Original Article

Effect of Nicotine On Oral Mucosa

To Investigate the Effects of Nicotine on Morphology of an In-Vitro Reconstituted Model of Normal Healthy Uninflamed Oral Mucosa

Numan Sheikh[1], Sajid Hanif[2], Faisal Pasha[3], Mohammad Irfan[5] and Zahur Qayyum[4]

ABSTRACT

Objective: The objective of this study was to investigate the effect of nicotine on morphology of an uninflamed reconstituted oral mucosa in vitro when treated for 5 minutes and 24 hours respectively.
Study Design: Observational study.
Place and Duration of Study: This study was conducted at the Department of Oral Pathology Bart's and the London Queen Mary School of Med. and Dentistry Queen Mary, University of London from July 2018 to July 2019.
Materials and Methods: This study focuses on the effects of nicotine on an in vitro reconstituted model of oral mucosa. The reconstituted human epithelium model used in this study was prepared and supplied by Skin Ethic Laboratories, Nice, France. The effect of the different treatments of nicotine on tissue morphology was assessed using formalin fixed paraffin wax sections and heamatoxylin and eosin staining.
Results: It was found that the effect of nicotine after 5 minutes and 24 hours with working solutions (10µM and 1mM) used on uninflamed oral mucosal did not significantly effect on the gross morphology.
Conclusion: This study has confirmed that all the concentration of nicotine used after 5 minutes and 24 hours had no effect on tissue morphology.
Key Words: Tobacco**,** Nicotine, Oral mucosa, Morphology.

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INTRODUCTION

The Tobacco consumption is directly responsible for nearly 6 million deaths annually and a further 600,000 people die each year from exposure to second-hand smoke (SHS).[1]

Tobacco is killing 1 in 10 adults worldwide and its quantity of consumption is increasing globally especially in developing countries according to WHO statistical data.[2] The genus tobacco comes from a source named after Jean Nicot, a French ambassador that is being credited for the shipment of tobacco from Portugal to Paris in the year 1560.[3] The frequency of smoking in countries like Western Europe, Australasia, and the United States and the developing world is rising.[4] Eriksen et al. concluded that tobacco smoking has serious adverse health consequences in all of the countries of the world irrespective of social, economic, personal, and political influences in determination of the smoking prevalence and cessation patterns.[5]

People consume different types of tobacco products which can be smoked, chewed or sniffed.[6] These include products that are smoked such as cigars, cigarettes, pipe tobacco and roll- your-own or consumed smokeless as chewing tobacco and snuff.[7] Tobacco smoking is a very popular habit and is associated with the development of cancers in humans.[8] The incidence of oral cancer is correlated to the use of tobacco products.[9] Cigarette smoking and tobacco use are also associated with development of other cancers in humans, including cancer of the oesophagus and the lungs.[10] Nicotine is an important component of tobacco, it is the addictive substance in tobacco and the main reason why people continue to use tobacco related products and it is highly suggested that it may be associated with tobacco related diseases.[11] Nicotine addiction results in exposure to various carcinogens and bioactive compounds present in tobacco.[12] Nicotine (C10 H14 N2) is a naturally

[1.] Department of Oral Pathology, Baqai Dental College, Karachi Pakistan.
[2.]Department of Oral Pathology, KMDC. Karachi.
[3.]Department of Oral Medicine/ Oral & Maxillofacial Surgery[4], WM&DC Abbottabad
[5.] Department of Oral & Maxillofacial Surgery, LHR Peshawar.

Correspondence: Numan Sheikh Associate Professor Oral Pathology Baqai Dental College Karachi Pakistan.
Contact No: 0300 9207166
Email: sheikh_692000@yahoo.com

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occurring alkaloid present in the tobacco leaves and makes up about 5% of a tobacco plant by weight, and it acts as a botanical insecticide and is highly addictive.[13,14]

Tobacco smoking delivers rapid doses of nicotine into the brain following each inhalation, 15-20 minutes is its distribution half-life with a terminal half-life of two hours in the blood and nicotine has a penetrating effect on brain neurochemistry causing activation of nicotinic acetylcholine receptors, and releases dopamine in the nucleus accumbent.[15] Nicotine is a type of psychomotor stimulant and helps smokers to calm down when they are under stress and enables them to work more effectively and with a higher concentration.[15] Nicotine is associated with a variety of lesions within the oral cavity.[9] It is suggested that nicotine might be associated with the pathogenesis of oral white pre- malignant lesions.[16] Many Oral lesions and conditions associated with tobacco use include oral precancerous lesions such as leukoplakia, erythroplakia and smokeless tobacco keratosis, oral cancers such as squamous cell carcinoma of the tongue, floor of the mouth, lip and gingiva. It is further associated with verrucous carcinomas of the buccal mucosa gingival and alveolar ridge.[17]

The excessive consumption of tobacco has also been associated with other lesions within the oral cavity such as tooth stains, abrasions, smoker's melanosis, acute necrotizing ulcerative gingivitis, burns, keratotic patches, nicotinic stomatitis, peri-implantitis and other periodontal conditions including increased plaque and calculus depositions, gingival recession and alveolar bone loss.[18] Carcinogens found in the tobacco smoke are responsible for the developing of oral diseases and cancer.[19] Nicotine can contribute to cancer etiology when it is nitro sated and in turn, makes carcinogenic tobacco-sp. edifice nitrosamines.[12] In vivo studies showed that when 0.216M of nicotine is applied topically to the oral mucosa for a period of two hours leads to alterations within the epithelium like acantholysis and nuclear shrinkage.[20] Oral keratinocytes are the first cells in contact with tobacco components, thus keratinocyte inflammation has been stated as a critical step in tumor promotion.[21] Recent study has shown that when 6% nicotine alone or in combination of other tobacco-specific nitrosamines such as 0.01% NNN, 0.01% NNK was applied on hamster cheek pouch and gastric mucosa, off them the cheek pouch epithelium showed signs of hyperplasia, hyperkeratosis and moderate dysplasia.[22] Nicotine is associated with increase the permeability of oral mucosa to N-nitrosonornicotine.[23] Nicotine agents by acting on nicotine acetylcholine receptors, directly modulates the stimulated release of calcitonin gene related peptide (CGRP). This modulation can contribute to inflammatory processes within the oral cavity.[24] As oral keratinocytes are the first cells in contact with tobacco components, thus keratinocyte inflammation has been stated as a critical step in tumor promotion.[21]

MATERIALS AND METHODS

This study was conducted at Department of Oral Pathology Bart's and the London Queen Mary School of Medicine and Dentistry Queen Mary, University of London. The study focused on the effects of nicotine on an uninflamed stratified epithelial layer, when applied for a period of 5 minutes and over 24 hours respectively.

The reconstituted human epithelium model used in the study was prepared and supplied by Skin Ethic Laboratories, Nice, France. It is a three-dimensional tissue culture model obtained by culturing transformed oral keratinocytes (TR146) derived from a buccal carcinoma. The cells were seeded and cultivated in a defined medium for 14. The resulting culture formed a stratified epithelium with 5-7 cell layers devoid of stratum corneum (Fig 1). Skin Ethic Laboratories also supplied maintenance medium (MCDB 153 containing 5μg/ml insulin and 1.5mM ca2+) for use in the experiments. The Model cultures were transferred into a new 24 well culture plates (Costar, UK) containing 500μl maintenance medium per well and incubated for 2 hours at 37ºC in 5% CO2 in a humidified atmosphere. The cultures were transferred to a new 24 well plate containing fresh media for all experiments. Working solutions (10μM and 1mM) of nicotine were prepared from a 2.5M stock solution (Sigma, UK). The working solutions were diluted in phosphate buffered saline immediately before use. The morphology of the stratified oral mucosal model was examined using formalin fixed paraffin processed tissue. The culture was fixed by submerging in excess neutral buffered formalin for 24 hours at room temperature. The epithelium, with supporting polycarbonate membrane was dissected out of the inserts and the tissue processed to paraffin wax using an automatic tissue processor (Shandon Hyper Centre II). 5μm sections were cut and stained with hematoxylin and eosin, examined by light microscopy whilst the image was recorded with digital photography.

RESULTS

In order to confirm the effect of nicotine on an epithelial layer of the different treatments on tissue morphology was assessed using formalin fixed paraffin wax sections and hematoxylin and eosin staining. All the samples of nicotine treated uninflamed tissue were stratified with the presence of 10-15 epithelial layers and the absence of a stratum corneum (Figure 2-5) respectively.

The results showed no evidence of damage or alteration to the surface layers or the basal layer of the stratified squamous epithelial models.

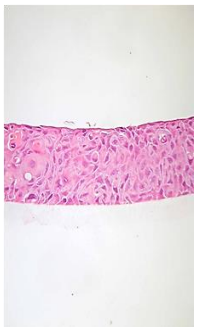

Figure No.1: Haematoxylin and eosin stained section of mucosal model (original magnification x63)

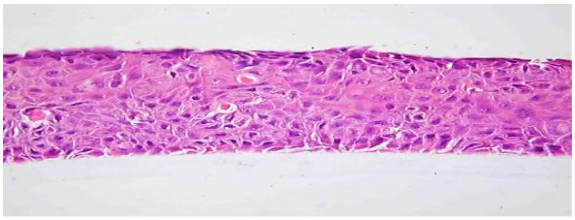

Figure No.2: Heamatoxylin and eosin stained section of Mucosal model (original magnification x63)

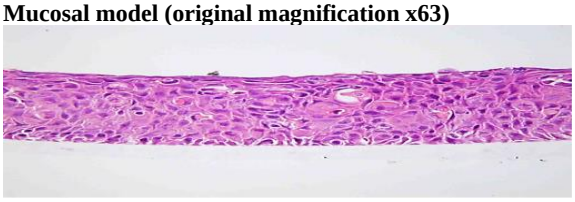

Figure No.3: Heamatoxylin and eosin stained of uinflamed mucosatreated with 1mM nicotine for 5 minutes; original magnification x63

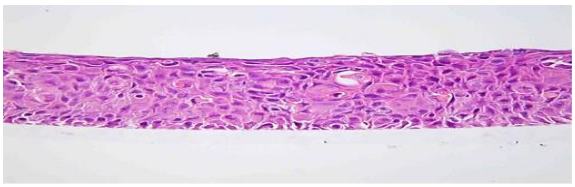

Figure No.4: Heamatoxylin and eosin stained section of uninflamed mucosatreated with 10µM nicotine for 24 hours; original magnification x63

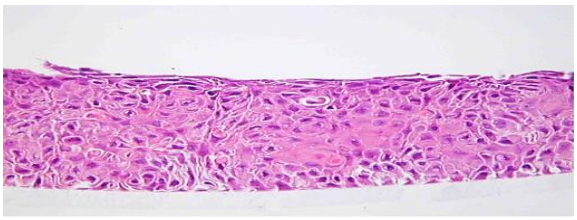

Figure No.5: Heamatoxylin and eosin stained section of uninflamed mucosa treated with 1mM nicotine for 24 hours; original magnification x63

DISCUSSION

The aim of this study has been to investigate the effect of nicotine on an uninflamed reconstituted oral mucosa**.** The epithelial model allowed us to consider the effect of nicotine on an epithelial layer in the absence of any influence from mesenchyme. Stratified cultures were treated for 5 minutes and 24 hours respectively. Tissue morphology was assessed using formalin fixed paraffin wax sections and hematoxylin and eosin staining. The results from morphology studies suggested that nicotine treatment of uninflamed reconstituted oral mucosa after 5 minutes and 24 hours respectively had no significant effect on the morphological structure of the epithelium.

The results from morphological studies suggest that nicotine induces only a subtle change in membrane integrity, and also of important note was the fact that there were no gross changes in the appearance of the epithelium. This was surprising, in fact, nicotine has been shown to alter viability and morphology. In a previous in vivo study by Anderson and Warfving,[25] revealed that nicotine exerts its biological effect on the oral mucosa and resulted in changes in the appearance of the epithelium. Alpar et al. in their study showed that 4mM nicotine dose caused significant morphological alterations of microtubules and vimentin filaments which than lead to atypical and vacuoles formation within the oral fibroblasts.[26]

However in a similar type of study conducted on a reconstituted oral mucosa by Kwon et al.[16] revealed that nicotine had no effect on the viability of the cells although decreased, dose-dependently, mucosal epithelial thickness at 10µM , and 100µM concentration, but nicotine reduced cell viability in the epidermal keratinocyte at a concentration 100µM. Previously Alpar et al.[26] had also linked higher doses of nicotine (10.5-15.5mM) to be responsible for causing irreversible changes in morphological appearance of the cells. Squier and Johnson [27], also showed that when 0.2M nicotine was applied topically to the oral mucosa, after 2 hours it induced acantholysis and nuclear shrinkage within the epithelium. There may be some factors that may limit the significance of the findings in this study. It may be due to the permeabilizing effect of nicotine on mucosa, and it may be due to the reason that we used in vitro tissue culture models, whereas most of the studies were conducted in vivo. Alternatively, these results could suggest that it was not possible to quantify the amount of mitochondrial disruption by nicotine at the concentration range used.

CONCLUSION

In these experiments the tissue morphology was assessed by conventional light microscopy. Results showed that application of nicotine after 5 minutes and 24 hours treatment on uninflamed tissue with different concentrations used, did not have a significant effect on gross morphology. None of the treatments caused a

significant effect on the morphological structure of the epithelium.

Author's Contribution:

Concept & Design of Study:	Numan Sheikh
Drafting:	Sajid Hanif, Faisal Pasha
Data Analysis:	Mohammad Irfan, Zahur Qayyum
Revisiting Critically:	Numan Sheikh, Sajid Hanif
Final Approval of version:	Numan Sheikh

Conflict of Interest: The study has no conflict of interest to declare by any author.

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