The 3rd ASEAN Plus and Tokushima Joint International Conference

Theme:
“Strategic Achievement of Oral Sciences and Promotion of Quality of Life and Professional Education for Oral Hygienists by Using Information and Communication Technology”

Organized by:

Faculty of Dentistry
Hasanuddin University
Makassar, Indonesia

Faculty of Dentistry
The University of Tokushima
Tokushima, Japan

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Program and Proceeding Book

The 3rd ASEAN Plus and Tokushima Joint International Conference on "Strategic Achievement of Oral Sciences and Promotion of Quality of Life and Professional Education for Oral Hygienists by Using Information and Communication Technology"

Organized by

Collaboration,
Faculty of Dentistry The University of Tokushima
Faculty of Dentistry Hasanuddin University

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# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preface Dean of Faculty of Dentistry The University of Tokushima</td>
<td>2</td>
</tr>
<tr>
<td>Preface Dean of Faculty of Dentistry Hasanuddin University</td>
<td>3</td>
</tr>
<tr>
<td>Greetings from the Chairman of Organizing Committee</td>
<td>4</td>
</tr>
<tr>
<td>Organizing Committee</td>
<td>5</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>7</td>
</tr>
<tr>
<td>Schedule of Conference</td>
<td>8</td>
</tr>
<tr>
<td>Abstracts of Oral Presentation</td>
<td>25</td>
</tr>
<tr>
<td>Abstracts of Poster Presentation</td>
<td>50</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>89</td>
</tr>
</tbody>
</table>
LCFW was heated at 95°C for 15 min (BLCFW) or dialyzed against water using dialysis tube with a molecular weight cutoff of 14,000 (DLCFW).

Results: Acinar atrophy was detected in sublingual (SL) glands from senescent but not young rats. LCFW, BLCFW, or DLCFW-administration restored this age-dependent atrophy to normal histology like young rats. In SL glands from senescent rats, LCFW-administration induced more than 10-fold increase in proline-rich protein genes (Prp2, Sgp158) and cystatin S gene (Cyss) and 2-fold reduction in granzyme B gene (Gzmb) compared to water-administration. BLCFW-administration induced more than 10-fold increase in proline-rich protein gene (Prp1), amylase gene (Amy1a) and cystatin 10 gene (Cst10) compared to LCFW-administration.

Conclusion: LCFW-administration restores the age-dependent atrophy and hypofunctions of SL glands. Ingredients of whey inhibiting age-dependent atrophy and gene expression changes of SL glands were heat-resistant compounds with molecular weight more than 14,000.

Session 3.4
Quantification Of Cultivable Bacteria In Chronic Apical Periodontitis Before And After Chemomechanical Preparation With 6% Sodium Hypochlorite

Maria Tanumihardja
Department of Conservative Dentistry, Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia.

This clinical study was conducted to quantify cultivable bacteria in teeth with chronic apical periodontitis before and after chemomechanical preparation with 6% sodium hypochlorite and 6% warm-sodium hypochlorite. Twenty two root canal samples from twenty two patients were selected and allocated into two groups of eleven (Group A and Group B). Samples were collected before (S1) and after chemomechanical preparation (S2). Culture techniques were used to determine the colony-forming unit (CFU), and Gram staining was used to identify the bacteria. All initial samples of both groups showed the presence of bacteria with a median concentration of 35 x 10³ CFU/mL and 51 x 10³ CFU respectively. At S2, mean bacterial load reduction of Group A was 14 x 10³ CFU/mL or 49.41%, and Group B was 17 x 10³ CFU/mL or 61.44%. Non-Parametric Mann-Whitney U test was used to evaluate the significance of bacterial load reduction of both groups. There was no significant difference between Group A and Group B, although the bacterial load reduction was higher in Group B. The predominant bacteria found in both groups before and after chemomechanical preparation with 6% sodium hypochlorite and 6% warm-sodium hypochlorite. It can be concluded from this study that chemomechanical preparation with 6% sodium hypochlorite or 6% warm-sodium hypochlorite was moderately effective against bacteria in chronic apical periodontitis.
Quantification Of Cultivable Bacteria In Chronic Apical Periodontitis
Before And After Chemomechanical Preparation With 6% Sodium Hypochlorite

INTRODUCTION

Bacteria is the main cause for the development of pulpal and periapical diseases as demonstrated in animal models and human studies (1-3). Eradication or controlled of bacteria through endodontic treatment is the primary goal to achieve success, however elimination of bacteria from infected root canal systems is challenging (4). Chemomechanical debridement with advanced instruments, irrigation techniques and intra-canal medicaments have been developed to optimize the eradication of bacteria, however bacterial-free root canal is almost impossible to achieve due to the complex anatomy of the root canal system (5, 6).

Many irrigants have been usually used in endodontic therapy, and the most recommended is sodium hypochlorite (NaOCl) which is bactericidal agent and also organic tissue solvent. Lately, higher concentration is recommended to improve its antibacterial effectiveness, and warmer sodium hypochlorite have been proposed to enhance the disinfection process.

Clinical evaluation of high concentration of 6% sodium hypochlorite irrigation on its antibacterial activity following root canal treatment of chronic apical periodontitis (CAP) is still scarce.

This clinical study was aimed to evaluate and compare the efficacy of irrigation with 6% warm and non-warm sodium hypochlorite on antimicrobial effect during treatment of primary infected root canals of teeth with chronic apical periodontitis.
MATERIAL and METHODS

Patient selection

Twenty two patients consulting at the clinic of Endodontic Department, Oral and Dental Hospital, Hasanuddin University, were included when they had clinical diagnosis of Chronic Apical Periodontitis (CAP). The age of patients ranged from 16-38 years. Diagnostic criteria included the presence of apical lesion detected by periapical radiography in upper anterior teeth with clinical determination of non-vital pulp, and had indication of endodontic treatment. Exclusion criteria included marginal periodontal diseases, defined by the absence of clinical attachment loss (≥ 2 mm), increased probing depths (≥ 3 mm), systemic illness or previous antibiotics or non-steroid anti-inflammatory treatment during the 3-month period prior to the study. All the protocols and procedures were approved by the Ethics Committee of Medical Faculty (0892/H4.8.4.5.31/PP36-KOMETIK/2014), Hasanuddin University, and informed consents were obtained from all individuals.

Clinical procedures

The teeth were isolated with a rubber dam, disinfection of their external surfaces and the surrounding structure field was carried out by using 30% hydrogen peroxide, followed by 2.5% NaOCl. The solutions were inactivated with 5% sodium thiosulfate to avoid interference with bacteriologic sampling. The sterility of external surfaces of the crown was checked by taking a swab sample from the crown surface and streaking it on blood agar plates. The access preparation was performed with sterile saline and high-speed diamond bur. Before entering the pulp chamber, the access cavity was disinfected again following the protocol described above.
The root canal was prepared by using hand K-files with back-and-forth alternated rotation motion (size 15/40 and 45/80; FKG Dentaire, Switzerland). The working length was established with an apex locator and confirmed by radiographs. Master apical files ranged from 30-40, depending on both root anatomy and initial apical files. Before canal preparation, a sterile paper point (size 20; Dochem, China) was introduced into the full length of the canal, and retained in position for 60 seconds for bacterial sampling (S1). Chemomechanical preparation was completed at the same appointment in all cases. The samples were allocated into two groups. The root canals of Group I were irrigated with 3 mL of 6% sodium hypochlorite (local medical supplies) using a 27-gauge needle after each instrument size. The canals were then flushed with 2 mL of sterile aquadest solution, dried, and a sterile paper point (size 20; Dochem, China) was introduced into the full length of the canal, retained in position for 60 seconds. All the paper point were placed in each screw-cap container containing Stuart-transport medium and were directly sent to the microbiology laboratory for microbial cultivation (S1). The same procedure was repeated for Group II, but the canals were irrigated with 3 mL of 6% warm sodium hypochlorite (using infant bottle warmer, the syringes contained sodium hypochlorite were left for 1 minute). A sterile paper point (size 20; Dochem, China) was again introduced into the full length of the canal, retained in position for 60 seconds. All the paper point were placed in each screw-cap container containing Stuart-transport medium and were directly sent to the microbiology laboratory for microbial cultivation (S2).

The canal was medicated with calcium hydroxide paste (Ultradent, Utah, USA) which was placed by means of lentulo spiral fillers and packed with a cotton pellet at the level of canal entrance. A radiograph was taken to ensure proper placement of the calcium hydroxide paste in the canal. Access cavity was filled with a temporary filling, Cavit-G (3M, ESPE, USA).
Isolating and detection of species

Each paper point was taken out from the container and inserted into the other container filled with enhancement medium of Brain-Heart Infusion Broth (BHIB), vortexed for 60 seconds to remove all bacteria and spread on BHIB. One mL of BHIB was added with 9 mL NaCl 0.9%, diluted and repeated in 3 series until it reached $10^3$ CFU bacteria. One mL was swabbed on a sterile petri dish contained Blood Agar (BA) and incubated anaerobically for 24 h at 37°C in gas vac. Another 1 mL was swabbed on a sterile petri dish contained Nutrient Agar (NA) to detect facultative anaerob bacteria. Appeared colonies were counted visually.

Results

<table>
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<th>Before</th>
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<tr>
<td>Median</td>
<td>NaOCl 35 x 10$^3$ CFU/mL</td>
<td>14 x 10$^3$ CFU/mL</td>
</tr>
<tr>
<td></td>
<td>NaOCl (warm) 51 x 10$^3$ CFU/mL</td>
<td>17 x 10$^3$ CFU/mL</td>
</tr>
<tr>
<td>Range</td>
<td>NaOCl 14 to 290 x 10$^3$ CFU/mL</td>
<td>0 to 250 x 10$^3$ CFU/mL</td>
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<tr>
<td></td>
<td>NaOCl (warm) 5 to 246 x 10$^3$ CFU/mL</td>
<td>1 to 57 x 10$^3$ CFU/mL</td>
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Median showed decreased CFU following irrigation with either 6% sodium hypochlorite or 6% warm sodium hypochlorite

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<th></th>
<th>Before</th>
<th>After</th>
<th>p-value</th>
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<tr>
<td>Mean</td>
<td>NaOCl 78.27 x 10$^3$ CFU/mL</td>
<td>35.64 x 10$^3$ CFU/mL</td>
<td>.003</td>
</tr>
<tr>
<td></td>
<td>NaOCl (warm) 62.91 x 10$^3$ CFU/mL</td>
<td>18.82 x 10$^3$ CFU/mL</td>
<td></td>
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<tr>
<td>P-value</td>
<td>.763</td>
<td>.797</td>
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Mann-Whitney U test (Non parametric)
No significant difference was noted on CFU decreased between irrigation with 6% sodium hypochlorite or irrigation with 6% warm sodium hypochlorite ($p>0.05$). Significant difference was noted on CFU decreased before and after irrigation with 6% sodium hypochlorite or irrigation with 6% warm sodium hypochlorite ($p<0.05$).
Discussion

Successful root canal therapy relies on the combination of proper instrumentation, irrigation, and obturation of the root canal. Of these three essential steps of root canal therapy, irrigation of the root canal is the most important determinant in the healing of the periapical tissues. The primary endodontic treatment goal must thus be to optimize root canal disinfection and to prevent reinfection.

The ideal requirements of root canal irrigants should have broad antimicrobial spectrum, able to dissolve necrotic pulp tissue remnants, able to inactivate endotoxin, able to prevent the formation of a smear layer during instrumentation or to dissolve the latter once it has formed, nontoxic when they come in contact with vital tissues, and noncaustic to periodontal tissues, and with little potential to cause an anaphylactic reaction.

Sodium Hypochlorite (NaOCl) has an extensive history in medicine and dentistry and continues to be popular even today. During World War I, the chemist Henry Drysdale Dakin and the surgeon Alexis Carrel extended the use of buffered 0.5% NaOCl solution to the irrigation of infected wounds. (6) NaOCl acts as an organic and fat solvent, degrading fatty acids and transforming them into fatty acid salts (soap) and glycerol (alcohol), which reduces the surface tension of the solution. NaOCl neutralizes amino acids forming water and salt. With the exit of hydroxyl ions, there is a reduction of pH. When hypochlorous acid, a substance present in NaOCl solution, comes in contact with organic tissue it acts as a solvent and releases chlorine, which combines with the protein amino group to form chloramines. Hypochlorous acid (HOCl⁻) and hypochlorite ions (OCl⁻) lead to amino acid degradation and hydrolysis. (9) The chloramination reaction between chlorine and the amino group (NH) forms chloramines that interfere in cell metabolism. Chlorine (a strong oxidant) has an antimicrobial action, inhibiting
bacterial enzymes and leading to an irreversible oxidation of SH groups (sulphydryl group) of essential bacterial enzymes. The most effective irrigation regimen is reported to be 5.25% at 40 min \(^{(10)}\) irrigation with 1.3% and 2.5% NaOCl for this same time interval is ineffective in removing \(E\ faecalis\) from infected dentin cylinders.\(^{(11)}\) NaOCl was moderately effective against bacteria but less effective against endotoxins in root canal infection.\(^{(12)}\) This was confirmed by the result of this study which showed higher CFU of obligate Gram – bacteria (unpublished data).

Efficacy of sodium hypochlorite is proposed by altering the pH, hypochlorites at a lower pH possess greater antimicrobial activity, and increase its temperature. A rise in temperature by 25°C increased NaOCl efficacy by a factor of 100. The capacity of a 1% NaOCl at 45°C to dissolve human dental pulps was found to be equal to that of a 5.25% solution at 20°C. However no significant difference in bacterial CFU decreased was noted in this study when sodium hypochlorite was warmed. The use of ultrasonic agitation increased the effectiveness of 5% NaOCl in the apical third of the canal wall, while passive ultrasonic irrigation with a nickel-titanium tip produced superior tissue-dissolving effects as compared to sonic irrigant activation. Kuruvilla \textit{et al} suggested that the antimicrobial effect of 2.5% NaOCl and 0.2% chlorhexidine (CHX) used in combination was greater than that of either agent used separately. During instrumentation canals should be irrigated using copious amounts of the NaOCl solution. Once the shaping procedure is completed, canals can be thoroughly rinsed for at least 1 min using 5 to 10 ml of sodium hypochlorite.
CONCLUSION

Under the limitations of this study, it can be concluded that efficacy of warmed sodium hypochlorite has the ability to decrease bacteria CFU from the root canal systems. Future research should be performed on the use of combination of irrigants to evaluate its antibacterial efficacy.
REFERENCES


