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The Effectiveness of Antimicrobial Agents in Commercialised Oral Rinses in Controlling Dental Plaque Microbes

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Abstract

A thin film of microorganisms on the tooth surfaces is known as dental plaque, which can contribute to the development of dental caries and periodontal disease. It has been widely known that the usage of antimicrobial oral rinses plays an important role in maintaining oral hygiene mainly by reducing the numbers of dental plaque microbes. The study was carried out to compare the effectiveness of antimicrobial compound in commercialized oral rinses, chlorhexidine (Oradex®) and hexetidine (Bactidol®) in controlling the numbers of dental plaque microbes. In addition, the aim was also to determine the duration of effects in suppressing and reducing plaque microbes with the usage of the commercialized oral rinses. The antibacterial effects of chlorhexidine and hexetidine-containing oral rinses were evaluated in healthy adults, which were not on any antibiotic treatment for the past 6 months. The oral rinses were tested on volunteers for a period of 30 min interval for up to 120 min. Volunteers were required to suspend normal oral hygiene habits and on the sampling day, the exposed tooth surfaces of each volunteer were swabbed, followed by rinsing with the specified oral rinse. The tooth surfaces were swabbed again after 30, 60 and 120 min. Swab samples were serially diluted and plated on BHI agar. Phenotypic appearance and the colony forming units (CFU/mL) were obtained. The study showed that immediately after rinsing with the respective oral rinses, hexetidine acted faster than chlorhexidine in reducing the number of plaque microbes. However, the suppression effect could not be maintained for a longer duration compared to chlorhexidine, which exhibited longer magnitude of suppression of dental plaque microbes.

Introduction

Plaque is a tenaciously adherent deposit that forms on tooth surface. It consists of an organic matrix containing a dense concentration of bacteria (Socransky and Haffajee, 2002). Plaque is normally classified according to the location of plaque accumulation such as subgingival plaque and supragingival plaque. Accumulation of plaque supragingivally can cause caries on enamel surface meanwhile subgingival plaque can cause gum disease likes gingivitis and periodontitis.

The importance of effective dental plaque control has been emphasized over the years. As an adjunct to mechanical methods for dental plaque elimination, oral rinse products containing chemotherapeutic agents with a variety of antimicrobial mechanism have been beneficial and desirable. For many years, mouth rinse has been formulated in an attempt to improve plaque control provided by the mechanical cleaning method. Medicated oral rinse usually contains antimicrobial agents, such as chlorhexidine (CHX), hexetidine (Hx), etc. Using mouth rinse frequently will influence the reduction of dental plaque bacterial attachment, proliferation or retention on the tooth surfaces, as oral rinse are capable of lowering the rate of dental plaque formation to a statistically significant degree. However, the information on the inhibitory duration effect on the plaque is currently insufficient (Christine et al., 2000).
Therefore, this study was carried out with the objectives of comparing the effectiveness of the antimicrobial agents (CHX and HX) in reducing the numbers of plaque microbes and also to determine the duration of effect in reducing and controlling plaque microbes by using oral rinses.

Materials and Methods

Oral rinses

Commercialized oral rinses used in the experiment are Oradex® that contains 0.12% chlorhexidine gluconate as the active compound, and Bactidol® that contains 0.1% hexetidine as its active compound. Distilled water was used in the experiment as a negative control.

Rinsing procedures

The antibacterial effects of chlorhexidine and clove extract on dental plaque microbes were evaluated on dentally healthy adults which were not on any antibiotic treatment for the past 6 months. The used rinsing volume of each of the oral rinse was based on the suggested instructions on the manufacturer’s product labels. Volunteers were required to suspend normal oral hygiene habits daily, which include the routine toothbrushing every morning after getting up from bed and before bedtime.

Sampling procedures

On the sampling day, the tooth surfaces of the buccal, occlusal and lingual sides of each volunteer were swabbed with sterile cotton swab, followed by rinsing with the specified oral rinse. The selected tooth surfaces were swabbed again after 30, 60 and 120 min. Swab samples were vortexed for 30 seconds to dislodge all microbes. Following this, samples were serially diluted and plated on Brain Heart Infusion (BHI) agar. All plates were incubated at 37°C for 18-24 h.

Bacterial identification

Pure colonies were isolated and Gram stained for cell morphology identification. The isolates were then subjected to bacterial identification procedures using the API Identification System (Bio Merieux, France).

Results and Discussion

Two different types of bacterial colonies were successfully cultured on the BHI agar plates. Colonies were white and cream in colour. The cream colour colonies with diameter ranging from 1-2mm were identified as Streptococcus species, and the white colonies which were around 3-5mm in diameter were identified as Candida species. This study has shown that dental plaque community was composed of a variety of different microorganisms which is in agreement to other reported results elsewhere (Socransky and Haffajee, 2002). Maturated dental plaque is the initiation of gingivitis. This is agreed from a number of studies which showed high positive correlation between the amount of supragingival plaque and the development of gingivitis including between removal of bacterial plaque and the resolution of gingival inflammation. Therefore, dental plaque is considered the major aetiological factors in the development of gingivitis and periodontitis (Kornman, 1986).
Daily usage of oral rinse is important to maintain the oral hygiene and prevent dental plaque accumulation. Chemotherapeutics oral rinses have been reported to act as an adjuvant to mechanical removing of supragingival plaque (Robert and Addy; 1980). Oral rinse containing antimicrobial properties are considered effective and safe, and play an important role in maintaining oral health by killing cariogenic organisms and preventing plaque, gingivitis, and bad breath (Wade and Addy, 1989). Similarly, this study has proven that chlorhexidine and hexetidine are both effective in reducing dental plaque microbes. The antimicrobial effects of all rinses showed a drastic reduction of supragingival plaque microbes (Table 1). Chlorhexidine has showed a steady reduction throughout the tested period compared to hexetidine and distilled water. In contrast, dental plaque microbes were observed to slowly increase with time when using hexetidine and distilled water (Figure 1). It is in agreement with a study done by Robert and Addy (1986) in comparison of in vivo and in vitro antibacterial properties of mouthrinses containing chlorhexidine and hexetidine.

<table>
<thead>
<tr>
<th>Oral rinse Time</th>
<th>0.12% Chlorhexidine (x 10^3)</th>
<th>% reduction (compared to before rinsing)</th>
<th>0.1% Hexetidine (x 10^3)</th>
<th>% reduction (compared to before rinsing)</th>
<th>Distilled water (x 10^3)</th>
<th>% reduction (compared to before rinsing)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before rinsing</td>
<td>170.3</td>
<td></td>
<td>160.6</td>
<td></td>
<td>96.7</td>
<td></td>
</tr>
<tr>
<td>0 min (immediately after rinse)</td>
<td>128.7</td>
<td>24.41%</td>
<td>97.2</td>
<td>39.54%</td>
<td>81.5</td>
<td>15.74%</td>
</tr>
<tr>
<td>30 min</td>
<td>118.8</td>
<td>30.25%</td>
<td>121.1</td>
<td>24.56%</td>
<td>74.2</td>
<td>24.7%</td>
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<tr>
<td>60 min</td>
<td>108.4</td>
<td>36.34%</td>
<td>128.9</td>
<td>19.73%</td>
<td>99.2</td>
<td>-2.3%</td>
</tr>
<tr>
<td>120 min</td>
<td>88.2</td>
<td>48.2%</td>
<td>128.1</td>
<td>20.20%</td>
<td>101.3</td>
<td>-4.8%</td>
</tr>
</tbody>
</table>

Table 1: The Effects of rinsing with chlorhexidine, hexetidine and distilled water on supragingival microflora

![Figure 1: The mean CFU/ml of supragingival plaque following a single rinse with CHX, HX and distilled water](image)

Figure 1: The mean CFU/ml of supragingival plaque following a single rinse with CHX, HX and distilled water
Chlorhexidine was observed to exhibit a longer duration of antimicrobial effect as the dental plaque population was not increased compared to before rinsing. This was due to the active antimicrobial agent that could destabilize and penetrate bacterial cell membranes and interfere with membrane function by inhibiting oxygen utilization that led to cell death.

Although hexetidine was found to immediately reduce dental plaque microbes, this agent was less effective than chlorhexidine in controlling and maintaining the antimicrobial effect for a longer period of duration.

This result was in agreement with other reports that chlorhexidine performed almost equal killing effect to other antimicrobial agents in most oral rinses for up to 30 min after rinsing, but continued its microbicidal effect for a longer duration period.

Conclusion

The result of this study has demonstrated that supragingival microflora can be successfully suppressed by the use of mouthrinses. Chlorhexidine and hexetidine mouthrinses are able to reduce the microbial load of supragingival plaque. From the reduction in CFU of the supragingival microflora, it was clearly shown that both rinses were effective in reducing dental plaque microbes. However, chlorhexidine has a longer suppression effect on controlling dental plaque microbes compared to hexetidine.

References


