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Original Article

# Nicotine is a risk factor for dental caries: An *in vivo* study

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ABSTRACT

*Background/purpose:* *Streptococcus mutans* is an important pathogen in the development of dental caries. Many studies have focused on the relationship between nicotine and *S. mutans in vitro*. The aim of this study was to investigate the effect of nicotine on the growth of *S. mutans* and its cariogenic potential *in vivo*.

*Materials and methods:* Sixteen male Specific-pathogen-free Wistar rats were divided into 2 groups (nicotine-treated and nicotine-untreated group) and infected with *S. mutans*. The *S. mutans* suspension was treated with 1 mg/mL nicotine in the nicotine-treated group. The Keyes method was used to evaluate sulcal caries of rats, and dental plaque on molar teeth was observed by scanning electron microscopy (SEM).

*Results:* Incidence of sulcal caries was higher in nicotine-treated group compared to nicotine-untreated group ($42.7 \pm 1.7$ vs $37.3 \pm 4.9$, $P = .009$). Severity of caries increased with nicotine treatment. The slightly dentinal caries scores and moderate dentinal caries scores were higher in the presence of nicotine ($P < .001$). Increased number of *S. mutans* cells attached to dental surface was observed under SEM in the nicotine-treated group.

*Conclusion:* Nicotine would promote the attachment of *S. mutans* to dental surface, and further increase the incidence and severity of dental caries. Therefore, nicotine might be a risk factor for smoking-induced caries.



## 1. Introduction

Dental caries is a major health problem that affects 60–90% of school-age children and most adults,[1] and is second only to the common cold in humans.[2] Caries is a complex and multifactorial condition which results in demineralization and progressive destruction of dental hard tissue. Many factors, such as microorganisms, environment and food, are associated with dental caries.[3] Dental plaque is the mainly responsible for the formation and development of caries. *Streptococcus mutans* is thought to be a crucial pathogen involved in the formation of dental caries and the presence of *S. mutans* is 70 times higher in caries-affected subjects than in caries-free subjects.[4] The ability of *S. mutans* to synthesize extracellular polysaccharide (EPS) and produce acids leads to the establishment and development of highly cariogenic dental biofilms.[5,6] And the tolerance to low pH helps *S. mutans* survival in oral ecosystem.[7,8] Taken together, all these characteristics make *S. mutans* cariogenic.

That tobacco smoking is harmful to human health has been well demonstrated. It leads to cardiovascular disease, cancers and other systemic diseases.[9–11] Oral cavity is inevitably affected by smoking, since it is the first part exposed to tobacco smoke. The incidence of periodontal diseases and oral cancer is much higher in smokers than in non-smokers.[12,13] In recent years, more and more studies have found a close correlation between smoking and dental caries. In England, exposure tobacco products for years significantly increased coronal and root caries.[14] In USA, there was a dose-dependent association between tobacco shewing and root-surface caries.[15] In Japan, existence of smokers in the home and the number of smokers in the family are significantly associated with early childhood caries (ECC).[16] An *in vivo* study demonstrated that cigarette smoke exposure and viral infections can synergistically

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increase the susceptibility of mice to secondary bacterial invasion.[17] Another *in vivo* study showed that exposure to cigarette smoke expands the caries-affected area in the maxillary molars of rats.[18] Consequently, the relationship between tobacco smoking and dental caries is unambiguous. Nicotine is an alkaloid and the component of cigarette smoke. Lots of studies focused on the relationship between nicotine and *S. mutans in vitro*. Our previous *in vitro* studies demonstrated that nicotine has the promotion effect on *S. mutans* growth, metabolic activity, cell aggregation, acids production and EPS synthesis.[19–21] However, no reports ever concerns about their relationship *in vivo*. It would be of great interest to investigate the effect of nicotine on the growth of *S. mutans* and its cariogenic potential *in vivo* and further verify our previous *in vitro* studies.

## 2. Materials and methods

### 2.1. Ethics statement

The study was performed with the approval of the West China Hospital of Stomatology Institute Review Board (WCSHIRB) ethics committee and all experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

### 2.2. Chemicals, bacterial strains and growth conditions

*S. mutans* UA159 (ATCC 700610, a cariogenic bacterial pathogen) was inoculated into brain heart infusion (BHI) broth with or without 1 mg/mL nicotine (Sigma-Aldrich, St Louis, MO, USA) and incubated overnight. Our previous study demonstrated that the minimum inhibitory concentration (MIC) of nicotine to *S. mutans* was 16 mg/mL,[22] and the physiological concentration of nicotine in the saliva of a smoker ranges from 70 to 1560 μg/mL.[23] In this study, 1 mg/mL nicotine was chosen as an appropriate concentration. For each treatment, the concentration of bacteria was adjusted to $1 \times 10^9$ colony-forming units (CFU)/mL. The bacteria were incubated in an atmosphere of 5% $CO_2$ at 37 °C.

### 2.3. In vivo models of dental caries

Rats have been used as a model to establish dental caries since 1922.[24] Sixteen male Specific-pathogen-free Wistar child rats aged 21 days were randomly and averagely divided into 2 groups (n = 8), one treated with nicotine and the other without nicotine. The selection of rats' age was according to the article that Bowen WH et al.[25]

During the first three days, any indigenous oral microorganism was removed by feeding the rats a diet containing antibiotics (chloramphenicol, ampicillin, carbenicillin, 1.0 g/kg diet).[26] *S. mutans* was incubated in culture medium (BHI) with or without 1 mg/mL of nicotine for 24 h and then inoculated to the rats' teeth. Each rat was subsequently challenged with 400 μL of $1 \times 10^9$ CFU/mL *S. mutans* suspension (the suspension contained 1 mg/mL of nicotine in nicotine-treated group) for the following three consecutive days (twice a day, interval of 30 min, no food or water for 1 h after inoculation) and then either sterile BHI plus 1 mg/mL nicotine (nicotine-treated group) or sterile BHI (nicotine-untreated group) every four days until the rats were sacrificed. The schedule is shown in Fig. 1. All rats were provided with the National Institutes of Health cariogenic diet 2000 and 5% sucrose water.[27] The experiment lasted for 24 days and then the rats were sacrificed. The jaws were aseptically dissected and processed for Keyes caries scoring[28] and scanning electron microscopy (SEM). We randomly selected 7 rats processing for Keyes caries scoring and 1 rat (4 jaws) for SEM in each group.

The methods for Keyes caries scoring: Four jaws of each rat were stained in 0.4% ammonium salt solution for 16 h, protected from light. The jaws were rinsed, dried and hemisectioned, and finally observed by a stereo microscope. The caries lesions were stained in red and Keyes scoring rules were used to assess the caries of each rat. The depth and size of red ammonium salt solution disseminated into tooth represent the severity and impact area of caries lesions. According to the Keyes method, caries are divided into four grades: enamel only (E), slightly dentinal (Ds), moderate dentinal (Dm), and extensive dentinal (Dx). Ds caries involve approximately 1/4 of the dentin between the enamel and pulp chamber. Dm caries include involvement of approximately 1/4–3/4 of the dentin, while the penetration beyond 3/4 of the dentin region is labelled Dx caries. Sulcal caries lesions of every molar tooth at each level (E, Ds, Dm, and Dx) in every rat (selected rats proceeded for Keyes caries scoring) were scored. Then we added the E scores of every molar tooth in a rat up and the total number of E scores in a rat was the data we collected to statistically analyze. The same calculus was applied to collect the Ds, Dm, Dx scores data. Since dental decay starts from the enamel regions and gradually progresses to the dentin regions, The E scores represents the incidence of caries and the Ds, Dm, Dx scores represent the severity of caries.[28]

### 2.4. Scanning electron microscopy (SEM)

We randomly chose 1 rat in each group processing for SEM. After removing extra bones and flesh surrounding the jaws, the jaws were washed twice with phosphate buffer saline (PBS) and fixed overnight with 2.5% glutaraldehyde at 4 °C. The jaws were subsequently washed twice with distilled water, dehydrated by a series of ethanol rinses (30, 50, 70, 80, 85, 90, 95 and 100%), immersed for 10 min in hexamethyldisilazane and dried in a desiccator. After sputter coating with gold-palladium, samples were imaged at least three times on randomly selected positions in

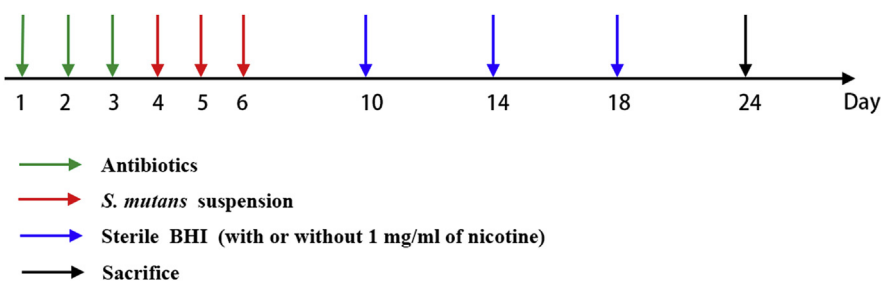


**Fig. 1. Challenge schedule of rats.** Rats were fed with antibiotics for the first three consecutive days and then were challenged with a *S. mutans* suspension for next three consecutive days and sterile BHI (with or without 1 mg/mL of nicotine) every four days until the rats were sacrificed.

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