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Kseniia Ostrovska
SHEE Ivano-Frankivsk National Medical, University, Ivano-Frankivsk, Ukraine. Department of Phtisiology and Pulmonology with the Course of Occupational Diseases

Nataliia Ivanyshyn
SHEE Ivano-Frankivsk National Medical, University, Ivano-Frankivsk, Ukraine. Department of Phtisiology and Pulmonology with the Course of Occupational Diseases

Iryna Savelikhina
SHEE Ivano-Frankivsk National Medical, University, Ivano-Frankivsk, Ukraine. Department of Phtisiology and Pulmonology with the Course of Occupational Diseases

Oleksandr Varunkiv
SHEE Ivano-Frankivsk National Medical, University, Ivano-Frankivsk, Ukraine. Department of Phtisiology and Pulmonology with the Course of Occupational Diseases

Mariana Kulynych-Miskiv
SHEE Ivano-Frankivsk National Medical, University, Ivano-Frankivsk, Ukraine. Department of Phtisiology and Pulmonology with the Course of Occupational Diseases

Stepan Ornat
SHEE Ivano-Frankivsk National Medical, University, Ivano-Frankivsk, Ukraine. Department of Phtisiology and Pulmonology with the Course of Occupational Diseases

Galyna Korzh
SHEE Ivano-Frankivsk National Medical, University, Ivano-Frankivsk, Ukraine. Department of Phtisiology and Pulmonology with the Course of Occupational Diseases

Mykola Ostrovskyy
SHEE Ivano-Frankivsk National Medical, University, Ivano-Frankivsk, Ukraine. Department of Phtisiology and Pulmonology with the Course of Occupational Diseases

Correspondence
Kseniia Ostrovska
SHEE Ivano-Frankivsk National Medical, University, Ivano-Frankivsk, Ukraine. Department of Phtisiology and Pulmonology with the Course of Occupational Diseases

C-reactive protein and morphological changes in the lungs in patients with severe COPD: Focus on roflumilast

Kseniia Ostrovska, Nataliia Ivanyshyn, Iryna Savelikhina, Oleksandr Varunkiv, Mariana Kulynych-Miskiv, Stepan Ornat, Galyna Korzh and Mykola Ostrovskyy

Abstract
COPD is a serious chronic disease, which is increasingly being recognized as having an inflammatory component. New treatments are required that reduce inflammation and stop disease progression, and inhibition of PDE4 represents a promising mechanism to treat COPD, given the resulting effects on inflammation and associated underlying disease processes.

Keywords: Chronic obstructive pulmonary disease, CRP, roflumilast, morphological remodeling

1. Introduction
Chronic obstructive pulmonary disease (COPD) is one of the most important causes of morbidity and mortality all over the world. It is characterized by cellular inflammation and structural remodeling of small airways and progressive deterioration of lung function due to airway obstruction [1-4] Although primarily affecting the lungs, the chronic inflammatory process of COPD does have systemic repercussions. C-reactive protein (CRP) is an ancient highly conserved molecule and a member of the pentraxin family of proteins. CRP is secreted by the liver in response to a variety of inflammatory cytokines. CRPs shows a 1000-fold or more increase in concentration during the occurrence of an injury, inflammation or tissue death [5]. Thus, the measurement of CRP is widely used to monitor various inflammatory states.
Systemic inflammation is associated with, and appears to be a risk factor for, a variety of symptoms and conditions including weight loss, muscle wasting, atherosclerosis, malignancy, osteoporosis, diabetes, and anemia. One novel class of compounds that may deliver therapeutic Benefit in COPD is 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-cGMP to their respective inactive nucleotide 5-monophosphates [6]. Eleven distinct PDE families have been identified, although most of the anti-inflammatory activity is believed to result from the inhibition of PDE 4, for which there is clinical precedent. Roflumilast is synthesized in five steps from 3-cyclopropylmethoxy-4-hydroxybenzald-ehyde [7, 8].
The Global Initiative for Chronic Obstructive Lung Disease (GOLD) has included roflumilast as a new treatment option in its COPD management guidelines. A section on the new class, phosphodiesterase 4 (PDE4) inhibitors, describes the efficacy of roflumilast in patients with COPD [9, 10].

2. Materials and methods of research
The levels of CRP were studied in 61 patients in bronchoalveolar fluid (BALF) in patients with severe COPD. The patients were divided into groups based on the treatment assignment.
Group I - 85 patients who received maintenance treatment without roflumilast.
Group II - 66 patients was divided into:
II-a subgroup - 31 patients who as a part of maintenance treatment used roflumilast 500 micrograms (one tablet) once daily 30 days.
II-b subgroup - 24 patients, who as a part of maintenance treatment used roflumilast 500 micrograms (one tablet) once daily 90 days.

II-c subgroup - 11 patients, who as a part of maintenance treatment used roflumilast 500 micrograms (one tablet) once daily 180 days. There were 15 healthy persons examined (PHP). Maintenance treatment included: M-long-acting anticholinergics, β-2 agonists, short-acting inhaled and systemic glucocorticosteroids.

The C-Reactive Protein test is based on the principle of the latex agglutination. When latex particles complexed human anti-CRP are mixed with a patient's serum containing C reactive proteins, an visible agglutination reaction will take place within 2 minutes.

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. Plasmolemma 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 kariopicnoti 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.

Before treatment the CRP levels were increased in 4.7 times ($p<0.05$) compared to the PHP. This rate in GOLD group D was in 2.7 times higher ($p<0.05$) than in GOLD group C. After 180 days treatment without roflumilast the positive dynamics of CRP level was not achieved. The BALF concentration of CRP in II-a subgroup became $(5,1 \pm 0,4)$ g / l.

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 monocitoide ones. Blood vessels of microhemocircular bed had an ordinary picture of the build of their wall without blood corpuscles in the lumen with all normalk 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 plasmolemma. 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.

It was also reduction of CRP levels in patients of II-b subgroup observed. This measurement after treatment was (4,7 ± 0,33) mg / L and it was in 2.9 times higher than in the control group and in 1.3 times lower than in patients of I group and in 1.1 times lower than in II-a subgroup (p<0.05).

However, the maximum beneficial effect we still obsereved in patients in II-c subgroup. CRP level at the end of treatment in II-c subgroup was (4,1 ± 0,3) mg / L and it was in 1.5 times lower compared with the I group, but still remained in 2.5 times higher compared the rate of the PHP group.

4. Conclusions

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

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