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# Effect of Saccharin on Antibacterial Activity of Chlorhexidine Gel

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Although chlorhexidine is the most effective agent against dental plaque it is extremely bitter. To prepare formulations, it is necessary to use flavoring and sweetening, which can inhibit the antibacterial effect of chlorhexidine. Saccharin has been considered a compatible substance to use in chlorhexidine rinse or gel preparations; however, the effect of a range of concentrations has not been studied. To evaluate the effect of different concentrations of saccharin on the antibacterial activity of chlorhexidine gel, hydroxy-ethyl-cellulose gels containing 1.0% chlorhexidine digluconate and 0.0 to 1.0% sodium saccharin were prepared. Activity against *Streptococcus mutans* was evaluated using the agar diffusion method and determination of MIC values. The inhibitory zones of growth were 7.83 ± 0.54 mm when no saccharin was added to the chlorhexidine gel and 7.75 ± 0.50, 7.63 ± 0.48, 6.21 ± 0.40, 4.13 ± 0.38, when the concentrations of saccharin in the gels were 0.02, 0.10, 0.5, and 1.0%, respectively. The range of MIC values was 1-2 µg/ml, with saccharin concentrations of 0%, 0.02, and 0.1%. In contrast, the MIC values were 4-8 and 8-16 µg/ml with saccharin concentrations of 0.5% and 1.0%, respectively. The paired "t" test showed that 0.5 and 1.0% sodium saccharin inhibit the antibacterial activity of 1% digluconate chlorhexidine gel. These *in vitro* results suggest that saccharin may inhibit the efficacy of chlorhexidine against mutans streptococci, depending on the concentration.

Key Words: chlorhexidine, saccharin, *Streptococcus mutans*, dental plaque, antibacterial.

## Introduction

Chlorhexidine (CHX) is recognized as the primary agent for chemical plaque control (Jones, 1997). It can be used in mouthrinses and gels (Cummins and Creeth, 1992). Mouthrinse formulations are effective in reducing gingivitis (Grossman et al., 1986) and gels are potent chemotherapeutic agents against mutans streptococci and caries (Emilson, 1994). The major advantage of CHX over most other compounds lies in its oral substantivity (Adams and Addy, 1994), because it is a cationic substance that binds to soft and hard tissues of the mouth (Rolla and Melsen, 1975) as well as to bacterial cell walls (Jones,

1997). However, when formulations are prepared the availability of CHX can be impaired. While dentifrices are considered inappropriate vehicles to deliver CHX because of the detrimental interactions between CHX and the foaming and abrasive agents used (van der Ouderaa and Cummins, 1989), mouthrinses and gels are considered compatible (van der Ouderaa, 1991).

Because CHX has an extremely bitter taste, it is often necessary to flavor and sweeten mouthrinses and gel products. Saccharin is considered compatible with CHX (van der Bijl and Dreyer, 1982) and has been used in gel preparations (Ostela et al., 1990; Tenovuo et al., 1992). However, when a 1% CHX gel regime suggested by Maltz et al. (1981) was applied in volunteers using removable dentures we were not able to show salivary mutans streptococci reduction (Rocha et al., 1999). Our explanation was that the *in vivo* antibacterial activity of CHX was inhibited by saccharin added to improve the taste of the gel. Considering that saccharin has not been recognized as a substance incompatible with CHX (Gardner and Gray, 1983), we decided to test the hypothesis that this inhibition could be an effect of concentration.

## Material and Methods

The antibacterial activity was evaluated by the agar diffusion method using brain heart infusion (BHI, Difco, Detroit, MI, USA) and Mueller Hinton (Difco) agars. The microorganism was seeded by pour plate. *Streptococcus mutans* Ingbritt 1600 (kindly donated by Eastman Dental Center, Rochester, NY, USA) actively growing in BHI broth was subcultured onto BHI agar plates (100 x 20 mm) for 18-24 h at 37°C in a 10% $CO_2$ environment (IG 150 incubator, Jouan S.A., Saint-Herblain, France). Isolated colonies were suspended in 5.0 ml of sterile 0.85% NaCl solution, homogenized in a vortex mixer and the suspension was adjusted spectrophotometrically to match 0.5 of turbidity according to the McFarland standard. A volume of 0.5 ml was mixed with 50 ml BHI agar at 45°C and poured onto a Petri dish (150 x 25 mm) containing a previous set layer of MH agar. The inoculum procedure was appropriate to provide a semi-confluent bacterial growth (Phillips, 1991). To evaluate the CHX gel antibacterial activity, 5 sterile stainless cylinders (8.0 x 10.0 mm) were placed onto each inoculated agar plate. The cylinders were filled with 1% CHX gels (pharmacy prepared, containing 1.0 g chlorhexidine digluconate (Medichem SA, Spain); 1.0 g hydroxy-ethyl-cellulose 250 (Galena Química e Farmacêutica Ltda, Campinas, SP, Brazil); 0.0, 0.02, 0.1, 0.5 or 1.0 g sodium saccharin (KD Feddersen & Co, Germany), and deionized water to 100 g). The plates were incubated at 37°C for 24 h in an incubator containing 10% $CO_2$ and the zones of growth inhibition around the cylinders were measured using a digital caliber rule (± 0.01 mm).

For statistical analysis, 6 replicates were made and the inhibitory zones, in mm, were evaluated by paired "t" test at $p<0.05$. In order to check if the gel formulation affected the diffusibility of the active agent (CHX), all of these preparations were also diluted in sterile deionized water to reach 0.12% of CHX and tested for antibacterial activity following the same procedures described above. An aliquot of 100 µl of the preparation was added to

stainless cylinders and incubated as previously described. Chlorhexidine digluconate (C-9394, Sigma Chemical Co., St. Louis, MO, USA) diluted to 0.12% was used as a positive control.

In addition, the minimum inhibitory concentration (MIC) of diluted CHX gel preparations was determined. The assay concentration of *S. mutans* was 1-2 x $10^5$ colony forming units/ml (CFU/ml) (Barry et al., 1983; Phillips, 1991). Tubes containing BHI broth inoculum and a two-fold dilution series of CHX formulations (concentrations ranging from 0.25 to 16 µg of CHX/ml) were incubated at 37°C, 10% $CO_2$, for 24 h. After incubation, bacterial growth was assayed spectrophotometrically by measurement of absorbance at 660 nm ($A_{660}$). MIC was defined as the lowest concentration of CHX that restricted growth to a level <0.05 $A_{660}$ (no visible growth). Six replicates were made for each analysis.

## Results and Discussion

The values of inhibitory zones are shown in Table 1. At concentrations of 0.02 or 0.1%, saccharin did not significantly inhibit the antibacterial activity of 1% CHX gel. However, at concentrations of 0.5 and 1% saccharin significantly reduced ($p<0.05$) the anti-mutans activity of 1% CHX gel, as demonstrated by the smaller inhibitory zones. These data were confirmed by analyzing the MIC values (Table 2). The addition of 0.5% and 1% saccharin reduced the antibacterial activity 4-8 and 8-16 times, respectively, when compared to the CHX standard (Sigma), whereas 0.02 and 0.1% saccharin did not significantly affect the anti-mutans activity of CHX. The range of MIC values (1-2 µg/ml) obtained for CHX standard (Sigma) is in agreement with previous studies (Osawa et al., 1992; Järvinen et al., 1993; Drake et al., 1993). Therefore, these results demonstrate that depending on the

Table 1 - Means (± SD) of the inhibitory zone of *S. mutans* Ingbritt 1600 growth according to the formulations evaluated.

| Formulations | Inhibitory zone (mm) Gel preparation | Inhibitory zone (mm) Diluted gel* |
|---|---|---|
| 1% CHX | 7.83 ± 0.54[a] | 6.50 ± 0.30[a] |
| 1% CHX + 0.02% Saccharin | 7.75 ± 0.50[a] | 6.42 ± 0.29[a] |
| 1% CHX + 0.1% Saccharin | 7.63 ± 0.48[a] | 6.33 ± 0.25[a] |
| 1% CHX + 0.5% Saccharin | 6.21 ± 0.40[b] | 5.00 ± 0.30[b] |
| 1% CHX + 1.0% Saccharin | 4.13 ± 0.38[c] | 3.25 ± 0.26[c] |

*The gel preparations were diluted in sterile deionized water to reach a concentration of 0.12% CHX. The inhibitory zone of bacterial growth of CHX standard (Sigma) at a concentration of 0.12% was 6.54 ± 0.26.

Different superscript letters indicate statistically significant differences ($p<0.05$) among the formulations.

Table 2 - The range of MIC values of different formulations of CHX for *S. mutans* Ingbritt 1600.

| Formulations | Range of MIC values (μg/ml)* |
|---|---|
| 1% CHX | 1-2 |
| 1% CHX + 0.02% Saccharin | 1-2 |
| 1% CHX + 0.1% Saccharin | 1-2 |
| 1% CHX + 0.5% Saccharin | 4-8 |
| 1% CHX + 1.0% Saccharin | 8-16 |

*Two-fold dilution series of CHX formulations at concentrations ranging from 0.25 to 16 μg of CHX/ml. The range of MIC values of CHX standard (Sigma) was 1-2 μg/ml.

concentration, saccharin can inhibit CHX antibacterial activity *in vitro*. These data explain our previous *in vivo* study when we were not able to show reduced mutans streptococci when 1% CHX gel containing 1% saccharin was used (Rocha et al., 1999).

The present *in vitro* evaluation supports the observations of van der Bijl and Dreyer (1982) who prepared a mouthrinse containing 0.2% CHX and 0.01% saccharin, in combination with 0.1% cyclamate to improve its taste. If saccharin had been the only sweetener added at a higher concentration, CHX may have been inhibited. The results also support the observations of Ostela et al. (1990) and Tenovuo et al. (1992) who reported a reduction of salivary mutans streptococci when 1% CHX gel containing 0.02% saccharin was applied in volunteers.

The sweetness acceptability of a therapeutic product may be variable depending on regional and cultural differences. In Brazil, most oral hygiene products contain 0.2 to 1% saccharin. Particularly for CHX gel formulations, 1% saccharin is added to make the gel acceptable. Therefore, when saccharin is used, the detrimental effect of concentration on CHX activity should be considered or another non-complexing sweetener should be used. When we repeated our previous study using CHX gel containing aspartame as sweetener there was a reduction in salivary mutans streptococci in volunteers using removable dentures (Rocha et al., 1999).

The results of this *in vitro* study clearly showed that, depending on the concentration, saccharin inhibits the antibacterial activity of CHX. It is more difficult to understand this inhibition because the concentrations are in percentage. However, considering that in the gel containing 1% chlorhexidine digluconate and 1% sodium saccharin there were 10 millimoles of CHX for 46 millimoles of saccharin, it is easier to understand the inhibition. This can be explained by stoichiometric binding of CHX by saccharin (Sac) according to the equilibrium:

$$K = \frac{[CHX(Sac)_2]}{[CHX]\,[Sac]^2} \qquad CHX^{2+} + 2Sac^{-} \rightleftharpoons CHX(Sac)_2$$

In fact, the data suggested that insoluble salts $[CHX(Sac)_2]$ were formed between CHX and saccharin which may be through an interaction between the positively charged groups of CHX and the sulfonyl group of saccharin. Precipitated insoluble products were

observed when the gel formulations were suspended in water. It was possible to note that the 1% CHX gel containing 0.5 and 1% of saccharin are cloudy while the gel without saccharin is clear. Even the gel containing 0.1% of saccharin is not totally transparent, but the CHX available still showed a significant antibacterial activity. There is a precedence for this in the literature; zinc, a well-known antibacterial agent can be complexed by saccharin (Christie et al., 1991).

In conclusion, these *in vitro* data suggest that depending on the concentration of saccharin used to make CHX acceptable, the *in vivo* antibacterial activity of this CHX may be reduced.

## Resumo

Cury JA, Rocha EP, Koo H, Francisco SB, Del Bel Cury AA: Efeito da sacarina na atividade antibacteriana da clorexidina. Braz Dent J 11(1): 29-34, 2000.

Embora clorexidina seja reconhecida como o agente antimicrobiano mais eficiente contra placa dental, seu gosto extremamente amargo é uma limitação nos preparos farmacêuticos. Substâncias adoçantes e flavorizantes usadas para preparar formulações podem inibir a atividade antibacteriana da clorexidina. Sacarina tem sido considerada uma substância compatível para ser usada em enxaguatórios bucais ou géis, entretanto o efeito da concentração deste adoçante não tem sido estudado. A atividade antibacteriana de géis de clorexidina a 1%, contendo sacarina de 0,0 a 1,0%, foi avaliada a partir de preparações farmacêuticas formuladas. Atividade contra *Streptococcus mutans* foi avaliada através da inibição do crescimento em ágar e determinação da concentração inibitória mínima (CIM). Os halos de inibição de crescimento foram de 7,83 ± 0,54 mm, na ausência de sacarina, e de 7,75 ± 0,50, 7,63 ± 0,48, 6,21 ± 0,40 e 4,13 ± 0,38 quando da presença de sacarina a 0,02, 0,10, 0,5 e 1%, respectivamente, nos géis de clorexidina a 1%. A faixa de CIM foi de 1-2 µg/ml quando da presença de 0,0, 0,02 e 0,1% de sacarina nos géis. Quando o gel de clorexidina a 1% continha sacarina a 0,5 e 1% a CIM foi de 4-8 e 8-16 µg/ml, respectivamente. Teste "t" pareado mostrou que sacarina sódica nas concentrações de 0,5 e 1% inibiu a atividade anti *mutans* de digluconato de clorexidina a 1% em gel. Estes resultados *in vitro* sugerem que sacarina pode inibir a eficácia de clorexidina contra streptococcus do grupo mutans, dependendo da concentração usada.

Unitermos: clorexidina, sacarina, *Streptococcus mutans*, placa dental, antimicrobianos.

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