Effects of Caffeine and Theophylline on the Development of Dental Caries in Rats

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The purpose of this study was to evaluate the influence of caffeine and theophylline on the development of dental caries in rats. Six Wistar dams (spf), mutants streptococci free, were obtained, each with six male pups. The dams were infected by Streptococcus sobrinus 6715 and divided into three groups which received during the lactating period: (1) diet 2000; (2) diet 2000 plus caffeine (2 mg/100 g) and (3) diet 2000 plus theophylline (0.57 mg/100 g). After weaning, the pups were infected by S. sobrinus, placed in a König-Höfer programmed feeder machine, and received 17 meals daily at hourly intervals, for five weeks. During this time the pups were fed with the same diet that their dams were. The percentage of S. sobrinus relative to total flora was significantly higher in the theophylline group. The results for slight (D) and moderate (DM) dentine lesions, for smooth-surface and sulcal scores were statistically higher for the theophylline group than the other groups. Salivary assays did not demonstrate significant inorganic alterations in salivary composition. Caffeine and theophylline groups showed the highest ulcer score. It is concluded that caffeine does not affect the cariogenic potential of the diet, however theophylline can increase the development of dental caries, and this effect may be related to organic alterations of salivary composition.

Key words caries; caffeine; theophylline; saliva; peptic ulcer

Dental caries is a transmittable infectious disease that involves a triad of indispensable factors: bacteria (dental plaque), carbohydrates (the diet) and susceptible teeth (the host). The essential process involves demineralization of tooth enamel by high concentrations of organic acids produced by bacteria in dental plaque from dietary carbohydrates.1

Saliva has a major role in the maintenance of oral health.1) Severe reduction of salivary output considerably alters the quality of life of sufferers, who show trauma, taste alteration, ulceration of the oral mucosa, oral infections including candidosis and rapidly progressive dental caries and also difficulty in eating, swallowing and speaking. A large number of medications used specially by the elderly, have been related as a major cause of salivary gland hypofunction and xerostomia. Many drugs that induce hyposalivation can affect autonomic control; a typical example being propranolol.2

Non-prescribed drugs, such as caffeine (1,3,7-trimethylxanthine), are highly consumed because of their presence in a large number of beverages such as coffee, tea, soft drinks and over-the-counter medicines. In Brazil, a study on caffeine consumption in a large city demonstrated that the daily medium-ingestion potential of caffeine was 2.74 mg/kg body weight (b.w.) and coffee was the major source of caffeine.3 Caffeine stimulates the central nervous system (CNS) even in a moderate dose (200 mg/d), or the equivalent of three cups of coffee, and also has weak diuretic action, although tolerance may develop.4 Caffeine also has a stimulatory effect on gastric secretion.5 Animal studies have shown that lactating dams fed with a diet supplemented with caffeine (2 mg/100 g b.w.) can alter the mineral composition of the first molars of newborn rats,6,7,8 although Batirbagyi et al.9 did not find the same results when caffeine was administered to adult rats.

Theophylline (1,3-dimethylxanthine), another xanthine, used as a potent bronchodilator for the treatment of acute asthma, and as a medullar respiratory stimulator,10,11 also has effects on the circulatory system, increasing perfusion of some organs and diuresis. The usual dose is 400 mg/70 kg, up to a maximum dose of 800 mg/d. About 6% of a theophylline dose is converted in caffeine in adults, due to hepatic metabolism. In premature infants about 50% of an oral dose is recovered in the urine unchanged; the rest is metabolized to caffeine, and this drug can accumulate in blood up to 30% of the initial dose of theophylline.11

These pharmacokinetic data suggest that theophylline, like caffeine, can possibly increase the development of dental caries, an effect that could be explained by its diuretic action. Thus, the main purpose of this study was to evaluate the effect of chronic administration of xanthines on the development of dental caries in rats subjected to a cariogenic challenge.

MATERIALS AND METHODS

Animals Six Wistar female rats, specific pathogen free (spf) with six male pups each were obtained from Cemib/Unicamp (Centro de Bioterismo, ICILAS Monitoring/Reference Center, Campinas, Brazil).

Infection The dams were screened for indigenous mutants streptococci in MSA (Mitis Salivarius Agar, Difco Laboratories Ltd., São Paulo, Brazil) and MSB (Mitis Salivarius Agar, Difco Laboratories Ltd., São Paulo, Brazil plus bacitracin, Sigma Chemical Co., St. Louis, MO, U.S.A.), and then infected with an active growing culture of Streptococcus sobrinus 6715.12 The pups were weaned when 22 d old, screened for mutants streptococci and infected on three successive days (23, 24 and 25 d old) with an active growing culture of S. sobrinus 6715 re-isolated from a desalivated rat.12 Infection was confirmed by cotton swabbing on MSS (Mitis Salivarius Agar plus streptomycin, Sigma Chemical Co., St. Louis, MO, U.S.A.).

Groups The dams were divided into three groups that

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received, during the lactating period (until 22 d old): group (1) diet 2000 and sterile distilled water (sdw) ad libitum, (2) diet 2000 plus caffeine (Sigma Chemical Co., St. Louis, MO, U.S.A., 2 mg/100 g b.w.) and sdw ad libitum, (3) diet 2000 plus theophylline (Sigma Chemical Co., St. Louis, MO, U.S.A., 0.57 mg/100 g b.w.) and sdw ad libitum. At age 26 d, the pups were placed into three experimental groups of 12 animals each, and received the same diet as the dams.

Each group received its respective diet via a König-Höfer programmed feeding machine, being fed with 17 meals daily, beginning at 4 pm. The groups also received sdw ad libitum. This investigation continued for 5 weeks; the number of meals consumed by each animal was monitored daily, and the animals' body weight, weekly. The dose regimen was readjusted weekly, according to the medium body weight of each group.

Saliva Tests The day before sacrifice of the animals, saliva was collected from each rat approximately 2 h after the last meal to minimize any interference of residual food or absorbed nutrients in saliva composition. Saliva was collected by anesthetizing the rats with 90 mg/kg b.w., of ketamine (Ketalur® Parke-Davis Laboratories, São Paulo, Brazil, Ketamine HCl inj. USP 100 mg/ml) given intraperitoneally. Then 0.2% pilocarpine in saline (Pilocarpine Hydrochloride, Sigma Chemical Co., St. Louis, MO, U.S.A., 10 mg/kg b.w.) was given intraperitoneally to stimulate salivary secretion. After 1 min, the rats were placed head down, and saliva was collected on plastic disposable beakers for 15 min to calculate salivary flow rates, expressed as ml/min, and for over 15 min to obtain enough volume for the other assays. The initial pH of saliva was analyzed using a potentiometer (Procyon® SA 720, Orion Research Inc., Boston, MA, U.S.A.). Salivary buffering capacity was assayed as follows: 0.25 ml of saliva and 0.75 ml of HCl 5 m were homogenized and the pH values were expressed as salivary buffering capacity.

Microbial Assays and Caries Scores Animals were killed by ether asphyxiation and immediately decapitated. The lower-left jaws were dissected aseptically and suspended in 5.0 ml of 0.9% sterile saline solution and treated in a 200 W Thornton T50 Sonic Cleaner (Thornton Inpec Ltd., São Paulo, Brazil) for 20 min. The resulting suspension was diluted to 1 : 100 and 1 : 1000 and inoculated in plates with MSS medium for S. sobrinus recovery and sheep-blood agar (Blood Agar Base, Difco Laboratories Ltd., São Paulo, Brazil) to estimate total microbiota. MSS and blood agar plates were incubated under 10% CO2, at 37°C for 48 h. Blood agar plates were also incubated aerobically at 37°C for 24 h. The upper and lower jaws were defleshed i.e., had all their soft tissues removed and prepared for scoring by the Keyes’ method, as modified by Larson.

Gastric Ulceration Score Alterations on the gastric mucosa were analyzed after chronic exposure to xanthines. Just after sacrifice, the stomach of each animal was removed, cut open along the greater curvature, and the mucosa was washed under running tap water. The number and severity of lesions were determined using a light microscope Steini SV6 (Carl Zeiss Jena GmbH, Jena, Germany). The ulcer score was calculated from the number of ulcers and their sizes, and from other parameters such as edema, petechiae, hemorrhage and others, as described by Souza-Formigoni et al.

Statistical Analysis All data were analyzed by ANOVA and Tukey-Kramer HSD test, using software for statistical visualization, JMP version 3.1 (SAS Institute Inc., Cary, NC, U.S.A.). Analyses of certain outcome measures were performed using transformed values of the measures in order to stabilize variances. Smooth-surface and sulcal caries scores were expressed as proportions of maximum possible values (124 and 56, respectively), and the arcsine transformation was applied.

Ethics This study was scientifically and ethically approved by the Pharmacology Graduate Committee, and the ethical guidelines issued in the Declaration of Helsinki were applied.

RESULTS

Weight Gain and Meals The animals remained in apparent good health during the experimental period. All animals gained weight during the five-week-experimental period, although rats fed with a diet supplemented with caffeine showed lower weight gain (p<0.01). There were no significant differences in the number of daily meals consumed by the groups (p<0.01) throughout the entire experiment (Fig. 1).

Caries Scores Total smooth-surface caries scores were not statistically different among the groups (p<0.09). Total sulcal score of the theophylline group was not statistically different from the control group, but higher than the caffeine group and statistically different (p<0.01). The results for slight (Ds) and moderate (Dm) dentine lesions were higher and statistically different for the theophylline group than caffeine and control groups (Figs. 2 and 3) for smooth-surface and sulcal scores (p<0.01).

Microorganism Recovery Total cultivatable microbiota and S. sobrinus populations recovered from the jaw suspensions are shown in Fig. 4. The percentage of S. sobrinus relative to total microbiota was significantly higher in the theophylline group than the caffeine and control groups (p<0.03).

Salivary Parameters Figure 5 shows salivary parameters. There were no statistically significant differences in salivary flow rate, pH and salivary buffering capacity between the control and theophylline groups. The results of salivary flow rate in the caffeine group were not statistically different from the theophylline group. Although the pH value for the
Fig. 2. Influence of the Treatments on Smooth-Surface Caries Scores and Its Severity
Values followed by different letters (a, b) in a horizontal line are statistically different.

Fig. 3. Influence of the Treatments on Sulcal Caries Scores and Its Severity
Values followed by different letters (a, b) in a horizontal line are statistically different.

Fig. 4. Influence of the Treatments on Total Cultivable Microbiota, S. sobrinus Populations and Percentage of S. sobrinus
Values followed by different letters (a, b) in a horizontal line are statistically different.

Fig. 5. Influence of the Treatments on Salivary Parameters
Values followed by different letters (a, b) in a horizontal line are statistically different.

Fig. 6. Influence of the Treatments on Gastric Ulceration Score
Values followed by different letters (a, b) in a horizontal line are statistically different.

The caffeine group was statistically higher than the other groups, the results of salivary buffering capacity showed that the caffeine and theophylline groups were not statistically different from the control group \( (p<0.01) \).

**Gastric Ulcer** Chronic administration of xanthines resulted in gastric ulceration, as shown in Fig. 6. The ulcer scores for the caffeine and theophylline groups were statistically higher than the control group \( (p<0.01) \).

**DISCUSSION**

The animals in this study were subjected to a severe cariogenic challenge experimental model.\(^{10}\) The frequency of ingestion and the number of meals consumed by the groups are directly related to the caries scores obtained, as demonstrated in an animal study of caries development\(^{20}\) when rats consumed at least 13 meals daily. There were no statistically significant differences on the number of meals consumed among the groups, which were approximately 16 meals daily (Fig. 1). This means that all groups were subjected to the same cariogenic challenge and exposed to the treatments. This was possible due to the use of the König-Höfer programmed feeding machine.\(^{13}\) In other caries studies, caffeine consumption was not controlled since the treatments were offered \( \textit{ad libitum} \).\(^{6,7,9}\)

All animals gained weight, although the caffeine group had the lowest weight gain. These results are similar to those of other studies,\(^{7,9}\) which demonstrated that suckling pups of lactating dams fed with a diet containing caffeine had lower body weight due to decreasing mineral deposition on the tissues. The caffeine group also showed the highest ulcer score, which could have affected the weight gain resulting in a lower body weight.

The caffeine group was not statistically different from the other groups in relation to total smooth-surface caries scores; sulcal caries scores did not differ from the control group, although they were lower and statistically different from the theophylline group. Nakamoto \textit{et al.}\(^{6}\) found that rats which received caffeine during the lactating period had significantly higher caries scores than the control group, but only in the first molars. These authors claimed that this effect could be related to developmental differences in the first-molar-tooth germ growth, that initiates in the 13th day \textit{in utero}, when caffeine exposure could have impaired tooth mineralization. In this research, caffeine did not affect caries scores relative to the control group.

There were no statistically significant differences among
the groups for total smooth-surface caries scores. However, slight (Ds) and moderate (Dm) dentine lesions were higher and statistically different for the theophylline group compared to the caffeine and control groups for smooth-surface and sulcal scores. These results are related to significantly higher colonization by *S. sobrinus* in the theophylline group, which shows that probably theophylline offered better conditions to the development of *S. sobrinus* and enhanced the virulence of this microorganism. Furthermore, as discussed by van Houte,11 dental caries must be seen as a dynamic relationship among the dental plaque microbiota, dietary carbohydrate, saliva, and the pH-lowering and cariogenic potential of dental plaque. Thus, saliva plays a major role on the regulation of the exposure of tooth surfaces to etiologic agents for caries.

The diuretic effect of the xanthines12 could have affected salivary flow rates and saliva composition, specially decreasing bicarbonate secretion and increasing the risk of caries development. In this experiment, there were no statistically significant differences in the results of salivary flow rate, pH and salivary buffering capacity between the control and theophylline groups. The results for salivary flow rates in the caffeine group were lower than the other groups but not different from the theophylline group. Although the pH value for the caffeine group was statistically higher than for the others, the results of salivary buffering capacity showed that both caffeine and theophylline groups were not statistically different from the control group. These findings demonstrate that the diuretic effect of caffeine and theophylline does not alter salivary parameters. It is possible however, that tolerance to the diuretic effect of the xanthines due to chronic and controlled administration may have occurred.10 The treatments were given by the König-Höper programmed feeding machine,15 17 times daily, so that plasma concentrations could be maintained, which may have favored the appearance of tolerance.

Since caffeine and theophylline apparently did not affect the inorganic composition of saliva, alteration of the secretion of organic compounds may have occurred. Watson et al.22 showed that the administration of propranolol, an adrenergic β-blocker, to rats under cariogenic challenge did not alter salivary flow rates, but decreased expression of prolamine-rich proteins (PRPs) and concurrently enhanced susceptibility to dental caries. Further studies21,22 also detected alterations in the expression of other antimicrobial factors such as histatins and statherins.

Denny et al.23 demonstrated that an adrenergic β-agonist like isoproterenol administered to mice increased the secretion of mucin, a salivary protein. Theophylline, like isoproterenol, inhibits phosphodiesterase and increases cAMP concentrations, resulting in similar effects on mucin secretion. Mucins are glycoproteins that have two major functions: it favors bacterial aggregation and clearance by deglutition,24 and also mediates bacterial adhesion to the tooth surface, enhancing the formation of dental plaque.25

Two major mucins, MG1 and MG2, have been identified in human saliva. Ioncheva et al.26 detected the formation of heterotypic complexes between MG1 and other salivary proteins such as amylase, PRPs, histatins and statherins. Immunological studies have shown that MG1, amylase, PRPs and statherin are major precursors of the early in vivo acquired pellicle enamel. The formation of protein complexes suggests that enamel pellicle could possibly be formed by binding of heterotypic complexes rather than single proteins. Salivary amylase enzymatically digests carbohydrates and binds a selected group of oral Streptococci with high affinity.25 Amylase in a MG1 amylase complex bound to tooth surfaces could potentially release carbohydrates from MG1 and provide a short-term source of nutrients for bound microorganisms, enhancing bacterial colonization.

Microbial data (Fig. 4) and caries scores (Figs. 2 and 3) showed a positive correlation, suggesting that the highest colonization by *S. sobrinus* in the theophylline group could be related to an increased concentration of salivary mucins provided by the action of theophylline, as described by Watson et al.23 for isoproterenol.

Higher toxic effects on gastric mucosa due to chronic administration of xanthines were observed in the treated groups, when compared to the control group. This effect could be explained by their mechanism of action: the inhibition of central adenosine receptors,27 inhibition of phosphodiesterase, and an increase of cAMP and calcium concentrations,28 that could result in higher gastric secretion. Van De Venter et al.29 found that caffeine ingestion provides decreased lower esophageal sphincter pressure, higher gastric acid and gastrin secretions, enhancing the formation of gastric ulcers. The lower gastric ulcer score in the control group is probably due to the stress caused by the experimental conditions of the programmed feeding machine.

It is concluded that theophylline increases the cariogenic potential of sucrose in this rat model, when compared to caffeine and sucrose only, and this effect may be possibly related to alterations in the salivary composition, probably in mucin secretion. Caffeine does not alter the cariogenic potential of sucrose, and the chronic administration of xanthines enhances the development of gastric ulcers.

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