COMPARISON OF ANTIBACTERIAL EFFECTS OF 0,1% AND 0,3% CHLORINE DIOXIDE SOLUTIONS AGAINST *ENTEROCOCCUS FAECALIS* ATCC 29212

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ABSTRACT
Chlorine dioxide is a broad-spectrum antibacterial agent that operates on the same principle as sodium hypochlorite, oxidizing bacterial cells and leading to cell death. Unlike sodium hypochlorite, which is toxic to periradicular tissues, chlorine dioxide is considered more biocompatible and has no toxic effects on humans up to a concentration of 3000 ppm (0.3%). The concentration of 0.1% is commonly used in commercial mouthwashes, while 0.3% represents the highest non-toxic concentration of chlorine dioxide. This study aims to determine the comparative antibacterial effects of chlorine dioxide at concentrations of 0.1% and 0.3% on the growth of *Enterococcus faecalis*. This research is an in vitro experimental laboratory study. The test groups used chlorine dioxide concentrations of 0.1% and 0.3%, sodium hypochlorite 2.5% as a positive control, and aquades as a negative control. The antibacterial efficacy of chlorine dioxide was tested against *Enterococcus faecalis* using the disc diffusion method to determine the zone of inhibition. The results of the zone of inhibition measurements were then analyzed statistically using one-way ANOVA and Post Hoc tests. This study revealed that chlorine dioxide at 0.3% has a larger mean zone of inhibition (14,72 mm) than chlorine dioxide at 0.1% (12,19 mm), although it is still lower than sodium hypochlorite at 2.5% (18,83 mm). Chlorine dioxide at a concentration of 0.3% exhibits a higher antibacterial effect than chlorine dioxide at 0.1% against *Enterococcus faecalis.*

Keywords: antibacterial, chlorine dioxide, *Enterococcus faecalis*, inhibitory power, sodium hypochlorite

INTRODUCTION

Antibacterial properties are essential for any irrigant used in root canal treatment. The most commonly used synthetic antibacterial agent for root canal irrigation is sodium hypochlorite.[1] Besides its antibacterial properties, sodium hypochlorite can also dissolve necrotic tissue.[1,2] However, despite its advantages, sodium hypochlorite has many drawbacks, including its corrosive nature towards metals, can irritate skin and eye, unpleasant taste, strong odor, tendency to be unstable, toxic to periradicular tissues, and its potential to induce permanent damage to dental follicles, peripheral tissues, and oral mucosa.[2,3] Therefore, when used as a root canal irrigant, sodium hypochlorite must be applied carefully to avoid extrusion into the periapical area.[3]

Chlorine dioxide is a broad-spectrum disinfectant that has been popular and widely used since the late 19th century. It has numerous applications, including as a surface disinfectant solution, water treatment, food processing, animal care, dental practice water treatment, and a commercially available mouthwash.[4] Chlorine dioxide is a powerful oxidizing agent, with effectiveness comparable to or even greater than other oxidizers such as ozone or chlorine.[5] It can dissolve necrotic tissue, though not as effectively as sodium hypochlorite.[1]

Chlorine dioxide is a strong candidate for use as an irrigant due to its potent antimicrobial properties while being biocompatible.[3] Chlorine dioxide does not have toxic effects on humans at concentrations up to 3000 ppm (0.3%), does not cause allergic reactions, and is not carcinogenic as its reactions only produce minimal trihalomethanes or even not at all.[2,4] In contrast, sodium hypochlorite produces carcinogenic byproducts such as trihalomethanes and haloacetic acids.[1,6] Chlorine dioxide can eliminate various types of pathogenic microorganisms, including Gram-negative and Gram-positive bacteria, fungi, spores, and viruses.[4,5]

Enterococcus faecalis is a Gram-positive bacterium that plays a significant role as a predominant organism causing persistent periradicular lesions following root canal treatment.[7] Chlorine dioxide has been reported to inhibit the growth of *Enterococcus faecalis*. Kalay et al. found that 0.3% chlorine dioxide has higher antibacterial activity against *Enterococcus faecalis* than 2.5% sodium hypochlorite (NaOCl), 2% chlorhexidine gluconate (CHX), and 30% ethanol propolis extract.[8] Deka et al. showed that 0.1% chlorine dioxide had lower antibacterial activity against *Enterococcus faecalis* than 3% sodium hypochlorite (NaOCl) and 2% chlorhexidine gluconate (CHX).[1] This differs from Herczegh et al., who found that after 2 and 5 days, canals irrigated with 0.12% chlorine dioxide had the lowest reinfection rates compared to 5.25% sodium hypochlorite and 2% chlorhexidine gluconate.[4] Earlier studies have demonstrated inconsistent results in the growth-inhibiting capability of chlorine dioxide compared to sodium hypochlorite. No research has compared chlorine dioxide concentration levels to the pathogenic root canal bacterium *Enterococcus faecalis* growth. This study aims to compare the effectiveness of different concentrations of chlorine dioxide against the growth of the root canal pathogen *Enterococcus faecalis.*

MATERIALS AND METHODS

This study is in vitro experimental research that has received approval from the Medical and Health Research Ethics Committee of the Faculty of Medicine, Sriwijaya University (Protocol No. 321-2023). Six Petri dishes containing Mueller Hinton Agar media were prepared according to the number of repetitions. The Petri dishes were labeled and divided into four sections for each treatment, which included 0.1% chlorine dioxide, 0.3% chlorine dioxide (CDS Laboratoire Boreal), 2.5% NaOCl (OneMed), and distilled water. The 0.1% chlorine dioxide used in the study was prepared by diluting 0.3% chlorine dioxide (CDS Laboratoire Boreal) with sterile distilled water. A suspension of *Enterococcus faecalis* ATCC 29212, prepared beforehand, was taken using a sterile loop and evenly spread on the Mueller Hinton Agar media with a sterile cotton swab.[9] Discs were then impregnated with 0.01 ml of the treatment solutions and placed on the agar surface in their designated areas. The Petri dishes were incubated anaerobically at 37°C for 48 hours. The formed inhibition zones were measured manually using a caliper in millimeters (mm) (Figure 1). The antibacterial strength was determined according to the inhibition zone categories by Davis and Stout: >20 mm (very strong), 10-20 mm (strong), 5-10 mm (moderate), and <5 mm (weak).[10]

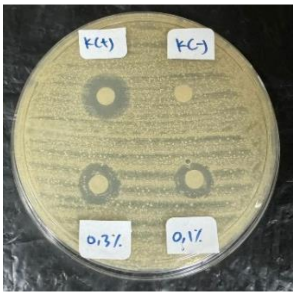

Figure 1. Inhibition zone of sodium hypochlorite (K+), distilled water (K-), chlorine dioxide 0,3% (0,3%), and chlorine dioxide 0,1% (0,1%)

Statistical Analysis

The data were analyzed using the Shapiro-Wilk test for normality and Levene's test for homogeneity. Data analysis proceeded with one-way ANOVA followed by *Post-Hoc* testing to determine statistical differences between groups using SPSS for Windows version 24 (IBM, Armonk, USA).

RESULTS

The antibacterial activity test results in Table 1 show that 2.5% sodium hypochlorite has the highest antibacterial efficacy in inhibiting the growth of *Enterococcus faecalis,* followed by 0.3% chlorine dioxide, 0.1% chlorine dioxide, and distilled water, with significant differences ($p < 0.05$). Table 2 indicates a highly significant difference in antibacterial activity between each treatment group ($p < 0.001$).

Table 1. Inhibition Zone of The Treatment Group and Control Group against *E.faecalis*

Group	Average ± SD (mm)
Chlorine Dioxide 0,3%	14.71 ± 0.32
Chlorine Dioxide 0,1%	12.19 ± 0.26
Sodium Hypochlorite	18.83 ± 0.25
Distilled water	0.00 ± 0.00

Table 2. *Games-Howell Post Hoc* Test for Comparison between Groups tested against *E.faecalis*

Group	*Chlorine Dioxide* 0,1%	*Chlorine Dioxide* 0,3%	NaOCl 2,5 % (K(+))	Aquades(K(-))
Chlorine Dioxide 0,1%		< 0,001*	< 0,001*	< 0,001*
Chlorine Dioxide 0,3%			< 0,001*	< 0,001*
NaOCl 2,5 % (K(+))				< 0,001*
Aquades(K(-))				

*) *highly significant*

DISCUSSION

Sodium hypochlorite (NaOCl) is a chlorine-releasing agent commonly used as an irrigant in root canal treatment due to its broad-spectrum antibacterial activity, ability to kill viruses and spores, and capacity to dissolve necrotic tissue.[11] However, sodium hypochlorite has several drawbacks, including its corrosiveness to metals, unpleasant taste, strong odor, and instability. It is also cytotoxic to vital tissues and can produce carcinogenic byproducts such as trihalomethanes and haloacetic acids.[6] Sodium hypochlorite has potential side effects and safety issues. Further research is needed into alternative substances to eliminate and eradicate *Enterococcus faecalis* with fewer side effects. Sodium hypochlorite functions through the ionization of its molecules, which triggers redox reactions with bacterial molecules. These reactions lead to protein denaturation, oxidation of lipids in the cell membrane/wall, enzyme deactivation, and DNA damage, ultimately resulting in bacterial cell death.[12]

Chlorine dioxide (ClO2) is a potent oxidizing agent that also belongs to the group of chlorine-releasing agents.[13] Chlorine dioxide is considered more biocompatible, does not have toxic effects on humans at concentrations up to 3000 ppm, and does not cause allergic reactions. Its bactericidal capability remains relatively constant within a pH range of 3-8 and can be applied in either gas or solution form.[14,15] Chlorine dioxide works by binding electrons from microbial structures such as cell walls, membranes, organelles, and genetic material, leading to the leakage of DNA and protein structures. This results in molecular imbalance and disrupts cellular homeostasis, ultimately causing microorganism death.[5,14,16]

Based on the inhibition zones formed, 0.3% chlorine dioxide has a higher antibacterial effect than 0.1% chlorine dioxide against *Enterococcus faecalis* (Table 1). This finding is aligned with the concentration-dependent principle, where the impact of a substance increases with its concentration.[17] Both concentrations of chlorine dioxide used in this study exhibit lower antibacterial effects than that of 2.5% sodium hypochlorite. However, according to Davis and Stout, all three are categorized as strong. This result may be due to the use of chlorine dioxide solutions with lower purity levels in this study.

High-purity (hyper-pure) chlorine dioxide solutions are more effective than commercially available solutions. Unlike chlorine dioxide processed by other methods, hyper-pure chlorine dioxide does not contain acid byproducts during its production process. As a true gas solution, hyper-pure chlorine dioxide is highly volatile and has a short contact time but remains reactive.[18] Additionally, chlorine dioxide's oxidative capacity can reach up to five electrons per microbial species per molecule.[5] Therefore, hyper-pure chlorine dioxide can kill bacteria without harming human cells.[18]

Previous studies have shown varying results related to the differences in the chlorine dioxide solution preparations. Research by Szabo et al. and Herczegh et al., which used hyper-pure chlorine dioxide, demonstrated antibacterial effects equal to or even more significant than 5.25% sodium hypochlorite despite using a lower concentration of 0.12%.[4,18] In contrast, Deka et al., using a commercial mouthwash containing 0.1% chlorine dioxide, found lower antibacterial effects than 3% sodium hypochlorite. Meanwhile, Kalay et al., using agricultural irrigation solutions containing 0.3% stabilized chlorine dioxide, observed higher antibacterial effects than 2.5% sodium hypochlorite.[8] These studies lacked measurements of purity levels and additional details about any preservatives contained in the solutions because they use different types of formulations, which could introduce bias and affect the observed antibacterial effects.

This study was conducted following scientific procedures but still has limitations. It used pure chlorine dioxide solutions but did not provide additional information from the manufacturer and fabricator about the purity levels of the chlorine dioxide. Furthermore, chlorine dioxide remains challenging to obtain due to limitations in producers and distributors in Indonesia.

CONCLUSION

Chlorine dioxide at a concentration of 0.3% has a higher antibacterial effect than 0.1% against *Enterococcus faecalis*. However, the antibacterial efficacy of 0.3% chlorine dioxide is still lower than that of 2.5% sodium hypochlorite.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

1. Deka A, Abhijit Anil T, Barua P, Paul R. An Ex Vivo Comparative Study Determining The Bactericidal Activity of 3 Different Irrigants Against Enterococcus faecalis. International Journal of Oral Health Dentistry. 2017;3(2):87–8.
2. Aberna R, Mohan G, Saranya S. Enterococcicidal Activity of Chlorine Dioxide and Lemon Extract Endorse Them as Contemporary Root Canal Irrigants. Archives of Medicine and Health Sciences. 2019;7(2):169.
3. V SN, K S. An In Vitro Study of Chlorine Dioxide as A Root Canal Irrigant. IJOCR. 2013;1(2):10.
4. Herczegh A, Ghidan Á, Friedreich D, Gyurkovics M, Bendo Z, Lohinai Z. Effectiveness of A High Purity Chlorine Dioxide Solution in Eliminating Intracanal Enterococcus faecalis Biofilm. Acta Microbiol Immunol Hung. 2013 Mar 1;60(1):64–8.
5. Andrés CMC, Lastra JMP de la, Andrés Juan C, Plou FJ, Pérez-Lebeña E. Chlorine Dioxide: Friend or Foe for Cell Biomolecules? A Chemical Approach. Vol. 23, International Journal of Molecular Sciences. 2022; 23:20-1.
6. Rodríguez PYA. Comparison of the Antimicrobial Effect of Chlorine Dioxide, Sodium Hypochlorite and Chlorhexidine, on Bacteria Isolated from the Root Canal. J Dent Oral Sci. 2022 Oct 10;4(4):2.
7. Krishnaraj S, Mds S, Kl Md S, Rao M, Phd KG. The Effect of Chlorine Dioxide on Enterococcus faecalis in Exponential, Stationary and Starvation Phases in Extracted Human Teeth - an In Vitro Study. Int J Med (Dubai). 2014;2(2):63.
8. Kalay TS, Kara Y, Karaoglu SA, Kolayli S. Evaluation of Stabilized Chlorine Dioxide in Terms of Antimicrobial Activity and Dentin Bond Strength. Comb Chem High Throughput Screen. 2021 Aug 16;25(9):1429–31.
9. Mirna Kuntari L, Hadriyanto W, Ema M. Perbedaan Daya Antibakteri Klorheksidin 2% dan Berbagai Konsentrasi Sodium Hipoklorit Kombinasi Omeprazole 8,5% terhadap Enterococcus Faecalis. J Ked Gi. 2014;5(2):141.
10. Alfredi Anis Fadhila, Welly Darwis, Risky Hadi Wibowo, Sipriyadi, Rochmah Supriati. Antibacterial activity of the ethanolic extract of Sembung Rambat (Mikania micrantha Kunth) leaves against Bacillus subtilis. Natural Science: Journal of Science and Technology. 2021;10(1):8.
11. Topbas C, Adiguzel O. Endodontic Irrigation Solutions: A Review. International Dental Research. 2017 Dec 31;7(3):55.
12. Widiastuti D, Fikria Karima I, Setiyani E. Efek Antibakteri Sodium Hypochlorite terhadap Staphylococcus aureus Antibacterial Effect of Sodium Hypochlorite to Staphylococcus aureus. Vol. 11, Jurnal Ilmiah Kesehatan Masyarakat. 2019;11(4):305-6.

13. Riedel S, Morse SA, Mietzner T, Miller S. Jawetz Melnick & Adelbergs Medical Microbiology 28th Edition [Internet]. 28th ed. McGraw-Hill Education; 2019:64.
14. Eryilmaz M, Kaskatepe B, Kiymaci ME, Erol HB, Simsek D, Gümüstas A. In Vitro Antimicrobial Activity of Three New Generation Disinfectants. Tropical Journal of Pharmaceutical Research. 2016 Oct 1;15(10):2193.
15. Wexler P. Encyclopedia of Toxicology 3rd Edition. Vol. 1. Elsevier; 2014;864.
16. Ersoy ZG, Dinc O, Cinar B, Gedik ST, Dimoglo A. Comparative Evaluation of Disinfection Mechanism of Sodium Hypochlorite, Chlorine Dioxide and Electroactivated Water on Enterococcus faecalis. LWT. 2019 Mar 1;102:208–12.
17. Bernier SP, Surette MG. Concentration-dependent activity of antibiotics in natural environments. Vol. 4, Frontiers in Microbiology. Frontiers Research Foundation; 2013;4(20):4.
18. Szabó EV, Huszta B, Polyák M, Ruksakiet K, Bernáth R, Ghidán Á, et al. Antimicrobial efficacy of sodium hypochlorite and hyper-pure chlorine dioxide in the depth of dentin tubules in vitro. BMC Oral Health. 2023 Dec 1;23(1):2-7.