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EXPERIMENTAL OUTBRED RATS MODELING OF PULMONARY FIBROSIS

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Resume: The impact of COVID-19 on lung function is the most important in the post-coronavirus period. The effects of experimentally induced inflammation of the respiratory tract have not been thoroughly studied. The pathogenesis of the development of fibrosis in the lungs is local and systemic production of proinflammatory cytokines, followed by narrowing of the respiratory tract. In the post-COVID years, there are many studies describing the signs of pulmonary fibrosis, such as increased fibroblast proliferation and deposition of extracellular matrix in the late stages of acute lung inflammation. This article describes the modeling of fibrosis in an experiment induced by daily exposure to nitrogen dioxide in the upper respiratory tract of experimental animals.

Thus, it should be noted that the modeling we have chosen seems possible and effective, which allows us to study the mechanisms of pulmonary fibrosis development and ways of early diagnosis, timely treatment and prevention.

Key words: experimental model, pulmonary fibrosis, outbred rats, modeling of pulmonary fibrosis in rats.

INTRODUCTION

The incidence of idiopathic pulmonary fibrosis (IPF) has been reported in several studies worldwide and appears to be increasing, but different case ascertainment methodologies and classification systems have prevented reliable comparisons between studies. Several review articles from different countries on incidence and prevalence have been reviewed, but current evidence suggests that there is no statistical analysis of overall incidence and mortality data worldwide [Hutchinson J. (2015)].

Work has been conducted on the mediastinal lymph nodes of patients with IPF, revealing differential expression profiles than those of patients with lung cancer, indicating distinct immune-mediated pathways regulating fibrogenesis and carcinogenesis. PD-1 expression in mediastinal lymph nodes is consistent with that in lung tissue. Low-dose pembrolizumab may have an antifibrotic effect. Clinical trials aimed at endotyping patients based on mediastinal lymph node profiling and, accordingly, the introduction of targeted therapies such as PD-1 inhibitors are highly anticipated. With the gradual understanding of the pathogenesis of IPF, new progress has been made in the treatment of IPF [Karampitsakos T. (2023)].

However, there is room for progress before satisfactory efficacy can be achieved. Recent experiments have confirmed that the PD-1/PD-L1 pathway can interact with various cell types and pathways and is involved in promoting fibrosis and immune regulation in IPF. Simultaneously, animal experiments have shown that the use of PD-1/PD-L1 inhibitors

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reduces the symptoms of pulmonary fibrosis. In this brief review, we present the effects of PD-1/PD-L1 in IPF; ongoing studies suggest that this may offer a new direction for future IPF therapy [Tan J. (2024)].

Enlarged mediastinal lymph nodes (LNs) are common in idiopathic pulmonary fibrosis (IPF) and are known to be associated with the severity of pulmonary fibrosis. However, the relationship between mediastinal LNs and IPF prognosis is not entirely clear to date [Sin S. (2017)].

Another study was performed on a population-based study of 152 patients with IPF, of whom 135 (89%) received antifibrotic treatment for IPF during the study follow-up. Among the 62 patients with LNE who underwent follow-up chest CT and received antifibrotic treatment, 57 (92%) had persistent evidence of mediastinal LNE over time [Sgalla G. (2020)]. The results showed that diffuse mediastinal lymph node involvement predicts clinically significant functional deterioration in patients with IPF [de Souza Xavier Costa N, (2017)].

Currently existing treatments for IPF have limited efficacy and the prognosis of the disease remains disappointing. The latest advances in understanding several interrelated pathogenetic mechanisms of IPF is the identification of various molecular phenotypes resulting from complex interactions between genetic, epigenetic, transcriptional, post-transcriptional, metabolic and environmental factors. For accurate and early diagnosis of IPF and improvement of the prognosis of the disease, it is necessary to develop and validate diagnostic and prognostic biomarkers [Namakanova O.A. (2024)]. Antifibrotic therapy with potentially new drugs requires the use of biomarkers that make it possible to prognosticate the course of the disease and assess the effectiveness of drug therapy, which in turn contributes to a wider use of personalized therapy [Anaev E.Kh. (2017)].

In this work, we attempted to create a model of pulmonary fibrosis on white mongrel rats, which will show how a fibrosis-like condition is formed in the lungs in an experiment, which makes it possible to study the relationship of this pathology with other organs and systems, provoked by the introduction of nitrogen dioxide into the respiratory tract.

MATERIALS AND METHODS

In the experiment, sexually mature white mongrel rats weighing 180-220 g were used to model pulmonary fibrosis. All ethical requirements were met and approved by the protocol of the Ethics Committee of the Ministry of Health of the Republic of Uzbekistan ((No. 6/16-1699 dated September 27, 2022) [Barnoev A.I., 2023] .

Pulmonary fibrosis itself was provoked by passive introduction of nitrogen dioxide into the respiratory tract of experimental animals. For this purpose, experimental rats were placed in a special box and exposed to NO_2 30-40 mg/m^3 using a specially adapted fan with an outlet pipe through which toxic substances are released into the open atmospheric air. On the opposite side there is a vent that is tightly closed during poisoning of experimental animals. The role of the toxic substance was performed by nitrogen dioxide obtained by the chemical reaction of nitric acid with zinc metal. To create a concentration of the toxic substance in the range exceeding the MAC (single concentration in the air of 0.4 mg/m3), namely 30-40

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mg/m3 (the model was proposed by L.N. Danilov et al., 2009), the reaction of nitric acid with zinc inside the box was used.

The reaction used concentrated nitric acid and Zn 0.08 mg, respectively. The concentration of vapors of the toxic substance in the air was derived using chemical and mathematical equations. As a result of the reaction of nitric acid with metal, vapors of the toxic substance nitrogen dioxide are released, which, with prolonged poisoning for 90 days, daily cause inflammatory connective tissue changes in the lung parenchyma. A fan was mounted inside the chamber to create the same concentration of NO_2 throughout the entire volume of the box. The animals were exposed to the toxic substance daily for 30 minutes three times a day with an interval of 15 minutes between poisonings. The ventilation intervals of the chamber were made to remove the accumulated carbon dioxide released during the breathing of the animals. The box was ventilated by exhaust through an exhaust pipe with an open vent in the chamber. After each ventilation of the chamber, the toxic substance was re-injected.

As a result, it was found that after 60 days, the surface of the visceral membrane in the lungs, the drainage function of the lymph nodes, sharply decreases. As a result, varying degrees of narrowing of the lymphatic vessels that have reached a morphofunctional passive state lead to varying degrees of tissue fluid retention in the interstitial tissue of the lung tissue, chronic obstructive and inflammatory diseases of the lungs. At later stages from 60 to 90 days, significant changes in the lung tissue were formed, such as vascular fibrosis and perivascular fibrosis, as well as interstitial and interalveolar fibrosis. With these clinical and morphological changes in the bronchopulmonary lymph nodes, functional changes of varying degrees occur.

The experimental animals were divided into 2 groups by age, 6- and 9-month-old, and correspondingly control groups.

The removed lung tissue samples were fixed in 10% formalin, embedded in paraffin, and after hardening, standard 6-7 μm thick sections were prepared. The sections were stained with hematoxylin and eosin and histochemically with alcian blue. The prepared histological preparations were studied under an NLCD-307B binocular microscope (Novel, China). The study materials were subjected to statistical processing using parametric and nonparametric analysis methods. Accumulation, adjustment, systematization of the initial information and visualization of the obtained results were carried out in Microsoft Office Excel 2010 spreadsheets. Statistical analysis was performed using the IBM SPSS Statistics v.23 program (developer - IBM Corporation).

RESULTS AND DISCUSSION

The model we proposed was used to determine and study morphofunctional and structural changes in the lung tissue and regional lymph nodes, in particular axillary and bronchopulmonary. Under experimental conditions, pulmonary fibrosis is manifested by a continuous change of inflammatory processes, pathological processes and reparative regeneration processes. A significant part of the experiment was modeled to study changes in the lymph nodes, while long-term changes in lung tissue were studied superficially.

In our study, we used a model of pulmonary fibrosis induced by passive introduction of a toxic substance into the respiratory tract of a rat. The proposed model allows us to evaluate

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stepwise changes in the acute inflammatory period, chronic obstructive stage and chronic inflammation stage, as well as the later period of fibrosis formation.

The simulation lasted 90 days, with an intermediate study of lung tissue and regional lymph nodes on the 30th and 60th days. The results obtained from white outbred rats in the group with the simulation of pulmonary fibrosis, but not subjected to correction, were studied. Secondary follicles developing in the germinal center are revealed. As a result, foci of diffuse hyperplasia are revealed in the lymphoid follicles of the cortex. In the secondary lymphoid follicles, lightly stained cells consist of the reproductive center (germinative) and a cluster of lymphocytes with a dark-colored crown around it. Intensively growing B-lymphocytes, lymphoblasts, macrophages, dendritic cells and lymphocytes are found in the germinal center of the lymphoid follicles. The following results were obtained in a study of the bronchopulmonary lymph nodes of white outbred rats with experimentally induced pulmonary pneumosclerosis.

It was found that at the initial stage of modeling (day 30) a small amount of collagen was deposited, which indicates only the formation of fibrosis. Accordingly, a longer period will be required for the accumulation of a larger amount of collagen in the lung tissue, taking this conclusion into account, we continued the modeling up to 90 days. The same conclusion was made regarding changes in the bronchopulmonary lymph nodes.

In conclusion, it was found that long-term exposure of the respiratory tract of experimental animals to nitrogen dioxide leads to the expected changes, namely, typical signs of pulmonary fibrosis with the deposition of collagen fibers in the lung tissue, and also gave informative results in the study of regional lymph nodes. All of the above leads us to a clear understanding of the problem of pulmonary fibrosis, which in turn may help us completely rid humanity of this destructive disease in the future.

CONCLUSION

The presented modeling is noninvasive , reproducible and quite feasible, which was carried out by introducing nitrogen dioxide into the respiratory tract of rats. A more thorough study of the pathogenetic processes of pulmonary fibrosis reveals an understanding of this pathology in general and possible complications, and also makes it possible to find new ways in early diagnosis, therapy and prevention, which is the main advantage of our study.

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