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Epithelial–stromal interactions in salivary glands of rats exposed to chronic passive smoking

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ABSTRACT

Objectives: Cigarette smoke leads to precancerous and cancerous lesions in the mouth even when the exposure is passive. The salivary glands are amongst the tissues exposed to the smoke but it is unclear whether or not passive cigarette exposure is related to detectable changes in these tissues. The objective of this study was to observe the tissue architecture of the parotid and submandibular glands in rats after passive cigarette exposure and to measure any changes that occurred.

Design: Twenty Wistar rats were divided into 10 non-smoking animals and 10 animals exposed to cigarette smoke. After 6 months of smoke exposure samples were collected from both exposed and unexposed salivary glands for histological examination under both transmitted and polarized light microscopy.

Results: Changes in the glands of exposed animals included involution of the cytoplasm and nucleus of the acinar cells and the presence of an inflammatory infiltrate. There was an abnormal accumulation of type I collagen in the stroma and an enlarged interacinar space filled with extracellular matrix.

Conclusion: Passive smoking led to substantial structural changes in the salivary glands which could significantly affect function.

1. Introduction

Tobacco use has become a habit in the Western world, including the American continent. The tobacco, especially cigarette smoking, is the leading cause of preventable death. Although evidence indicates a decline in the number of smokers over the last 30 years, this addiction continues to be an important public health problem.[1–3]

More than 4000 substances can be isolated from cigarettes, with nicotine being responsible for the dependence-forming properties of smoking, amongst other harmful effects.[4–6] The effects of active smoking on the oral cavity and associated structures predispose to the occurrence of precancerous and cancerous lesions.[7–14] The salivary glands are one of the most important associated structures. These glands consist of a secretory epithelium and a glandular stroma. The stroma forms a microenvironment of extracellular matrix that is fundamental for the homeostasis of this organ.[15,16] The salivary glands are also targets of the effects of cigarette smoking. For example, the observation of cellular DNA damage demonstrates the potential carcinogenic action of nicotine, one of the components of tobacco smoke.[17] Other organs are also affected by active smoking, which destructures the epithelium and adjacent connective tissue, consequently impairing the interaction between these tissue compartments. Some investigators have dem-

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onstrated that cigarette agents induce extracellular matrix alterations.[18,19]

In addition to the systemic and local effects of active smoking described in the literature, passive smoking has shown a possible relationship with dental problems, decreases in salivary pH, alterations in salivary flow rate, buffering capacity and protein levels, harmful effects during pregnancy, amongst others.[20–22] Chatzimichael et al. studying 240 children with bronchitis aged 6 months to 2 years, found that 50.8% of the infants presented severe symptoms of the disease when exposed to tobacco smoke.[23] Similarly, an association was observed between respiratory tract infections and tobacco exposure in 87.3% of children aged 2–12 years.[24] However, the mechanisms underlying this damage remain unclear. One possible cause is the production of reactive oxygen species as a result of the accumulation of nicotine and heavy metals in the cells, causing genetic alterations, amongst others.[25,26]

In contrast to these findings, Hassan et al. studying 808 patients with pancreatic adenocarcinoma, found no significant increase in the risk of this disease amongst passive smokers.[27] These results indicate the need for further studies regarding the association between passive smoking and cellular effects in different tissues, especially in the salivary glands. Thus, the objective of this study was to observe the tissue architecture of the parotid and submandibular glands in rats after passive cigarette exposure and to measure any changes that occurred.

2. Materials and methods

2.1. *Animals and tissue preparation*

Twenty 12-week-old male Wistar rats, weighing on average 400 g, obtained from the Multidisciplinary Centre for Biological Research of the State University of Campinas (CEMIB, certified ICLAS/UNICAMP) were divided into two groups: 10 non-smoking rats (control group) and 10 animals exposed to cigarette smoke (exposed group). The animals were maintained under standard conditions of housing, feeding and treatment at the Sector of Laboratory Animal Experimentation (SEA), Department of Morphology and Basic Pathology, Faculty of Medicine of Jundiaí.

Animals were exposed to passive medium-tar cigarette smoke (10 mg) in a cage containing two orifices, one where the smoke entered and another where the smoke was removed. The animals were allowed to circulate uniformly and continuously in the cage for 1 h/day, 7 days/week, for 6 months, similar as described previously.[28] To simulate the treatment conditions, control animals were manipulated daily in another environment to avoid contamination with cigarette residues. Water and pelleted chow (Nuvilab CR1, São Paulo, Brazil) were available *ad libitum* during the experimental period and food and fluid intakes were measured daily. The variation in body weight was calculated as the difference between the final and initial weight of the animals in the two groups. After the treatment period, the animals were anaesthetized with ketamine/xylazine (1:1) at a dose of 0.1 ml/29 g body weight and salivary gland samples were collected for transmitted and polarized light microscopy analysis. All procedures were performed in accordance with the ethical guidelines on laboratory animal experimentation of the Brazilian College of Animal Experimentation (COBEA) and were approved by the Institutional Ethics and Research Committee.

Samples of the parotid and submandibular glands were fixed in Bouin's solution (picric acid solution), embedded in plastic paraffin (Paraplast Plus, Oxford Lab, USA), and stained with haematoxylin/eosin (HE). Some of these samples were stained with picrosirius red (saturated aqueous solution of picric acid supplemented with 0.1 g Sirius red F3B, Bayer) for polarized light microscopy of fibrillar components of the extracellular matrix.[29,30]

2.2. *Stereology: three-dimensional analysis of tissues*

The nuclear and cytoplasmic volumes of acinar cells of the parotid and submandibular glands were determined in HE-stained histological sections by transmitted light microscopy. For this purpose, 40 cells were analysed per animal (corresponding to 400 acini per experimental group) by the point counting method described by Weibel.[31] Only intact cells and spherical or ellipsoid nuclei with defined limits were considered for this study. In addition, the main types of collagen components were determined (types I–III) and the spatial volume density of these components was analysed under polarized light and calculated as the mean of four regions in each histological section by the point counting method.[31–33] The relative area occupied by the epithelium and glandular stroma was measured with the Image J 1.39 image analysis system (Image Processing and Analysis in Java, National Institutes of Health, MD, USA). All analyses were performed with a Nikon Eclipse microscope using 20×, 40× and 100× planachromatic objectives for transmitted light microscopy and birefringent lenses for polarized light microscopy. The microscope was coupled to the SD-3.3 CCD image acquisition system of the Department of Morphology and Basic Pathology, Faculty of Medicine of Jundiaí.

2.3. *Statistical analysis*

The results are reported as the mean ± standard deviation for the determination of body weight variation, food and fluid intake, and as the mean for nuclear and cytoplasmic volume of acinar cells of the parotid and submandibular salivary glands (μm^3), relative area of the secretory epithelium (%), relative area of glandular stroma (%), and volume density of collagen fibres (μm). Data were compared by analysis of variance (ANOVA), complemented by the Kruskal–Wallis test for pairwise comparison.[34,35] The level of significance was set at 5% for all tests.

3. Results

3.1. *Variation in body weight and food and fluid intake*

No significant differences in body weight variation (final weight – initial weight) were observed between the animals