl has an antimicrobial function, even when pathogens are organized in biofilms and dentinal tubules; has the unique ability to dissolve organic tissue; and few reports of allergic reactions[4]. On the other hand, NaOCl has a toxic effect on tissues directly related to its concentration; however, accidents are relatively rare and usually considered iatrogenic[4]. Even though NaOCl covers more of the requirements for endodontic irrigant than any other known compound, other approaches have been proposed to improve root canal disinfection either by replacing conventional chemo-mechanical procedures or by supplementing their effects. Photodynamic Therapy (PDT), Passive Ultrasonic Irrigation (PUI), and ozone therapy are some examples of these new procedures[5-7].

Based on the idea that ozone (O_3) quickly dissociates into water and releases a reactive form of oxygen that can oxidize cells, ozone therapy is believed to be effective against microorganisms without inducing to drug resistance[8]. This oxidation mechanism breaks the integrity of the cell membrane allowing ozone to penetrate inside microorganisms, therefore oxidizing glycoproteins and glycolipids, and blocking bacterial enzymatic function[9]. In endodontics, ozone therapy has been investigated in chemomechanical canal preparation and used solely as an alternative to NaOCl or as a complementary disinfection

source. Some researchers found that ozone therapy is as effective as NaOCl in decreasing bacteria[6,11], while others found that it is less effective[8,13,14]. However, most of those previous studies that determined the benefits of the use of ozone therapy, failed to adequately compare ozone therapy and NaOCl, incorrectly associating variables that could lead to biased conclusions [6,10]. Therefore, this study aimed to evaluate, the microbial reduction in root canals irrigated with ozonized water in comparison to NaOCl solution, after root canal preparation. The null hypothesis tested was that there would be no differences between the solutions regarding microbial reduction.

Material and methods

Sample size calculation

The sample size was calculated with an alpha-type error of 0.05 and a power of 95%, based on a pilot study that demonstrated an effect size of 2.6 and determined a sample size of 5 per group. To overcome possible losses during the experimental procedures a final sample size of 8 per group was used.

Selection and standardization of specimens

For this study, 40 single-rooted bovine teeth with similar anatomy (based on radiographic images) and without evident pathological changes were selected. The elements were stored for 6 months in saline solution. The crowns were sectioned with diamond disks (KG Sorensen, São Paulo, SP, Brazil), standardizing all roots at 16 mm in length. The 40 teeth were instrumented using 50, 55, 60, and 70 K-files (Dentsply Sirona, Ballaigues, Switzerland). At each instrument change, the teeth were irrigated with 2 mL of water to remove debris.

Before the sterilization of the samples, the teeth had their apexes covered with OpalDam (Ultradent, South Jordan, UT, USA) which was polymerized for 1 minute. Then, they were sealed with

colorless nail polish throughout their exterior, individually introduced into polypropylene tubes containing distilled water, and autoclaved at 121 °C for 40 min. To verify the effectiveness of sterilization, each specimen in the negative control group was irrigated with 1 mL of sterile saline, and samples were taken sequentially by introducing three size 15 sterile paper points into the root canals. Samples were plated on Mitis salivarius agar (Difco, Maryland, USA) and incubated at 37 °C for 48 hours. After this process, each tooth was placed under pressure in a sterile rubber stopper (with a hole in the middle), in the cervical portion of the root so that the elements were vertical and above the shelf for microtubes, in which they were kept for 21 days. All procedures with the samples after sterilization were performed within a laminar flow.

Contamination of specimens

The contamination of the specimens was carried out according to a previous study[15]. A pure culture of *Enterococcus faecalis* (ATCC 29212) was grown to contaminate the root canals. The cell suspension was adjusted to ± 1.0 McFarland standard to ensure that the number of bacteria was 3 x 10^8 Colony-Forming Units (CFU ml). Sterile pipettes were used, under laminar flow, to inoculate each specimen with 100 µL of the bacterial suspension. A sterile 15 K-file was used to spread the bacterial suspension along the entire length of the root canal. The samples were kept at 37 ºC for 21 days and every 2 days, 100µL of freshly prepared BHI (Brain Heart Infusion) were added to the samples to maintain the biofilm.

After the contamination period, the root canal of each specimen was irrigated with 1 mL of sterile saline solution and the first bacterial sample (S1) was obtained by sequentially introducing three sterile size medium paper points (Dentsply Sirona) into the root canal and keeping them inside for 1 min. The collected samples were transferred individually to 1.5 mL Eppendorf tubes containing 1 mL of sterile saline solution. CFU counts were performed as described [15] in the quantification of bacterial load.

Irrigation of specimens

The elements were separated into 5 groups according to the irrigating solution used (n=8 per group):

- **Positive control group:** In this group, the root canals were contaminated but did not receive irrigation with any type of substance.

- **Negative control group:** The root canals of this group were not contaminated with *E. faecalis*, but they were irrigated nonetheless.

- **NaOCl Group:** Irrigation was performed using a sterile syringe, containing 20 mL of 0.5% NaOCl for 20 minutes, and then the NaOCl solution was maintained resting for 5 minutes. Following that, each canal received a 2 mL of saline and a 5-minute irrigation of 2.5 mL of 10% sodium thiosulfate (0.5 mL of sodium thiosulfate were refurbished inside the root canals every minute for 5 minutes). The NaOCl neutralizing process was finished after the 5-minute cycle by irrigating the canals with 2 mL of sterile saline once more.

- **Saline solution group:** Irrigation was performed using a sterile syringe containing 20 mL of sterile saline solution for 20 minutes, and then the irrigant solution was maintained resting for 5 minutes. After that, the root canal was irrigated with 6.5 mL of saline, in the same way, described in the neutralization of the NaOCl process.

- **Ozonated water group:** Ozonated water was obtained through the Ozone Generator (Philozon Medplus, Nova Esperança, SC, Brazil) using double-distilled water as a base and following the manufacturer's instructions, 40 µg/mL of ozone concentration were for every 250 mL of double-distilled water. Irrigation was performed with 20 mL of the solution for an uninterrupted period of 20 minutes, and then the irrigant solution was maintained at rest (5 minutes). After this period, each root canal was

irrigated with 6.5 mL of sterile saline, in the same way, described in the neutralization of the NaOCl process.

Irrigation was performed using a 30G Navitip needle (Ultradent, South Jordan, UT, USA) attached to a 5 mL hypodermic syringe and 26.5 mL of solution was standardized for each root canal in all experimental groups.

Microbiological analysis

After each group received their irrigation protocols, a sterile size 40 Hedstrom file (Dentsply-Sirona) was used to perform 3 scraping movements on the walls of the root canal, and the samples were collected using size medium paper points, for a total of 3 cones per specimen. The cones were introduced individually and kept inside the root canals for 1 minute each. The paper points were introduced into an Eppendorf tube containing 1 mL of sterile saline solution.

The Eppendorf tubes containing the collected samples were vortexed for 1 minute and a dilution of the sterile saline solution up to 10^{-5} were prepared by serial dilution. A volume of 10 µL of each dilution was inoculated onto Mitis salivarius agar plates (Difco), incubated at 37 °C for 48 h, and then subjected to bacterial counts.

Statistical methods

The data normal distribution was rejected by the Shapiro–Wilk test ($P<0.05$). The S1 sample collection were compared using Kruskal Wallis test to confirm the hypothesis of similar microbial counts amongst the groups. The Kruskal Wallis analysis followed by Dunn test was used to compare the results amongst the groups in S2 and the percentage of microbiological reduction data. The intra-group analysis was performed by Wilcoxon (signed-rank test). The level of significance was set at $\alpha = 5\%$ (Biostat, Analyst soft; Walnut, CA, USA).

Results

While the microbial growth in the specimens in the positive control group (C+) proved the bacterial viability throughout the whole experimental process, the sterility of the specimens in the negative control group (C-) confirmed that the aseptic chain was maintained throughout the experiment.

One hundred percent of the samples from the initial sample collection (S1) indicated the bacterial presence using the plate culture method. Following the irrigation protocol, the second collection (S2) resulted in a significant reduction in the microbial counts in all groups in comparison to the first sample (S1) ($P<0.05$) (Table 1).

The analysis of the inter-group microbial counting data showed that in S1 the bacterial load was similar ($P>0.05$). In S2, a significant difference occurred between NaOCl and ozonated water groups, as well as between NaOCl and saline groups ($P<0.05$), with the NaOCl group having less microbial counts than the other two, with no differences between ozonated water and saline groups ($P>0.05$) (Table 1).

There were significant differences between NaOCl and ozonated water groups and between NaOCl and saline groups ($P<0.05$) in terms of the percentage of microbial reduction, with the NaOCl group having a higher percentage of microbial reduction than the other groups (Table 1).

TABLE 1 · Mean, minimum and maximum values of microbial count and the percentage of reduction in NaOCl, Ozone and Saline groups in the different sample collections (S1 and S2).

	S1	S2	% reduction
NaOCl	14650 (8600-62000)^{Aa}	0 (0-16.6)^{Ba}	100 (99.8-100)^{a}
Ozone	12800 (6600-43300)^{Aa}	2215 (1330-4000)^{Bb}	81.7 (72.5-96.1)^{b}
Saline	17300 (660-38300)^{Aa}	2295 (300-3660)^{Bb}	87.7 (50.0-99.2)^{b}

*Different upper-case letters refer to significant statistical differences in each group in correlation to microbial count in the different sample collections ($P<0.05$). Different lower-case letters refer to significant statistical differences among groups in any given sample collection ($P<0.05$).

Discussion

The use of ozonated water as a possible alternative or complementary antimicrobial agent during root canal treatment is currently being discussed. Despite some studies demonstrated the antimicrobial potential of ozonated water as an antimicrobial agent[6,16-19], some limitations in studies about the potential of root canal disinfection using ozone therapy were pointed out in a recent systematic review[21] and motivated the elaboration of this study. The failure in equivalence of parameters between control and experimental groups[6,11], the limited sample size with the absence of sample size calculation[9-14,19], the absence of relevant information regarding distributions of groups[10,12], and the presentation of results[12] were cited in the systematic review, and all of them were overcome in the present study with the current methodological design.

The present study evaluated the microbial reduction in contaminated root canals irrigated with ozonated water in comparison to NaOCl and saline solutions. During the elaboration of the study design, some attempts were made to eliminate the risk of bias, as such, (i) the samples were only contaminated after root canal instrumentation, to eliminate the bias of microbial reduction being connected with mechanical preparation and (ii) the same irrigation volume and time were employed across the different solutions.

The analysis of the inter-group microbial counts revealed a significant statistical difference at S2, with the NaOCl group having the smallest counts, while ozonated water and saline groups presented similar results, evidencing the inability of ozone to eliminate microorganisms organized as a biofilm. Therefore, the null hypothesis tested was rejected. This result is corroborated by many others which showed that ozone, when used alone, was not able to yield similar results to NaOCl [10-14]. It is important to emphasize that no differences were demonstrated between the ozonated water and saline groups, proving

that ozone was completely ineffective in the current investigation. Despite the inferior results when compared to NaOCl, both saline and ozonated water solutions reduced the number of bacteria, which is probably justified by the mechanical action of the irrigant flow. The microbial counts between sample collections in each group revealed a considerable reduction in the microbial load from S1 to S2 in all three groups ($P<0.05$). In this study, the irrigation process was performed after root canal instrumentation and the methodology was similar to that described by De-Deus *et al.*[20] (2022). The irrigation process was performed in two distinct phases: the flow phase, during which a flowing movement occurs over the entire length of the root canal (20 minutes), and the resting phase, during which the irrigant solution is deposited and maintained at rest (5 minutes). Both phases are streamlined and optimized by the larger shape of the root canals. The canal is filled with the irrigant solution and kept at a stop during the resting phase to enable NaOCl to perform to its fullest ability inside the small canal space microenvironment.

The way microbial sampling takes place is one limitation of the method chosen to evaluate microbial reduction. In this methodology[22-24], microbial sampling was performed by means of paper points and endodontic instruments, which only served to offer information on the microbial condition of the lumen, the root canal walls, and potentially locations outside the main root canal in each tooth[25]. Future investigations might use cryopulverization, which has been advocated for a wider range of endodontic microbiota analyses[26].

Even though ozone is being used in several areas of dentistry, the results of the present study reinforce previous results[10-14], indicating that such therapy should not replace the irrigating substances conventionally used in the treatment of root canal.

Conclusion

Despite the limitations of the present study, it concludes that ozonated water did not prove to be a viable substance as a replacement for NaOCl. Future studies should focus on the evaluation of other presentations of ozone, such as gas, oil, and the use of different agitation techniques.

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The authors deny any conflicts of interest related to this study.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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A água ozonizada é uma alternativa adequada ao NaOCl no preparo do canal radicular?

Resumo

Este estudo avaliou a capacidade de desinfecção da água ozonizada em comparação com a solução de NaOCl em canais radiculares infectados com Enterococcus faecalis. Quarenta dentes bovinos uniradiculares foram padronizados e preparados com limas K. Os canais radiculares foram contaminados por 21 dias e a primeira amostra microbiana foi coletada dos canais radiculares (S1). Os dentes foram divididos em 5 grupos: solução salina, NaOCl 0,5%, água ozonizada e 2 grupos controle. Os canais radiculares foram irrigados com 20 mL da solução selecionada por um período ininterrupto de 20 minutos, a solução irrigante foi mantida em repouso por 5 minutos e, a seguir, foram coletadas amostras dos canais radiculares (S2). Os dados foram avaliados pelos testes de Kruskal Wallis e Wilcoxon (P<0,05). O grupo NaOCl apresentou menor contagem microbiana (P<0,05) e maior percentual de redução microbiológica do que os outros grupos (P<0,05). Foi determinado que a água ozonizada não era um substituto adequado para o NaOCl.

PALAVRAS-CHAVE: Desinfecção; Endodontia; Ozônio; Hipoclorito de sódio

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