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Morphological Changes in Mucous Membrane of Bronchi After 180-day roflumilast treatment in subjects with severe COPD

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Abstract

Chronic obstructive pulmonary disease (COPD) is a chronic respiratory disease characterized by a decline in lung function over time and accompanied by respiratory symptoms, primarily dyspnea, cough, fatigue, and sputum production. Consequently, COPD is associated with a significant economic burden, including frequent hospitalization and disability. By 2020 COPD is predicted to be the third-leading cause of death worldwide (from sixth in 1990) and fifth leading cause of years lost through early mortality (12th in 1990).

For 9 people with severe COPD before treatment and after 180 days treatment with roflumilast were performed biopsies of the mucous membrane of the bronchi. Verification of the diagnosis and its formulation confirmed with the orders of Ministry of Health of Ukraine № 128 from 19.03.2007. "On approval of clinical protocols of medical care in the specialty pulmonology" ^[1] and the Order of Ministry of Health of Ukraine № 555 of June, 27, 2013 ^[2].

Keywords: Chronic obstructive pulmonary disease, airway remodelling, roflumilast.

1. Introduction

COPD is a complex, multifactorial, and progressive disease associated with significant morbidity and mortality worldwide. COPD has a major impact on daily life and quality of life that goes beyond airway limitation. ^[3, 4]. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) guideline recommends a combined assessment of COPD using 'ABCD' classification model, which, apart from spirometry, assesses future risk and current impact of disease using questionnaire data ^[5]. Although its pathogenesis is incompletely understood, generally effective in asthma, inhaled corticosteroids (ICSs) provide relatively modest benefit in COPD, chronic inflammation plays an important part and so new therapies with a novel anti-inflammatory mechanism of action may be of benefit in the treatment of COPD ^[3]. Although one novel class of compounds that may deliver therapeutic advantage in COPD are phosphodiesterase (PDE) 4 inhibitors.

PDE is a generic term that describes a large superfamily of enzymes that catalyze the breakdown of cyclic adenosine-3',5'-monophosphate (cAMP) and/or cyclic guanosine-3',5'-monophosphate (cGMP) to their respective inactive nucleotide 5'-monophosphates. Phosphodiesterase-4 (PDE4) is a vital enzyme in the metabolism of cyclic adenosine monophosphate (cAMP) and inhibition of PDE4 can inactivate immune and inflammatory cells via increase cAMP ^[6]. It is recommended by the GOLD guideline that a combination of PDE4 inhibitor and long-acting bronchodilator can be considered as an alternative treatment in patients with severe COPD due to the effective improvement of lung functions ^[7]. Roflumilast, a phosphodiesterase (PDE) 4 inhibitor, is a novel oral anti-inflammatory agent that, in clinical studies, has been shown to reduce exacerbations in patients with severe and very severe COPD who have symptoms of chronic bronchitis and a history of exacerbations. There were many clinical trials performed that showed that roflumilast could significantly improve post-bronchodilator forced expiratory volume in one second (FEV₁) (0.097±0.018, *P*<0.0001) and post-bronchodilator forced vital capacity (FVC) (0.114±0.031, *P*=0.0002), and decrease incidence of acute exacerbations (28% vs. 35%, *P*=0.0114) compared with placebo ^[8], which were further demonstrated by a

subsequent meta-analysis of seven trials with 9675 patients but without improving health-related quality of life by St George's Respiratory Questionnaire (SGRQ) (mean difference (MD) -0.70 , 95% confidence interval (CI) $-2.65\sim 1.26$, $P=0.49$) or decreasing mortality rate (risk ratio (RR) 0.90 , 95% CI $0.63\sim 1.29$, $P=0.56$)^[9].

In other trial 743 patients with moderate-to-severe COPD were randomized into roflumilast plus tiotropium and tiotropium groups and they reported a significant improvement in Shortness of Breath Questionnaire (SOBQ) (MD -2.6 , 95% CI $-4.5\sim -0.8$, $P=0.0051$) in roflumilast plus tiotropium^[10].

Many phase II and III randomized controlled trials (RCTs) have been initiated to explore the efficacy and safety of the PDE₄ inhibitors roflumilast in patients with COPD. As a result, roflumilast was recently approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for reducing the risk of exacerbations in patients with severe COPD and a history of exacerbations^[11, 12].

2. Material and Methods

For 9 people with severe COPD before treatment and after 180 days treatment with roflumilast was performed fibrobronchoscopy with biopsy of bronchial mucosa of the bronchi. The material of the research was bronchoalveolar lavage and bronchial biopsy materials received on the level of bifurcation of proximal bronchi to segmental bronchi during fiberoptic bronchoscopy.

3. Results

Before treatment we have observed, that in the pseudostratified epithelium of the mucous membrane of a bronchus a thickening of the basement membrane was observed. The thickness of epithelium on the perimeter was not the same. The areas with low epithelium alternated with pavement epithelium and a complete absence of epithelial cells and denudation of the basement membrane. In the epithelial layer it was difficult to separate basal and intercalary cells. Goblet cells were barely distinguished. On the surface of epithelium in the preserved areas the cilium were not found and the clots of mucus were localized.

In lamina propria of mucous membrane of a bronchus it was observed a great number of mighty bundles of collagen fibers. Cell population was presented by a great number of myofibroblasts that were defined by an irregular shape with pointed processes, basophilic cytoplasm, and a big rounded nucleus with hyperchromic chromatin. Myofibroblasts are inclined to torsion in deep areas of lamina propria. Between them there are a lot of macrophages (often with granules in cytoplasm) and lymphocytes. There are few fibroblasts. Inflammatory polymorphocellular infiltration was not identical in the visual field. The lumen of blood vessels is dilated and often contained blood corpuscles, their wall is thinned. In the vessels of microcirculation bed there are stases. Often the cellular infiltration dominates in perivascular areas. The electronic microscopic picture was characterized by the signs of chronic inflammatory process. In epithelial cells that were found in the section, a picture of necrosis was often observed. Plasmalemma of an epithelial cell did not contour and its content conjugated with amorphous substance of the subordinate connective tissue. In cytoplasm there are numerous transparent vacuoles, remains of organelles, amorphous structures. On the transverse section it was observed dilatation and weakening of intercellular contacts with dilatation of intercellular spaces. In one viewing field it

was observed a section of cells in their nuclear and anucleate parts. Separate nuclei contained nucleoli. Cytoplasm is homogeneous, organelles are hard to identify. Goblet cell is in the necrobiotic condition with karyopyknotic nucleus. In its enclosing epithelial cells are deeply damaged.

In fibroblasts of the subordinate connective tissue the nucleus is big, chromatin is moderately and evenly condensed throughout the whole nucleus. In cytoplasm there are identified mitochondria, endoplasmic reticulum, and beyond the cells – the products of fibrillogenesis – pro- and microfibrils, elements of amorphous substance. Such fibroblasts occur not often. More widespread in the connective tissue are myofibroblasts. These cells are localized in the shape of groups of a few cells. The cells bodies are of lengthened fusiform form, the nuclei are lengthened with peripheral condensation of chromatin. In cytoplasm there are identified numerous microfilaments, and separate mitochondria. In the environment of the cells it is observed thick collagen fiber bundles oriented in different directions.

In the inflammatory infiltration there are quite a lot of macrophages. In the nucleus there is a peripheral condensation of chromatin. It contains two fragments of a nucleus. Plasmalemma does not have contours clarity. Numerous twisted processes are coming from the body and that is why there are a lot of their fragments near the cells. In cytoplasm there are big phagosomes one of which is a giant one. Their content is fragments of membrane organelles and thickened osmiophil material. In other areas the cytoplasm is homogeneous, single organelles are barely seen (cisterns of endoplasmic reticulum, mitochondria with shortened cristae). Around the macrophage there is the basic (amorphous) substance of the soft connective tissue in the state of edema.

What attracts attention is that among the cells of inflammatory infiltration in the connective tissue there are few plasmocytes. Plasmocytes show considerable destructive changes. In a nucleus it can be often observed an edema with eccentric displacement of chromatin. In cytoplasm degranulation of rough endoplasmic reticulum, single mitochondria. Primary lysosomes are identified seldom, more often secondary – phagosomes and tertiary - residual corpuscles. Plasmalemma is contoured not clearly.

In many blood capillaries in dilated lumen there is a stasis of blood corpuscles mostly erythrocytes. The basement membrane is thickened and damaged in some places. In the nucleus of endotheliocyte there is a peripheral condensation of chromatin. In the peripheral area of the endotheliocytes cytoplasm is difficult to identify the organelles, but mitochondria and phagosomes are distinguished. Around the capillaries there are considerable layers of collagen fibers. In the surrounding of the capillary there are observed myofibroblasts and their processes, bundles of collagen fibers that have different directions and maturity.

Sometimes the thickening of the basement membrane and surrounding of the capillary by collagen fibers is considerably expressive. The capillaries have fissural lumen and are "embedded" into the bundles of connective tissue that have circular localization. Endotheliocyte of the capillary wall has pyknotic nucleus and thinned electronically thickened cytoplasm. In lamina propria of mucous membrane there is an evident pulmonary fibrosis, there are myofibroblasts and mighty bundles of collagen fibers.

In some capillaries the lumen is free from blood corpuscle but endotheliocytes are in the state of destruction (necrosis). Their cytoplasm is electronically thickened and has vacuoles. The

basement membrane is identified only in separate areas. The capillary is surrounded by collagen fibers situated as tight laminae. From the outside of them there are collagen fibers on the transverse section, i.e. collagen fibrous structures have two layers – circular and longitudinal. Near the nucleus-containing area of endotheliocyte the collagen fibers have reticular form and are less dense and thick.

A six-month therapy with the use of roflumilast gave its positive results. In the pathohistological picture of a bronchus mucous membrane there were outlined positive changes in the epithelial tissue as well as in the connective tissue of lamina propria of mucous membrane.

Microscopically there was observed the renewal of the epithelium on rather long areas of a bronchus surface. On these areas the epithelium normalized and in it there could be distinguished basal, ciliated, high inserted cells covered with cilia. That is, in some places the epithelium acquired the form of a renewed pseudostratified ciliated epithelium. Ciliated cells had mostly cubic form and were intimately adjacent to the basement membrane. The latter had different thickness. Nuclei of epithelial cells were coloring actively basophilic; they were characterized by euchromatin. Anyway, the goblet cells seemed to be absent.

In the connective tissue of lamina propria there was also outlined positive progress. Polymorphocellular infiltration decreased and in some places disappeared. Bundles of collagen fibers are thin; between them there are cells of fibroblastic and macrophage rows.

Fibroblasts looked like elongated cells with elongated nuclei. Micro-fibroblasts were localized one by one or by small groups. They had little processes with basophilic cytoplasm. Sometimes there occurred mast cells with granules in small number. Considerable attention was paid to mast cells by Gh. Nini *et al.* (2012). Authors established that mast cells are present in lamina propria of mucous membrane of a bronchus before and after the treatment of COPD, but before the treatment 90% of mast cells displayed the signs of degranulation. Mast cells in the state of degranulation after the treatment were observed in the center of inflammatory nidus of infiltration and this explains their participation in the cellular immune response. After the treatment the number of mast cells decreased. Among them occurred granulated and degranulated cells. Their granules were immature. Macrophages were mostly related mature macrophages and rarely to monocitoid ones. Blood vessels of microhemocircular bed had an ordinary picture of the build of their wall without blood corpuscles in the lumen with all normal definitions of the wall of microhemovessels. Fibroblasts of normal build often are determined in connective tissue of lamina propria of mucous membrane. The form of these cells is elongated. A nucleus is rounded with not deep invagination. In a nucleus equally is represented euchromatin and moderately condensed heterochromatin. Karyolemma is contoured clearly. In cytoplasm there are mitochondria in which there are clearly identified cristae and matrix. Cisterns of granular endoplasmic reticulum are localized near mitochondria. Granular endoplasmic reticulum is represented by numeral flat cisterns. Around the fibroblast there is the amorphous substance in which elements of newly created, young collagen fibers are distinguished.

In the electronically microscopic picture of a bronchus wall there happened a lot of changes. First of all, this concerns the cells of superficial epithelium. Epithelial cells in a larger amount than in the previous term and before the treatment had

cilia on their apical surface. The cilia were not of great thickness but they had all signs of a normal build. In their basis there were observed basal corpuscles that transferred to free protrusions with an axoneme inside and covered with plasmalemma. Cytoplasm of the ciliated epithelial cell contains mitochondria. The mitochondria have different build – from small dark to larger ones with determined cristae and somewhere cleared matrix. Vacuoles are practically absent.

On the transverse section through basal pole of epithelial cells there were observed a few cells with centrally situated nucleus. The nuclei had invaginations, which confirms the active state of the cells. Chromatin of the cells is moderately condensed. Karyolemma is contoured clearly. In cytoplasm there are mitochondria, cisterns of granular and vesicles of agranular endoplasmic reticulum, small vacuoles, canalicular apparatus, single lysosomes and (in some) phagosomes. Intercellular unions were getting stronger and the cells were situated close one to another connected by simple unions and with invagination of the “lock” type. Among epithelial cells there is identified a cell with rounded granules with osmiophil content - endocrine cell that belongs to dissociated endocrine system. In it there appear mitochondria, cisterns of granular endoplasmic reticulum, Golgi complex with vesicular component.

Inclusion of roflumilast in the complex of pharmacological therapy provided positive dynamics of structural morphological changes of bronchial mucosa. These arguments allow us to recommend the proposed therapies for intensive distribution in the clinical practice.

4. Conclusions

Inclusion of roflumilast in the complex of pharmacological therapy provided positive dynamics of structural morphological changes of bronchial mucosa. These arguments allow us to recommend the proposed therapies for intensive distribution in the clinical practice.

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