ORIGINAL ARTICLE

NEW POSSIBILITIES FOR MODIFYING THE COURSE OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE: THE EFFECT OF TIOTROPIUM BROMIDE ON CERTAIN PATHOGENETIC LINKS OF NEOCOLLAGENOGENESIS AND LOCAL IMMUNE DEFENCE OF THE BRONCHIAL TREE

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ABSTRACT

The aim: To evaluate the dynamics of the interferon and collagen-IV systems in bronchoalveolar lavage in the treatment of chronic obstructive pulmonary disease using the tiotropium bromide medication.

Materials and methods: The study involved 60 COPD patients with bronchial obstruction of the II degree before and on days 30 and 60 of therapy using conventional treatment regimens and inhalations of tiotropium bromide a the dose of 18 mcg once a day. The collagen-IV levels in bronchoalveolar fluid were determined by means of enzyme-linked immunoassay using "StatFax 303 Plus" analyzer and "Biotrin Collagen IV EIA" reagents. The level of IFN-γ was identified with the help of enzyme-linked immunoassay using "StatFax 303 Plus" analyzer and "ProKon" reagents (LLC "Protein Contour", Russia) in bronchoalveolar fluid obtained during fiber-optic bronchoscopy.

Results: When examining Group I patients on the 30th day we found out that the content of collagen-IV in the bronchoalveolar fluid had decreased by only 10.29% ($p < 0.05$). Detection of collagen-IV indices in Group II patients on the 30th day of tiotropium bromide use showed the 29.43% ($p < 0.05$) decrease in its content as compared to the initial indices. In Group III patients, the concentration of collagen-IV had a maximum tendency to normalize and made up (24.72 ± 1.15) ng/ml, and decreased by 2.44 times ($p < 0.05$) as compared to the initial indices. Our examination of 12 patients from the comparison group I on the 60th day of treatment revealed even a slight increase in the content of collagen-IV in the bronchoalveolar fluid, as compared with the data obtained on the 30th day. The identified IFN-γ deficiency is indicative for the COPD of the II degree of bronchial obstruction, and its indices were 2.29 times lower than those observed in people from the control group. On day 30, we found out that the content of IFN-γ in Group I patients increased by only 10.29% ($p > 0.05$). Detection of IFN-γ in Group II patients showed 42.27% ($p < 0,05$) increase in its content as compared to the initial indices. The most favorable dynamics of IFN-γ levels in bronchoalveolar contents was observed in Group III patients, and at the time of observation it made up (1.16 ± 0.08) pg/ml, having 2 times ($p < 0.05$) increased as compared to the initial indices. However, in contrast to those taking tiotropium bromide, we examined 12 patients from Group I on the 60th day of treatment and found no significant positive dynamics of IFN-γ content in bronchoalveolar fluid as compared to the indices obtained on day 30.

Conclusions: The obtained findings indicate the effect of tiotropium bromide on the reduction of interferon-γ and reduce of collagen-IV levels, which depend on the duration of its use.

KEY WORDS: chronic obstructive pulmonary disease, tiotropium bromide, collagen-IV, IFN-γ

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is one of the most serious diseases in terms of disability and economic costs, the second most common infectious disease in the world, ranks fourth in the structure of mortality and is characterized by the persisted tendency for its constantly increasing prevalence rates due to the spread of bad habits on the one hand and extending life expectancy on the other [1, 2]. In the "European Lung White Book" Ukraine is presented as one of the countries with the highest mortality rate due to respiratory pathologies among men [3]. During the period of 2015–2016, the incidence of chronic bronchitis in Ukraine has increased by 0.51%, which puts this pathology in the category of strategic issues of domestic medicine [4]. Despite the fact that the development of treatment methods for COPD is well financed, it still continues to be one of the leading causes of death in the world.

The course of COPD becomes especially aggressive in the II degree of bronchial obstruction with the onset of morphological changes of the airways (sclerosis, fibrosis, bronchospasm, edema, hypercrinia, dyscrinia), resulting in bronchial remodeling and subsequent galloping progres-

sion of its systemic manifestations. Respiratory remodeling is a pathological process observed in chronic inflammatory and obstructive respiratory diseases [5].

COPD is characterized by asymptomatic or paucisymptomatic onset of the disease with subsequent progressive increase in the severity of the condition, as well as steadily progressive reduction of the pulmonary function (PF), which is the most specific and prognostic sign of the disease. Unfortunately, this pathology is often diagnosed at later stages, when the most advanced treatment programs are not able to slow down the steady progression of the disease.

The UPLIFT (Understanding Potential Long-term Impacts on Function with Tiotropium) findings show that selective M3 cholinolytic tiotropium has demonstrated a long-term improvement of the lung function in the four-year treatment of COPD patients [6]. While studying the group of patients who were administered tiotropium it became possible to observe the decrease of the mortality risk by 16 % ($p = 0.016$), positive effect of such treatment on survival rate ($p = 0.034$), prevention of disease exacerbation ($p < 0.001$) and significant decrease in the COPD destabilization risk, requiring further hospitalization (risk ratio 0.86; $p < 0.002$) as compared with the control group [6]. Additionally, large-scale clinical studies suggest that the use of long-acting muscarinic antagonist tiotropium in the treatment of COPD patients delays airway deterioration in dynamics up to 4 years, which should probably contribute to the involution of morphological changes in the bronchi. [6-9].

In our opinion, the effectiveness of treatment of any pathology depends on the deep knowledge of all stages of its pathogenesis. Therefore, the study of complex and multisystem processes that occur in the body in COPD, against the background of the therapeutic standards with the inclusion of tiotropium bromide medication with its characteristic effects, is quite relevant and will contribute to modern science and understanding of the need for a long-term basic treatment by both patients and healthcare professionals.

THE AIM

The goal of this study is to evaluate the dynamics of the interferon and collagen-IV systems in bronchoalveolar lavage in the treatment of chronic obstructive pulmonary disease using the tiotropium bromide medication.

MATERIALS AND METHODS

The study involved the examination of 60 patients with COPD II degree of bronchial obstruction. The diagnosis was verified and formulated in accordance with the order №555 of the Ministry of Health of Ukraine from June 27, 2013 "On approval and introduction of medical and technological documents for standardization of care in chronic obstructive pulmonary disease" [1, 10]. The investigation was performed before the start of treatment and on days 30 and 60 of therapy which involved the use of conventional treatment regimens and the administration of tiotropium bromide by inhalation of 18 mcg, once a day.

In order to study the effectiveness of the suggested treatment programs for COPD II degree of bronchial obstruction, all patients were divided into groups depending on the prescribed treatment.

Group I – 20 patients who did not receive the selective M3 long-acting cholinolytic in their comprehensive therapy [1];

Group II – 40 patients who were prescribed a 30-day course of tiotropium bromide on the background of comprehensive treatment of the exacerbation phase.

Group III – 21 patients who were prescribed a 60-day course of tiotropium bromide on the background of comprehensive treatment of the exacerbation phase.

The control group consisted of 15 apparently healthy individuals (AHI) without any signs of diseases of the respiratory tract and other pathologies of the internal organs.

The material of study was bronchoalveolar lavage, obtained considering the localization of inflammatory lesions of the lungs during fiber-optic bronchoscopy by Clements (1967) method in modification of Ramires (1980). The collagen-IV levels in bronchoalveolar fluid were determined by means of enzyme-linked immunoassay using "StatFax 303 Plus" analyzer and "Biotrin Collagen IV EIA" reagents. The level of IFN-γ was identified with the help of enzyme-linked immunoassay using "StatFax 303 Plus" analyzer and "ProKon" reagents (LLC "Protein Contour", Russia) in bronchoalveolar fluid obtained during fiber-optic bronchoscopy.

RESULTS

The analysis of findings, obtained by fiber-optic bronchoscopy that was performed to 60 patients with COPD II degree of bronchial obstruction before the start of treatment, showed that mostly identified signs were: severe catarrhal inflammation – in 48.8% of patients and purulent inflammation – in 28.8% of the examined individuals. Occasionally, moderate catarrhal inflammation was observed in 12.8% of patients, and 6.4% of the examined individuals had atrophy of the bronchial mucosa; the structure of bronchial mucosa was close to normal – in 3.2% of patients. Repeated fiber-optic bronchoscopy, which was performed to 20 patients from Group I on the 30th day of treatment, showed mainly ($p<0.05$) moderate catarrhal inflammation in 60.0% of patients, while the marked form of catarrhal inflammation was observed in 40.0% of cases.

Particularly, we would like to emphasize the obtained findings of fiber-optic bronchoscopy performed to 20 patients from Group II on the 30th day of treatment. We revealed a significant decrease ($p<0.05$) in the proportion of severe catarrhal inflammation by 31.3% in patients from Group II, which was 1.9 times lower than among patients receiving basic treatment regimens. However, the proportion of moderate catarrhal inflammation has increased to 68.8% ($p <0.05$) among Group II patients. At the same

Table I. Indices of collagen-IV (ng/ml) in bronchoalveolar fluid of patients with chronic obstructive pulmonary disease II degree of bronchial obstruction during treatment with tiotropium bromide

Study group	Indices of collagen-IV in bronchoalveolar fluid			p
	before treatment	on day 30 of treatment	on day 60 of treatment	
I, n=20	60.21±1.08	55.21±1.12	57.08±1.27	< 0.05
II, n=40	62.86±1.25	44.36±1.19		< 0.05
III, n=21	59.07±1.33		24.72±1.15	< 0.05
AHI, n=15	9.87±0.52			

Table II. Idices of IFN-γ (pg/ml) in bronchoalveolar fluid of patients with chronic obstructive pulmonary disease II degree of bronchial obstruction during treatment with tiotropium bromide

Study group	Indices of IFN-γ in bronchoalveolar fluid			p
	before treatment	on day 30 of treatment	on day 60 of treatment	
I, n=20	0.59±0.06	0.68±0.12	0.71±0.07	> 0.05
II, n=40	0.56±0.05	0.97±0.07		< 0.05
III, n=21	0.58±0.03		1.16±0.08	< 0.05
AHI, n=15	1.28±0.11			

time, fiber-optic bronchoscopy of Group III patients on day 60 from the start of treatment, showed that the proportion of moderate catarrhal inflammation increased to 82.6% ($p<0.05$), and the proportion of marked catarrhal inflammation made up only – 17.4% ($p<0.05$). Thus, the obtained findings indicate higher effectiveness of comprehensive treatment of patients with COPD II degree of bronchial obstruction, who were administered tiotropium bromide medication as compared to the patients whose treatment regimens did not include this medication. The use of tiotropium bromide medication in the treatment of patients with COPD II degree of bronchial obstruction has also resulted in faster regression of clinical symptoms of the pathology, positive changes in the data of spirography and laboratory dynamics.

The studies have revealed that the collagen-IV level in bronchoalveolar lavage of healthy individuals was: (9.87 ± 0.52) ng/ml, and IFN-γ (1.28 ± 0.11) pg/ml. On admission of patients with COPD II degree of bronchial obstruction, there was a significant increase in levels of collagen-IV in bronchoalveolar fluid – up to (60.71 ± 1.18) ng/ml ($p <0.05$) on the background of IFN-γ depression to (0.56 ± 0.07) pg/ml ($p<0.05$).

The examination of Group I patients on the 30th day of treatment, we found out that the content of collagen-IV in bronchoalveolar fluid decreased by only 10.29% ($p<0.05$). Detection of collagen-IV levels in Group II patients with COPD II degree of bronchial obstruction on the 30th day of tiotropium bromide use showed the decrease in its content by 29.43% ($p <0.05$). A real qualitative breakthrough in the dynamics of collagen-IV levels in bronchoalveolar lavage was observed in Group III patients, who received tiotropium bromide for 60 days. At the end of observation, the concentration of collagen-IV in bronchoalveolar fluid had maximum tendency to normalize and made up (24.72 ± 1.15) ng/ml, that was 2.44 times ($p<0.05$) lower than the indices observed in group with baseline therapy regimens. The examination of 12 patients from the comparison group I on day 60 revealed even a slight increase in collagen-IV levels in bronchoalveolar fluid, as compared to the data obtained on day 30 (Table I).

Deficiency of IFN-γ in bronchoalveolar fluid of patients with COPD II degree of bronchial obstruction is indicative and its indices were 2.29 times lower as compared to those observed in the control group (Table II). On day 30, we found out that the content of IFN-γ in bronchoalveolar fluid of Group I patients increased only by 10.29% ($p>0.05$). Detection of IFN-γ indices in Group II patients with COPD II degree of bronchial obstruction on the 30th day of tiotropium bromide use showed an increase in its content by 42.27% ($p<0.05$), as compared to the indices observed with the use of baseline therapy regimens. As in case of collagen-IV levels, the dynamics of IFN-γ levels in bronchoalveolar fluid was the most favorable in Group III patients who received tiotropium bromide for 60 days. At the end of observation, the concentration of IFN-γ in bronchoalveolar fluid had the maximum tendency to normalize and was (1.16±0.08) pg/ml, and was 2 times ($p<0.05$) lower as compared to the indices observed with the use of baseline therapy. However, in contrast to those taking tiotropium bromide, we examined 12 patients from Group I on day 60 and found out no significant positive dynamics of IFN-γ in bronchoalveolar fluid, as compared to the data obtained on day 30 (Table II).

Tendency for regenaration of local IFN-γ levels in patients with COPD II degree of bronchial obstruction treated with tiotropium bromide is worth special attention, since it substantiates the whole cascade of sanogenetic mechanisms for resolving pathological changes in the inflammatory process of the bronchial tree. Currently, interferons are isolated into a special class of cytokines, which were previously considered exclusively as antiviral factors, but afterwards their antitumor and immunomodulatory activities were discovered. [11]. The antibacterial property of interferons is associated with their increased phagocytic activity, increased production of immunoglobulins and increased cytotoxicity of natural killer cells [11]. The interferon system has neither specialized cells

nor specialized organs, however, the biggest producers of interferon are immunocompetent cells. When cells are stimulated by an inducer (infectious agent), the genes encoding interferon proteins are activated and the production-translation of these proteins occurs. [11]. Thus, cells start producing interferon, which, on the one hand, inhibits the proliferation of infectious agents, and on the other – enhances the expression of molecules of the major histocompatibility complex class I on the surface of altered cells and causes activation of NK cells, which initiate cytolysis of damaged cells and produce IFN -γ, which directs the development of the immune response by T-helper-1 type [11]. Macrophages, activated by the contact with antigen and IFN-γ, produce IL-12, which stimulates the differentiation of immature CD4 lymphocytes into type 1 T-helpers, which after antigen presentation are activated and produce a number of cytokines (IL-2, TNF-α, IFN -γ) [11]. The effect of these transmitters is the activation of cytotoxic CD8 lymphocytes, which provide cytolysis of the corresponding target cells, culminating in the elimination of pathogens and sanation of the inflammatory process in the bronchi [11].

DISCUSSION

The study has also showed the reduction in collagen-IV content in bronchoalveolar fluid in patients with COPD II degree of bronchial obstruction which were treated with tiotropium bromide. In order to understand the significance of this effect, it is necessary to focus on the role and localization of collagen in the human body. It is a well-known fact that a special layer is visualized between the epithelial membrane and the underlying connective tissue, it is located directly at the base of the epithelial cells and is called the basement membrane. [11]. Typical basement membrane (40 - 120 nm thick) consists of two layers of different origin [12-14]. The first layer of the basement membrane is the basal plate – a separate homogeneous electron-dense layer with a thickness of 50-100 nm, which is located just above the "felt-like" network of reticular fibers, which actually form the second layer [13]. It has been established that the basal plate always follows the contours of the basal surface of epithelium and the distance between them makes up 40 nm [14]. Basal plates may be connected not only with epithelium but also with nerve and muscle fibers [13].

The basement membrane performs two main functions: an elastic framework and a barrier for filtration and diffusion of substances (low molecular weight compounds, intercellular fluid) [13]. It is characterized by a unique form of collagen – type IV. These structures are synthesized by fibroblasts (Latin "fibra-" meaning the fiber, and Greek "blastos-" meaning the embryo). In addition, fibroblast-related cells such as osteoclasts and chondroblasts are also capable of synthesizing collagen. [14].

Currently, ten types of collagen are identified, and they are different in composition of α-chains and a number of functions. The most studied are: Type I – connective tissue proper (loose and dense) and bone tissue, Type II – hyaline cartilage, Type III – skin of the fetus, arteries, Type IV – basement membrane, Types V - VI collagen is involved in ossification, chondroprotection. Types IX - X may play a role in the maturation of connective tissue proper [12-14].

The three α-chains are identical in all types of collagen, except for Type I [14]. However, the collagen of the basement membrane (type IV) contains much more carbohydrate side chains, as well as more hydroxylysine and hydroxyproline [13]. The presence of hydroxylysine ensures the stability of collagen fibers of the basement membrane, due to the cross-linking of collagen molecules. The hydroxylysine molecule promotes the attachment of short carbohydrate residues – galactose and glucose.

Collagen IV is synthesized in the form of a precursor – procollagen. Synthesis of α-chains of procollagen occurs by means of polyribosomes of the granular endoplasmic reticulum [14]. The synthesized chains are 13 nm longer. A certain number of included proline and lysine residues are hydroxylated within 3 min in the synthesized chain, while the synthesis of the whole chain lasts from 5 to 6 min. The residual tail regions of α-chains are cleaved by means of peptidase enzyme on the cell surface of the fibroblast, so the procollagen molecule is converted into a tropocollagen molecule, which is further transformed into collagen fibrils. [12-14]. However, type IV collagen does not lose residual peptides after the secretion by fibroblasts. These peptides promote the formation of long fibrils by means of lateral condensation "side by side" [14]. First, the two chains are joined by C-terminal divisions to form dimers that associate with the N-terminals with three other molecules and thus spreading wider. [14]. The result of this association is a mobile multilayer structure stabilized by disulfide and other covalent bonds. [14]. The composition of the basement membrane, in addition to collagen Type IV, also includes heparan sulfate proteoglycan (perlecan) and glycoproteins (laminin and entactin) [14].

Thus, we think that the increase in collagen-IV content in bronchoalveolar fluid in COPD is the evidence of increased fibroblast activity (against the background of microcirculation disorders, activation of lipid peroxidation and hypoxia), and thus manifests the thickening of basement membranes, as well as the violation of both metabolic processes in their own biological systems and the dissociation of a number of dosage forms, especially inhalations. The decrease in collagen-IV content under the influence of treatment with tiotropium bromide is a prominent evidence of physiological course of repair processes, inhibition of neocollagenogenesis in the bronchi and the implementation of a cascade of effects of remodeling inhibition.

CONCLUSIONS

1. Verification of COPD II degree of bronchial obstruction, as compared with apparently healthy individuals, is accompanied by depression of IFN-γ levels (2.29 times, $p < 0.05$) with an increase in collagen-IV level

(6.15 times, p<0.05) in bronchoalveolar lavage.
2. The use of tiotropium bromide as the background therapy of COPD II degree of bronchial obstruction provides a significant reduction in collagen-IV levels in the bronchoalveolar lavage, which depends on the duration of tiotropium use and is the manifestation of antisclerotic activity of this medium.
3. The use of tiotropium bromide as the background therapy of COPD II degree of bronchial obstruction along with the stabilization of clinical and laboratory indices is accompanied by the restoration of IFN-γ levels in the bronchoalveolar lavage, which increases with the prolongation of tiotropium administration, eliminates antigen load and contributes to the physiological sanitation of the inflammatory process in the bronchi.

The prospect for the follow-up studies: is to visualize morphological changes at both cellular and subcellular levels, which would allow us to evaluate the obtained data on the effects of background treatment of COPD.

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The Authors declare no conflict of interest.

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