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In Vitro Effects of Nicotine, Cigarette Smoke Condensate, and *Porphyromonas gingivalis* on Monocyte Chemoattractant Protein-1 Expression from Cultured Human Gingival Fibroblasts

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Abstract

Background: Monocyte chemoattractant protein-1 (MCP-1) is an inducible protein that attracts monocytes to areas of injury and infection. Studies have shown that it is produced by gingival cells in periodontal diseases and that its incidence increases with the severity of the disease. The aim of the present study was to investigate the effects of nicotine, cigarette smoke condensate (CSC), and ( ) on MCP-1 expression from human gingival fibroblasts (HGFs) .

Methods: HGFs were exposed for 72 h to 250 µg/mL of nicotine, 100 µg/mL of CSC, 10% supernatant, supernatant with nicotine, or supernatant with CSC. A control group comprised HGFs without any treatment. The conditioned media was then collected for MCP-1 analysis by enzyme-linked immunosorbent assay (ELISA).

Results: There were significant differences in MCP-1 level between the (=0.0432) and with CSC (=0.0037) groups when compared to the control group.

Conclusions: stimulates an inflammatory response in periodontal tissues by increasing MCP-1, which helps attract host cells to combat the bacterial infection. Tobacco usage can mask the inflammatory responses normally seen in periodontal diseases by reducing the levels of MCP-1, thus allowing the bacteria to grow somewhat undetected. This could be one factor that explains why smoking is a major contributing factor to the initiation, development, and progression of periodontal diseases.

Keywords: Smoking; Periodontal disease; Monocyte chemoattractant protein-1; *P gingivalis*

Introduction

Periodontal diseases, among many other prevalent chronic diseases, have been constantly linked to smoking and tobacco use. The correlation between smoking and periodontal tissue destruction results mainly from the smoking-associated impairment of normal immunological surveillance or defensive mechanisms. Periodontal diseases are initiated by bacterial colonization that activates tissue mechanisms resulting in a series of inflammatory and immunological changes leading to connective tissue and bone destruction. *Porphyromonas gingivalis* (*P. gingivalis*) is one of the principal pathogens responsible for the development of adult periodontitis. Several reports have provided evidence to implicate P. gingivalis in the local destruction of periodontitis [1-4].

Monocyte functions are considered determinants to the periodontal breakdown in periodontal diseases. Monocyte chemoattractant protein-1 (MCP-1) is an inducible protein that has chemotactic activity for lymphocytes and monocytes, and is considered a major signal for the chemotaxis of mononuclear leukocytes [5-6]. MCP-1 is secreted by various cell types such as leukocytes, fibroblasts, kerotinocytes and endothelial cells in response to different endogenous and exogenous stimuli [7]. Overexpression of MCP-1 in the periodontal tissues of patients with periodontitis has been reported in the literature [8,9]. In addition, the association between MCP-1 and host responses was suggested to play a role in aggressive periodontitis [10]. All these studies suggest that the MCP-1 levels may be an important factor that affects the progression and the severity of the periodontal diseases. The aim of the present study was to investigate if nicotine, cigarette smoke condensate (CSC), and/or *P. gingivalis* would alter MCP-1 expression from human gingival fibroblasts *in vitro*.

Materials and Methods

Cell cultures and treatment

Human gingival fibroblasts (HGFs) were cultured from clinically healthy gingival tissues removed from patients undergoing crown-lengthening surgery as described previously 11 with Institutional Review Board approval. The cells were grown in Dulbecco's modified

Eagle's medium (DMEM) supplemented with 10% bovine growth serum, 4 mM L-Glutamine (HyClone), antibiotics (100 U/ml of penicillin, 100 U/ml of streptomycin and 0.25 µg/ml of fungi zone). Cultures were maintained at 37°C in a humidified atmosphere of 5% CO2 and 95% air. Confluent cells were detached with 0.25% trypsin and aliquots of separated cells were sub-cultured. Cells between the third and eighth passages were used.

Nicotine and CSC were purchased from Sigma (St. Louis, MO) and Murty Pharmaceuticals (Lexington, KY), respectively. *P. gingivalis*

CSC. These differences were significant between the control group and the *P. gingivalis* (p=0.0432), and *P. gingivalis* with CSC (p=0.0037). These results support the hypothesis that both smoking and *P. gingivalis* infection affects the levels of MCP-1 produced by HGFs and thus may contribute to inflammatory process and the development of periodontal diseases. The significantly increased level of MCP-1 expression from HGFs infected with *P. gingivalis* indicates the virulence property of this pathogen that was previously reported to induce increased expression of other inflammatory cytokines at the periodontal tissues [15-17].

The results of this study are in accordance with previous reports. A study by Pradeep et al. [18] examined the gingival crevicular fluid (GCF) levels of MCP-1 in periodontal health and disease and indicated