THE EFFECT OF DIFFERENT CONCENTRATIONS OF THE PROTON PUMP INHIBITOR OMEPRAZOLE MIXED WITH SODIUM HYPOCHLORITE ON ENTEROCOCCUS FAECALIS: AN IN VITRO STUDY

Abeer Saba*

ABSTRACT

Objective: The aim of this study was to evaluate in vitro, the antibacterial effectiveness of different concentrations of one brand of commercially available proton pump inhibitor omeprazole (Omez), mixed with 5.25% sodium hypochlorite (NaOCl) on Enterococcus faecalis.

Methods: Four concentrations (6%, 8%, 10% and 12%) of omeprazole were prepared and mixed with 5.25% NaOCl. The agar diffusion test was used to measure the antibacterial activities of these agents against E faecalis as compared to 2% CHX and 5.25% NaOCl. The size of zones of inhibition was measured in mm and the results were statistically analyzed. The significance level was set at P ≤ 0.05.

Results: The 2% CHX showed statistically significant more antibacterial effect than 5.25% NaOCl. The four concentrations of omeprazole mixed with 5.25% NaOCl had similar antibacterial effect to the 2% CHX with best results obtained by the 6% omeprazole/NaOCl mix which was more efficient than the 2% CHX although no significant difference was detected.

Conclusions: Results of this study showed that mixing omeprazole with 5.25% NaOCl did improve the antibacterial effect of NaOCl against E faecalis as compared to 5.25% NaOCl alone. The study suggested an easy and reproducible method of preparing omeprazole/NaOCl mix for clinical use.

KEYWORDS: Proton pump inhibitor, omeprazole, sodium hypochlorite, chlorhexidine, Enterococcus faecalis, agar diffusion test.

INTRODUCTION

Persistence of microorganisms in the root canal is believed to be one of the main causes of endodontic treatment failure (1, 2, 3). In spite of different intracanal disinfection measures; chemomechanical preparation techniques and intracanal medications, few bacterial species might still survive (4, 5). Enterococcus faecalis is a facultative anaerobic Gram-positive bacterium that has been found in a high percentage of endodontic failures and

* Lecturer of Endodontics, Department of Endodontics, Faculty of Oral and Dental Medicine, Cairo University, Egypt.
cases showing persistent periapical lesions after treatment (6, 7, 8). It has the ability to withstand intracanal disinfecting measures used during treatment and endure starvation in cleaned and obturated canals (9). E faecalis has also demonstrated high resistance to several medicaments including calcium hydroxide (9, 10). Hence, once established in the root canal, its eradication by conventional means might become difficult (11).

The ability of E faecalis to survive adverse challenges is attributed to its ability to elicit variety of stress responses that enhances its survival and recover. Among these responses is a functioning proton pump in its cell membrane with the ability to acidify the cytoplasm when E faecalis is exposed to high pH levels, thus pH homeostasis is maintained so that enzymes and proteins continue normal function (12, 13).

Omeprazole is the first of a class of drugs, the proton pump inhibitors (PPIs), whose main action is the reduction of gastric acid production through selective and irreversible inhibition of the gastric H+K+ ATPase system (proton pump) (14, 15). Omeprazole is used in combination with antibiotics for eradicating Helicobacter pylori bacteria which, together with stomach acid, causes ulcers of the stomach and duodenum through reducing acid secretion and increasing the bacterial sensitivity to antibiotics by direct blocking of its proton pump (16). Consequently, Wagner et al (17) used a combination of calcium hydroxide and omeprazole as an intracanal medication and showed that association of omeprazole with calcium hydroxide resulted in a better repair of rat periapical lesions; moreover, they suggested that this combination might have a selective activity over endodontic microbiota when compared with conventional calcium hydroxide dressing. To date, no study has evaluated the effect of omeprazole when mixed with sodium hypochlorite on E faecalis.

The objectives of this study was to evaluate in vitro the effect of omeprazole when used in different concentrations (6%, 8%, 10% and 12%) mixed with 5.25% sodium hypochlorite as compared to 2% chlorhexidine and 5.25% NaOCl on E faecalis using the agar diffusion test.

**MATERIALS AND METHODS**

The irrigants tested were 2% chlorhexidine (CHX; Consepsis, Ultradent, USA), 5.25% sodium hypochlorite (NaOCl) and four concentrations (6%, 8%, 10% and 12%) of one brand of commercially available omeprazole (Omez, Pharaonic Pharmaceuticals, Egypt) mixed with 5.25% NaOCl. Sterile saline solution served as the negative control and 5.25% NaOCl as positive control.

Omeprazole used in this study was supplied as hard gelatin capsules; each contains omeprazole 20 mg as enteric coated pellets. To prepare omeprazole solutions, pellets were grinded into powder and diluted to different concentrations by sterile saline solution. 0.5 ml from each concentration was mixed with 15 ml 5.25% NaOCl. Solutions were freshly prepared at the time of the experiment.

The microorganism selected for this study was Enterococcus faecalis (ATCC 29212). Culture of the experimental strain was allowed to grow for 24 hours at 37° C in brain heart infusion (BHI) broth and density was adjusted to 0.5 McFarland turbidity standard scale (1.5 × 10^8 CFU/ml). The resulting suspension was spread with sterile swabs over the entire BHI.

Eight BHI agar plates were prepared. For each plate, seven sterile paper disks, 6 mm in diameter, were soaked with 20 μl of either the six test solutions or saline solution (control group) and placed on its position in the plate. The plates were incubated under aerobic conditions for 24 hours. The resulting microbial zones of inhibition were measured across the diameter in millimeters using a transparent ruler. For reproducibility, the test was repeated three times such that, a total of 168 disks were used for the study (n=24 for every test solution).
**Statistical analysis**

The results were represented as mean ± standard deviation values. The one-way analysis of variance (ANOVA) was used for comparison between the groups followed by Tukey post hoc test. The significance level was set at $P \leq 0.05$.

**RESULTS**

The mean and standard deviation values of the inhibition zone of the tested materials in mm are presented in Tables 1 and 2. The results of the study showed that the positive control group (5.25% NaOCl) was effective against *E. faecalis* with a distinctive zone of inhibition, while the negative control (saline) was ineffective.

The 6% omeprazole/NaOCl mix, 2% CHX and 8% omeprazole/NaOCl mix showed statistically significant larger zones of inhibition compared with 5.25% NaOCl with no statistically significant difference between them. The 6% omeprazole/NaOCl mix had also statistically significant larger zones of inhibition than the 12% and the 10% mix. Whereas no statistically significant differences were found between the 8%, the 12% or the 10% mixes. Regarding the 12% and the 10% omeprazole/NaOCl mixes, there was no statistically significant difference between them or the 5.25% NaOCl or the CHX groups.

| TABLE (1) Mean ± Standard Deviation values (mm) of the inhibition zone of the tested materials. |
|-----------------------------|-----------------------------|-----------------------------|
| **Mean ± SD**              | **P value**                | **Post hoc category**      |
| 2% CHX                     | 1.48 ± 0.11                | <0.001                      |
| 5.25% NaOCl                | 1.24 ± 0.12                | B                           |
| 12% Omeprazole/NaOCl       | 1.38 ± 0.26                | B,C                         |
| 10% Omeprazole/NaOCl       | 1.36 ± 0.26                | B,C                         |
| 8% Omeprazole/NaOCl        | 1.46 ± 0.27                | C,D                         |
| 6% Omeprazole/NaOCl        | 1.64 ± 0.41                | D                           |
| Saline                     | 0.0 ± 0.0                   | A                           |

*Values with the same letters are not significantly different at $P \leq 0.05$ level.*

| TABLE (2) P values of Pairwise comparisons of Tukey post hoc test. |
|-----------------------------|-----------------------------|-----------------------------|
| **5.25% NaOCl**             | 0.015*                      |                             |
| 12% Omeprazole/NaOCl        | 0.771                       | 0.457                       |
| 10% Omeprazole/NaOCl        | 0.618                       | 0.618                       |
| 8% Omeprazole/NaOCl         | 1                           | 0.026*                      |
| 6% Omeprazole/NaOCl         | 0.195                       | <0.001*                     |
| Saline                     | <0.001*                     | <0.001*                     |

*Significant at $P \leq 0.05$
DISCUSSION

The agar diffusion test is one of the most frequently used methods to test the antibacterial activity of dental materials including endodontic irrigants. It is a simple, standard, reproducible and relatively inexpensive test. Despite that antibacterial activity of this in vitro environment may be affected by some factors as the pH of the substrates in the plates, incubation period, solubility and diffusion ability of the test material through the agar, the agar diffusion test can still provide a reliable prediction of the response of a certain bacterial isolate to a particular test material (18, 19, 20).

In this study two irrigants, NaOCl and CHX, were selected for reliable comparison with the omeprazole mixes on \textit{E. faecalis}. NaOCl is the most commonly used endodontic irrigant and is known to have antibacterial and tissue dissolving ability (21, 22). The antibacterial effectiveness of NaOCl is based on its high pH (pH > 11) which according to Estrela et al (23), interferes in the cytoplasmic membrane integrity causing irreversible enzymatic inhibition and alterations in cellular metabolism and phospholipid degradation. Moreover, hypochlorous acid (HOCl) present in sodium hypochlorite solution when comes in contact with organic tissue acts as a solvent and releases chlorine, which is a strong oxidant, that causes an irreversible oxidation of sulphydryl group of essential bacterial enzymes resulting in the killing of bacterial cells (23, 24). The antibacterial action of NaOCl is proportional to its concentration (25, 26, 27). Previous studies had shown the ability of \textit{E. faecalis} to survive the antibacterial action of NaOCl (21, 27, 28). This was partly attributed to the inability of NaOCl to reach areas where \textit{E. faecalis} might hide as dentinal tubules or inaccessible areas of root canal system or, because of the buffering effect of dentin (29, 30). The presence of a functioning proton pump in the cell membrane might be another possible explanation for the survival of \textit{E. faecalis} in the high alkaline environment of NaOCl (13).

Chlorhexidine is another endodontic irrigant known with its excellent antibacterial activity beside many other advantages including substantivity, lower tissue toxicity than NaOCl and efficient clinical performance (31, 32). The antibacterial effect of CHX is related to its cationic molecule which is capable of binding to negatively charged sites on bacterial cytoplasmic membrane and causing loss of osmotic balance. At low concentrations (0.2%), CHX causes low molecular weight substances, mainly potassium and phosphorous, to leak out resulting in a bacteriostatic effect. At higher concentrations (≥2%), CHX has a bactericidal effect due to precipitation of bacterial cytoplasmic contents resulting in cell death. CHX also binds to hydroxyapatite and soft tissues, causing changes in their electrical field to compete with bacterial binding (26, 33). In this study, 2% liquid form of CHX was used which has been shown in previous studies to be effective against \textit{E. faecalis} (26, 34). Unfortunately, CHX lacks tissue-dissolving property which limits its uses (31). Attempts to adjunct the antibacterial benefit of CHX to NaOCl by combining both, resulted in formation of a toxic precipitate (para-chloroaniline) that tends to occlude dentinal tubules (35, 36).

Obviously, addition of a medication that could enhance the antibacterial activity of NaOCl against \textit{E. faecalis} would be advantageous. Looking back to the survival mechanisms of \textit{E. faecalis}, brought the idea of using PPIs to block the bacterial functional proton pump present in its cell membrane which is thought to be one of the main causes of resistance of \textit{E. faecalis} to high pH changes created by calcium hydroxide and NaOCl (13, 17). According to Evans et al (13), in an alkaline medium, bacterial cells maintain pH homeostatis by pumping cations/protons into the cell to lower the internal pH. The PPI omeprazole is a weak base that has acceptable stability under alkaline conditions, it easily crosses the cell membrane and rapidly degrade in acid media to shut down the pump (14, 15).
In this study Omez, one of the commercially available over-the-counter omeprazole preparations was used. The selection depended on its availability and the absence of known compounds that could interact with NaOCl from its list of excipients. In previous studies, omeprazole with only 8.5% concentration was used in combination with calcium hydroxide or NaOCl. In this study, four concentrations (6%, 8%, 10% and 12%) of omeprazole were prepared and tested. To our knowledge, no other studies tested these concentrations mixed with 5.25% NaOCl. During preparation of the mixes, when NaOCl (pH 11) was added to omeprazole, the pH of the mix was markedly reduced to pH 8, continuous addition of NaOCl until pH 11 was regained and ensured in all of the concentrations prepared, and that was 15 ml of NaOCl added to 0.5 ml of each concentration. The mixes prepared were stable for approximately 90 minutes then the pH gradually decreased. It is worth mentioning that omeprazole which is known to be slightly soluble in water, was completely soluble in NaOCl.

The results of this study showed that 2% CHX was significantly more effective against *E. faecalis* than 5.25% NaOCl. These results were consistent with other studies in spite of the differences in the used methodologies of these studies. When considering the use of PPIs, the omeprazole/NaOCl mix did improve the antibacterial effect of 5.25% NaOCl. The four concentrations of omeprazole tested in this study, when mixed with 5.25% NaOCl showed similar antibacterial effect to the 2% CHX with best results obtained by the 6% omeprazole/NaOCl mix which was more efficient than the 2% CHX although no significant difference was detected. The findings of the present study confirm those obtained by Wagner et al and Gandi et al although these studies used omeprazole with different formulation, concentration (8.5%) and ratio (1:1). Apparently, addition of PPIs to a material that can provide high alkaline environment as calcium hydroxide and NaOCl, might contribute in further reduction of endodontic microbiota with selective action on species possessing a functioning proton pump as *E. faecalis*.

This study suggests an easy and reproducible method of preparing omeprazole/NaOCl mix for clinical use. It is worth mentioning that care should be taken during the selection of omeprazole formulation. Some commercially available brands might contain compounds in their list of excipients that might interfere with the action of NaOCl such as disodium edetate (EDTA).

Despite the promising results of incorporating PPIs in endodontics yet, further studies are needed to determine the cytotoxicity of omeprazole/NaOCl mix, whether this mix might form any toxic precipitate, substantivity of omeprazole, different formulations, other possible concentrations and effect on other bacterial strains.

**ACKNOWLEDGMENTS**

*The author deny any conflicts of interest related to this study*

**REFERENCES**


