An In Vitro Evaluation of the Antibacterial Efficacy of Chlorine Dioxide on *E. faecalis* in Bovine Incisors

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Abstract

This study investigated the ability of chlorine dioxide to eliminate *Enterococcus faecalis* from dentinal tubules of bovine incisors. Thirty-seven extracted bovine incisor roots were sectioned into seventy-four 5 mm disks. Standardized lumens were filled with either sterile Brain Heart Infusion Broth (contamination controls, $n = 10$) or BHI containing *E. faecalis* (1.0×10^8 cfu/ml). Disks were incubated in 5% CO_2 at 37°C for 72 h. To simulate endodontic instrumentation the lumens were again enlarged. Sixty disks were randomly divided into four experimental groups and filled with one of the following irrigants: 10% Clidox-S (chlorine dioxide), 13.8% BioClenz (chlorine dioxide), 5.25% Clorox, or saline. The disks were incubated for 30 min and were then frozen, pulverized, serially diluted in phosphate buffered saline, and plated on BHI plates in triplicate. Total colony forming units were counted macroscopically. Statistical analysis of the data was performed with a Kruskal-Wallis one-way ANOVA on ranks ($p < 0.05$, $n = 60$). Bacterial counts, expressed in $\log_{10}$ cfu/disk were as follows (">" denotes significant differences): Saline > Clidox-S = BioClenz > Clorox. All negative controls were sterile. Chlorine dioxide and NaOCL were both effective in eliminating *E. faecalis* from the dentinal disks within 30 min.

Key Words

Irrigants, chlorine dioxide, sodium hypochlorite, *E. faecalis*

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The removal of pulpal and dentinal debris and the elimination of viable microorganisms from the root canal system are of paramount importance during endodontic therapy. Bacteria are the main factor in pulpal and periapical inflammation, and failure to effectively eliminate them and their by-products could result in persistent irritation and impaired healing (1, 2). It has been widely reported that viable bacteria can remain within the canal system even after chemomechanical preparation (3, 4). A variety of irrigants have been used in endodontics in an attempt to eliminate these bacteria.

Instrumentation and irrigation with 5.25% sodium hypochlorite can reduce bacterial concentrations, but cannot eliminate *Enterococcus faecalis* from the canal system (5). *E. faecalis*, a gram-positive bacterium, is often used to investigate the effectiveness of endodontic irrigants and medicaments. This microorganism is frequently isolated from previously obturated canals that exhibit chronic periapical pathology (6–9). Many authors have demonstrated *E. faecalis* ' ability to resist intracanal medicaments and to survive as a single organism within the canal system (10–12).

An ideal endodontic irrigant should be antimicrobial, nontoxic, capable of dissolving tissue or debris, able to lubricate the canal, and helpful in removing the smear layer (13). Sodium hypochlorite is currently one of the most commonly used irrigants in endodontic therapy, and its antimicrobial and tissue dissolving properties have been widely reported (14–16). In addition it is inexpensive, has a long shelf life, and is readily available. The disadvantage of using 5.25% sodium hypochlorite as an endodontic irrigant is its extreme cytotoxicity. If extruded into the periapical tissues during therapy, it can cause excruciating pain, immediate swelling, and profuse bleeding (17, 18). The ensuing local tissue necrosis and ecchymosis will persist for several days. Although most cases are self-limiting, some require surgical intervention to contain the tissue destruction (19). Therefore, the search for a less toxic but equally effective endodontic irrigant should continue.

Chlorine dioxide, much like sodium hypochlorite, is used to eliminate contaminants from drinking water. Its disinfectant properties have been recognized since the early 1900s, and it was registered with the EPA in a liquid form for use as a disinfectant and sanitizer in 1967. Current uses include food processing, water treatment, veterinary care, surface disinfection, and dental waterline treatment. Its powerful oxidizing properties enable it to kill bacteria by disrupting the transport of nutrients across the cell wall (20). This strong antibacterial activity makes it a potentially useful endodontic irrigant.

The purpose of this study is to evaluate the antibacterial efficacy of chlorine dioxide as an endodontic irrigant.

Materials and Methods

A modified version of Haapasalo and Ørstavik's in vitro model for testing the antibacterial activity of medicaments in the dentinal tubules of bovine incisors was used in this study (12). Unlike the original model, the cementum was not removed from the root surfaces, and only the internal lumens of the bovine incisors were exposed to *E. faecalis*.

Thirty-seven intact bovine central incisors were extracted from frozen jaws and stored for 72 h in 5.25% sodium hypochlorite (NaOCl) for surface disinfection and soft tissue dissolution. The apical 5 mm was removed with a diamond saw (Isomet, Buehler LTD, Evanston, IL) at slow speed (5000 rpm) with water coolant. The roots were further sectioned into cylindrical dentinal disks 5 mm in height, and the lumens were standardized to 2.5 mm with an ISO 025 round bur (Brasseler, Savannah, GA).

TABLE 1.

	E.faecalis, log cfu	*SD*	*n*	SE	Antilog	% Kill
Saline	6.2	0.8	15	0.28	1584893	0
BioClenz	5.1	0.8	15	0.28	125892	100
Clidox	5.4	0.4	15	0.14	251188	100
NaOCl	2.4	2.9	15	1.01	251	100

The smear layer was removed by immersing the specimens in an ultrasonic bath first with 17% Ethylenediaminetetraacetic acid (EDTA) for 4 min, and then with 5.25% NaOCl for 4 min. The disks were next placed in distilled water and sterilized in a steam autoclave for 15 min at 121°C. All remaining manipulations of the disks were performed under a laminar flow hood (NUAIRE, Plymouth, MN) to avoid contamination from outside organisms.

The dentin disks were dried with sterile gauze and mounted in individual 22 mm diameter tissue wells (Corning Cell Wells, Corning Glass Works, Corning, NY) on a base of rope wax approximately 5 mm tall. The lumens of the dentin disks were filled with 25 μl Brain Heart Infusion (BHI) broth (Difco, Detroit, MI) containing 1.0×10^8 cfu/ml of *E. faecalis* (ATCC 29212) and incubated at 37°C in 5% CO_2 for 72 h. Saline was added to the tissue wells surrounding the wax base to maintain a humid environment. The lumens were replenished with sterile BHI every 24 h. A contamination control group consisting of ten disks was treated as above, except they were filled with 25 μl sterile BHI broth.

The disks were removed from the tissue wells, and the BHI broth was flushed from the lumens with approximately 2 ml of sterile saline. The lumens were enlarged to 2.9 mm with a sterile slow speed ISO 029 round bur to mimic clinical endodontic instrumentation. Each disk was rinsed with sterile saline to remove any gross dentin shavings and then patted dry with sterile gauze. The dentin disks were remounted in sterile individual 22 mm diameter tissue wells on a rope wax base approximately 5 mm tall.

Sixty disks were randomly divided into four experimental groups and filled with one of the following irrigants:

Group 1: 10% Clidox-S (Pharmacal Research Laboratories, Naugatuck, CT)
Group 2: 13.8% BioClenz (Frontier Pharmaceutical, Melville, NY)
Group 3: 5.25% Clorox (The Clorox Co., Oakland, CA)
Group 4: Saline

The chlorine dioxide products, Clidox-S and BioClenz, were prepared according to the manufacturer's instructions. The sodium hypochlorite solution for group 3 was prepared by diluting 6.11% Clorox with sterile water.

The lumens were then filled with one of the test irrigants, saline was added to the tissue wells to maintain a humid environment and the plates were incubated at 37 °C in 5% CO_2 for 30 min. Each six well plate contained five experimental disks and one contamination control disk.

The disks were removed from the tissue wells and dried by patting with sterile gauze. Each disk was transferred to a labeled, sterile 10 ml vial and frozen in a −72°C freezer. The disks were weighed, pulverized in liquid nitrogen with a mortar and pestle, transferred to sterile vials containing 2 ml PBS (Phosphate Buffered Saline), and vortexed for 5 min. Serial dilutions (1:10, 1:100, 1:1,000, 1:10,000, and 1:100,000) were made, and a 100-μl aliquot of each dilution was plated on BHI plates in triplicate. The BHI plates were incubated in 5% CO_2 at 37°C, and the number of bacterial colony forming units were counted at 24 h. Dilutions that were used on plates containing between 30 and 300 bacterial colonies were used preferentially for data analysis.

Statistical analysis was performed with a Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks with an all-pairwise multiple comparison procedure (Bonferroni *t* test) ($p < 0.05$, $n = 60$).

Results

All contamination controls were sterile. Bacterial counts, expressed in $\log_{10}$ cfu/disk, for each irrigant were as follows (">" denotes significant differences): Saline (6.2 ± 0.8) > Clidox-S (5.4 ± 0.8) = BioClenz (5.1 ± 0.4) > Clorox (2.4 ± 2.9) (Table 1). Sodium hypochlorite was significantly more effective at eliminating *E. faecalis* than any of the other irrigants. BioClenz and Clidox-S were significantly better than saline, which was ineffective at eliminating *E. faecalis*. There was no significant difference between the chlorine dioxide groups (Fig. 1).

Discussion

Haapasalo and Ørstravik's model for in vitro infection and disinfection of dentinal tubules was modified for this study to more closely resemble clinical conditions (12). The cementum was left intact, only the internal lumens were inoculated with a known quantity of the bacteria for a 72-h period and the lumens were instrumented after the disks were infected to mimic clinical endodontics. Another important modification developed by Behnen et al. was the use of quantitative microbiology to more accurately compare the efficacy between experimental groups (21).

E. faecalis was selected for this experiment because it is the most commonly isolated bacteria in failed endodontic cases (6, 7). It can survive chemomechanical preparation and remains viable even after calcium hydroxide therapy. McHugh et al. found that pH 10.5 to 11.0 retards growth of *E. faecalis*, whereas at pH 11.5 or greater *E. faecalis* is destroyed (22). Lynne et al. found that 10% $Ca(OH)_2$ may be more effective than Peridex or 10% $Ca(OH)_2$ in Peridex for the elimination of *E. faecalis* from dentin tubules (23). *E. faecalis* is capable of surviving as a single species within the canal, which makes it ideal for this study (6). The selection of a 72-h inoculation period was based on a previous study that showed complete penetration of the bovine tubules with this period (24). To eradicate all of the bacteria, an irrigant must possess powerful antimicrobial properties and be highly diffusible.

Chlorhexidine has gained favor as an antimicrobial irrigant. Lin et al. found that Peridex showed significantly larger zones of inhibition compared with CaOH and no statistically significant difference was found between Peridex and the combination of $Ca(OH)_2$ and Peridex (25). A study by Podbielski et al., however, supports the use of a combination of $Ca(OH)_2$ and chlorhexidine for faster eradication of *Peptostreptococcus micros* and *Streptococcus intermedius* from infected root canals (26). Evanov et al. found that raising the temperature of 10% $Ca(OH)_2$ or 0.12% Chlorhexidine gluconate to 46°C enhanced the antibacterial action of both irrigants (27). Other researchers have placed Chlorhexidine in slow releasing devices, Lin et al. (28), or mixed it with ProRootMTA to enhance it's antimicrobial activity Stowe et al. (29).