Effects of Nicotine on the Growth and Protein Expression of Porphyromonas gingivalis

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(Received April 25, 2011 / Accepted September 15, 2011)

Tobacco smoking is considered one of the most significant environmental risk factors for destructive periodontal disease. The effect of smoking on periodontopathogenic microbiota has not yet been elucidated, as previous studies failed to identify a concrete relationship between periodontopathogenic microorganisms and smoking. However, it is likely that smoking, as an environmental stress factor, may affect the behavior of dental plaque microorganisms, ultimately leading to alteration of the host-parasite interaction. The goal of this study was to examine the effect of nicotine, a major component of tobacco, on the growth and protein expression of the crucial periodontal pathogen Porphyromonas gingivalis. The growth of P. gingivalis 381 was measured after bacterial cells were cultivated in liquid broth containing various nicotine concentrations. First, P. gingivalis cells were allowed to grow in the presence of a single dose of nicotine (the single exposure protocol) at 0, 1, 2, 4, and 8 mg/L, respectively. Second, P. gingivalis cells were exposed to five consecutive doses of nicotine (the multiple exposure protocol) at 0, 1, 2, and 4 mg/L, respectively. Bacterial growth was measured by optical density and protein expression was analyzed by SDS-PAGE and 2-D gel electrophoresis. In the single nicotine exposure protocol, it was observed that the growth of P. gingivalis 381 was inhibited by nicotine in a dose-dependent manner. In the multiple nicotine exposure protocol, the growth rate of P. gingivalis increased with each subsequent nicotine exposure, even though bacterial growth was also inhibited in a dose-dependent fashion. SDS-PAGE and 2-D gel electrophoresis analyses revealed a minor change in the pattern of protein expression, showing differences in proteins with low molecular weights (around 20 kDa) on exposure to nicotine. The results of this study suggest that nicotine exerts an inhibitory effect on the growth of P. gingivalis, and has a potential to modulate protein expression in P. gingivalis.

Keywords: smoking, nicotine, protein expression, virulence, periodontal disease, oral anaerobic bacterium

Introduction

Periodontal disease is arguably one of the most common infectious diseases affecting humans, and this chronic affliction can lead to destruction of the supporting structures of dentition and, ultimately, tooth loss (Williams, 1990). It has been established that dental plaque is an etiological agent of periodontal disease. Dental plaque is a biofilm consisting of more than 700 different bacterial species and their products (Kroes et al., 1999; Paster et al., 2001). Immunological and inflammatory responses by the host to dental plaque biofilm via host-parasite interaction are manifested by signs and symptoms of periodontal disease. The outcome of this interaction could also be modulated by other components known as risk factors or disease modifiers. These factors, either inherent (genetic) or acquired (environmental), may in turn significantly affect the initiation and progression of periodontal diseases of different types (Page et al., 1997; Kinane and Hart, 2003; Loos et al., 2005).

Tobacco smoking is considered to be one of the most important environmental risk factors for the initiation and the progression of periodontal disease (Palmer et al., 2005). Over the past several decades, numerous epidemiological data have demonstrated that smoking represents an increased risk for periodontal disease (Albandar, 2002; Rivera-Hidalgo, 2003). The exact pathogenic mechanisms whereby smoking exerts its effect on periodontal disease are not completely understood, although it has been suggested that changes in the host immune and inflammatory responses may be partially responsible for periodontal disease in smokers (Obed and Bercy, 2000).

The relationship between smoking and dental plaque microorganisms has not yet been clearly delineated. Although the effect of smoking on dental plaque development has not been clearly determined, it appeared that the rate of plaque formation was not affected by smoking (Bergstrom and Preber, 1986; Bosstrom et al., 2001). Some studies observed higher emergence of certain bacterial species in smokers, including Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia (Zambon et al., 1996; Umeda et al., 1998; Kamma et al., 1999), while others failed to detect differences in the microbiota between smokers and non-smokers (Darby et al., 2000; Bosstrom et al., 2001; Van der Velden et al., 2003). P. gingivalis is a Gram-negative, non-

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pore-forming, anaerobic, black-pigmented bacterium, and has long been considered an important pathogen involved in the initiation and progression of periodontal disease, due to frequent recovery of this species from the lesion, and the presence of potent virulence factors (O’Brien-Simpson et al., 2004; Holt and Ebersole, 2005), including fimbriae, hemagglutinins, a broad spectrum of proteases and other enzymes, lipopolysaccharides, and capsule. Bacterial pathogens are equipped with sophisticated mechanisms for adapting to complex environmental changes and thereby ensure adequate growth and survival within the host (Finlay and Falkow, 1989, 1997). Recent studies showed that expression of virulence factors of P. gingivalis is regulated in response to environmental changes. It was found that fimbrial gene (fimA) activity was decreased by approximately 50% in response to hemin limitation and the presence of serum or saliva in the growth medium (Xie et al., 1997). Iron and heme utilization by P. gingivalis adopts various schemes to ensure optimal nutrient uptake in response to environmental changes (Olczak et al., 2005). However, the effect of smoking, as an environmental stress factor, may affect the behavior of P. gingivalis, leading to modification of the host-parasite interaction.

The purpose of this study was to assess the effects of nicotine, a major component of tobacco smoking, on modulation of the growth pattern and overall protein expression of P. gingivalis.

**Materials and Methods**

**Bacterial strain and growth conditions**

P. gingivalis 381 was purchased from ATCC (USA) and maintained on anaerobic blood agar plates (Anaerobe Systems, Hardy Diagnostics, USA) at 37°C under anaerobic conditions (80% N₂, 10% CO₂, 10% H₂), in a Forma 1025 Anaerobic Chamber (Thermo, USA). For liquid growth, bacterial cells were cultured in 3% (w/v) trypticase soy broth supplemented with 0.5% yeast extract, 5 mg/L hemin, and 1 mg/L menadione. E. coli DH5α was obtained from Promega (USA) and grown in LB medium, as described elsewhere. Nicotine (Acros Organics, Belgium) was added to the liquid broth at final concentrations of 0, 1, 2, 4, and 8 mg/L. The growth rates of P. gingivalis 381 were determined by measuring optical density at 600 nm, using a spectrophotometer.

**The growth of P. gingivalis 381 after exposure to nicotine**

In the single nicotine exposure scheme (Fig. 1), 5×10⁷ P. gingivalis cells were added to the 20 ml liquid broth containing varying nicotine concentrations, i.e 0, 1, 2, 4, and 8 mg/L, and cultivated for 5 days. The effect of nicotine on...