Morphometric characteristics of distal airways of guinea pigs sensitized with ovalbumin

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Introduction
There is a constant increase in the incidence of bronchial asthma (BA) in Ukraine and the world today, associated with environmental pollution, increased allergization of the population, deterioration of the gene pool of nations [7]. BA is considered today as a genetically determined disease with neuroendocrine and immune mechanisms of bronchial hypersensitivity development [4, 6, 10]. According to modern concepts, the development of bronchial hyperreactivity is the leading pathophysiological mechanism of the development of bronchial asthma, the degree of which correlates with the severity of the disease [8].

The morphological basis of bronchial hypersensitivity in bronchial asthma is allergic chronic inflammation of the airways, which is observed at all stages of the disease, regardless of its severity and course [11, 12]. Despite its importance, the problem of studying the morphogenesis of allergic inflammation of the distal airways often remains outside the field of vision of scientists. The number of works, devoted to the study of bronchial morphogenesis in various pathological conditions [9, 13], including allergic inflammation in the chronobiological aspect, is insignificant. Considering the above, the study of morphological changes in the distal airways with allergic inflammation in the chronobiological aspect is the urgent issue in experimental medicine.

The aim of this work is to study the morphometric parameters of the distal airways of guinea pigs sensitized with ovalbumin.

Materials and methods
This research is a part of the research work of Zaporizhzhya State Medical University "Immunomorphological
characteristics of internal organs under the influence of endo- 
and exogenous factors on the body* state registration 
№ 0118U004250.

The object of the experimental study was lung, removed 
from 48 sexually mature male guinea pigs weighing 450- 
600 g, which were kept in standard conditions of the 
vivarium of the Zaporizhzhya State Medical University. All 
manipulations were carried out in compliance with the basic 
principles of working with experimental animals in 
accordance with the provisions of the European Convention 
for the Protection of Vertebrate Animals used for 
Experimental and Other Scientific Purposes (Strasbourg, 
1986), the General Ethical Principles for Animal Experiments 
adopted by the First National Congress on Bioethics (Kyiv, 
2001), the Law of Ukraine “On the protection of animals 
from cruelty” (from 21.02.2006).

Allergic airway inflammation was induced by 
subcutaneous sensitization and followed challenging by 
intranasal inhalation with ovalbumin (OVA) (Sigma Aldrich, 
USA). Guinea pigs were actively sensitized by 
subcutaneous injections into the interscapular region of 
ovalbumin (0.5 mg/mL) with alum (10 mg/mL in saline) as 
an adjuvant (AluVax Hydroxide vaccine adjuvant, OZ 
Biosciences France) on days 0, 7 and 14. From 21 to 28 
days of the experiment, guinea pigs were challenged for 
15 min with inhalation of either OVA (10 mg/mL in saline) 
via a nebulizer (Little Doctor International, Singapore, 
LD-211C) coupled to a plastic box. The animals were 
divided into 6 groups (8 animals in each group). The first 
four groups are animals sensitized and challenged OVA, 
withdrawn from the experiment, respectively, on the 23rd, 
30th, 36th and 44th days after its start; 5 - control group, 
received injections and challenged with saline only; 6 - intact 
group. For the purpose of rational presentation of the obtained 
data and their interpretation, we conditionally distinguish 
the early (23rd, 30th days of the experiment) and late (36th 
and 44th days after the start of the experiment) periods of 
the development of allergic inflammatory process in lung.

The animals were withdrawn from the experiment by an 
overdose of thiopental anesthesia (50 mg/kg) according to 
the established terms (23rd, 30th, 36th and 44th days of the 
experiment). Histological sections were stained with 
hematoxylin-eosin. Masson staining was carried out to 
assess the organization of collagen fibers, alcian blue - to 
determine the dynamics of the distribution of 
glycosaminoglycans, the PAS reaction - to determine the 
dynamics of the distribution of glycoproteins.

The sections were viewed and photographed by a 
compound binocular light microscope (Primo Star, Zeiss, 
Germany). The thickness of mucosal layer, muscular layer 
and adventitial layer was determined to assess 
morphometric parameters of bronchioles and terminal 
bronchioles.

The research results were processed by modern 
statistical methods of analysis on a personal computer 
using the standard software package Microsoft Office 2010 
(Microsoft Excel) and STATISTICA® for Windows 6.0 (StatSoft 
Inc., USA, license 46 No. AXXR712D833214FAN5). We use 
the Shapiro-Wilk test and the Kolmogorov-Smirnov test 
of consistency testing the hypothesis about the normal 
distribution of the studied parameters. We use the 
Kolmogorov-Smirnov homogeneity criterion testing the 
hypothesis that two independent samples belong to the 
same distribution law. The arithmetic means (M) and 
standard errors of the mean (±m) were calculated. The 
statistical significance of intergroup differences according 
to the data obtained was established using the parametric 
Student’s t-test (p*) and the nonparametric U-Whitney-
Mann test (p**). The obtained indicators were compared 
between the median and interquartile range Me (Q1; Q3). Differences between the compared values at the level of 
95% (p<0.05) were considered statistically significant.

Results

There are the thickening of the wall of bronchioles and 
terminal bronchioles of guinea pigs sensitized with 
ovalbumins, showed by histological analysis. There are 
also changes in the structure of alveoli, the increase in the 
number of peribronchial and perivascular lymphoid 
nodules, compared to the control group (Fig. 1). The degree 
of the manifestation of inflammatory changes increases 
with the decrease of the diameter of bronchi, reaching its 
maximum in the terminal bronchioles. We also observe 
the bronchiolar epithelium desquamation into the airways 
lumen, partial exposure of the basement membrane, 
eosinophilic peribronchial infiltration (Fig. 1b).

Noteworthy is the uneven thickening of the muscular 
layer of bronchioles due to muscular hyperplasia (Fig. 1a, 
b). The lumen of some bronchioles is narrowed (Fig. 1d) 
or even obstructed (Fig. 1c), which is a morphological 
confirmation of bronchospasm in animals after OVA-
sensitization and challenge. There are disorganization of 
fibrous elements, accumulation of PAS + positive material 
and numerous of mast cells in the connective tissue stroma 
by the adventitial layer of bronchioles (see Fig. 1c).

Morphological changes in the structural elements of 
bronchioles and terminal bronchioles, appeared at the light-
optical level, have their own regular morphometric display.

There was no statistically significant difference between 
the indices of the thickness of the mucosal layer 
of bronchioles in animals of the intact and control groups 
(p*/*>0.05), which indicates that the experimental protocol 
does not itself affect the changes in the morphometric 
parameters of bronchioles. There is a tendency to the 
decrease in the thickness of the mucosal layer of 
bronchioles after OVA sensitization and challenge in the 
early period of development of experimental ovalbumin-
duced allergic inflammation in lung. In animals of the 
first experimental group, the mucosal layer of bronchioles 
is 40.46±0.44 μm, which is 1.3 times less than that in the 
control group. The statistically significant thinning of the 
mucosal layer of bronchioles is also manifested on the
30th day of the observation in animals of the second experimental group - 42.29±0.53 μm, which is 1.2 times less than the same indicator in the control group (Table 1).

There is a significant tendency towards the mucosal layer thinning of bronchioles in the late period of the development of allergic inflammation. A statistically significant decrease (p**<0.05) in the mucosal layer thickness of bronchioles, compared to the control group, was observed in animals of the 3rd experimental group - 43.38±0.55 μm (see Table 1). There is a tendency for the indicator of the mucosal layer thickness in bronchioles riches those in the control group on the 44th day of observation.

There are no statistically significant differences between the muscular layer thickness of bronchioles of animals in the intact and control groups (p*/**>0.05). There is a tendency to the increase of the muscular layer thickness of bronchioles in the early period of the development of experimental ovalbumin-induced allergic inflammation in lung. A statistically significant thickening of the muscular layer of bronchioles is manifested from the 30th day of the experiment in animals of the 2nd experimental group - 25.59±0.23 μm, the increasing coefficient is 1.5 compared to the same indicator in the control group. In the late period of the development of allergic inflammation in guinea pigs' lung, a statistically significant muscular layer thickening by 1.7 times compared to the control appears in the third experimental group on the 36th day of observation - 29.1±0.46 μm (see Table 1). The tendency to the muscular layer thickening in bronchioles persists to the 44th day of the experiment in the fourth experimental group and is 24.73±0.12 μm, which is 1.5 times more than in the control group (p*/**<0.05).

The adventitial layer thickness in bronchioles is...
Table 1. Morphometric indicators of the wall of bronchioles of guinea pigs sensitized with ovalbumin.

<table>
<thead>
<tr>
<th>Group</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40.46±0.44 **</td>
<td>20.57±0.29</td>
<td>26.12±0.18 **</td>
</tr>
<tr>
<td>2</td>
<td>42.29±0.53 **</td>
<td>25.59±0.23 **</td>
<td>29.68±0.31 **</td>
</tr>
<tr>
<td>3</td>
<td>43.38±0.55 **</td>
<td>29.11±0.45 **</td>
<td>30.01±0.32 **</td>
</tr>
<tr>
<td>4</td>
<td>48.63±0.49</td>
<td>24.73±0.12 **</td>
<td>25.93±0.18 **</td>
</tr>
<tr>
<td>5</td>
<td>52.04±0.66</td>
<td>17.0±0.28</td>
<td>22.19±0.25</td>
</tr>
<tr>
<td>6</td>
<td>53.74±0.49</td>
<td>17.17±0.35</td>
<td>22.26±0.28</td>
</tr>
</tbody>
</table>

Note: * - p<0.05 (Student’s t-test); ** - p<0.05 (Whitney-Mann U-test) compared to the control group. M±m, (N=8); I - the mucosal layer thickness (μm); II - the muscular layer thickness (μm); III - the adventitial layer thickness (μm).

Table 2. Morphometric indicators of the wall of terminal bronchioles of guinea pigs sensitized with ovalbumin.

<table>
<thead>
<tr>
<th>Group</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21.23±0.2 **</td>
<td>11.76±0.14</td>
<td>15.13±0.2</td>
</tr>
<tr>
<td>2</td>
<td>26.24±0.42</td>
<td>13.53±0.34 **</td>
<td>14.02±0.09</td>
</tr>
<tr>
<td>3</td>
<td>32.17±0.39 **</td>
<td>13.67±0.17 **</td>
<td>18.76±0.17 **</td>
</tr>
<tr>
<td>4</td>
<td>28.44±0.4</td>
<td>11.73±0.18</td>
<td>13.97±0.1</td>
</tr>
<tr>
<td>5</td>
<td>24.92±0.22</td>
<td>10.42±0.07</td>
<td>12.98±0.2</td>
</tr>
<tr>
<td>6</td>
<td>24.8±0.36</td>
<td>10.08±0.14</td>
<td>13.08±0.19</td>
</tr>
</tbody>
</table>

Note: * - p<0.05 (Student’s t-test); ** - p<0.05 (Whitney-Mann U-test) compared to the control group. M±m, (N=8); I - the mucosal layer thickness (μm); II - the muscular layer thickness (μm); III - the adventitial layer thickness (μm).

22.26±0.28 μm in the intact group. There is no statistically significant difference between these parameters in animals of the intact and control groups. There is a tendency to the increase of the adventitial layer thickness in bronchioles during all periods of observation after OVA sensitization and challenge (see Table 1). In animals of the 1st experimental group, the thickness of the adventitial layer in bronchioles is 26.12±0.18 μm, which is 1.2 times more than in the control group. A statistically significant thickening of the adventitial layer in bronchioles, compared to the control group, is also present in the animals of the 2nd experimental group - 29.68±0.31 μm, which is 1.3 times more than in the control group. The maximal thickening of the adventitial layer in bronchioles of guinea pigs reaches in the late period of the development of allergic inflammation on the 36th day of observation - 30.01±0.32 μm, the increasing coefficient is 1.4, compared to the same indicator in the control group.

There was no statistically significant difference between the mucosal layer thickness in terminal bronchioles in animals of the intact and control groups (p””>0.05). After OVA sensitization and challenge in the early period of the development of allergic inflammation in the lungs, there is a tendency to the decrease in the mucosal layer thickness in terminal bronchioles. In animals of the 1st experimental group the mucosal layer thickness in terminal bronchioles is 21.23±0.2 μm, which is 1.2 times less than that of the control group (Table 2).

The gradual magnification in the thickness of the mucosal layer in terminal bronchioles is observed, starting from the 30th day of the experiment. A statistically significant thickening of the mucosal layer of terminal bronchioles appears on the 36th day of observation in animals of the 3rd experimental group - 32.17±0.39 μm, which is 1.3 times more than the same indicator in the control group.

There are no statistically significant differences between the parameters of the thickness of the muscular layer in terminal bronchioles in animals of the intact and control groups (p””>0.05). There is a tendency to the increase in the muscular layer thickness in terminal bronchioles in the early period of the development of experimental ovalbumin-induced allergic inflammation in lung of guinea pigs. A statistically significant thickening of the muscular layer in terminal bronchioles appears from the 30th day of the experiment in animals of the second experimental group - 13.67±0.17 μm, the increasing coefficient of 1.3 compared to the same indicator in the control group. In the late period of the development of allergic inflammation in the guinea pig lungs, a statistically significant thickening of the muscular layer of terminal bronchioles, compared to the control group, appears in the 3rd experimental group on the 36th day of observation - 13.67±0.17 μm (see Table 2). On the 44th day of observation, there is a tendency for the thickness of the muscular layer in terminal bronchioles approaches those in the control group.

In the intact group, the adventitial layer thickness in terminal bronchioles is 13.08±0.19 μm. There is no statistically significant difference between these parameters in animals of the intact and control groups. There is a tendency to the increase in the adventitial layer thickness of terminal bronchioles during all periods of observation after OVA sensitization and challenge (see Table 2). The statistically significant thickening of the adventitial layer in terminal bronchioles, compared to the control group, in animals of the 3rd experimental group is 18.76±0.1 μm, which is 1.5 times more than in the control group.

Discussion

Airways hyperresponsiveness develops as a result of bronchial allergic inflammation, caused by OVA sensitization and challenge [10]. The mechanism of inflammation is a cascade of processes involving neuroendocrine and immunocompetent cells, cytokines and mediators, the interaction of which forms the inflammatory process and the bronchial remodeling caused by it [14, 15].

Thus, this study determines the regularity of the dynamics of the structural elements of the wall of bronchioles and terminal bronchioles. The most significant and reactive changes in morphometric parameters of the wall of bronchioles were established on the 23rd and 30th days of observation - the thinning of the mucosal layer and on the 36th day - the thickening of the muscular layer and...
the adventitial layer. In our opinion, the fact of the thinning of the mucosal layer in bronchioles is associated with alternative phenomenon in the early period of the development of airways allergic inflammation, namely the epithelial destruction and desquamation. Muscular hypertrophy in bronchioles is the morphological confirmation of the development of bronchial hyperreactivity as a result of OVA sensitization and challenge, which is also confirmed by the narrowing of the bronchial lumen, probably due to bronchospasm. The increase in the thickness of the adventitial layer of bronchioles is a confirmation of the development of the inflammatory process in the connective tissue, and, as a consequence, the development of stromal edema and disorganization of fibrous elements in the connective tissue of the bronchial wall. A similar trend of morphological changes in the bronchioles is observed in the studies of other scientists [1, 2, 3, 16]. The most significant changes in the terminal bronchioles, confirmed statistically, found on the 36th day of the experiment in the form of thickening of the mucosal and adventitial layers, is a consequence of muscular hypertrophy and edema of the connective tissue stroma in terminal bronchioles. It should also be noted the multidirectional reaction of the mucosal layer in terminal bronchioles in the early and late periods of the development of allergic inflammation.

In the future, we aim to study ultramicroscopic changes of epithelial cells, basement membrane, connective tissue stroma of bronchioles and terminal bronchioles of guinea pigs with allergic inflammation.

Conclusions

1. It has been established that OVA sensitization and challenge of experimental animals cause morphological and functional changes in the structural elements of the wall of bronchioles and terminal bronchioles, which have the staged, mainly multidirectional character and correspond to the main morphological manifestations of allergic inflammation.

2. Confirmation of the development of hyperreactivity of bronchioles and terminal bronchioles in experimental animals is muscular hypertrophy and narrowing of their lumen, which are most pronounced in the late period of development of allergic ovalbumin-induced inflammation (36th day of the experiment).

References


МОРФОМЕТРИЧНА ХАРАКТЕРИСТИКА ДИСТАЛЬНИХ ВІДДІЛІВ ДИХАЛЬНИХ ШЛЯХІВ МОРСЬКИХ СВИНОК, СЕНСИБІЛІЗОВАНІ ОВАЛЬБУМІНОМ

Попко С.С., Євтушенко В.М.

Актуальною проблемою морфології та медицини в цілому, а також одним із недостатньо досліджених явищ у вивченні морфологічних змін дихальних шляхів при алергічному запalenні в хронобіологічному аспекті залишається реакція структурних компонентів малих бронхів та термінальних бронхіол. Мета роботи - дослідити морфометричні параметри дистальних відділів дихальних шляхів морських свинок, сенсибілізованих овальбуміном. За допомогою гістологічного, морфометричного та статистичного методів вивчили легені 48 самців морської свинки в умовах експериментального овальбумін-індукованого алергічного запалення, яке моделювали шляхом триразової підшкірної сенсибілізації та наступної 8-денної інтрназальної інгаляції овальбуміном. Для дослідження морфометричних показників структурних елементів малих бронхів і термінальних бронхіол визначали товщину їх слизової, м'язової, та адвентиційної оболонок. Встановлено реактивні зміни метричних показників стінки малих бронхів на 23 і 30 доби спостереження у вигляді потоншення слизової оболонки та на 36 добу у вигляді потовщення м'язової пластинки слизової та адвентиційної оболонок, що супроводжувалося зменшенням просвіту бронхів. Найбільш значними зміни у термінальних бронхіолах, підтверджені статистично, виявлені на 36 добу експерименту у вигляді потовщення слизової та адвентиційної оболонок, що є наслідком гіпертрофії гладеньких міоцитів і набряку сполучнотканиної строми термінальних бронхіол. Таким чином, сенсибілізація та алергізація овальбуміном експериментальних тварин викликають в структурних елементах стінки малих бронхів і термінальних бронхіол морфофункціональні зміни, які мають стадійний, переважно різнонаправлений характер і відповідають основним морфологічним проявам алергічного запалення з максимальними змінами протягом пізнього періоду розвитку (36 доба експерименту).

Ключові слова: дихальні шляхи, алергічне запалення, овальбумін, морська свинка.