CD117 EXPRESSION IN RATS TREATED WITH NICOTINE SKIN
“A study Immunohistochemical and Histopathological”

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ABSTRACT

In this study, 14 adult Sprague-Dawley rats weighing 240-250 gm (±10 gm) were used as experimental animal. The rats were divided into 2 groups as: the rats of ‘Group Experimental’ (n= 6) were systemically nicotinized with nicotine sulphate, 2mg/kg subcutaneously, daily 28 days; their skin was removed and placed in 10% formaline. Sections were stained with Hematoxyline-Eosine, Trichrome-Masson and observed under light microscope. Immunohistochemical staining with antibodies to melanocytes studied the expression of CD117. Histopathological examination of the skin revealed the reduction of the epidermal thinkness, the decrease in size, of the hair follicles compared with control/nicotine rats. Nicotine, pigmentation of the epidermis, the dermis, hair follicles, blood vessels and skin affected by histopathological examination showed accelerated aging.

Key words: Nicotine, rat, CD117.

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Introduction

Nicotine(NIC) can enter the human body by smoke inhalation, ingestion, intranasal spray, transdermal patch, topical cream, or enema. Nicotine can be absorbed through the oral cavity, lung, bladder, gastrointestinal tract, and skin. Seventy to 80% of nicotine absorbed from the gastrointestinal tract is converted to its principal metabolite, cotinine, via first-pass hepatic metabolism[1]. The precise ways in which tobacco smoke damages or changes skin are not fully understood, though scientific studies have produced evidence about a number of possible ways. Studies suggest that tobacco smoke exposure decreases capillary and arteriolar blood flow, possibly damaging connective tissues that help maintain healthy skin[2]. Nicotine is absorbed through skin and mucous membranes in a dose-dependent manner. The half-life of nicotine is approximately two hours[3]. In vivo studies show that mice exposed to smoke develop atrophy of the epidermis, reduced thickness of the subcutaneous tissue, and scarcity of hair follicles. Cellular apoptosis is also seen in the hair bulbs[4]. Smoking affects the dermal hair papilla microvasculature and leads to DNA damage of the hair follicle[5]. Topical effects of nicotine to skin by smoking have been examined in previous studies. c-KIT (CD117) is a transmembrane tyrosine kinase receptor that functions in melanocyte development and proliferation[6,7,8,9]. In this study, cells and melanocytes in the epidermis of nicotine, depending on the implementation of the hair follicles in the dermis and connective tissue structure were investigated by immunohistochemical and histological methods.

Materials and methods

The study protocol was approved by the Animal Research Committee of Dicle University,
Turkey. 14 adult Sprague-Dawley rats weighing 240-250gm (±10gm) were used as experimental animal. The animals were group-housed (7 per cage) under standard conditions (21 ± 2°C) in the Animal Health and Research Center of Dicle University. The animals were fed ad libitum with water and standard laboratory animal diet, under the care of trained wardens. The rats were divided into 2 groups as: the rats of ‘Group Experimental’ (n=6) were systemically nicotinized with nicotine sulphate, SIGMA, 2mg/kg subcutaneously, daily for 28 days. The rats of ‘Group Control’ (n= 6) were used as control and received no NIC, but were maintained in similar environment and food. At the end of the study, animals were a piece of skin of face made the anesthetic ketamine. For sample preparation under light microscopy after fixation of tissues by formaldehyde 10% solution, they were directly dehydrated in a graded serious of ethanol and embedded in paraffin. Then, sections of 5µm in thickness were cut and made into slides. These were processed for Hematoxylin-Eosin and Trichrom Masson staining, carried out according to conventional procedures.

**Immunohistochemistry staining**

Sections were incubated with 0.3% hydrogen peroxide in methanol for 20 min to block endogenous peroxidase activity and microwave-treated for antigen retrieval in citrate buffer solution at pH 6.0. After overnight incubation with the primary antibody (polyclonal rabbit antihuman KIT protein, CD117 DAKO; Dakopatts, Glostrup, Denmark) at a 1:500 dilution in PBS, the sections were incubated with the secondary antibody and then with the streptavidin–peroxidase complex. The sections were then immersed for 12 min in DAB chromogenic substrate solution (diaminobenzidine 0.02% and H2O2 0.001% in PBS), counterstained with Harris hematoxylene. Olympus BH2 light microscope was used for microscopic examination.

**Statistical analysis**

Data are presented as means with standard deviations or as percentages. The significance of the difference between groups was calculated with two tailed Student’s t-tests for independent samples.

**Result**

The epidermal thickness was defined as the distance between the stratum corneum and the basement membrane, the hair follicle size, measured by selecting the cross-sections. Histopathological examination of the skin revealed the reduction of the epidermal thickness, and the decrease of the hair follicles in size compared with control/nicotine rats (P < 0.01)(Table 1)

<table>
<thead>
<tr>
<th></th>
<th>Control Group n= 6</th>
<th>Nicotine Group n= 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>The thickness of the epidermis</td>
<td>22.73 ± 9.41</td>
<td>17.93 ± 9.94</td>
</tr>
<tr>
<td>Mean</td>
<td>15.45</td>
<td>12.41</td>
</tr>
<tr>
<td>St.Dev</td>
<td>3.67</td>
<td>3</td>
</tr>
<tr>
<td>Diameter of the hair follicles</td>
<td>48.21 ± 30.3</td>
<td>32.39 ± 16.61</td>
</tr>
<tr>
<td>Mean</td>
<td>37.42</td>
<td>24.68</td>
</tr>
<tr>
<td>St.Dev</td>
<td>15.62</td>
<td>5.47</td>
</tr>
</tbody>
</table>

Table 1: Epidermal thickness and hair follicle diameters were measured.

Epidermal thickness had decreased statistically in comparison with the control group. Cross-section of the control group, consisted of densely packed, variably oriented, thick bundles of collagen. Clusters of hair follicles and associated sebaceous glands were evident, and hairs in all phases of development were observed. Arrector pili muscles were easily identifiable. (Fig. 1) Nicotine group, Masson’s trichrome staining dermis arranged in scattered thin collagen fiber bundles were seen. Congestion and vascular wall changes with extravasated erythrocytes were observed (Fig. 2).

**Figure1a and b:** Epidermis and dermis were seen to have normal histological appearance. Hair follicles (arrow), Arrector pili muscles and sebaceous glands (*)Trichrome-Masson stain(a)Hematoxylen-Eosin(b)
Immunohistochemical light microscopic examination of the skin, proved that melanocytes in the basal layer of epidermis cells were positive for CD117 expression. Subepithelial mast cells also stained positively (Non nicotine group). Cross-section of the nicotine skin treated group showed a decrease in CD117 expression in melanocytes. Nicotine group compared to the control one showed a lower the density of CD117 expression in hair follicles. There was no expression of mast cells in the dermal layer of the sections in the nicotine group.

**Discussion**

Nicotine in liquid form is a known poison. It can be absorbed in liquid form through ingestion or through contact with skin. The damaging effects of smoking on the skin vasculature and on oxygenation have been documented in both human and animal models. Nicotine does not appear as the only one factor of vasoconstriction and hypoxemia. Nicotine induces vasoconstriction, higher in skin than in gingiva, associated with local hyperaemia. Smoking is associated with many dermatological conditions, including poor wound healing, premature skin aging, squamous cell carcinoma, melanoma, oral cancer, acne, psoriasis, and hair loss. Considerable teratogenic effects of nicotine were observed histologically on newborn rat skin: increased mitotic activity in the basal cells, induction of hypertrophic epithelial cells in the epidermis.

A study on light and electron microscopy observation indicates a delayed exocytosis from keratinocytes. Keratinocyte migration inhibitory effect of nicotine in an attempt. Nicotine which is connected to the damage at the cellular level, the decrease in expression of CD117 in the melanocytes, hair follicles, is the result of hypopigmentation. The major function of cytokeratins is to serve as cytoprotectors by preventing apoptosis and providing mechanical integrity to cells. In this study, the effect of nicotine was found to be a reduction in the content of the epidermis and the hair follicle cytokeratin. Congestion and vascular wall changes with extravasated erythrocytes were observed, suggesting the presence of circulatory disturbance.

Depending on the effect of nicotine, vasoconstrictor and decrease collagen fibers, is thought to bind the keratinocyte differentiation, and migration of skin aging. The use of nicotine, affects pigmentation of the epidermis and hair follicles, caused by changes in the structure of collagen is thought to accelerate aging of the skin.
References


