Comparison of chlorine dioxide and dichloroisocyanurate disinfectants for use in the dental setting

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ABSTRACT

Aim: The aim of this study was to compare the antimicrobial properties of a slow release noncorrosive chlorine dioxide with those of sodium dichloroisocyanurate to establish their possible use in the dental settings.

Materials and methods: Disinfectant solutions were prepared according to manufacturers' instructions and tested against *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus mutans* NCTC 1044, *Candida albicans* ATCC 90028, *Bacillus subtilis* ATCC 15244 spores, *Mycobacterium tuberculosis* ATCC 25177, *Mycobacterium avium subsp. avium* ATCC 25291 and Hepatitis B virus using the Standard quantitative suspension test. The shelf-lives of the disinfectants were also determined.

Results: Both disinfectants killed all the test organisms within 30 seconds. *B. subtilis* spores were killed in 2 and 2.5 minutes by chlorine dioxide and sodium dichloroisocyanurate respectively. When diluted solutions of these disinfectants were stored in screw cap bottles, they retained their activity for at least 30 days.

Conclusions: Chlorine dioxide and sodium dichloroisocyanurate containing disinfectants can be used in the dental settings for surfaces and heat sensitive instruments. However, chlorine dioxide is advantageous because it is noncorrosive and the effective concentration is lower than that recommended for sodium dichloroisocyanurate.

Key words: Disinfectant; Hypochlorite; sodium dichloroisocyanurate; Chlorine dioxide; HBV; Mycobacteria, chlorine

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INTRODUCTION

Chlorinated compounds are often used in dental clinics and laboratory environment due to their broad spectrum of antimicrobial activity, low toxicity, low cost and efficacy in biofilms.[1] However, they corrode metals and are inactivated by organic matter at high concentrations. A slow-release chlorine compound, sodium dichloroisocyanurate is used in healthcare settings; however, it too is corrosive. Slow-release chlorine dioxide disinfectants have been developed containing corrosion inhibitors which are extensively used in the industrial settings.

The use of chlorine dioxide containing products in dentistry has been explored. Studies have shown that in mouthrinses it is effective for the management of chronic atrophic candidiasis, denture stomatitis, and the control of plaque accumulation, periodontal pathogens and oral malodor.[2-5] The efficacy of sodium dichloroisocyanurate for disinfection of radiographic films and irreversible hydrocolloid impression material has also been established.[6,7] None of the above studies have tested the efficacy of these disinfectants against Mycobacteria and Hepatitis B virus. In addition, anti hepatitis B virus activity of chlorine dioxide has not been established.

This study compared the antimicrobial effect of a chlorine dioxide and a chlorine generating disinfectant on the contaminants commonly present on dental instruments and in the dental surgery.

METHODS

Disinfectants

Chlorine dioxide (Aseptrol®) tablets manufactured by Engelhard Aseptrol technology, USA and supplied by Waylor Trading and Logistics cc, South Africa were used in the study. A 48ppm solution of ClO2 was prepared by dissolving one 1.5g tablet of Aseptrol® in 2.5 litres of tap water according to the manufacturer's instructions. The mixture was allowed to stand for 20 minutes. A further two- fold dilution of the disinfectant was prepared in tap water to give a final concentration of 24ppm solution.

Sodium dichloroisocyanurate (PreSept®, Johnson and Johnson, South Africa) solution was prepared by dissolving seven 2.5g tablets in one litre of tap water, according to the manufacturer's instruction. This concentration is generally used for blood spillage and gave a final solution of 10 000ppm available chlorine. Both disinfectant solutions were prepared shortly before each experiment.

Test organisms and inocula

The antimicrobial properties of both disinfectants were tested against *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus mutans* NCTC 1044, *Candida albicans* ATCC 90028, *Bacillus subtilis* (ATCC 6633) spores, *Mycobacterium tuberculosis* (ATCC 25177), *Mycobacterium avium subsp. avium* (ATCC 25291) and Hepatitis B virus (HBV). Stock cultures of *S. aureus*, *P. aeruginosa, S. mutans, C. albicans* and *B. subtilis* were stored in semisolid agar and subcultures were prepared as required. *M. tuberculosis* and *M. avium subsp. avium* were stored at -70°C in aliquots. Hepatitis B virus was obtained from the immunology laboratory, National Health Laboratory Services, Johannesburg, South Africa.

The test organisms were cultured on the selective media to obtain a primary culture for preparation of inoculum suspensions. *S. aureus* and *P. aeruginosa* were grown on Tryptone Soy agar and incubated at 37°C for 24 hours and *S. mutans* on Blood agar and incubated at 37°C for 48 hours under carbon dioxide. *C. albicans* was grown on Sabouraud Dextrose agar and incubated at 37°C for 48 hours. The resultant growth was harvested using a sterile wire loop, which was then suspended in 20ml sterile distilled water and the optical density was adjusted to 0.2 McFarland standards. *M. tuberculosis* and *M. avium subsp. avium* were grown in BACTEC MGIT 960 (7ml of modified Middlebrook 7H9 Broth base with supplements) incubated at 37°C until it became positive. The number of organisms in the inoculum was determined for each experiment using serial dilutions.

B. subtilis was plated on Tryptone Soy agar which contained 2mg/l $MnSO_4.4H_2O$ to enhance sporulation and was incubated at 37°C for 7 days.[8] The Schaefer and Fulton's method for staining spores was used to confirm >90% spore production.[9] The sporulating culture was suspended in 20mL of sterile distilled water and placed in a water-bath at 70°C for 20 minutes in order to kill the vegetative cells. The optical density of the resultant spore suspension was adjusted to 0.2 McFarland standards (approximately 10^6 test organisms per ml) and used as the inoculum.

Test procedures for percentage kill

S. aureus, P. aeruginosa, S. mutans, C. albicans and B. subtilis

Standard quantitative suspension tests or exposure tests were performed.[10] Two millilitres of 48 and 24ppm noncorrosive chlorine dioxide and 10000 ppm sodium dichloroisocyanurate solutions were inoculated with 20µl of inoculum, containing approximately 10^6 test organism per milliliter, and with 10µl of skim milk, used to simulate organic material. After inoculation, 20µl of the inoculated test compound was removed every 30 seconds for 5 minutes, neutralised with a universal neutraliser (quarter strength Ringer's solution, 0.5% Tween 80 and 0.5% sodium thiosulphate), diluted and spread on an appropriate medium to determine the number of surviving microorganisms. The culture plates were incubated at 37°C for 24 or 48 hours depending on the type of organisms. The colony count for each plate was determined and log reduction was calculated using the inoculum count. The time taken to kill the challenged organisms was recorded against the log reduction. Each test was repeated five times.

M. tuberculosis and *M. avium subsp. avium*

The quantitative suspension test described by Hernández *et al*. was modified and used in this study.[11] Cultures were grown on Mycobacteria Growth Indicator Tube 960 medium at 37°C until it became positive. A viable bacterial count was obtained using a serial dilution technique. 20µl of this prepared suspension and 10µl of skim milk was added to 2ml of disinfectant. After inoculation, 20µl of inoculated test compound was removed every 30 seconds for five minutes, neutralised with a universal neutraliser, and spread on Middlebrook agar plate and incubated at 37°C for four to six weeks. The colony count for each plate was determined and the log reduction was calculated for each contact time period. Each experiment was repeated five times.

Hepatitis B virus

Viral particles (1.0 x 10^5 IU/ml) were obtained from the immunology laboratory of the National Health Laboratory Services, Johannesburg and used as an inoculum. Two millilitre of the test disinfectant solutions were inoculated with 20µl of inoculum and 20µl of disinfectant was removed every 30 seconds for five minutes, neutralised and transferred into 2ml HBV-negative human EDTA-plasma. Skim milk was not added because human plasma contains organic material. The samples were processed on a COBAS® AmpliPrep/COBAS® Taqman® system (Roche Molecular Systems, Inc, Branchburg, NJ) using the Roche COBOS TaqMan HBV Test for quantitation of hepatitis B virus. COBAS AmpliPrep-COBAS Taqman HBV Test is an in vitro real-time PCR amplification and detection test used for the quantitation of Hepatitis B virus (HBV) DNA in human plasma.[12] This test is FDA approved and can detect a HBV DNA range from 12 to 110 000 000 IU/ml. In each test the intact HBV was determined and the number of degraded HBV DNA was calculated. A quantitation standard was run for each sample to compensate for inhibition and to control the preparation and amplification processes.

Table 1: Log reduction of the test organisms by chlorine dioxide and chlorine releasing disinfectants.

Disinfectant	Test org.	Mean No. of challenged org. Log cfu or iu/ml	Log reduction and contact time period. Mean ± SD. n=5				
			½ min	1 min	1½ min	2 min	2½ min
Aseptrol® 48ppm available chlorine dioxide	*S. aureus*	6.67	6.67±0	*	*	*	*
	P. aeruginosa	6.04	6.04±0	*	*	*	*
	S. mutans	6.14	6.14±0	*	*	*	*
	C. albicans	4.78	4.78±0	*	*	*	*
	M. tuberculosis	5.7	5.7±0	*	*	*	*
	M. avium complex	4.6	4.6±0	*	*	*	*
	Hepatitis B virus	5.0	5.0±0	*	*	*	*
	***B. subtilis* spores**	6.83	0.42±0.09	1.56±0.49	2.05±0.45	6.83	6.83
Aseptrol® 24ppm available chlorine dioxide	*S. aureus*	6.67	6.67±0	*	*	*	*
	P. aeruginosa	6.04	6.04±0	*	*	*	*
	S. mutans	6.14	6.14±0	*	*	*	*
	C. albicans	4.78	4.78±0	*	*	*	*
	M. tuberculosis	5.7	5.7±0	*	*	*	*
	M. avium complex	4.6	4.6±0	*	*	*	*
	Hepatitis B virus	5.0	5.0±0	*	*	*	*
	***B. subtilis* spores**	6.83	0.39±0.11	1.51±0.61	1.85±0.00	6.83±0	6.83±0
Presept® 10000ppm available chlorine	*S. aureus*	6.67	6.67±0	*	*	*	*
	P. aeruginosa	6.04	6.04±0	*	*	*	*
	S. mutans	6.14	6.14±0	*	*	*	*
	C. albicans	4.78	4.78±0	*	*	*	*
	M. tuberculosis	5.7	5.7±0	*	*	*	*
	M. avium complex	4.6	4.6±0	*	*	*	*
	Hepatitis B virus	5.0	5.0±0	*	*	*	*
	***B. subtilis* spores**	6.83	0.55±0.38	1.21±0.78	2.05±0.45	2.05±0.44	6.83±0

* Results same as ½ min for relevant cultures

Shelf-life test

Chlorine dioxide and sodium dichloroisocyanurate solutions were prepared and stored in screw cap bottles. Approximately 10^6 cfu/ml of *S. aureus, P. aeruginosa, S. mutans, C. albicans, M. tuberculosis* and *M. avium subsp. avium* were exposed to disinfectant for 30 and 60 seconds to determine the survival of these organisms. This procedure was repeated every five days for two weeks and thereafter every day for 37 days, using the same disinfectant solutions.

RESULTS

Both chlorine dioxide and sodium dichloroisocyanurate killed the challenged number of bacteria, fungi and viruses within 30 seconds of exposure (Table 1) and *B. subtilis* spores in two to 2.5 minutes. The shelf-life test showed that chlorine dioxide killed all the test microorganisms within 30 seconds for up to 27 days whereas sodium dichloroisocyanurate was still effective after 37 days (Table 2).

Table 2: Antimicrobial shelf-life of chlorine dioxide and chlorine releasing disinfectants.

Organisms	Disinfectant	Effectiveness (days) Time taken to kill	
		30 seconds	60 seconds
S. aureus	Aseptrol	27	27
	Presept	>37	>37
P. aeruginosa	Aseptrol	35	>37
	Presept	>37	>37
S. mutans	Aseptrol	35	>37
	Presept	>37	>37
C. albicans	Aseptrol	30	>37
	Presept	>37	>37
M. tuberculosis	Aseptrol	>28	>28
	Presept	>28	>28
M. avium complex	Aseptrol	>28	>28
	Presept	>28	>28

DISCUSSION

The efficacies of a chlorine dioxide and a chlorine generating disinfectant were tested against common microorganisms including HBV and mycobacterium that exist in the dental settings. Both the disinfectants proved to be effective on all the vegetative form of test organisms and HBV within 30 seconds and *B. subtilis* spores after 2.5 minutes which suggests that both these compounds can be considered intermediate to high-level disinfectants which can be used in the dental setting. Although chlorinated disinfectants are known to have a low sporicidal activity, this study showed good sporicidal property. Similar results were found by Coates[13] who suggested the use of 140 ppm chlorine dioxide in the semi-critical conditions such as endoscope decontamination. Although isolates of *M. avium* (environmental contaminants) are known to be resistant to chlorine dioxide,[14,15] in our study the solution effectively killed *M. avium* within 30 seconds. Results obtained by Hernández

et al.[16] also showed that chlorine dioxide wipes at 200 ppm can kill *M. avium* within 30 seconds. However, our effective test concentrations were much lower concentration (24 and 48ppm). The present study also established an *in vitro* anti-HBV activity of chlorine dioxide. Glutaraldehyde is known to have similar efficacy against a broad spectrum of organisms but the use of chlorine dioxide has been suggested over glutaraldehyde due to the chemical health hazards posed by the latter such as skin and mucosal sensitivity.[17]

Although chlorinated compounds are generally known to have antimicrobial activity against a wide range of organisms,[18-19] they are considered an intermediate level disinfectant because they have low sporicidal activity, are relatively unstable and their activity is dependent on pH.[20] Demand-release chlorine compounds, such as the chlorine dioxide tested in this study, were developed because chorine is then retained longer, exerting a more prolonged bactericidal effect and the compound is stable. Chlorine dioxide is also more rapidly effective against microorganisms than is chlorine.[21] In this study both disinfectants were shown to exhibit good sporicidal properties and could therefore be used as for intermediate to high level disinfection of semi-critical and non-critical instruments such as dental mouth mirrors, amalgam condenser, reusable impression trays, dental handpieces, radiographic head cone and radiographic films[22] and as a sterilant for semi-critical heat sensitive instruments. Chlorine dioxide has an additional advantage because it is non-corrosive as claimed by the manufacturer and effective disinfection is achieved at low concentrations. Products containing chlorine dioxide concentrations of 150 to 1100ppm have shown antimicrobial and sporicidal activity.[13,21,23] However our results showed that even at 24ppm chlorine dioxide exerts an antimicrobial effect including activity against spores, HBV and mycobacteria.

In the dental surgery chlorine dioxide can be used as a pre-soaking solution for dirty instruments and for the decontamination of radiographic films before processing. Quantitative carrier tests were not performed on these compounds because similar tests were performed by Rweyendela *et al.*[7] who tested the efficacy on contaminated impression material blocks and found similar results. However, they did not test the efficacy of chlorine dioxide on HBV and Mycobacteria.

Housekeeping surfaces such as hospital and surgery floors, bedside units, dental chairs and light handles are often contaminated and generally require EPA registered hospital disinfectant/detergent depending on the nature of the surface and the type and degree of contamination.[24] Sodium dichloroisocyanurate has been recommended for this type of surface[25] at 1000 ppm. Our results show that even at 24ppm chlorine dioxide will eliminate most of the contaminants including mycobacteria and HBV within 30 seconds which suggests that lower concentrations could be used for housekeeping surfaces. Furthermore, in our study, organic material (skim milk) did not compromise the efficacy of disinfectants which suggests that body fluids will not interfere with the disinfection process. However, the pre-cleaning of heavily contaminated instruments is advantageous as suggested by Isomoto *et al.*[26]

Although the manufacturers of chlorinated compounds recommend the daily preparation of solutions, our results showed that both disinfectant solutions can be effective for up to 27 days. Although the recommended concentration of chlorine dioxide is 48ppm, our results showed that 24ppm was just as effective. These results have financial as well as time implications because the daily preparation of disinfectant solution can be avoided. Similar results were found by Rutala et al. who showed that brown screw cap bottles were ideal for the storage of chlorinated disinfectants.[27]

CONCLUSIONS

In conclusion this study has shown that a non-corrosive slow release chlorine dioxide and chlorine releasing sodium dichloroisocyanurate disinfectants are microbiocidal after 30 seconds exposure and sporicidal after two to three minutes in the presence of organic material. Both the disinfectant solutions were effective for 27 to 37 days if stored in screw cap bottles. They have the potential to be used in the dental setting as a surface disinfectant and a sterilant for semi-critical heat sensitive instruments. Chlorine dioxide has an additional advantage because it is non-corrosive and the effective concentration is much lower than that required for sodium dichloroisocyanurate.

Clinical significance

Chlorine dioxide has a broad-spectrum antimicrobial property and therefore it can be used in the dental settings for surfaces and heat sensitive instruments as a sterilant.

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