ORIGINAL ARTICLE

The antibacterial effect of four mouthwashes against streptococcus mutans and escherichia coli

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Abstract

Objective: To evaluate the antimicrobial properties of several mouthwash concentrations on oral Streptococcus mutans and Escherichia coli.

Methods: The study was conducted at Shiraz Medicine School in 2011. Serial dilutions of Chlorohexidin, Oral B and Persica and Irsha (2,4,8,16,64,128) were prepared in Muller-Hinton media. Minimum inhibitory concentration was visually determined and defined as the lowest concentration of each oral washing which inhibited ≥ 95% growth reduction compared to the growth control well.

Result: Chlorhexidine, Oral B and Irsha mouthwash inhibited Streptococcus mutans even with diluted concentrations. Also, Chlorhexidine and Oral B prohibited Escherichia coli with different potencies. But Persica had no antimicrobial activity against either Escherichia coli or Streptococcus mutans.

Conclusion: Chlorhexidine, Irsha, and Oral B mouthwashes can be used for antimicrobial effects, especially on Streptococcus mutans. This chemical activity of mouthwashes is an adjuvant for mechanical removing of plaque. However, the antimicrobial effect of Persicaremains controversial.

Keywords: Laboratory research, Chlorhexidine, Persica, Listerine, Oral B, Streptococcus mutans, Escherichia coli. (JPMA 65: 350; 2015)

Introduction

Improvement of oral health can influence the life quality of people, so development of the new preventive and treatment methods and products which are safe, effective and economical is necessary. Mouthwashes can inhibit dental plaque, and are widely used to maintain oral hygiene. Dental plaque formation begins with the accumulation of gram-positive streptococci, developed by gram-negative anaerobic bacteria aggregation.[1]

Mouthwashes are non-sterile aqueous solutions. They are used for reducing oral bacteria, cleaning food remnants and for decreasing oral malodour. Since many people cannot remove dental plaque properly and mechanical plaque control alone is not enough, chemical plaque controlling such as mouthwash can be suggested.[2,3]

Irsha (an Iranian brand) has equivalent chemical formula of Listerine. It contains phenol and essential oil. It affects the bacterial cell wall and their proliferation. A study evaluated the antiseptic effect of essential oil containing mouthwash (Listerine) on Streptococcus mutans (S.mutans). The results showed that using Listerine can reduce streptococcal colonisation in both saliva and dental plaque.[4]

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Chlorhexidine (from the biguanidin group) is one of the most common mouthwashes prescribed for plaque control.[5] Review of literature showed Chlorhexidine decreased oral S.mutan colonies.[5-9]

Persica is a herbal mouthwash prepared from Salvadora Persica, Achilleamilefolium and Mentaspicata. It does not have side effects of chemical mouthwashes. It is safe for children and pregnant women.[5,10] There are some reports of antimicrobial effects of Persica on oral S.mutans.[5,6,11]

Oral B is an alcohol-free mouthwash consisting of cetylpyridinium chloride and fluoride.[12]

Oral cavity ecosystem represents a dynamic pattern and it is not advisable to eliminate all bacterial micrflora. The ideal condition is removing most cariogenic and periodontopathic agents from dental plaque.[13]

S.mutans is a facultative anaerobiccoccus-shaped, gram-positive bacteria commonly found in the oral cavity and has a major role in tooth decay formation. S.mutans metabolise sucrose to lactic acid.[14,15] There are 25 species of oral streptococci in human oral cavity. Each of them develops specialised properties for colonisation in oral sites and constantly changes conditions to fight competing bacteria. Oral diseases can be initiated by imbalances in the microbial flora. In specific conditions, streptococci can change to opportunistic pathogen that can initiate the disease and damage the host. Oral streptococci is mentioned both as

harmless and harmful bacteria.[16]

A US study showed that there was positive correlation between the concentration of S.mutans in saliva and its isolation from the smooth surface of the teeth; on the other hand there was no positive relation.[17]

Escherichia coli (E.coli) are gram-negative, anaerobic rods that can be found in the lower intestine. Most strains of E.coli are harmless, but in humans, some serotypes may cause serious food poisoning.[18]

The current study was conducted to evaluate antimicrobial properties of several concentrations of these mouthwashes on oral S.mutans and E.coli. The study can help dentists in prescribing the most effective mouthwash with minimal side effects. Also, the results can suggest the proper concentration of these mouthwashes.

Materials and Methods

The experimental study was conducted in the Department of Microbiology, Shiraz Medicine School in 2011, using standard species of S.mutans (ATCC000) and E.coli (ATCC25922). Minimum inhibitory concentrations (MICs)were determined using the broth micro-dilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) with some modifications.[19] To determine the antibacterial activities, serial dilutions of Chlorohexidin, Oral B and Persica (2,4,8,16,64,128) were prepared in Muller-Hinton media (Merck, Darmstadt, Germany). S.mutans and E.coli strains were suspended in Muller Hinton media and cell densities were adjusted to 0.5 McFarland standards at 530nm wavelength using a spectrophotometer method (this yields stock suspension of 1-1.5×108 cells/ml of bacteria). Then 100µl of working inoculums was added to 100µl of various concentration of Chlorohexidin,Oral B and Persica in the microtiter plates which were incubated in a humid atmosphere at 37ºC for 24 hours. Later, 200µl of uninoculated medium was included as a sterility control (blank). In addition, growth controls (medium with inoculums but without three oral washings) were also included. The growth in each well was compared with that of the growth control well. MICs were visually determined and defined as the lowest concentration of each oral washing which inhibited ≥95% growth reduction compared with the growth control well. Each experiment was performed in triplicate.

Result

Chlorhexidine inhibited S.mutans at several diluting concentration up to 1:128 (1:2, 1:4, 1:16, 1:32, 1:64 and 1:128). Also, this mouthwash prohibited E.coli at these diluting concentrations, including 1:2, 1:4, 1:16, 1:32 and 1:64.

The growth of both E.coli and S.mutans were inhibited by

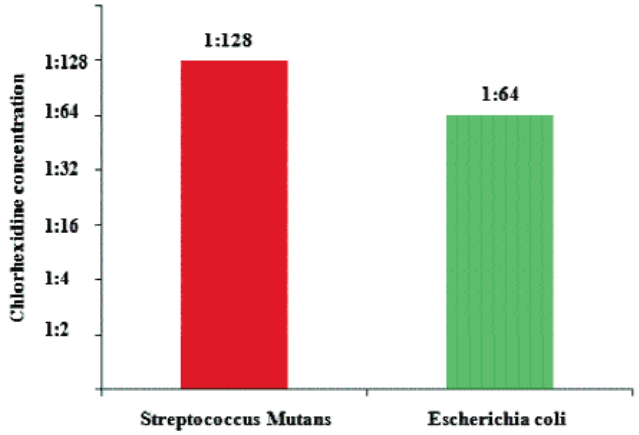

Figure-1: Antibacterial effect of Chlorhexidine.

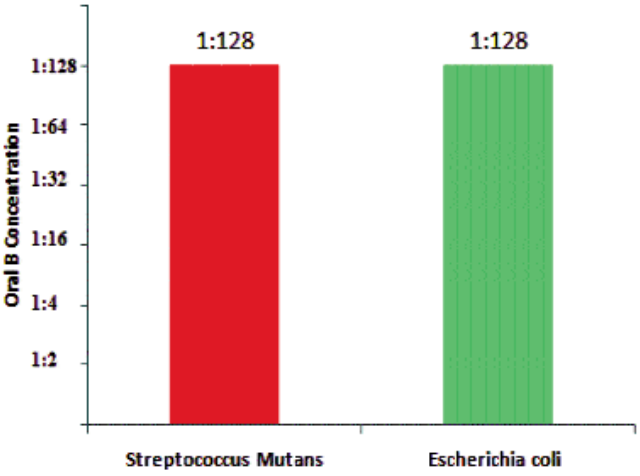

Figure-2: Antibacterial effect of Oral B.

Oral B mouthwash at concentrations of 1:2, 1:4, 1:16, 1:32, 1:64 and 1:128.

The antimicrobial effect of Irsha against S.mutans was showed at diluting concentrations of 1:2, 1:4, 1:16, 1:32, 1:64 and 1:128. But this mouthwash had no antimicrobial effect against E.coli at any concentration.

Against other mouthwashes, Persica did not inhibit either E.colior S.mutans at any concentration.

Each diluting concentration of 1:2, 1:4, 1:16 of mouthwashes decreased the bacterial count from 1-1.5×10^8 cells/ml of bacteria to zero. Diluting concentration of 1:32, 1:64 and 1:128 decreased the bacterial count to 10,100 and 1000 bacteria which seems to be significant; these counts being the average of triple repeat of examinations.

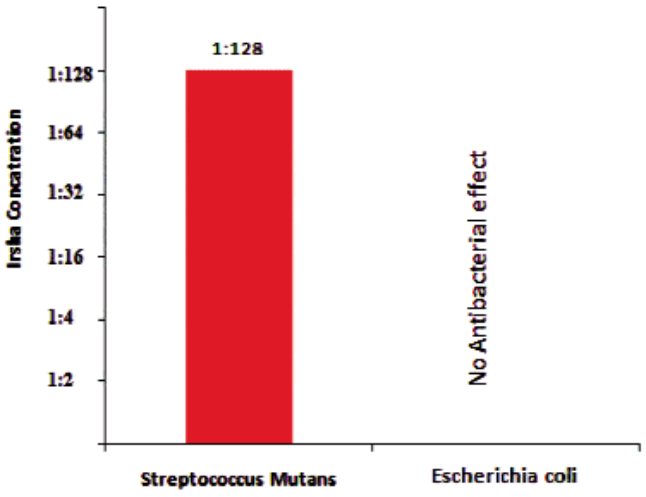

Figure-3: Antibacterial effect of Irsha.

Discussion

Chlorhexidine, Oral B and Irsha mouthwashes inhibited S.mutans even with their diluted concentration in this study. Also, Chlorhexidine and Oral B prohibited E.coli with different potency. But Persica had no antimicrobial activity against both E.coli and S.mutans.

Studies have reported different results. One reported more considerable antimicrobial effect of essential oil-containing mouthwash (Listerine Antiseptic) compared to the amine fluoride/stannous fluoride mouthrinse.[20]

Another study evaluated the antimicrobial efficacy of Listerine mouthrinses. Listerine killed S.mutans completely in 10 to 30 seconds.[21]

One study investigated antimicrobial activities of herbal mouthrinse, Chlorhexidine 0.12% and essential oil mouthwash. Chlorhexidine was the most potent mouthwash. The herbal mouthrinse showed more antimicrobial activities than essential oil mouthrinse against S.mutans.[22]

Another study evaluated bactericidal effect of essential oil-containing mouthwash (Listerine) on S.mutans. It reported significant reduction in S.mutan colonies in both dental plaque and saliva.[4]

These studies focused on antimicrobial efficacy of essential oil mouthwashes alone or in comparison to others. In our study different concentration of mouthwashes were tested on oral S.mutans and E.coli. The results showed that Irsha could not affect E.coli colonisation, but could inhibit S.mutans growth. The results are in agreement with earlier findings.[4,21]

One set of researchers reported a significant reduction in S.mutans amount by rinsing Chlorhexidine gluconate mouthwash in patients with fixed orthodontic appliances.[8]

One study compared the antimicrobial efficacy of Chlorhexidine mouthwash (0.12%) and two other mouthwashes against S.mutans, Candida albicans and lactobacilli. S.mutans was isolated from unstimulated saliva of the patients. Chlorhexidine mouthwash (0.12%) showed the best anti-microbial efficacy against all of these organisms.[9]

A study evaluated the effect of Chlorhexidine and Persica on S.mutans in patients who had received fixed orthodontic treatment. Although Chlorhexidine was more potent, but Persica reduced the S.mutans colonies ($p<0.001$) without the side effects of Chlorhexidine.[5]

Another study compared some concentrations of herbal antimicrobial mouthwashes (miswak extract and Persica) in diluting concentration of 0.1, 0.05, 0.025% and 0.1, 0.05, 0.025, 0.0125%, with Chlorhexidine mouthwash in concentrations of 0.2, 0.1, 0.05 0.025%. Chlorhexidine demonstrated higher inhibitory effect on Streptococcus strains growth and herbal mouthwashes showed borderline antimicrobial efficacy.[6]

In US, a study compared antimicrobial activity of miswak extract 50% with some mouthwashes including Chlorhexidine. It reported that Chlorhexidine was the most effective agent against S.mutans among other tested mouthrinses.[7]

Our study demonstrated that Chlorhexidine, Oral B and Irsha mouthwashes inhibited S.mutans growth up to diluting concentration of 1:128. This finding is similar to earlier findings. Most of these researchers advised Chlorhexidine as the most powerful antimicrobial agent in preventing bacterial growth among other mouthwashes such as Persica, miswak extract and essential oil.[5-7,9]

A double-blind, cross-over trial in 2004 in New Zealand to compare the oral health efficacy of Persica mouthwash (extract of Salvadora Persica) with placebo. The results showed reduction in salivary concentrations of S.mutans ($p< 0.05$) by a three-week use of Persica, but this reduction was not achieved by placebo.[11] In contrast to what a study found, our results showed no antimicrobial effect of Persica on oral S.mutans and E.coli at any concentration.

A study evaluated the antimicrobial effect of some topical oral agents including Chlorhexidine (0.12%) with and without alcohol, baking soda-salt rinse, 0.4% stannous fluoride gel, 0.63% stannous fluoride rinse, calcium phosphate mouthrinse, and acemannan hydrogel (aloe vera) rinse on common oral microorganisms of patients with head and neck cancer who had received radiotherapy. In an in-vitro study E.coli, S.aureus, group B Streptococcus and C.albicans were

evaluated. The most potent antibacterial agent was Chlorhexidine (0.12%) with and without alcohol. Also E.coli was the most sensitive microorganism.[2] The results of our research are also confirmed by literature.[22]

One study evaluated the antimicrobial effect of methanolic extract of Algerian Hoggar Salvadora Persica (miswak) on some oral microorganisms isolated from dental plaque such as Streptococcus and Escherichia. Miswak showed a significant in vivo and in vitro antimicrobial effect.[2]

Beside miswak, the antimicrobial effect of Oral B against E.coli was reported, but Chlorhexidine was more potent.

Variable commercial brands of each mouthwash, different ingredients and maybe different study design can affect these results and can explain these diversities. Précising the concentration, ingredient, and commercial brand of tested mouthwashes may help the investigators to distinguish the cause of these differences.

Conclusion

Chlorhexidine, Irsha (Listerine), and Oral B mouthwashes can be used for their antimicrobial effects, especially on S.mutans. This chemical activity of mouthwashes is an adjuvant for mechanical removing of plaque. The antimicrobial effect of Persica is controversial and more investigations are required.

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