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Research Article

Effect of curcumin on acidogenicity, viable bacteria and biomass in experimental biofilm model on human tooth

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ABSTRACT

Dental caries is the most prevalent disease worldwide.The goal of the study was to determine the effect of curcumin on the acidogenicity, viable bacteria and biomass in an experimental biofilm caries model on human enamel. A validated caries model with Streptococcus mutans ATCC 3568 biofilms was used. Biofilms were formed on enamel slabs during five days. To copycariogenic challenges, slabs were exposed three times per day for five minutes to 10% sucrose followed by five additional minutes of exposure to different dilutions of curcumin(5 , 10 and 20 mg/ml) in dimethyl sulfoxide. Slabs were exposed to 10% sucrose followed by 0.9% NaCl served as caries-positive control.The acidogenicity of medium was measured twice per day by a pH meter. After the experimental phase, biofilms were recovered andthe biomass and viable bacteria were determined.Significant differences were observed in the number of viable bacterial cells and in the biomass ($P < 0.05$).Our findings suggest that curcumin significantly reduced the number of viable bacteria. Curcumin20 mg/ml appears to be the most effective dilution on the reduction of viable bacteria. All dilution of Curcumin decreased the amount of biomass in biofilms.

Keywords: Curcumin, Streptococcus mutans, Dental caries, Biofilm

INTRODUCTION

Dental caries is a major public health problem. It is one of the most prevalence disease in the world (1), affecting more than one-third of the population at all ages.(2)Dental caries results from

bacteria on teeth that breakdown foods and produce acid that destroys tooth enamel. (3)Mutans streptococci have been known as a main cause of dental decay in humans and experimental animals (4)Sucrose is the most cariogenic dietary carbohydrate and can lower the PH of plaque biofilm.(3, 5)Because of the high prevalence and its negative consequences of dental caries, new approaches are needed to decrease dental caries.As caries restorative treatment is expensive and dental health care has usually low coverage among the population in most countries,novel preventive approaches are required.

The antimicrobial effects of polyphenols have been widely reported. Polyphenols have the ability to inactivate bacterial toxins. The interest in the effects of natural products such as polyphenols in dental caries is increasing. The results of different studies support the hypothesis that bacteriahave animportant role in the etiology of dental caries.(6)

Curcumin, an active ingredient of turmeric, has shown powerful anti-inflammatory and antioxidant effects.Curcumin shows a variety of biological properties such as anti-proliferative activity against cancer cells and antioxidant activity.(7, 8)Moreover, it depicts antimicrobial activity in vitro,against a number of Gram-negative and Gram- positive bacteria such as Bacillus subtilis, Escherichia coli, Helicobacter pylori, Salmonella enterica serovar Typhimurium, and some Staphylococcus aureus strains.(9-12)Therefore, the present study aims to investigate the effect of curcumin on S. mutans and biofilm formation.

MATERIALS AND METHODS

Experimental designA previously validated caries model with biofilms of the cariogenic Streptococcus mutans (Strep. mutans) ATCC35668was used.(13). Strep. mutans biofilms were formed on human enamel slabs for 5 days. Three groups of Biofilms/slabs were exposed three times per day to a solution of 10% sucrose (w/v) for 5 min to create cariogenic conditions,and after that slabs exposed to different curcumin dilution for fiveadditional minutes. The number of slabs in each group was 10.The slabs of the first group were exposed to 10% sucrose + 5 mg/ml curcumin.

The slabs of the seccond group were exposed to 10% sucrose + 10 mg/ml curcumin and in the third group slabs were exposed to 10% sucrose + 20mg/mlcurcumin.Two groups of slabs were exposed to 10% sucrose+0.9% NaCl and 0.9% NaCl+0.9% NaCl as a positive caries control andnegative caries control, respectively. Culture medium was replenished twice per day, and the pH of the spent medium was measured to evaluate acidogenicity.Culture medium was replenished twice a day, before the first and after the last exposure. The exposure cycles were repeated to complete 5 days, a known time to induce enamel demineralization.After 5 days of the experimental phase, biofilms were recovered to assess total biomass and viable bacteria(14)

Biofilm acidogenicity

To verify acidogenicity of the biofilm, culture medium pH was measured. A pH meter was used to register the pH. Spent culture medium generated by the biofilm was read with the pH meter twice per day, before each medium change.(14)

Biomass

A 200 µl aliquot from the biofilm suspension was transferred to a pre-weighted tube and incubated with 100% ethanol at -20°C for 15 min, centrifuged (10 min at 5000 g and 4°C), and the resulting pellet was washed with 500 µl of 75% ethanol. After a second centrifugation, the pellet was dried for 24 h in a desiccator. Biomass was calculated by subtracting the fi Bi weight to the initial weight of the empty tube. Biomass dry weight of the biofilm was expressed as mg per ml of biofilm suspension. (15)

Viable bacteria in the biofilm

Serial dilutions from the 50 µl of the biofilm suspension in 0.9% NaCl (v/v) were inoculated in duplicate by the drop-counting technique on BHI

agar plates. Plates were incubated anaerobically for 24 h at 37°C, and the colony-forming units (CFU) were determined by colony-counter.Counting was corrected by the dilution factor and expressed as CFU mg^{-1} of biofilm dry weight.(16)

Statistical analysis

An ANOVA test was carried out to detect differences among all the experimental groups in each of the dependent variables under study.A post hoc test and Tukey HSDserved to detect statistical differencebetween each pair of experimental group. The SPSS version22 statistical software was used to manipulate the data, setting a 95% confidence level.

RESULTS

No differences among the groups were detected during the first 8 h ($P > 0.05$).After that the amounts of PH decreases in all groups except the caries-negative control. However, slabs exposed to the 20mg/mlcurcumin and the caries-positive control showed lower PH than the rest of the experimental dilutions.The critical demineralizing pH of enamel is lower than 5.5. Samples exposed to the 5 mg/mlcurcumin failed to reach the critical pH at any time.The amount of PH in the five days of experiment decreased in all groups except the caries- negative control. Reduction of PH is the result of bacteria activity that produces acid.

Table 1illustrates the characteristics of the biofilms of Strep. mutans retrieved from the slabs after the five days of experiment. When compared tothe caries-positive control group, all tested curcumindilutions decreased the number of Strep. Mutans (CFU ml^{-1}) .($P<0.001$).The highest decrease in the number of viable bacteria was seen in sucrose followed by 20 mg/ml curcumin exposure(third group). (1.2×10^{6} CFU mg^{-1}). All of the samples exposed to the curcumin with different dilution showed decrease in the numbers of viable bacteria in comparison with the caries-positive control ($P<0.001$).

Amount of biomass was lower in those slabs/biofilm which exposed to the different dilution of curcumin compared with the caries-positive control.($P<0.001$) The amount of biomass in biofilm withsucrose exposure followed by 10 mg/ml curcuminshowed similar behaviour than sucrose exposure followed by 20 mg/ml curcumin($P > 0.05$).

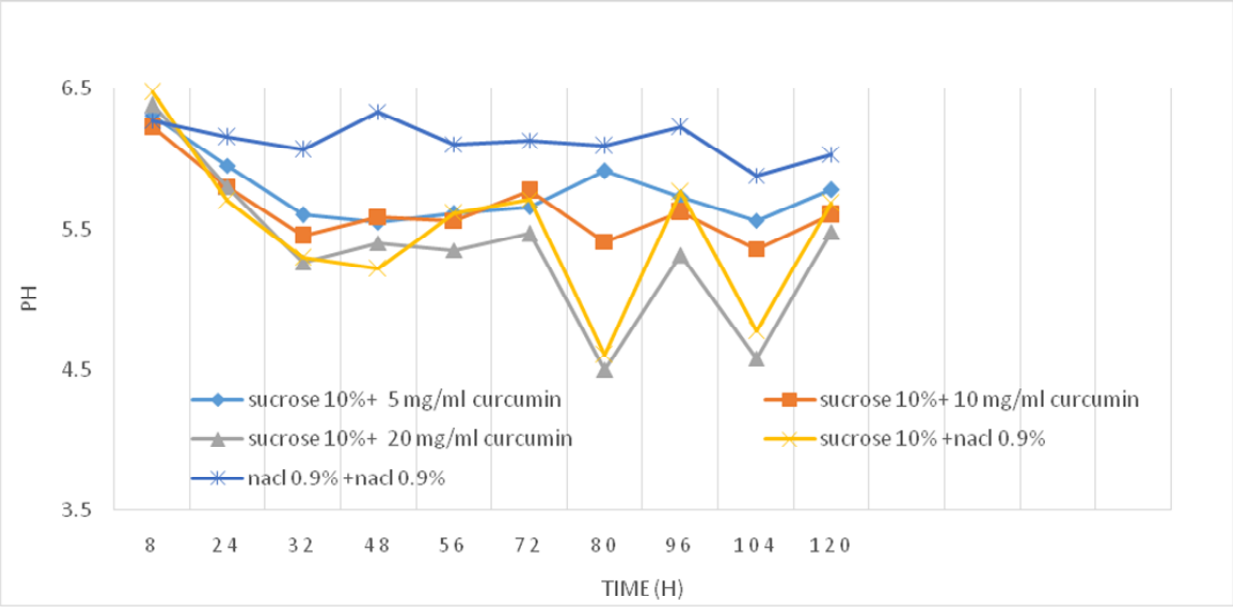

Figure 1 Acidogenicity elicited from the Streptococcus mutans biofilms formed on enamel in response to sucrose and dilutions of the Curcumin. Measurements were performed after 24 h of biofilm formation, twice per day, at defined times.

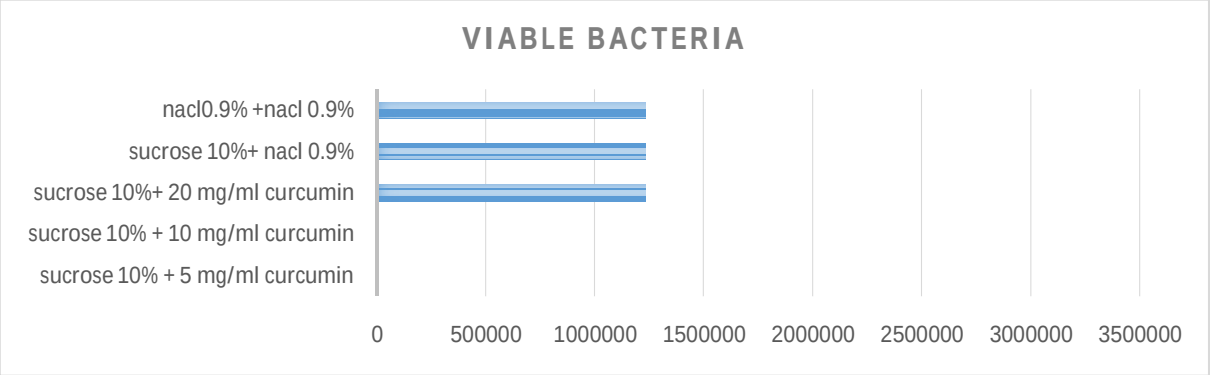

Figure 2 Serial dilutions from the biofilm suspension in 0.9% NaCl (v/v) were inoculated in duplicate by the drop-counting technique on BHI agar plates

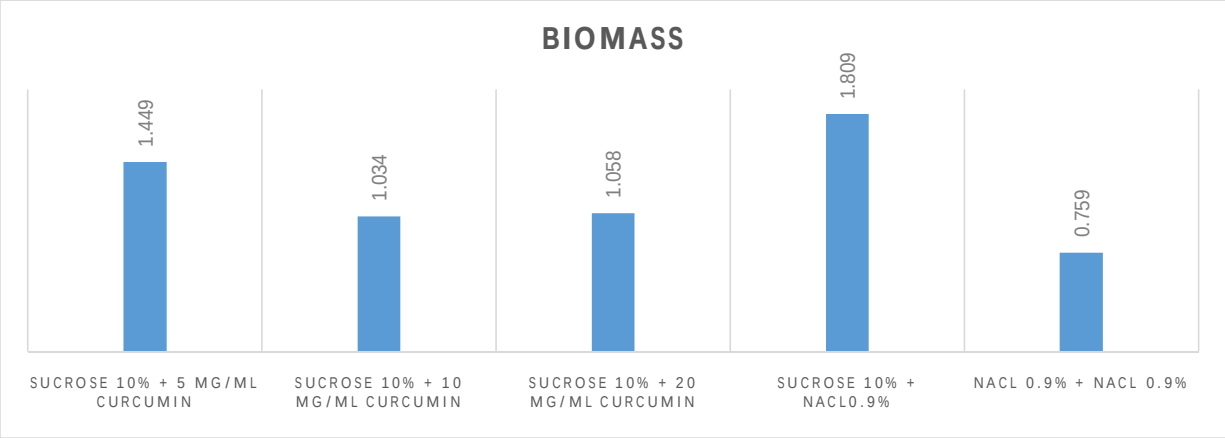

Figure 3 The aliquot from the biofilm suspension was transferred to a preweighted tube and after centrifugation, the pellet was dried biomass was calculated by subtracting the final weight to the initial weight of the empty tube.

Table1 Biofilm traits after exposure to sucrose followed by dilutions of curcumin.

Groups	5 mg/ml curcumin	10 mg/ml curcumin	20 mg/ml curcumin	Caries-positive control	Caries-negetive control	sig
Viable bacteria	1.6×10^{7}	2.01×10^{7}	1.35×10^{7}	2.39×10^{7}	2.95×10^{7}	P‹0.001
Biomass	1.499	1.034	1.058	1.809	0.759	P‹0.001
PH (in last days of the experiment)	5.78	5.60	5.48	5.68	6.03	P‹0.001

DISCUSSION

Previous studies have investigated the effects of polyphenolic compounds mainly extracted from teas, cranberries or propolis, for anti-cariogenic or anti-periodontitis purposes (17-19).In this study, we investigated the biological potential of curcumin, which existed in turmeric. In our investigation on anti-bacterial effect, the numbers of viable bacteria was lower in biofilms treated with curcumin.Qian et al. 2008 used Grape seed extract(GSE) consisted mainly of 97.8% proanthocyanidin (PA)in their study.(20) PA is potent antioxidants known to possess vasodilation, anticarcinogenic, anti-inflammatory, antibacterial and immune-stimulating effects.(21) High molecular mass PA (condensed tannin) from cranberry shows some effects on acid production and biofilm development by S. mutans. (19, 22, 23)Our results show thatcurcumin in the significant dilution tested in our study, decreases

the numbers of viable bacteria.The highest dilution of curcumin induces the most significant reduction in the number of bacteria. Our result are consistent with a previous study showing lower count of S.mutans colonies upon fluoridate-milk exposure (24) but are different from another investigation showing no activity against viable bacteria. (25) It was previously shown that curcumin did not affect the viability of S. aureus strain Newman, but inhibited microbial adhesion to cell surface. (26) In the experiment of Song et al. suggested curcumin as an anti-adherence agent against S. mutans rather than bactericidal agents. (27) Several studies have highlighted a range of natural products that inhibit acid production in cariogenic streptococci. These inhibitory effects appear to be related to a reduction in bacterial growth.(28)5 and 10 mg/ml curcuminsignificantly increase PH in the culture medium. Consistent with our findings, a study tested an extract of psidium cattleianum leaves on Strep. mutans.(29) While high concentration resulted in bacterial killing, low concentration of curcumin increased PH. According to the stady of Hirasawa et al (2004), Green tea catechins (EGCg) inhibited Acid production by S. mutans.(30) In conclusion, the findings suggest that curcuminsignificantlyreducethe number of viable bacteria. To our knowledge, biofilms treated with highest dilution of curcumin (20 mg/ml), showed lower number of viable microorganism and also lower biomass. Also curcumin decrease the amount of biomass in biofilms.

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