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Gene expression of human beta defensins-1 and -2 is significantly reduced in non-inflamed keratinized oral tissue of smokers

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ABSTRACT

Objective: The impact of smoking on the local innate immune response in the oral cavity, and, commonly, on oral health is actively discussed in the scientific literature. The aim of the present study was to evaluate possible effects of smoking on gene expression of human beta-defensin-1 and -2 in human gingival tissue.

Material and methods: Biopsies of keratinized gingival tissues were taken from donors (with written informed consent) undergoing routine surgical treatment. Prior to the sample collection, participants with clinically healthy periodontium were classified as smokers ($n = 9$) or non-smokers ($n = 9$). Gingival tissue was homogenized, and total RNA was extracted and analysed by real-time RT-PCR for human beta-defensins-1-, -2-, and interleukins IL-1β- and IL-6-, as well as GAPDH-mRNA. The data obtained were analysed for significant differences using the Mann–Whitney-*U* test.

Results: hBD-1- and hBD-2-, as well as IL-1β- and IL-6-mRNA were detected in all gingival samples. Expression of hBD-1 and -2 was significantly reduced by nearly 2.5-fold ($p < 0.05$; Mann–Whitney) in gingival samples of smokers compared to control group specimens (non-smokers). In contrast, no significant differences of the gene expression of IL-6 and IL-1β were observed in human gingival tissue of smokers and non-smokers.

Conclusion: The results presented here suggest that expression of human beta-defensins hBD-1 and -2, and, thus, the basal levels of innate immune defense reactions in the oral cavity are reduced by smoking.

1. Introduction

It is well documented that the primary etiological cause of periodontal diseases is the microbial biofilm, and that a number of additional risk factors contribute to the susceptibility of the individual to the pathogenesis and severity of the disease. The term ''risk factor'' refers to ''an aspect of personal behaviour or lifestyle, an environmental exposure, or an inherited or inborn characteristic, which, on the basis of

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epidemiologic evidence, is known to be associated with health-related conditions''.[1,2] The presence of a risk factor increases the probability of a disease to occur. While specific microorganisms are considered as potential periodontal pathogens, it is well established that pathogens are necessary but not sufficient for the development of the disease.[3] The initiation and progression of the periodontal disease is the result of the interactions of genetic, environmental, host, and microbial factors.[4–6]

Notwithstanding, one of the crucial factors for the genesis of periodontal disease is the presence of microorganisms. However, in particular the progression of the disease is related to host and environmental factors such as genetic, age, gender, smoking, socio-economic factors, specific systemic diseases, and local risk factors. The interactions between these various risk factors may influence the host responses generally and the immune responses specifically.[7] Numerous investigations, including cross-sectional and longitudinal studies, which have been performed during the past 15 years, showed ample epidemiological evidence that smoking is clearly a risk factor for progression of periodontal disease.[8–13] Furthermore, a great number of studies have been performed to elucidate the possible mechanisms of action of tobacco smoking to the periodontal tissues, and, thus, it appears that smoking may affect the vasculature, the humoral immune system, the cellular immune system, and the inflammatory system.[4]

Therefore, the impact of smoking on the local innate immune response in the oral cavity, in particular on the expression and the synthesis of antimicrobial peptides such as human beta-defensins (hBDs) and proinflammatory cytokines as a part of the innate defense mechanisms is of great interest. These genes have been well investigated and described by previous studies, and could be representative for many similar agents of the first-line defense barrier against microorganisms in the oral cavity.[14–17] hBDs are small, endogenous, and positively charged peptides that exhibit antimicrobial and chemotactic activity. The action of defensins is based on their physical properties, and depends on their ability to bind microbial membranes through electrostatic and hydrophobic interactions. This leads to an elimination of the microorganisms by disrupting their cell membranes.[18]

Previous research elaborated that nicotine or cigarette smoke extract alters gene expression of proinflammatory cytokines and mediators, such as interleukins, MCP-1, COX-2, and hBD-2 in different human cells and tissues.[17,19,20] However, expression regulation of hBDs is still not completely understood, and the impact of smoking on hBD-expression in vivo is unknown.

Therefore, the aim of the present study was to evaluate and to compare the gene expression of human beta-defensins (hBD-1 and hBD-2) and proinflammatory cytokines IL-1β, and IL-6 in the gingival tissues of smokers and non-smokers using quantitative real-time RT-PCR techniques. We hypothesized (H_0) that the gene expression of hBD-1, -2, IL-1 and -6 in the gingival tissue would be not affected by the cigarette consumption (regular smokers), if compared to the control group (non-smokers). This null hypothesis was tested against the alternative hypothesis of a difference.

2. Materials and methods

2.1. Samples

The keratinized gingival tissue biopsies were excised from patients during routine surgical treatments at the Department of Oral Surgery, CharitéCentrum 3, Charité - Universitätsmedizin Berlin, Germany. All patients had received printed information and signed a written consent according to the guidelines of the Central German Ethics Committee's referral, focusing on the use of body materials in medical research.[21] The patients were interviewed about smoking habits, and were divided into two groups of smokers ($n = 9$) and non-smokers ($n = 9$) (Table 1). All smokers were cigarette smokers, and those who reported the use of $10 \geq$ cigarettes per day were classified as smokers.[22] Patients classified as non-smokers had no history of tobacco smoking at all. For ethical reasons, smokers were informed about the possible negative health effects of tobacco smoking, and were encouraged to cease smoking. Patients with systemic diseases, patients who had taken any medication in the past 24 h before treatment, and patients with untreated periodontal diseases as well as occasional smokers were excluded from the study.

The gingival biopsies were obtained either with gingival tissue punch technique from direct implantation (Fig. 1), or from gingival excision prior to the endosseous implant exposure. The gingival tissue specimens were immediately frozen in liquid nitrogen using RNA stabilization reagent (RNAlater; Qiagen, Hilden, Germany), and stored at −80 °C.

2.2. RNA extraction and cDNA synthesis

Total RNA was extracted from homogenized gingival tissue using the QIAzol lysis reagent (Qiagen). The tissue was crushed using a rotor-stator homogenizer (TissueRuptor, Qiagen) with 1000 μl QIAzol reagent. The homogenate was separated in aqueous and organic phases by the addition of 200 μl chloroform, followed by centrifugation at 12,000 × *g* for 15 min at 4 °C. RNA was precipitated by addition of 500 μl isopropanol and centrifugation at 12,000 × *g* for 10 min at 4 °C. The resulting RNA was washed in 70% ethanol, and solubilized in diethylpyrocarbonate (DEPC) treated water. After elution, final RNA concentrations were determined photometrically (GeneQuant, GE Healthcare, Buckinghamshire, UK). Complementary cDNA was synthesized from 1 to 13 μl of RNA (solution concentration 1 μg/μl) by RT using 1 mM oligo-dT primers and 200 units of M-MLV reverse transcriptase (Promega, Mannheim, Germany) at 42 °C for 1 h.

Table 1 – Study population according to smoking, gender, and age (SD: standard deviation).

Smoking	*n*	Gender		Age (years)	
		Men	Women	Mean	SD
Smokers	9	3	6	45.3	14.3
Non-smokers	9	4	5	54.7	15.2