The Antibacterial and Antiplaque Effectiveness of Mouthwashes Containing Cetylpyridinium Chloride With and Without Alcohol in Improving Gingival Health

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Overview
This article briefly discusses the antibacterial action of cetylpyridinium chloride (CPC) and its efficacy in the removal of bacterial plaque as an adjunct to the mechanical cleaning of tooth surfaces. It reviews new studies on the effectiveness of mouthwash formulations containing CPC against two common oral bacteria species and in disrupting plaque biofilms. Finally, this article reviews three clinical studies which support that the daily use of mouthwashes containing 0.075% CPC, with and without alcohol, represents a valuable complement to daily mechanical plaque control.

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Introduction
Bacteria accumulation on teeth results in the formation of dental plaque. Left untreated, dental plaque can lead to gingivitis, which is characterized by redness, edema, and bleeding on probing. Gingivitis is the early and reversible form of periodontal disease with no permanent gum damage when treated. However, if not treated, gingivitis can lead to the development of periodontitis, which results in irreversible damage to the gums and underlying support tissues.

The focus of any attempt to prevent and control periodontal disease is the maintenance of an effective level of plaque control by the individual through his or her daily oral hygiene. The most important part of oral healthcare takes place at home. Mechanical cleaning by tooth brushing and flossing has been the cornerstone of oral hygiene and health. However, these mechanical routines, for various reasons, do not appear to be enough for the majority of people, as the incidence and prevalence of gum problems are high in both the developed and developing world. Many patients find it difficult to comply with this daily regimen. Inufficient and/or inadequate brushing and flossing, due to the lack of manipulative skills, can lead to plaque build-up. As these methods may be insufficient to achieve optimum results, a common strategy is to supplement mechanical plaque removal with a chemotherapeutic agent (surfactant) which adsorbs readily to oral surfaces.

Cetylpyridinium chloride (CPC) in mouthrinses has been studied extensively in clinical trials for its ability to control plaque and gingivitis. It is one of only two antimicrobial mouthrinse ingredients that has received a Category I recommendation from a United States Food and Drug Administration (FDA) advisory panel for safety and effectiveness in reducing supragingival plaque and gingivitis.

Antimicrobial Activity of CPC
CPC is a quaternary ammonium compound with broad spectrum antibacterial activity. It is a cationic surface active agent (surfactant) which adsorbs readily to oral surfaces. The molecule has both hydrophilic and hydrophobic groups, providing the possibility for ionic, as well as hydrophobic interactions. The positively charged hydrophilic region of the CPC molecule plays a major role in its antimicrobial activity, imparting a high binding affinity for bacterial cells whose outermost surface carries a net negative charge. The strong positive charge and hydrophobic region of CPC enable the compound to interact with the microbial cell surface and integrate into the cytoplasmic membrane. As a result of this interaction, there is disruption of membrane integrity resulting in leakage of cytoplasmic components, interference with cellular metabolism, inhibition of cell growth, and cell death. CPC has also been shown to inhibit the co-aggregation of bacteria thus interfering with plaque maturation, inhibit the synthesis of insoluble glucan by Streptococcus genticus and Streptococcus mutans biofilms. The ability of CPC to adsorb to pellicle-covered enamel imparts substantivity to the molecule, that is, retention in the mouth and continued antimicrobial activity for a period of time after rinsing. The antibacterial activity of formulations containing CPC has been well-documented.

Studies have investigated the reduction in numbers of salivary bacteria following a single and multiple rinsing with mouthwashes containing CPC. These short-term studies can provide an assessment of the product’s potential antiplaque effectiveness, as well as an indication of the persistence of action, or substantivity, of a formulation. In a randomized, double-blind, parallel study, a mouthwash containing 0.05% CPC and 0.05% sodium fluoride was compared to a control mouthwash containing 0.05% sodium fluoride for its ability to control supragingival plaque bacterial counts for 12 hours after a single treatment, and 12 hours after 14 days’ use. The CPC mouthwash significantly reduced supragingival plaque bacteria counts by 35.3% and 70.9% compared to the control fluoride mouthwash 12 hours after a single use and after 14 days of use, respectively. Additional studies have shown significant reductions in salivary aerobic and/or anaerobic bacterial counts for up to seven hours following a single rinse with a CPC-containing product. These studies...
support that mouthwashes containing CPC are effective in reducing the levels of bacteria in plaque and saliva, and thus will have an effect on plaque formation.

Efficacy on Plaque and Gingivitis

A number of short-term clinical studies using mouthwashes containing 0.05% to 0.1% of CPC have demonstrated a significant reduction in plaque ranging from 25% to 39%. In a six-week study by de Silva and co-workers, a mouthwash containing 0.05% CPC and 0.05% sodium fluoride was shown to significantly reduce plaque by 27.9% and gingivitis by 25.0% compared to a control fluoride mouthwash without CPC. This study clearly showed an effect on gingival health. However, in other short-term studies, the effects of CPC mouthwashes on gingivitis have been mixed, and likely due to the treatment duration and CPC availability.

Studies in which CPC mouthwashes were used for extended periods have shown that these mouthwashes can provide proven effectiveness against gingivitis as well as plaque. Three randomized controlled trials of six months duration have assessed the effectiveness of rinsing with a CPC mouthwash on plaque and gingivitis when used as an adjunct to tooth brushing with a fluoride toothpaste. The studies varied in the concentration of CPC and the time spent brushing and rinsing; all included a placebo mouthwash. In the study by Allen and associates, in which subjects rinsed with 15 mL of a 0.05% CPC mouthwash for 60 seconds, the CPC group had 28% less plaque, a 63% reduction in plaque severity, 24% less gingivitis, and 67% less gingival bleeding when compared with the placebo group. In the study by Mankodi and associates, in which subjects rinsed for 30 seconds with 20 mL of an alcohol-free 0.07% CPC rinse, the CPC group had 16% less plaque and 33% less gingival bleeding when compared with the control group. Finally, Stookey, et al. compared two alcohol-free mouthwashes, one containing 0.075% CPC and the other 0.1% CPC. Subjects rinsed with 20 mL for 30 seconds. The respective percentage reductions in plaque and gingivitis were 17% and 23% for the 0.075% CPC group and 19% and 20% for the 0.1% CPC group. Although differing in details, these studies showed the respective CPC formulations to have significant antiplaque and antigingivitis effectiveness.

Development and Validation of an Improved Alcohol-Free Mouthwash Formula with 0.075% CPC

Since the antimicrobial activity of CPC is dependent upon the positively charged hydrophilic region of the molecule, the clinical activity of CPC-containing mouthwashes is dependent upon the way in which the product is formulated; many ingredients are negatively charged and have the ability to form a complex with CPC, and thus deactivating it. In order to be effective against plaque and gingivitis, CPC-containing mouthwash formulations must provide a sufficient level of biologically active CPC. For this reason, formulations must not contain ingredients that could inactivate the molecule by interacting with the positively-charged region, or otherwise interfere with CPC activity. Formulations containing 0.05% CPC in the presence and absence of low levels (6%) of ethanol were developed and marketed globally by the Colgate-Palmolive Company. The ability of these mouthwashes to inhibit bacteria and to reduce supragingival plaque, gingivitis, and volatile sulphur compounds associated with bad breath has been reported. Recently, these products have been reformulated to increase the level of CPC to 0.075% to boost activity.

To improve the efficacy of CPC-containing mouthwashes, a new formulation containing 0.075% CPC and 0.05% sodium fluoride in a base without alcohol was developed. The efficacy of the formulation has been assessed using in vitro methods and was found to demonstrate antimicrobial activity. Additionally, clinical studies have been conducted to demonstrate the long-lasting antibacterial efficacy of the formulation and its ability to prevent plaque formation, gingivitis, and gum bleeding. These studies are presented in this Special Issue.

In the first article, Schaeffer and co-workers report the in vitro antibacterial efficacy of new mouthwash formulations containing 0.075% CPC with and without alcohol against two common oral bacteria species, Aggregatibacter (Actinobacillus) actinomycetemcomitans and Streptococcus mutans after a 30-second exposure. Compared to a negative control mouthwash without CPC, the new mouthwash formulations containing 0.075% CPC statistically significantly (p < 0.05) reduced bacteria levels by > 99.9%.

In the second article, Rao and co-workers describe the development and use of two static model multispecies oral biofilm systems to compare the antibacterial activity of the new alcohol-free mouthwash containing 0.075% CPC and a negative control mouthwash without CPC. The model systems were a 24-well glass-bottom microplate and a chamber slide system. Confocal Laser Scanning Microscopy (CLSM) and fluorometric analysis were used to assess the biofilms. CLSM demonstrated that the mouthwash containing 0.075% CPC resulted in the disruption of both biofilms. The disruption of the biofilm developed using the glass-bottom microplate was corroborated by fluorometric analysis. These models support the antibacterial effectiveness of the alcohol-free mouthwash containing 0.075% CPC.

In the third article, He and co-workers present results of a clinical study in which two mouthwash formulations containing 0.075% CPC, one in an alcohol-free base and the other containing 6% alcohol, were compared to a control mouthwash without CPC for their effect on bacteria in supragingival plaque 12 hours after a single use and 12 hours after 14 days of use. Both mouthwashes statistically significantly reduced plaque bacteria compared to the control mouthwash at each post-treatment time point. The CPC mouthwash in an alcohol-free base reduced bacteria by 35.3% and 70.9% compared to the control mouthwash after a 6% alcohol, were compared to a control mouthwash without CPC 12 hours after a single use and after 14 days of use, respectively. The CPC mouthwash in the 6% alcohol base reduced bacteria by 35.3% and 73.8% compared to the control mouthwash 12 hours after a single use and after 14 days of use, respectively. There were no statistically significant (p > 0.05) differences between the two CPC-containing mouthwashes at either of the post-treatment time points.

In the fourth article, Barnes and co-workers present results of a clinical study in which the antiplaque efficacy of the two mouthwashes containing 0.075% CPC, one in an alcohol-free base and another in a 6% alcohol base, was evaluated using the
Modified Gingival Margin Plaque Index (MGMPI) method.\textsuperscript{53} The CPC-containing mouthwashes were compared to a negative control mouthwash. In the study, participants rinsed twice within a 24-hour period, with the final reading 12 hours after the second rinsing. There was no statistically significant difference (p > 0.05) between the CPC-containing mouthwashes for their ability to reduce plaque re-growth. Both mouthwashes were statistically significantly (p < 0.05) more effective than the control in inhibiting plaque re-growth. Plaque re-growth was reduced by 35.1% and 27.4% for the CPC mouthwash in the alcohol-free and 6% alcohol base, respectively, compared to the control mouthwash.

In the fifth article, Ayad and co-workers present the results of a clinical study in which the alcohol-free mouthwash containing 0.075% CPC was compared to a control mouthwash without CPC on controlling established plaque and gingivitis after three and six months of use.\textsuperscript{54} The alcohol-free mouthwash with 0.075% CPC was statistically significantly (p < 0.05) better than the control mouthwash in reducing established plaque and gingivitis after three and six months. After three and six months, the alcohol-free mouthwash with 0.075% CPC provided statistically significant (p < 0.05) reductions in gingival, gingival interproximal, gingival severity, plaque, interproximal plaque, and plaque severity index scores of 25.0%, 22.3%, 38.9%, 26.1%, 22.4%, and 75.0%, respectively, as compared to the control mouthwash. After six months of product use, the alcohol-free mouthwash with 0.075% CPC provided reductions in gingival, gingival interproximal, gingival severity, plaque, interproximal plaque, and plaque severity index scores of 38.1%, 37.1%, 63.6%, 36.5%, 33.2%, and 78.5%, respectively, as compared to the control mouthwash.

In conclusion, the laboratory and clinical research presented in this Special Issue provide scientific evidence that a new alcohol-free mouthwash formulation containing 0.075% CPC provides effective antibacterial, antiplaque, and antigingivitis efficacy. The CPC in the mouthwash is highly bio-available. The mouthwash effectively kills representative oral bacteria in vitro in the planktonic state by > 99.9%, reduces bacteria in vivo after a single use and after 14 days of continuous use, and reduces plaque build-up, gum inflammation, and bleeding. The research, therefore, supports that daily use of mouthwashes with 0.075% CPC, with and without alcohol, represents a valuable complement to daily mechanical plaque control.

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References


29. Smith RN, Andersen RN, Kolenbrander PE. Inhibition of intergeneric co-
42. Ashley FP, Skinner A, Jackson P, Woods A, Wilson RF. The effect of a 0.1% cetylpyridinium chloride mouthrinse on plaque and gingivitis in adult sub-