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Smoking and COPD: Endothelium-Related and Neuro-mediated Emphysema Mechanisms

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Abstract

This chapter describes endothelium-related and neuro-mediated mechanisms of emphysema development in chronic obstructive pulmonary disease (COPD) and smoking on the basis of previously completed studies, literature data, and own researches. As components of neurogenic inflammation in the processes of tissue remodeling in emphysema, we describe the distribution and activity of the substance P, neurokinin-1 and its receptor, tissue metalloproteinases and their tissue inhibitors in the lungs during the entire experimental period, the modeling of COPD in rats with a smoking model. We also analyzed the content of neurokinin system markers, the localization, and markers of tissue metalloproteinases in human lung tissue structures. We have confidence that there is a special morpho-functional continuum of development of lower respiratory tract remodeling in response to chronic exposure to tobacco smoke and the development of inflammation in COPD. New data suggest that imbalance of neuro-mediated interactions, alteration of vasomotoric signaling mechanisms, secretion, mucociliary clearance, cytoprotection involving substance P-dependent components with impaired content, and development of dystopia of matrix metalloproteinases and their tissue inhibitors are involved in the initiation of morphological restructuring. Research in this direction should be continued to allow approaches to the development of preventive and therapeutic strategies for emphysema.

Keywords: COPD, smoking, emphysema, remodeling, neuro-mediated, endothelium, neurokinins, metalloproteinases

1. Introduction

Emphysema, or destruction of the gas-exchanging surfaces of the alveoli, is one of the typical manifestations of chronic obstructive pulmonary disease (COPD). Emphysema is a pathological term that is often used clinically, has great medical significance and describes only one of several structural abnormalities present in patients [1]. Many previous definitions of COPD have emphasized the terms “emphysema” and “chronic bronchitis,” which are not included in the definition used in the last GOLD report. In GOLD 2018, COPD was defined as a common, preventable, and treatable disease that is characterized by persistent respiratory

symptoms and airflow limitation that is due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases [1]. It was mentioned that chronic respiratory symptoms also exist in individuals with normal spirometry and a significant number of smokers without airflow limitation have structural evidence of lung disease manifested by the varying presence of emphysema, airway wall thickening, and gas trapping [2–4]. Really, smoking is a major risk factor for COPD, and it plays an important role in lung tissue destruction development. Some experiments prove that aggressive pollutants of tobacco smoke (benzopyrene, peroxyxynitrite, acrolein, cyanides, peroxides, etc.) can cause direct damage to endothelial cells due to expression of adhesion molecules on their surface and intensification of lipid peroxidation [2, 5–7]. But the main underlying cause of structural changes is chronic inflammation, which is confirmed by numerous studies [1, 4–6]. Even in mild COPD, or in smokers susceptible to emphysema [7, 8], there are significant abnormalities in pulmonary microvascular blood flow that worsen with disease progression [9].

It was proven that vascular endothelium actively participates in inflammatory reactions in COPD [10–13]. It was a systemized data about cigarette smoke as an endothelial toxin and activator [14]. Endothelium is one of the direct participants of development and maintenance of chronic inflammation. Oxidized lipoproteins in the tunica intima of the vessel work as attractants for chemotaxis of leukocytes and monocytes that start to produce pro-inflammatory cytokines in big amounts. These processes trigger systemic inflammatory response that leads to irreversible thickening of the vessel walls and deterioration of their mechanical properties. Chronic exposure to tobacco smoke and the products of combustion of tobacco lead to chronic system inflammatory reaction, oxidative stress, endothelial dysfunction, and morphofunctional damage of target organs. Nowadays the connection between endothelium-related mechanisms and emphysema forming, and progression in COPD is well known. Recent studies are approaching the description of the neuro-mediated mechanisms of emphysema development in COPD.

In this chapter we have analyzed data from researchers and shared our own research on the study of endothelium and the neuro-mediated mechanisms of emphysema development in COPD and smoking.

2. Endothelium-related and neuro-mediated mechanisms of emphysema development in COPD and smoking: research data

The participation of endothelial dysfunction and injury in emphysema development in COPD has been described since 2000 and early [15, 16]. And the interest of researchers to the problem of the involvement of the endothelium in the pathogenesis of COPD has not decreased over the past decades. So, for the request “endothelium + emphysema” (in the title and/or abstract), the well-known online resource of the library PubMed offers 117 publications, of which 23 after 2015. For the request “endothelium + COPD,” it offers 335 publications, of which 72 after 2015, and for the request “endothelium + smoking,” it offers 1859 publications, of which 188 after 2015. This indicates the relevance of this area of research and demonstrates the hopes of researchers finding new opportunities for therapeutic and prophylactic effects on this relationship in the pathogenesis of tobacco-related lesions, COPD, and emphysema [17–23].

All disorders begin with local and systemic inflammation, hypoxia and oxidative stress, and lead to an imbalance of proteases-antiproteases, loss of recovery and destruction of lung tissue. Activation and dysfunction of the endothelium involves, first of all, imbalance of the endothelium and its associated mechanisms, which

are due to the following disorders in the pathogenesis of COPD [4]. Separately, we would like to pay attention on some endothelium-dependent factors.

Prolonged damage to endotheliocytes by aggression factors (persistent inflammation, hypoxia, oxidative stress, an imbalance in the protease-antiprotease system, etc.) leads to their death and anatomical reduction of the capillary bed, which is a component of emphysematous lung changes. Pathobiology of small vessels in COPD, in addition to inflammatory and hypercoagulative changes, is characterized by intimal thickening, arteriole muscularization, a decrease in the number of capillaries, and a decrease in blood vessels [13, 15]. Delivering a VEGF receptor (VEGFR) antagonist to rats led rapidly to air space enlargement and pruning of the pulmonary arterial tree [23, 24]. VEGF is a trophic factor that is crucial for the survival of endothelial cells. The experiment demonstrated that prolonged blockade of VEGF receptors leads to apoptosis of septal endothelial cells and emphysema [16, 25]. Subsequent studies of emphysematous lungs confirmed that COPD patients have decreased lung levels of VEGF, reduced expression of VEGFR in pulmonary endothelial cells and apoptotic alveolar septal cells, and reduced expression of hypoxia-inducible factor-1 α (HIF-1 α), a transcription factor that drives the expression of genes involved in endothelial function including VEGFRs [26, 27]. It has been shown that along with the progression of emphysema, degenerative changes in the walls of the aorta develop, including its dilatation and aneurysmatization [28, 29]. These facts indicate that among other circumstances, an important role in the pathogenesis of emphysema belongs to endotheliocytes and VEGF. Moreover, it has been described that the presence of emphysema in patients with COPD is associated with a reduced content of circulated endothelial cells, an endothelial repair factor [11, 30].

Endothelial dysfunction and damage are also caused by the acute effects of cigarette smoke long before the development of emphysema in animal models. Brief exposure of mice to cigarette smoke exacerbates lipopolysaccharide- and *Pseudomonas aeruginosa*-induced acute lung injury in vivo, and cigarette smoke extract increases the permeability of endothelial monolayers in vitro [14, 27]. Moreover, a recent study identified cigarette smoke-induced apoptosis of endothelial cells in the lungs of mice exposed chronically to cigarette smoke and COPD patients [11]. Thus, data from both animal models of COPD and COPD patients and controls support the hypothesis that endothelial dysfunction and injury are key processes in the pathogenesis of emphysema.

In **Table 1** we composed information on key endothelium-related agents that take into account the mechanisms of emphysema development in COPD, well described previously [4, 10–13, 18–20, 22, 23, 26, 27].

The mechanisms of the inclusion of neurokinins and related substances in neurogenic inflammation and destruction at different stages of COPD are much less known. Moreover, in recent studies, descriptions of the neuro-mediated mechanisms for the development of emphysema in COPD and smoking have become recognized.

It is known that tobacco smoke is a powerful inducer of the destruction of the respiratory epithelium throughout, followed by its morphofunctional remodeling [4, 31, 32]. Initiation of cell and tissue injury processes in prolonged exposure to smoking can take place due to excess release of neurotransmitters from sensitive afferent nerve fibers of the nervous vagus system. A large proportion (75%) of such fibers belong to the type of nonmyelinated or C-fibers, the sources of which are small neurons of the knotted and jugular ganglia, which synthesize neuropeptide transmitters or neurokinins (such as substance P (SP), a peptide genetically related to calcitonin (CGRP) and neurokinin (A)) [33, 34]. Afferent influences are primarily aimed at maintaining the structural and functional homeostasis of the respiratory system by stimulating the secretion of mucus from the submucous

Components	Origin, localization	Physiological effects and potential role at pathogenesis emphysema in COPD
Nitric oxide (NO)	In main, endothelial cells and other cells	Vasorelaxation, vasoprotection, anti-inflammatory, anti-adhesion, reparation. Endothelin antagonist
Endothelinum-1 (ET-1)	Endothelial cells, bronchial epithelium, alveolar macrophages	Activates receptors on smooth cells, encouraging stable vasoconstriction and increase of endothelium adhesively
sPECAM-1	Endothelial cells, lymphocytes, platelets, macrophages, granulocytes, T/NK-cell megakaryocytes, fibroblasts, osteoclasts	Plays a basic role in lymphocyte adhesion to vascular wall with followed effects
Selectins: E-selectin (CD62E), P-selectin (CD62P), L-selectin (CD62L)	Activated endothelial cells	Regulation of leukocyte adhesion (strengthens the capacity to migration, leukocyte adhesion to activated endothelium in inflammation)
Thrombomodulin (CD141)	Endothelial cells	Interacts with thrombin, activates protein C, acts as anticoagulant across activation factors fVa, fVIIIa, fXa, and fXIIIa
Circulated endothelial cells	Endothelial cells from vascular wall, activated bone marrow	Can be as the factor of reparation according to inflammatory processes and as the factor of injury to the endothelium and other tissues due to activated phenotype
Vascular endothelial growth factor (VEGF)	Endothelial cells	Main inductor of angiogenesis, VEGF, provides their effects across receptors' endothelial cell and expression of VEGF regulated by hypoxia, chronic inflammation, hypercoagulation, etc. In chronic processes, function can be an imperfect character Stimulates apoptosis and phagocytosis and promotes development of emphysema
Neutrophil elastase (cathepsin G, proteinase 3)	Neutrophils, monocytes, T lymphocytes, endothelial cells, vascular smooth muscles cells	Decreases migration of T lymphocytes and neutrophil to inflammation area, factor of decelerating of phagocytosis. Function from protection to damaging. Can cause damage to tissues, development of emphysema, and mucus hypersecretion Involved in normal degradation of matrix proteins elastin, collagen, fibronectin, laminin, and proteoglycans
Matrix metalloproteinases (MMP-1, MMP-2, MMP-9)	Endothelial cells, macrophages, neutrophils, monocytes, fibroblasts, keratinocytes, osteoblasts	Contributes to release TNF- α from macrophages, that results to neutrophils recruiting and production neutrophil elastase, that leads to damage tissues, development emphysema. Involved in degradation of type IV collagen, fibronectin, and elastin

Table 1.
Endothelium-related Components of emphysema development in COPD and smoking [4, 10–13, 18–20, 22, 23, 26, 27].

glands and goblet cells, contractility of smooth muscles, vascular permeability, modulation of immune cascades, etc. [34–36]. It is known that afferent fibers (C-fibers) are extremely sensitive to the effects of irritants that make up tobacco smoke [37]. In a situation of prolonged and/or intense stimulation of sensory

Agents	Origin, localization	Physiological effects and potential role in emphysema pathogenesis in COPD	References
Neurokinins Substance P (SP)	Sensitive nerve endings. SP receptors on respiratory and glandular epithelial cells and endothelial cells. Neurokinin receptor-1 affixed to SP is found in submucous glands, and SP release from nociceptive nerves is responsible for secretion of glands	Belongs to the family of tachykinins, sensory peptides. Induces vasodilation and transudation of blood plasma in the respiratory tract. Induces chemotaxis of monocytes, neutrophils, and eosinophils and stimulates macrophages to produce mediators of inflammation and neutrophil elastase. Powerful mast cell stimulator, causing their degranulation. Sources and release of histamine and cytokine synthesis (IL-6). Takes part in neurogenic inflammation when stimulating the production of IgA from B lymphocytes and cytokines from T-helper cells. Enhances the release of acetylcholine from the postganglionic cholinergic nerves of the respiratory tract It causes smooth muscle contraction, secretion of submucous glands, vasodilation, and increased vascular permeability. Tobacco smoking inhibits the activity of the enzyme endopeptidase, which enhances the activity of SP	[40, 45, 48, 49]
Neurokinins Neurokinin A	The highest density in the nerve fibers around the arteries. Tachykinin receptor subtypes NK1, NK2, and NK3	Belongs to the family of tachykinins, sensory peptides. Contraction of smooth muscles; secretion of submucous glands; vasodilation; increased vascular permeability; stimulation of cholinergic nerves, mast cells, B and T lymphocytes, and macrophages; eosinophil chemoattraction; and adhesion of neutrophils in the vessels of the respiratory tract with activation following	[44, 50, 51]
Neuropeptide - γ	It is produced in some of the upper cervical ganglia and the bodies of the main palatine cells Sympathetic nerves contain either norepinephrine or norepinephrine and neuropeptide Y	Reduces the frequency of vibrations of ciliary cells induced by cholinergic nerves, providing an inhibitory effect on these nerves Sympathetic reflexes cause vasoconstriction and can also stimulate the secretion of certain glands in the trachea and bronchi	[38, 39, 48]
Receptor NK1	NK1 tachykinin receptor mRNA is found in the pulmonary arteries, veins, and human bronchi, in the endothelium of the veins and arteries, and in the smooth muscles of the bronchi, as well as in lymphocytes, macrophages, and mastocytes	The tachykinin receptor NK1, with the highest affinity for SP. SP, in turn, is a powerful mast cell stimulator, causing their degranulation, and induces the chemotaxis of monocytes, neutrophils, eosinophils, and stimulates macrophages to produce inflammatory mediators and neutrophilic elastase	[40, 42, 45]

Agents	Origin, localization	Physiological effects and potential role in emphysema pathogenesis in COPD	References
Receptor NK2 Receptor NK3	NK2 receptor mRNA is abundantly expressed in the human bronchi and weakly in the pulmonary veins and arteries NK3 tachykinin receptor mRNA is found in the pulmonary arteries, veins, and human bronchi	The tachykinin receptor NK2, with the highest affinity for neurokinin A. Tachykinin receptor NK3, with the highest affinity for neurokinin B The release of histamine from mouse mast cells is mediated through tachykinin NK2 receptors	[40, 42, 45, 52]

Table 2.
Potential neuro-mediated mechanisms of emphysema development in COPD and tobacco smoking.

fibers, excessive release of neuropeptide mediator is accompanied by a number of plastic and destructive processes due to a cascade of pathological reactions of neurogenic inflammation [38, 39]. In addition to substance P of neuronal origin, neuropeptides from cells of the immune system—eosinophils, basophils, monocytes, macrophages and lymphocytes—join the realization of neurogenic inflammation [38, 40, 41]. The obtained data indicate the role of the disturbance of the activity of the neurokinin system in the development and maintenance of morphofunctional changes in the pathology of the respiratory tract associated with smoking [42–46]. Chronic exposure to cigarette smoke has been shown to increase SP expression in neurons of the central nervous system [35–37] and simultaneously inhibits the activity of enzymes that metabolize neurokinins [39, 40, 43]. According to experimental study, even low concentrations of cigarette smoke significantly reduce the topical activity of neuronal endopeptidase and increase the concentration of CP in the respiratory tract [35]. The contribution of excessive sensitivity of NK1 receptors in the airways to the development of bronchoconstriction under the influence of tobacco smoke irritants and/or during bronchial asthma has been proven [42, 43, 47]. At the same time, the components and mechanisms of neurogenic inflammation in the development of emphysema associated with prolonged exposure to tobacco smoke are poor and fragmentary in the literature.

In **Table 2** we have tried to compose information about potential neuro-mediated mechanisms of emphysema development in COPD and tobacco smoking.

3. Mechanisms of neurogenic inflammation in the tissue remodeling processes in emphysema: own researches

3.1 Study of the neurogenic inflammation mechanisms in the emphysema formation in the experiment

To study the contribution of the components of neurogenic inflammation to the processes of tissue remodeling in pulmonary emphysema associated with smoking, an experimental model of long-term tobacco smoking in vivo in rats was reproduced. The experiment was performed in appliance with the model D. According to Zheng [53] in our own modification [54], the duration of exposure to tobacco smoke in terms of human life is 12 years. The features of the

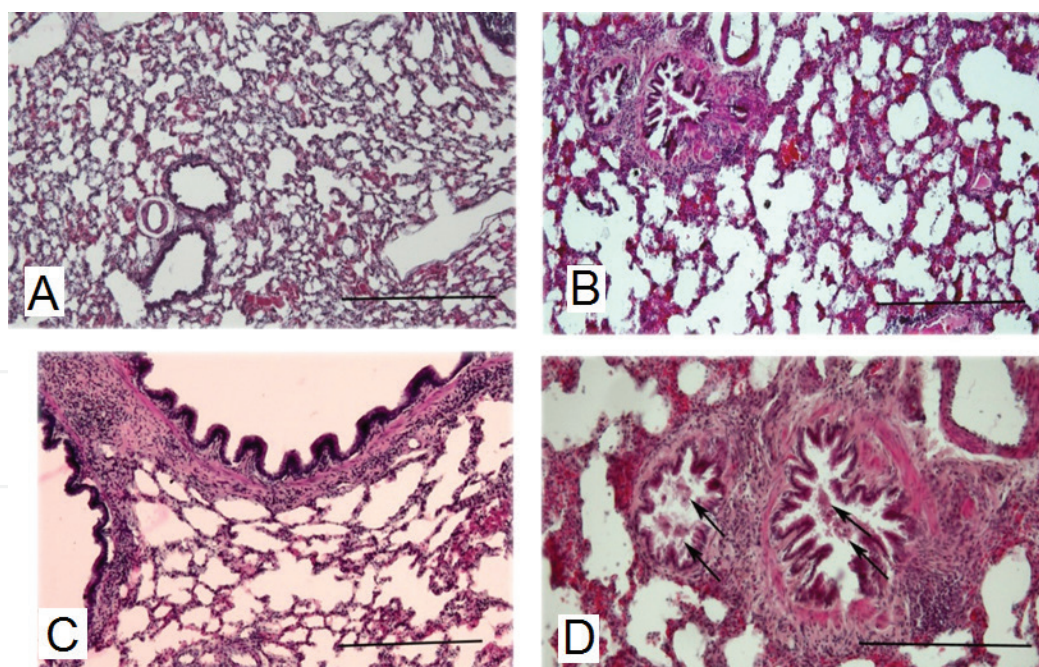


Figure 1.
 The lung (A, B) and bronchi (C, D) of animals in the control group (A, C) and rats with the DTC model (B, D). Coloring: hematoxylin-eosin. Scale: A, B (500 microns); C, D (100 microns).

distribution and activity of SP, NK1, MMP-2, MMP-9, and TIMP-2 in the tissues of the mucous membrane of the lungs were performed using the immunoperoxidase method on cryostat sections of 15 μm in thickness according to standard methods. The following primary antibodies were used: anti-SP (Abcam, ab 14184, 1:200, CIIA), anti-NK1 (Chemicon AB 5060, 1:500, USA), anti-MMP2 (rabbit polyclonal, ThermoScientific), anti-MMP9 (rabbit polyclonal, ThermoScientific, rb-9234-p, 1:200), anti-TIMP2 (rabbit polyclonal, Abcam, ab61224, 1:100), secondary biotinylated antibodies (ThermoScientific, 1:200), streptavidin peroxidase (ThermoScientific), and chromogen (Peroxidase Substrate Kit, VectorNovaRED, SK-4800). Morphological studies were performed in the laboratory of the Pacific State Medical University.

The results of morphological studies of the bronchopulmonary system of rats in the control group and with the model of long-term tobacco smoking are presented in **Figure 1**.

Morphological changes in the lungs of rats with a long-term tobacco smoking model are focal. Over the entire area of the slice of the lungs, there are fields with pronounced emphysematous changes, accompanied by loss of the integrity of the alveoli and the formation of large emphysematous expansions, an increase in the thickness of the interalveolar septa (**Figure 1B, D**). In other parts of the lung parenchyma, there are signs of swelling and/or hemorrhagic impregnation in peribronchial spaces. Cellular composition contains cells of immune inflammation—plasma cells, lymphocytes, and macrophages.

The distribution of the components of the neurokinin system of rats obtained by morphological examination of the bronchi and lungs coincides with the previously described data [55] and is shown in **Figure 2**. Nerve fibers secreting SP are presented in the subepithelial zones of the bronchi (**Figure 2A**); their penetration is recorded in the epithelial layer (**Figure 2B**), pulmonary parenchyma (**Figure 2D**), and adventitia of pulmonary vessels (**Figure 2E**).

The morphometry of the components of the neurokinin system in the bronchopulmonary system of rats was compared with the model of long-term tobacco smoking and control animals (**Table 3**).

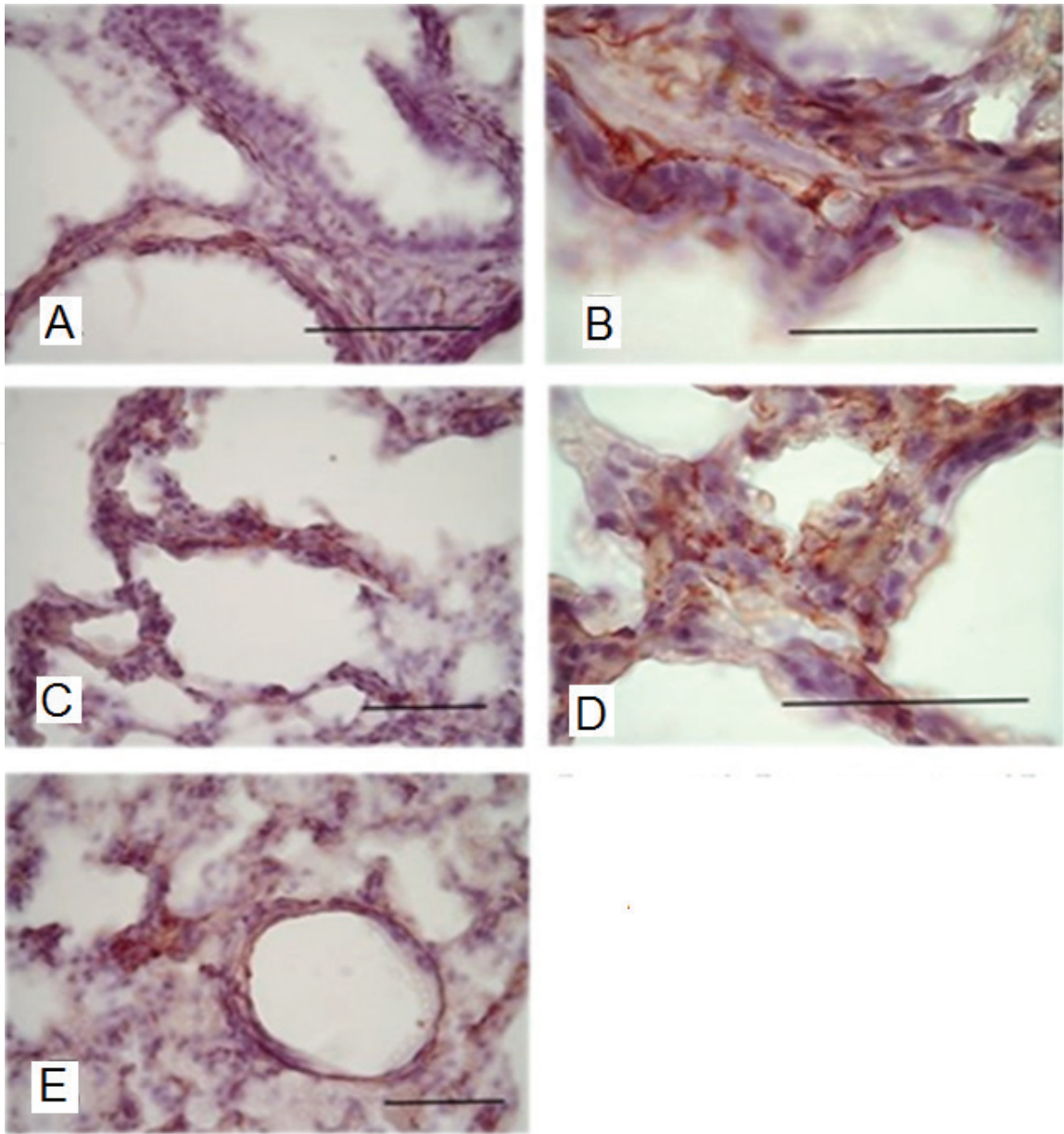


Figure 2.
Distribution of SP- and NK1-reactive structures in the bronchopulmonary system of rats. Coloring: immunoperoxidase reaction on SP; repainting, hematoxylin-eosin. Scale: A, 100 microns; B–E, 50 microns.

The content of SP-containing fibers and NK1-positive structures in the control group and in the model of long-term tobacco smoking shows an ambiguous pattern. An increase in the distribution area of SP-immunopositive nerve fibers in the lungs and bronchi of experimental animals was found to be 10.7 and 9.4%, respectively, compared with the control group. The most significant increase in fiber density was observed in the adventitia of pulmonary parenchyma vessels compared with other structures. Being a vasodilator, substance P increases vascular permeability and promotes adhesion and penetration of leukocytes into the surrounding tissues for the realization of local immune reactions involved in the development of destructive processes in the pulmonary parenchyma. Regarding NK1-positive elements, it should be noted that there is no change in their content in the lung tissue and a moderate increase in the density in the bronchial wall against the background of a decrease in the total number and NK1-immunoreactive tissue basophils. Obviously, substance P plays a key role in the implementation of neurogenic inflammation processes in chronic exposure to tobacco smoke. The established pattern of changes in NK1-positive structures can be explained by the ability of SP to cause mast cell degranulation and NK1 receptor-dependent release of histamine and serotonin involved in local inflammatory answer.

Indicator	Bronchi		Lungs	
	Control	Experiment (long-term smoking model)	Control	Experiment (long-term smoking model)
SP distribution area (%)	5.11 ± 0.36	5.59 ± 0.14*	4.60 ± 0.29	5.10 ± 0.34*
NK1 distribution area (%)	0.27 ± 0.03	0.31 ± 0.03*	0.16 ± 0.016	0.16 ± 0.02
NK1-positive mast cells (in 1 mm ³ of the tissue)			92.68 ± 19.26	76.39 ± 15.74*
General population of tissue basophils (in 1 mm ³ of the tissue)			129.53 ± 19.64	106.93 ± 7.64*

*The differences are significant with $p < 0.05$.

Table 3.
Morphometric characteristics of SP- and NK1-immunoreactive structures in the bronchopulmonary system of rats.

One of the leading stimulators of the synthesis of substance P in the model of long-term tobacco smoking is hypoxia which changes the humoral regulation of blood flow [55]. SP can adjust the change in vessel diameter at the unchanged vascular wall using an axon reflex for adequate blood flow at a given point in time. However, when the architectonics of the vascular wall changes, the SP loses its function as a regulator and can participate in both excessive vasodilation and paradoxical vasoconstriction.

In the development of the structural remodeling of the respiratory system, there are several morphological phenomena that accompany this process and are the basis for the development of reversible and irreversible morphofunctional changes. These include thinning of the epithelial layer, development of subepithelial fibrosis, an increase in smooth muscle thickness, an increase in the number and/or size of the submucosal glands, and the activation of angiogenesis processes [56]. In the pathogenesis of the changes taking place, great importance is attached to the activity of the enzymes of the extracellular matrix, which ensure the degradation of its interstitial proteins. Modern advances in proteomics have shown that for normal development, physiological renewal, restoration of healthy tissues, and the formation of pathological changes in tissue morphology, two groups of proteins are leading—MMPs and their tissue inhibitors [57, 58]. MMPs are a family of 20 zinc and calcium-dependent endopeptidases capable of cleaving almost all components of the extracellular matrix of connective tissues [59]. The level of synthesis and secretion of MMP into the extracellular space is regulated by transcription factors, and their proteolytic activity depends on the chemical transformations of the enzyme molecule in the interstitial space. As a result, either activation of the proenzymes or inhibition of their active forms can be observed. Depending on the type of protein metabolized, MMP can be divided into collagenases (MMP-1, MMP-8, and MMP-13), gelatinase (MMP-2 and MMP-9), stromalins (MMP-3 and MMP-10), etc. [60, 61]. In mammals there are four known TIMPs that inhibit all MMPs in a 1:1 ratio by strong covalent bonding [62]. It is believed that the balance of proteolytic and antiproteolytic mechanisms maintained in different tissues and organs is carried out by a specific set of intercellular matrix enzymes and their inhibitors [59, 63]. On the other hand, each process has a specific set of depressed matrix proteolytic enzymes. In this regard, great prospects in creating targeted therapy for many pathological processes are associated with the determination of the tissue specificity of the enzymes of the extracellular matrix and the identification of patterns of changes in their activity during the development of pathology.

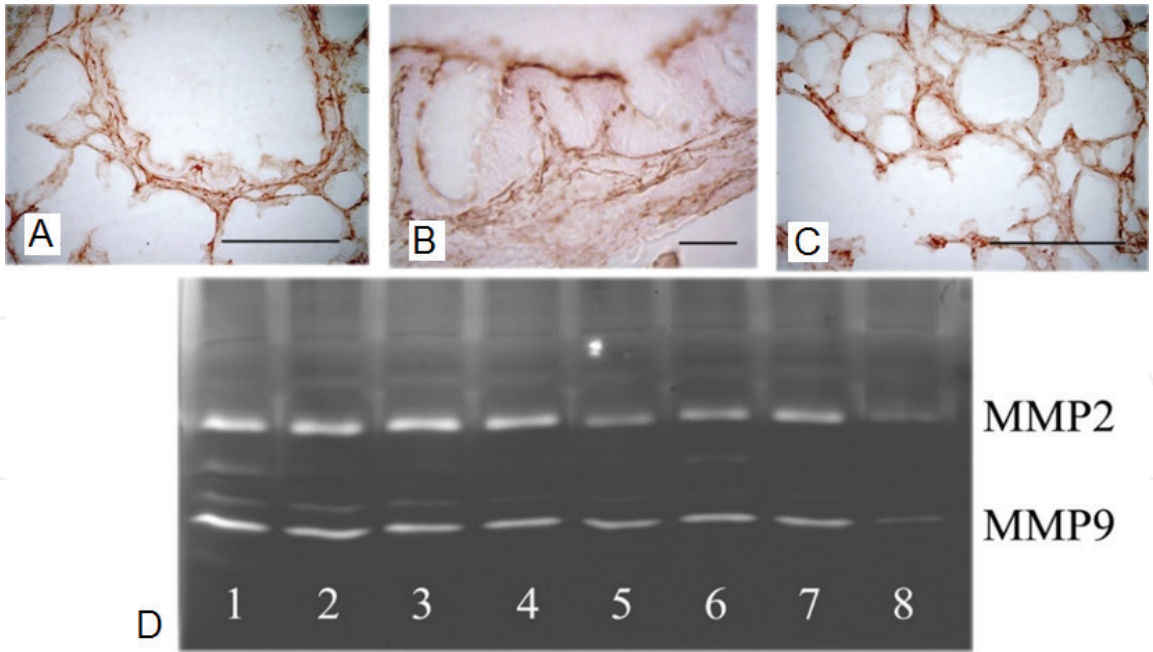


Figure 3. Immunohistochemical (A–C) and biochemical determination of MMP in the bronchopulmonary system of rats with a model of long-term tobacco smoking. Coloring—immunohistochemical reaction to MMP-2 and MMP-9. Scale: A, B, B—50 μ m. Localization of MMP-2 in the wall of the bronchus (A, B) and interalveolar septa (C). (D) Zymogram of MMP-2 and MMP-9. Lanes 1–4, lung homogenates of rats of the control group; lanes 5–8, lung homogenates of rats with a model of long-term tobacco smoking.

Indicators	Control	Experiment (long-term smoking model)	Control	Experiment (long-term smoking model)
	MMP-9		MMP-2	
Conventional density units	742689.75	450081.25*	367243.25	246414.75*
MMP-9/MMP-2	2.02 (control)		1.83 (experiment (long-term smoking model))	

**The differences are significant with $p < 0.05$.*

Table 4. The contents of MMP-2 and MMP-9 in the homogenates of the lungs of rats.

A number of studies have shown the role of individual types of MMP in the development of nicotine-associated pathology of the lungs and bronchi [57, 58]. To clarify the role of the leading MMP—MMP9 related to the inducible form and MMP2—considered as a constitutive variant of the enzyme in the development of pulmonary emphysema associated with long-term smoking, we studied the immunohistochemical and biochemical content of enzymes in the tissues and homogenates of rats with long-term smoking patterns (**Figure 3**).

In the bronchopulmonary system of rats, prolonged exposure to tobacco smoke is accompanied by ambiguous dynamics of the content of matrix metalloproteinases and their inhibitors. Normally, the activity of MMP-2 and MMP-9 is recorded in the cytoplasm and processes of bronchial and pulmonary fibroblasts, which form a thin MMP-positive strip in the lamina propria of the bronchial mucosa (**Figure 3A, B**) and in the interalveolar septa (**Figure 3C**). In the lungs and bronchi of rats with the long-term tobacco smoking model, a marked decrease in the immunohistochemical activity of MMP-2 and MMP-9 was observed. At the same time, in the acute phase of the experiment, the number and intensity of coloring of immunopositive structures on MMP-2 and MMP-9 are higher than in the control group. According to the quantitative

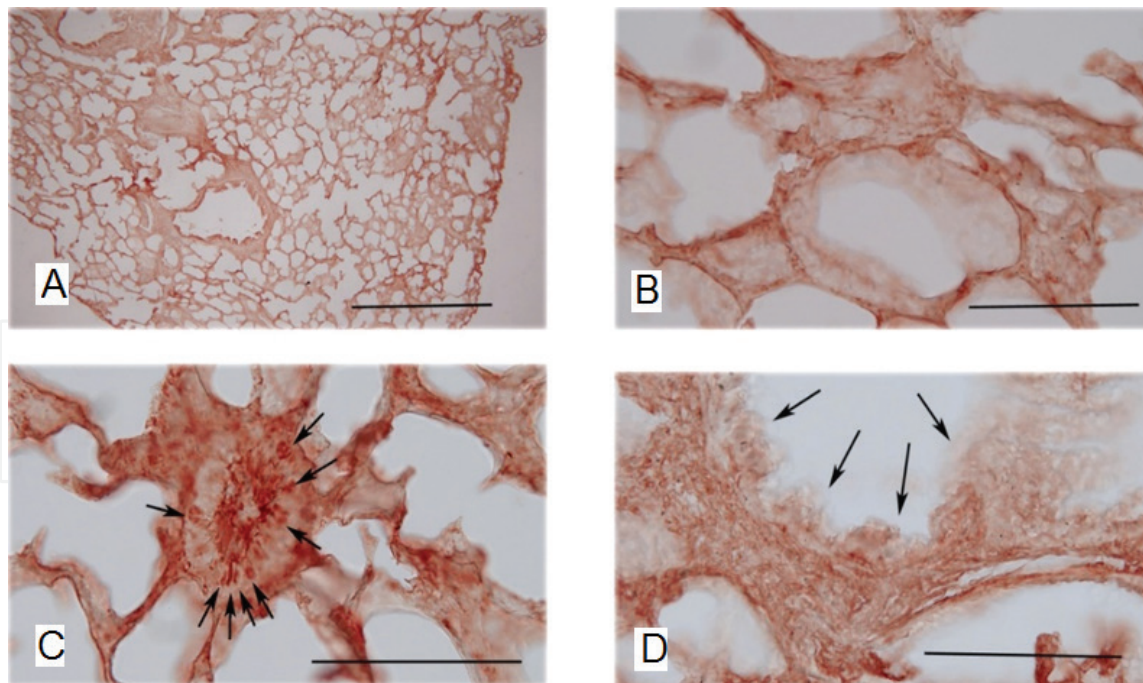


Figure 4.
 The distribution of the content of TIMP-2 in the lungs of rats of the control (A–C) and with the model of long-term tobacco smoking (D) groups. Coloring—immunohistochemical reaction to TIMP-2. Scale A, 100 microns; GD, 50 microns. A total enzyme content in the tissues of the bronchopulmonary system. B-TIMP-2-positive fibroblasts of the interalveolar septa. B-content of the enzyme in the epithelial cells of the small bronchus (indicated by arrows). G-reduction of the enzyme content in epithelial cells of the bronchus of rats with a model of prolonged smoking (indicated by arrows).

determination of enzymes in the homogenates of lung tissue (**Figure 3D**, **Table 4**), the resulting trend is confirmed. That is, with the development of pulmonary emphysema associated with prolonged smoking in the lung tissue, the content of both MMPs decreases evenly.

An analysis of the immunohistochemical composition of the tissue inhibitor of both MMP and TIMP-2 showed a marked decrease in its representation in the structures of the bronchopulmonary system of animals on a model of long-term smoking (**Figure 4**).

In intact animals, the enzyme localization occurs in the respiratory epithelial cells of the bronchial membrane (**Figure 4C**, indicated by arrows) and fibroblasts of the interalveolar septa (**Figure 4B**). In animals of the main group, the overall intensity of immunohistochemical staining of lung tissue decreases, and at high magnifications of the microscope, it is possible to fix a significant depression of the color or complete disappearance of the enzyme content in the epithelial lining cells of the bronchi and lung parenchyma tissue (**Figure 4**, indicated by arrows).

According to the data obtained, the immunolocalization of MMP-2, MMP-9, and TIMP-2 repeats the basic pattern of distribution of pro-inflammatory cells and coincides with the foci of the most noticeable rearrangements of the connective tissue of the bronchi and the pulmonary parenchyma. In the acute phase of the experiment, the activity of the markers is significantly higher compared to the control; after 6 months of exposure to smoke, there is a decrease of the proteolytic activity and at the same time the processes of its inhibition.

3.2 Study of the neurogenic inflammation mechanisms of the emphysema formation in humans

In addition to studying the processes of neurogenic inflammation and the contribution of matrix metalloproteinases to the development of emphysema in the

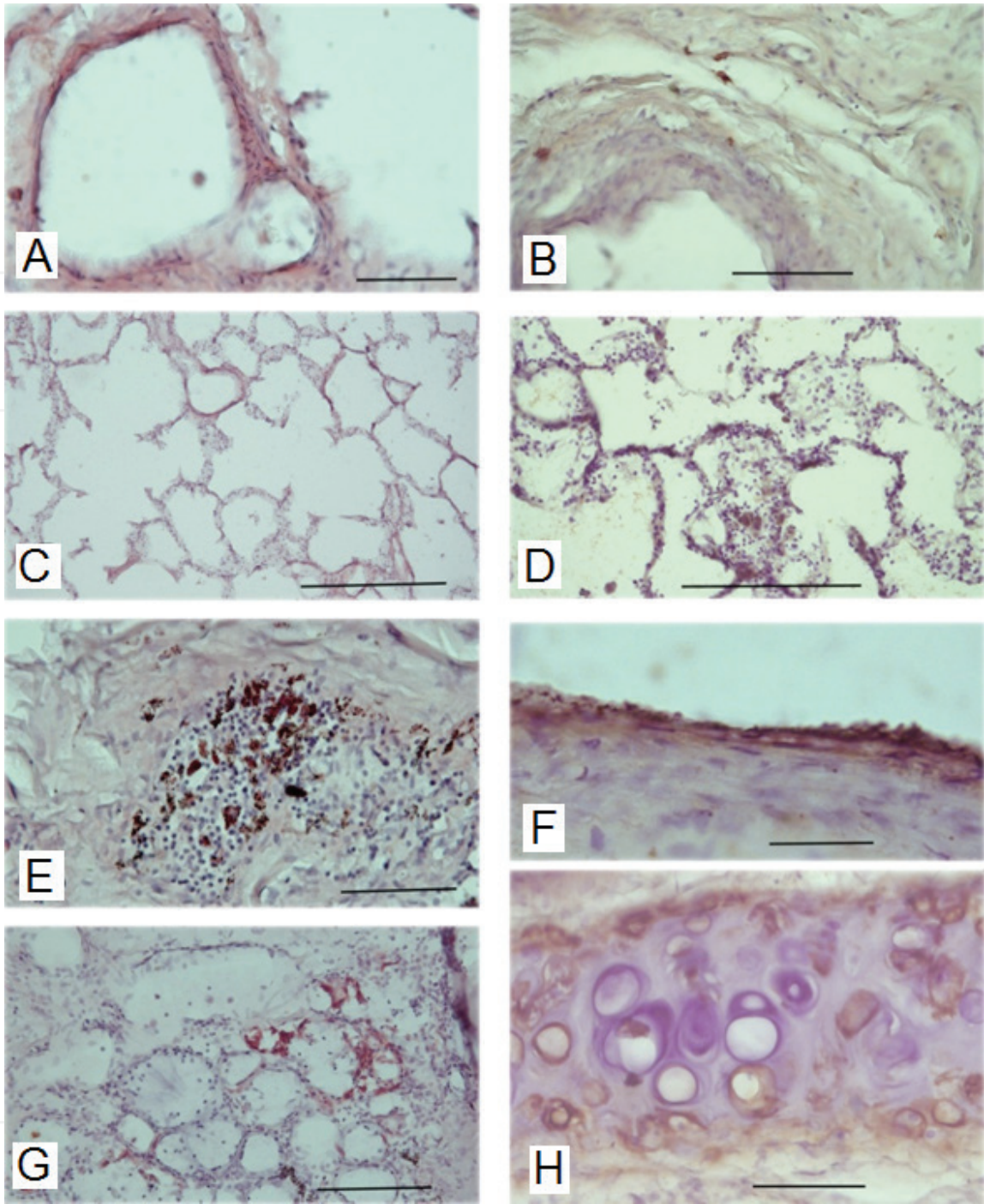


Figure 5. Distribution of the content of SP (A, C, E, G) and NK1 (B, D, F, H) in the bronchopulmonary system in persons with emphysema. Coloring: immunohistochemical reaction to SP and NK1, stained with hematoxylin. Scale: A, B, E, G (50 microns); C, D (100 microns); and F, H (20 microns). Nerve fibers innervating the wall of the bronchus (A) and interalveolar partitions (C). Localization of SP-positive macrophages in peribronchial infiltrates (E). SP-positive glandulocytes of the bronchial glands (G). Localization of neurokinin receptors on the surface of peribronchial macrophages (B), dust macrophages (D), vascular endothelial cells (F), and chondrocytes of the fibrocartilage membrane of the bronchi (H).

experiment, we analyzed the content of neurokinin system markers, the localization, and the content of MMP-2, MMP-9, and TIMP-2 in human lung tissue structures. Morphological studies of autopsy material were performed on 12 individuals aged 51–65 years and 3 women and 9 men, average age 61.5 ± 4.14 years, who died a sudden death outside the hospital. The long-term history of tobacco smoking was clarified from the close relatives and on the basis of the data of the outpatient card. Features of the distribution and activity of SP, NK1, MMP-9, and TIMP-2, in lung tissues were investigated using the immunoperoxidase method on cryostat sections

of 15 μm in thickness according to the standard procedure using the primary antibody line described above.

In lung tissue and bronchial wall of patients with pulmonary emphysema, positive SP immunoreactivity is found mainly in the nerve fibers (**Figure 5**). More common are single conductors having a uniform ribbonlike course and numerous varicose thickenings. With a successful coincidence of the cut plane with the spatial geometry of the fibers, it is possible to observe beams extending 300–500 μm . Fibers penetrate through the walls of the bronchi of medium and small caliber, spread around the perimeter of the submucosa (**Figure 5A, B**). In the interstitial tissue, lightweight fibers have a diameter of 0.5–1 microns, and sometimes they are grouped into clusters with the formation of numerous terminals. Probably, the latter are areas of the most dense accumulation of neuromuscular and glandular contacts. The morphological characteristics of the colored conductors allow them to be treated as mixed (afferent and motor) fibers. High SP expression is also detected in peribronchial leukocyte infiltrates (**Figure 5D**). Here, high immunoreactivity is observed for neurokinin receptors of type 1 (**Figure 5B, D**). The preferential localization of the NK1 surface of the membranes of the secretory epithelial cells of the bronchial glands, alveolar and stromal macrophages, microvascular endothelial cells, and elements of the fibrocartilage membrane (**Figure 5B, D, E, H**) should be emphasized.

Prolonged pathological effects of tobacco combustion products entail the formation of structural changes with the active involvement of the neurokinin innervation apparatus localized in the mucous membranes of the respiratory system. The increase in the number of macrophage cells and NK1-positive macrophages, and the direct interaction between them and afferent fibers, through terminals, suggests the involvement of sensory nerve fibers in the regulation of local immune,

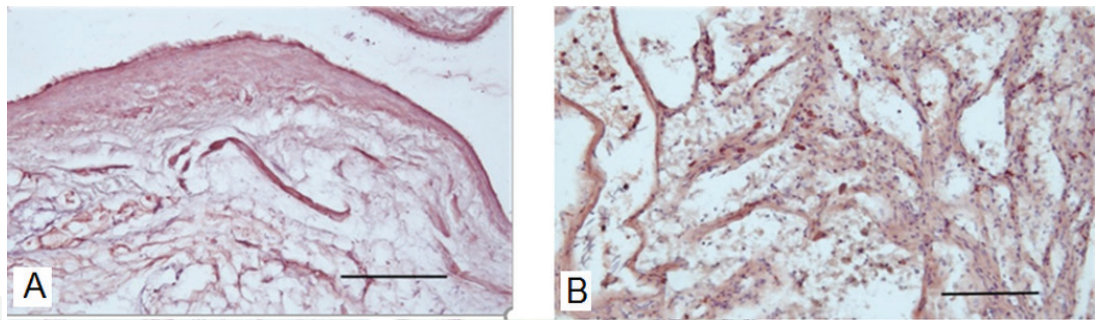


Figure 6. Localization of MMP-9 in the lungs (A, B) of a person. Coloring: immunohistochemical reaction to MMP-9, stained with hematoxylin. Scale: A (50 microns); B (100 microns).

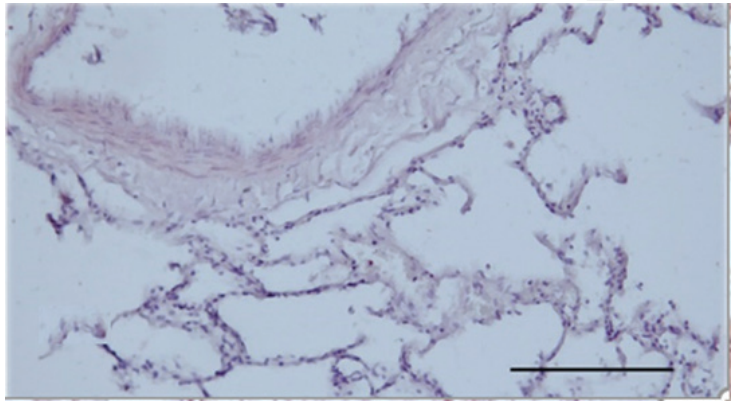


Figure 7. Localization of TIMP-2 pulmonary parenchyma in individuals with emphysema. Coloring: immunohistochemical reaction to TIMP-2, stained with hematoxylin. Scale: 100 microns.

inflammatory, and destructive processes in the lung tissue during smoking-induced emphysema.

In contrast to the experimental data, in individuals with long periods of smoking and emphysema, there is an increase in the immunohistochemical density of MMP-9 in the pulmonary parenchyma (**Figure 6**), while TIMP-2 is practically undetermined (**Figure 7**).

In this way, from the presented data of experimental modeling of emphysema associated with long-term smoking, as well as studies in people with pulmonary emphysema and long-term tobacco smoking experience, neurogenic inflammation takes an active part in the processes of remodeling of lung tissue. Markers of neuro-mediated inflammation activity are overexpression of SP-containing nerve fibers, the presence of NK-1-tagged macrophages, mast cell degranulation, and an immune-mediated pattern of inflammatory infiltrate. Pathomorphosis of pulmonary parenchyma destruction in nicotine-associated pulmonary emphysema is associated with dysregulation in the state of the family of matrix metalloproteinases. In the acute period of exposure to tobacco combustion products, overexpression of MMP-9 is observed with suppression of the activity of the tissue inhibitor TIMP-2, followed by depression of the tissue content of both MMP-2 and MMP-9 and an inhibitor of their activity TIMP-2. In individuals with pulmonary emphysema, the MMP-9 tissue pattern retains its excessive representation.

4. Conclusions and future directions

Results from human and animal studies indicate that endothelial dysfunction and injury contribute not only to the genesis and progression of pulmonary lesions in COPD (especially emphysema development) but may also contribute to some of the common comorbidities and systemic effects reported in COPD patients. Vascular endothelium initiates and modulates the main pathomorphic processes in COPD and smoking. In particular, endothelium activation is an important factor of initiation, development and persistence of inflammation, and vessel and tissue remodeling, in particular emphysema. It is not by chance that the relationship of emphysema of the lungs is described in violation of the mechanical properties of the aorta and excessive stiffness of other exponents' bloodstream [4, 6, 64]. At the basis of these pathological processes are common (genetically determined and pathologically determined) mechanisms associated with impaired collagen-elastin metabolism.

The latest studies are conducted in the direction of studying not simple, associated with the endothelium, but specific neuro-mediated mechanisms of emphysema development in COPD and smoking. Our studies presented in this chapter describe the study of the processes of neurogenic inflammation and the contribution of matrix metalloproteinases to the development of emphysema in the experiment and in humans.

We are confident that there is a special morphofunctional continuum in the development of lower respiratory tract remodeling in response to chronic exposure to tobacco smoke and the development of inflammation in COPD. New data suggest that imbalance of neuro-mediated interactions, alteration of vasomotoric signaling mechanisms, secretion, mucociliary clearance, cytoprotection involving substance P-dependent components with impaired content, and development of dystopia of matrix metalloproteinases and their tissue inhibitors are involved in the initiation of morphological restructuring. Future studies should also assess the extent to which endothelial dysfunction and injury, particularly neuro-mediated mechanisms, underlie emphysema in COPD and smoking as target to therapeutic and prophylactic impacts.

Conflict of interest

No any conflict of interests.

Acronyms and abbreviations

COPD	Chronic obstructive pulmonary disease
VEGF	Vascular endothelial growth factor
VEGFR	VEGF receptor
NO	Nitric oxide
ET-1	Endothelinum-1
sPECAM-1	Soluble platelet endothelial cell adhesion molecule
VEGF	Vascular endothelial growth factor
MMP	Matrix metalloproteinases
SP	Substance P

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