Overexpression of HIF-1α and Morphological Alterations in the Tongue of Rats Exposed to Secondhand Smoke

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Smoking is a risk factor for serious health problems and is associated with several changes in the tissues of the oral cavity. The aim of this study was to evaluate the collagen percentage, mast cells density, intensity of immunolabeled cells by anti-HIF-1α in the musculature lingual of rats exposed to secondhand smoke. Twenty-seven female Wistar albino rats were divided into three groups: rats not exposed to tobacco smoke inhalation (Control group) (n=7); rats exposed to smoke inhalation for 30 days (TAB 30) (n=10); and rats exposed to smoke inhalation for 45 days (TAB 45) (n=10). Subsequently, the animals were submitted to euthanasia and removal of the tongue for histological and immunohistochemistry processing and analysis. In the groups TAB 30 and TAB 45 there were a lower percentage of collagen, a higher density of mast cells and a greater intensity of anti-HIF-1α immunolabeled cells compared to Control group. There was also a positive and significant correlation between the percentage of collagen and mast cell density. There was no significant difference between TAB 30 and TAB 45 in any of the parameters evaluated. Therefore, the exposure of rats to secondhand smoke for 45 days causes decrease in perimysial collagen fibers, increase in the number of mast cells and increase in the immunolabeling for HIF-1α in lingual muscle cells. The present study was the first to evaluate the percentage of collagen, mast cell density and immunostaining for HIF-1α in rat tongues exposed to tobacco smoke.

Introduction

Smoking is the major modifiable risk factor associated with severe health problems such as cancer, cardiovascular disorders, respiratory diseases, infertility and one of the leading causes of preventable death (1).

The act of smoking affects not only active smokers but also affects people who are exposed to environmental smoke from cigarettes. The environmental smoke of cigarettes is a mixture of gases and particles from the burning of tobacco in the act of smoking. It is composed of the smoke that comes from the end of the cigarette when it is not being swallowed (side or secondary smoke) and the smoke exhaled by the smoker (main or exhaled smoke) (2).

The toxic substances of tobacco lead to the destruction of collagen fibers in the connective tissue (3), by increasing the expression of metalloproteinases (4). Connective tissue mast cells also suffer from the action of tobacco toxic substances, such as acrolein, which promotes mast cell degranulation with a consequent inflammatory reaction (5). In addition, exposure to long-term tobacco smoke increases the number of mast cells that produce important growth factors associated with angiogenesis (6) and fibrosis (7). Tissue hypoxia, induced by smoking and exposure to tobacco smoke, can also cause tissue damage (8). The hypoxia, in these cases, can be triggered by the action of nicotine, which stimulates the secretion of adrenocortical hormones that promote vascular contraction and reduction of local blood flow. Additionally, carbon monoxide contributes to the reduction in oxygen saturation (SpO2) and the formation of carboxyhemoglobin which also contributes in tissue hypoxia (9).

One way to assess whether the tissue is in hypoxia is to investigate the presence of hypoxia-induced transcription factor 1 (HIF-1) (10). HIF-1 is a heterodimeric protein composed of two subunits: HIF-1α and HIF-1β, the α subunit being expressed in dependence on the availability of oxygen. In hypoxia conditions, the HIF-1 complex translocates to the nucleus and induces the transcription of genes related to hypoxia-adaptive mechanisms triggering angiogenesis, oxygen transport, generation of ATP in the absence of oxygen, apoptosis and metastasis (10), which will assist in cell survival under these conditions (11).

Although some authors have shown epithelial changes in the tongue of rats exposed to tobacco smoke (12), no studies have been found describing changes in the lingual musculature due to inhalation of the main or exhaled smoke. Thus, we hypothesized that not only the epithelium, but also the lingual musculature could present tissue alterations...
resulting from main smoke. Knowing the importance of tongues in swallowing, chewing and speaking, it is justified to carry out the present study that aimed to evaluate the collagen percentage, mast cells density, the intensity of immunolabeled cells by anti-HIF-1α in the musculature lingual of rats exposed to secondhand smoke.

Material and Methods

The present study was elaborated according to the Guide for Care and Use of Laboratory Animals (NRC) and approved by the Ethics Committee on Animal Use of the University of Uberaba (CEUA), protocol nº 070/2017.

Animals and Groups

Twenty-seven female albino rats (Rattus norvegicus) of the Wistar lineage, adults, with a mean initial body mass of 150 g (± 10 g) supplied by the Biotério Central of the City Hall of Ribeirão Preto Campus of the University of São Paulo were used. The sample calculation was performed considering the loss rate of approximately 30% (13).

The animals were housed in standard cages at the Bioengineering Laboratory of the Medical School of Ribeirão Preto (FMRP / USP), where they remained during the whole experimental period under ambient temperature of 20 °C to 24 °C, with light cycles of 12 h clear and 12 h dark, with relative air humidity at 55% (± 10%) and without restriction to food or water.

The animals were randomly assigned to three experimental groups: female rats not exposed to the inhalation of tobacco smoke (control group) (n=7); female rats exposed to smoke inhalation for 30 days (TAB 30) (n=10); and female rats exposed to inhalation of tobacco smoke for 45 days (TAB 45) (n=10).

Exposure of Rats to Tobacco Smoke

The exposure of rats to tobacco smoke was performed according to methods previously described (13).

The animals were placed inside, wholebody a rectangular acrylic box composed by four cylindrical transparent acrylic tubes. In each tube was placed an animal. Each tube has one end with a controllable physical restrictor to prevent the animals from retreating and another tapered end that connects with a container that distributes the smoke interspersed with ambient air to the four tubes. The cigarette smoke “puffs” inside the box were removed by suction through a peristaltic pump (Provitec – model: AWG 5,000 AX-D, São Paulo, SP, Brazil), which has a timer that controls the time of injection of ambient air or smoke within the tubes in 30 s cycles of exposure to ambient air and 15 s of exposure to smoke. All equipment, except the pump, was placed inside the transparent acrylic box, connected to an exhaust hood that promotes the cleaning of ambient air. Four animals were simultaneously exposed to cigarette smoke.

All days, the percentage of carbon monoxide within each cylindrical chamber was measured with a portable carbon monoxide meter (Instrupemp, ITMCO-1500, São Paulo, SP, Brazil). The source of the smoke was Marlboro cigarettes (Phillips Morris, Santa Cruz do Sul, RS, Brazil), with each unit containing the following according to the product label: 0.8 mg of nicotine, 10 mg of tar and 10 mg of carbon monoxide.

The animals went through the set-up phase in the equipment for a period of 20 min without smoke, twice a day, with 6 h interval, in the morning and afternoon for seven consecutive days. The 20 min corresponded to the time of exposure to two cigarettes (cycles of 30 s of exposure to ambient air and 15 s of exposure to smoke). After periods of exposure to tobacco smoke the rats returned to their cages.

Later, the animals were submitted to adaptation to tobacco smoke for seven consecutive days by burning a cigarette twice a day (morning and afternoon), with a 6 h interval between exposures. After the adaptation period, the rats were exposed to the smoke of two cigarettes in the morning and two more cigarettes in the afternoon, with a 6 h interval, for 30 or 45 days according to the experimental groups. In order to guarantee similar exposure to tobacco smoke, once every seven days the percentage of carbon monoxide in each camera of the equipment was measured using a portable carbon monoxide meter (Instrutemp ITMCO-1500, São Paulo, SP, Brazil).

Evaluation of Weight, Euthanasia and Blood Sampling

The animals were weighed weekly with a semi-analytical balance (Digital Electronic Scale from 0 to 3 kg, TOLED, mod. 9094) with a capacity of up to 5200 g, with 0.1 g precision resolution.

At the end of the experiment the animals were submitted to euthanasia with excessive dose (3mL/100g) of Thiopental (sodium thiopentate, 1.25% solution) administered intraperitoneally. Then the blood samples were collected by means of open cardiac puncture and kept in boxes with ice. Subsequently, within two hours after the collection, these samples were centrifuged for separation of the plasma using a centrifuge (HETTICH, mod. EBA 280). Soon after, the plasma was stored at -20 °C until plasma cotinine was assessed in order to verify if plasma cotinine levels were the same as those found in passive smokers.

Dosage of Cotinine

Plasma cotinine dosage was performed by means of gas chromatography analysis, according to the routine methodology of the Laboratory of Forensic Toxicological
Analysis of the School of Philosophy, Sciences and Letters of Ribeirão Preto (FFCLRP/USP). Standard: cotinine in 1.0 mg/mL solution in methanol (Sigma-Aldrich, St. Louis, MO, USA).

The cotinine serum concentration ranging from 2.1 to 17.5 ng/mL was accepted as the cut-off level to characterize the animal exposure as the secondhand smoke (13), similar to levels found in human smokers (14).

After the dosage of cotinine, the heads of animals were placed in 10% formaldehyde and then transported to the Human Anatomy Laboratory of the Department of Structural Biology of the Federal University of Triângulo Mineiro (DBE/UFTM), where the fragments of tongues were collected.

Collection of tongues samples, histochemical and immunohistochemical processing

The tongue were collected by means of a medial incision in the region of the retromolar trigone and anterior region of the neck, followed by avulsion of the skin, resection of the musculature of the floor of the mouth and disarticulation of the temporomandibular joint. An incision was made in the region of the epiglottic vallecula with removal of the tongue and mandible, which were fixed in 10% formaldehyde for later lingual resection.

Fragments of the tongue were then collected by a longitudinal cut along the medial lingual groove, from the base to the apex. These fragments were processed histopathologically obtaining 5-μm-thick sections. Then, the fragments were stained with Toluidine Blue for determination of mast cell density; and Picrosirius red staining for quantification of collagen percentage. Additional sections were used for immunohistochemical processing for HIF-1α.

Determination of the Percentage of Collagen

For morphometric quantification of collagen, picrosirius red staining slides were polarized with a light filter on a standard Axio 4.1 light microscope (Zeiss, Berlin, Germany) and 20x objective. All fields of the slides containing muscle tissue were transmitted to the computer with the aid of an Axiocam image capture camera (Zeiss, Berlin, Germany). In the polarized image the collagen presented birefringence with yellowish, reddish or greenish coloration. The Axiovision 4.8 software (Zeiss) was used for morphometric analysis of the collagen percentage in each captured image. Using this software, a birefringent area of the image was marked with the computer cursor and immediately the software automatically marked all similar areas and determined the percentage of collagen in the analyzed field. Then the percentage of collagen of each field was sent in a spreadsheet for later statistical analysis. The analyses were performed separately in the apical, middle and posterior thirds.

Determination of Mast Cell Density

To determine the mast cell density, the blades stained by toluidine blue were analyzed using the 40x objective. The analyses were performed separately in the apical, middle and posterior thirds. With the aid of a micrometer blade, the area of each field (0.14 mm²) was calculated, being multiplied by the total number of fields analyzed to obtain the total area. With the total number of mast cells and the total area analyzed, the mast cell density was calculated and expressed in mast cells per mm².

Immunohistochemical Processing and Analysis of Anti-HIF-1α-Immunolabeled Cells:

For immunohistochemistry, the samples were deparaffinized in xylene and alcohol baths and then immersed in phosphate-buffered saline. For antigen retrieval and endogenous peroxidase inhibition, the sections were treated with 3% hydrogen peroxide in methanol for 10 min and then incubated in citrate buffer (0.01 mol/L, pH=6.0) for 30 min at 90°C. The samples were incubated in 2% bovine serum albumin for 30 min at room temperature to reduce nonspecific binding. Subsequently, the samples were incubated with the primary antibody anti-HIF-1α mouse monoclonal antibody (dilution 1:500 Sigma-Aldrich) diluted in bovine serum albumin dissolved in PBS (Sigma-Aldrich) at 4°C in a humid chamber for 18 h (overnight).

After overnight incubation, all slides were kept at room temperature for about 15 min, washed in PBS and incubated with a polymer detection system (Spring, Pleasanton, CA, USA) for 30 min at room temperature in a humid chamber. Posteriorly the reaction was developed by incubation with diaminobenzidine (Sigma-Aldrich). Finally, the sections were counterstained with hematoxylin. A negative control reaction was performed by omitting the primary antibody. As a positive control, human palatine tonsils were used, as suggested in the antibody package insert.

The immunohistochemical evaluation of the immunolabeled cells was performed semi-quantitatively using a microscope (Nikon Eclipse 80i) and a 40x objective. For this evaluation, the following scores were used: 1 - weak immunolabeling (up to 33% of the immunolabeled cells), 2 - strong immunolabeling (>33% of the immunolabeled cells) (15).

Statistical Analysis

The statistical analyses were performed using GraphPad Prism 5.0 and Bioestat 5.0 software. For the evaluation of the final weight of the animals, cotinine dosage and mast cell density, ANOVA and Tukey post-test were used,
since the data presented normal distribution. However, for the evaluation of the collagen percentage and the immunostaining for HIF-1α, the Kruskal-Wallis tests and the Dunn post-test were applied, since the data presented a non-normal distribution. To correlate the mast cell density and collagen percentage, the Pearson correlation test was used for the data that presented normal distribution and the Spearman correlation test for data that presented a non-normal distribution. All analyses were performed assuming a significance level of 5%.

Results

The analyzes were carried out on 25 animals because during the adaptation period two animals in TAB 45 group of the animals were lost.

The mean levels of carbon monoxide were similar at all measurements (338.79±1.16 ppm). Animals from all groups presented significant weight gain when comparing initial body weight and final body weight (p<0.0001). When the final weight of the animals was compared between the groups, it was observed that the groups exposed to tobacco had significantly lower final weight in relation to the control group (p<0.0001).

Type I and II collagen fibers were predominantly in the perimysium region and were red-orange in the polarized image. The percentage of collagen in the apical and posterior third of the tongue was significantly lower in the TAB 30 and TAB 45 groups than in the control group (p<0.0001). In the middle third, there was no significant difference between the groups in relation to the percentage of collagen (Fig. 2).

Mast cells with blue-stained intracytoplasmic granules were observed mainly in the connective tissue of the perimysium. The mast cell density was significantly higher in the TAB 30 and TAB 45 groups than in the control group, observed in the apical third (p=0.0225), in the middle third (p=0.0163) as well as in the posterior third of the tongue (p=0.0007) (Fig. 3).

Immunolabeling for HIF-1α in the lingual muscle cells and was significantly higher in the TAB 45 group than in the control group: in the apical third (p=0.0036), in the middle third (p=0.0029), and in the posterior third (p=0.0007). TAB 30 group showed significantly higher immunolabeling for HIF-1α than the control group in the posterior third of the tongue (p=0.0007). Immunolabeling for HIF-1α was observed in the muscle cell cytoplasm. There was no immunolabeling in the cell membranes or in the connective tissue of the perimysium or endomysium (Fig. 5).

No significant correlation was observed between mast cell density and the intensity of cells immunolabeled for HIF-1α (data not shown).

Discussion

The loss of 8% of the animals was probably due to intoxication from tobacco smoke, which was already foreseen in the exposure protocol (13).

Although animals of all groups showed weight gain, the final weight of the animals in the groups exposed to tobacco was significantly lower in comparison to the final weight of the control group animals. This finding corroborates another study that also observed lower body weight in rats exposed to secondary tobacco smoke (16). The decrease in weight gain in animals exposed to cigarette smoke may have been caused by the action of nicotine in the smoke that leads to reduced appetite and altered eating patterns, as already demonstrated in other studies (16).

Although we have not found studies that evaluated the percentage of collagen in the tongues of both humans and animals exposed to tobacco smoke, some authors have already shown an increase in the amount of collagen in the salivary glands (17) and in mammary tissue from rats.
Tongue of rats exposed to secondhand smoke

Figure 2. Comparison of the percentage of collagen in the lingual musculature among the control, TAB 30 and TAB 45 groups; Kruskal-Wallis test. A: apical third, p<0.0001; B: medium third, p=0.4752; C: posterior third, p<0.0001. D: Polarized image of collagen fibers in the apical third of lingual musculature (control group). E: Polarized image of collagen fibers in the apical third of lingual musculature (TAB 30 group). F: Polarized image of collagen fibers in the apical third of lingual musculature (TAB 45 group) (Picrossírius, 400×). * Indicates significant difference among the groups.

Figure 3. Comparison of mast cell density in the lingual musculature among the control group, TAB 30 and TAB 45 groups; ANOVA test. A: apical third, p=0.0225; B: mean third, p=0.0163; C: posterior third, p=0.0007. D: Mast cell density in the apical third of lingual musculature (control group). E: Mast cell density in the apical third of lingual musculature (TAB 30 group). F: Mast cell density in the apical third of lingual musculature (TAB 45 group) (Toluidine Blue staining, 400×). * Indicates significant difference among the groups.
(6) as a consequence of inhalation of tobacco smoke. In the present study, the percentage of collagen in the apical third and the posterior third of the tongue was significantly lower in TAB 30 and TAB 45 groups than in the control group. Our findings corroborate with other study that also found a reduction in the amount of collagen in the skin of Wistar rats (16) exposed to cigarette smoke.

The reduction in the percentage of collagen in the tongue of rats exposed to tobacco smoke may have been caused by nicotine and cotinine, leading to an increase in the expression of metalloproteinases, such as collagenases (6), that induce the destruction of collagen fibers type I, II and III (18). Moreover, the inhalation of tobacco smoke leads to an increase in the production of reactive oxygen species, such as superoxide and reactive nitrogen species, such as nitric oxide, which also cause destruction of the

![Figure 4](image1.png)

**Figure 4.** A: Correlation between the mast cell density and the percentage of collagen in the lingual musculature (TAB 30 group); Spearman correlation, rs=0.7472; p<0.0001. B: Correlation between the mast cell density and the percentage of collagen in the lingual musculature (TAB 45 group); Pearson’s correlation, r=0.7387; p<0.0001.

![Figure 5](image2.png)

**Figure 5.** Comparison of immunolabeling to HIF-1α among the control, TAB 30 and TAB 45 groups; Kruskal-Wallis test. A: apical third, p=0.0036; B: middle third, p=0.0029; C: posterior third, p=0.0007. D: Immunolabeling to HIF-1α in the posterior third of the lingual musculature (control group). E: Immunolabeling to HIF-1α in the posterior third of the lingual musculature (TAB 30 group). F: Immunolabeling to HIF-1α in the posterior third of the lingual musculature (TAB 45 group) (immunohistochemistry, 400×). * Indicates significant difference among the groups.
As there was a lower percentage of collagen found only in the apical third and the posterior third of TAB 30 and TAB 45 groups, we believe that these regions would be more exposed to the action of tobacco smoke. This is because during exposure to smoke, animals constantly open their mouths, which allows greater contact of the smoke with the apical third of the tongue. In addition, during inhalation the smoke penetrates the nasal region and passes through the oropharynx directly reaching the posterior region of the tongue, which could also justify the lower percentage of collagen in this region.

Collagen is known to participate in the organization, structure and mechanical properties of tissues (20) and in the tongue, the presence of collagen contributes to the constant maintenance of organ volume during muscle contractions (21). Therefore, although studies of lingual function in smokers are still necessary, we believe that decreasing the percentage of collagen in lingual musculature could impair the functions of this organ during chewing, swallowing and speaking.

Studies have shown that there is an increase in the number of mast cells in the ducts and lymph nodes of mammary tissue (6). These findings corroborate with the present study because we found significantly higher mast cell density in the apical, middle and posterior thirds of TAB 30 and TAB 45 groups in relation to the control group.

Mast cells are metachromatic cells that display granules in their cytoplasm and numerous surface receptors, which when stimulated release biologically active mediators such as proteases, proteoglycans, cytokines, chemokines and growth factors (7). Furthermore, it is known that toxic substances present in tobacco smoke, such as acrolein, provokes mast cell degranulation (5) and that some substances released during this degranulation cause recruitment of progenitor mast cells to the tissue, where they are finally matured (22). Thus, the increase in mast cell density in the groups exposed to tobacco smoke in the present study may have occurred because of chemical mediators released by these cells, which would be participating in the recruitment of progenitor mast cells from the bone marrow, contributing to the increase of mast tissue population in the lingual tissue.

Mast cells have been prominent because they are involved in several physiological and pathological processes, but one of the most well known functions of these cells is the participation in the process of collagen synthesis through fibroblastic activation (7). Study have shown a positive correlation between fibrosis and mast cell density in tongue of chronic chagasic patients of necropsied patients (23). This study corroborate our findings because we observed a positive and significant correlation between the percentage of collagen and the density of mast cells in the tongue. Thus, in the present study, the inhalation of the tobacco smoke would be causing an increase in number and mast cell degranulation with consequent release of fibrogenic factors. However, as there was a lower percentage of collagen in the groups exposed to tobacco smoke when compared to control group, we believe that the collagen destruction caused by the action of the smoke is overcoming the deposition process.

HIF-1 is a basic protein composed of two subunits: HIF-1α and HIF-1β. The α subunit is expressed in dependence on the availability of oxygen. This protein is involved in important adaptive responses to hypoxia, promoting cell survival under these conditions (10). Although studies have reported a decrease in HIF-1α in lung tissue of adult rats (24) and progeny of rats (25) exposed to tobacco smoke, our findings demonstrated that the immunolabeling anti-HIF-1α was significantly higher in the TAB 45 groups when compared to the control group, in the three thirds analyzed. In the posterior third, TAB 30 group presented significantly higher anti-HIF-1α immunolabeling than the control group. Our findings corroborate other studies that also found an increase in HIF-1α levels in the nasal tissue of rat models with eosinophilic rhinosinusitis exposed to the smoke of tobacco (26) and in orbital fibroblasts exposed to tobacco smoke extract (27).

In the present study, the increase in immunolabeling to HIF-1α in the groups exposed to tobacco smoke probably occurred due to the action of nicotine and carbon monoxide present in the smoke, which would cause both vasoconstriction and complex formation carboxyhemoglobin, which prevent the binding of the oxygen molecule to hemoglobin, dificulting the transport and supply oxygen to the cells (9). As TAB 45 group showed the highest immunolabeling to HIF-1α in relation to the control group in the three thirds analyzed, we believe that tissue hypoxia occurs with a longer exposure to tobacco smoke. However, as the posterior third of TAB 30 group also presented greater immunolabeling to HIF-1α when compared to the control group, we believe that this region was more exposed to the local action of the smoke.

Thus, after 45 days of exposure to tobacco smoke, there was an increase in the synthesis of HIF-1α, which is a transcription factor related to the production of growth factors (10). Therefore, in the present study, there would be an increase in the synthesis of growth factors with consequent multiplication of resident mast cells and an increase in the number of these cells in the lingual musculature. As discussed previously, although the increase in the number of mast cells is generally associated with increased collagen synthesis, in the present study we found a lower percentage of collagen in the groups exposed to...
cigarette smoke probably because the collagen destruction caused by the action of the smoke is overcoming the deposition process.

Although the increase in HIF-1α does not cause any clinical alteration in the tongue, we know that under hypoxic conditions, the HIF-1 complex translocates to the nucleus and induces transcription of genes related to adaptive mechanisms, triggering angiogenesis, transport oxygen and ATP generation in the absence of oxygen (10). Thus we suggest that HIF-1α would be associated with tissue protection by inducing angiogenesis and local neovascularization, which could explain the fact that we did not find relevant tissue lesions in the experimental groups. Thus, we believe that increased HIF-1α in lingual musculature would be an attempt by the body to decrease the damage caused by hypoxia. However, human studies are still needed to confirm this hypothesis.

In summary, the exposure of rats to tobacco main smoke causes decrease in perimysial collagen fibers, increase in the number of mast cells and increase in immunolabeling for HIF-1α in the lingual muscle cells. Knowing the importance of lingual musculature in swallowing, chewing and speaking, and of the limitations of the present study, new studies should be carried out to better understand the pathogenesis of lingual changes in the inhalation of tobacco smoke in humans. The present study was the first to evaluate the percentage of collagen, mast cell density and immunostaining for HIF-1α in rat tongues exposed to tobacco smoke.

In conclusion, secondhand cigarette smoke exposure to rats affected the morphology of the tongue musculature with reduction of perimisial collagen. In addition, the increase of HIF-1α suggests the presence of tissue hypoxia and would be triggering increased mast cell density. Similar events appear to be related to the time of exposure to tobacco.

Resumo

O tabagismo é um fator de risco para sérios problemas de saúde e está associado a diversas alterações nos tecidos da cavidade oral. O objetivo deste estudo foi avaliar a porcentagem de colágeno, densidade de mastócitos e intensidade de células imunomarcadas por anti-HIF-1α na musculatura lingual de ratos expostos passivamente à fumaça principal do cigarro. Vinte e sete ratos Wistar albinos foram divididos em três grupos: ratos não expostos à inalação da fumaça do tabaco (grupo controle) (n=7); ratos expostos à inalação da fumaça por 30 dias (TAB 30) (n=10); e ratos expostos à inalação da fumaça por 45 dias (TAB 45) (n=10). Posteriormente, os animais foram submetidos à eutanásia e remoção da musculatura lingual de ratos expostos passivamente à fumaça do tabaco. Houve diminuição do percentual de colágeno, maior densidade de mastócitos e maior intensidade de células imunomarcadas por anti-HIF-1α em comparação ao grupo controle. Houve também correlação positiva e significativa entre a porcentagem de colágeno e a densidade de mastócitos. Não houve diferença significativa entre TAB 30 e TAB 45 em nenhum dos parâmetros avaliados. Portanto, a exposição passiva de ratos à fumaça principal do cigarro por 45 dias provoca diminuição das fibras de colágeno perimisial, aumento do número de mastócitos e aumento da imunomarcção para o HIF-1α em células musculares linguais. O presente estudo foi o primeiro a avaliar a porcentagem de colágeno, densidade de mastócitos e imunomarcção para o HIF-1α em ratas expostas à fumaça do tabaco.
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