APPLICATION OF OZONE IN THE TREATMENT OF PURULENT ENDOBRONCHITIS IN EXPERIMENT

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ABSTRACT

Experimentally studied the impact of the ozonized physiological tincture for treatment of purulent endobronchitis. Research was conducted on 36-guinea pig, modelling endobronchitis transferred by the method of A.T. Sultanov. For morphological observation purulent were taken the bronchus in the order of 2 and 3, simple root and situated far from the main root zone. Endobronchitis application of ozonized physiological tincture leads to the positive dynamic index of cellular structure of endobronchialcytogram and microbe pollution, which is characterized as inflammation process decrease.

I. INTRODUCTION

The chronic suppurative lung disease (CSLD) of children is continuing to remain actual problem of medical science. The main reasons of dissatisfying results of treatment children with CSLD are existence of endobronchitis, increase of intoxication and inefficient endobronchial sanitation. [2,3,4,9]

Studying the cellular structure of bronchial content is the simple and informative method of morphological diagnostics, which allows to identify type and level of the inflammation process of mucous membrane, in local cellular protected condition and judge about the effectiveness of treatment [1,5,7,9,11].

Pathological conditions caused by cell hypoxia and infection are also at the heart of the pathology of suppurative lung diseases in children against the background of inhibition of the body's reactivity. In this regard, over the past few years, ozone-oxygen therapy has found a worthy application among many other methods of treatment [8]. The high oxidizing ability of ozone is manifested in the form of bactericidal, virucidal and fungicidal properties. In the available literature, we met a small number of works devoted to the use of ozone in pulmonary pathology, especially its endobronchial use, there are no data on the degree of permissible ozone ratio in therapeutic mixtures for children. [5,6,8,10].

Aim of the research
Studying the impact of endobronchitis ozone during the purulent endobronchitis observation in the way of research the dynamic endobronchial cytogram, bacteriology and bronchial ultrastructure

II. MATERIAL AND METHODS OF THE RESEARCH

The research was conducted on the 36-guinea pig at the age of 1-1.5 months, weight of body 120-140 gr. Modelling endobronchitis realized with the method of A.T Sultanov [6], which was based on using antibiotic-persistent strain of golden staphylococcus. Animals under ether anesthesia were slowly instilled into the nasal passages of 0.4-0.6 ml. microbial suspension with a concentration of 4-5 billion microbial cells in 1 ml. with parallel chest massage. After awakening, the animals were cooled in a bath at a temperature of 0 + 4 C. The moment when the animals stopped free swimming and began to perform sweeping movements with a simultaneous small tremor of the head and limbs was noted. These symptoms usually preceded termination. At this moment, the animals were taken out of the water and placed in a dry cage at a temperature of 18-20 C. Within 0.5-3 days, the animals developed signs of the disease, which were manifested by cyanosis of the nose and ears, sneezing with secretion from the nose, disheveledness, wool, refusal to eat, anxiety.

All the animals were divided into three groups. Healthy animals 1 group served as a controller. On the 3rd, 6th and 9th days, the animals of the 3rd group underwent endobronchial ozone therapy, which was performed by washing the bronchi with a bubbling saline ozone-oxygen mixture (with a concentration of 5 mg / l. OTRI-01 apparatus).

Animals of the 2nd group at the same time were washed out of the bronchial tree with physiological 0.9% sodium chloride solution.

On days 4.7 and 10, i.e. the next day after endobronchial treatment, animals of groups 2 and 3 were simultaneously withdrawn from the experiment.

For morphological examination, samples of bronchi of the 2nd and 3rd order, of the lung root and far from the root zone were taken. Cell for light-optical studies was fixed in 12% formalin solution, paraffin sections were stained with hematoxylin-eosin. For transmission electron microscopy (TEM), the cell was fixed in a 2.5% solution of glutaraldehyde in phosphate buffer, after dehydration in acetone alcohol, it was embedded in epon-araldite. Ultrathin sections after double contrasting were examined in TEM (Hitachi-H-600, Japan). For scanning electron microscopy (SEM), samples were similarly fixed, dehydrated by the critical point method (NSR-2 apparatus) and, after sputtering with ionic gold (-IB-3 apparatus), were examined in SEM (Hitachi S 405 A, Japan).

Diagnostic bronchoalveolar lavage was performed after slaughtering animals. To do this, the trachea is opened, a flexible sterile catheter is inserted into its lumen, and a sterile saline solution is washed in an amount of 2-3 ml.
The lavage fluid was collected in a sterile trap. The ratios of alveolar macrophages (AM), neutrophilic leukocytes (NL) and lymphocytes (L) were studied in smears from a centrifugate of broncho alveolar lavage fluid (BALF), stained according to Romanovsky-Giemsa based on counting 100 cells.

For bacteriological research, the homogenized material was inoculated on 5% blood agar and identified based on the study of morphological properties.

III. RESULTS AND DISCUSSION

On animals of second group in 4 days under the cytogram revealed the big amount of degenerated neutrophils and cellular detritus, not big amount of macrophages without functional activity. During the bacterial researches in BALF revealed staphylococcus, mud puppy, lambda and their combination, semination raised to $10^5$.

Contrasting character endobronchial cytogram on animals presented in the table.

### Table 1. Endobronchial cytogram on observed animals (on %)

<table>
<thead>
<tr>
<th>Cellular elements</th>
<th>Cont. health group</th>
<th>Three days after the modelling (before the treatment)</th>
<th>Endobronchial cytogram</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Three days</td>
<td>4 days</td>
</tr>
<tr>
<td>Neutrophil Lymphocyte (NL)</td>
<td>1,9±0,3</td>
<td>90,4±6,2</td>
<td>40,2±1,3</td>
</tr>
<tr>
<td>Lymphocyte (L)</td>
<td>1,7±0,3</td>
<td>7,5±1,3</td>
<td>13,5±0,63</td>
</tr>
<tr>
<td>Alveolar macrophage</td>
<td>35,6±5,6</td>
<td>2,1±0,4</td>
<td>36,3±1,49</td>
</tr>
</tbody>
</table>

Note: This statistic reliable on the contrast with II group ($P<0,01$).

From the table it is vivid that ozonotherapy causes significant change on endobronchial cytogram proving the reduction of inflammation process, raising the local cellular protection and activation of phagocytosis.

Histological research revealed existence of change inflammational character. Mucous membrane of bronchus puffed, unfiltered polymorph cellular elements becomes desquamation of epithelial cellular

SEM showed expansion of epithelial lining intercellular slots. Among epithelia occur pituitary cellular with domelike surface. On the surface of epithelial lining are being detected spinning tear and collected various migrated cellular of blood and stroma.
Cellular and spinning tear on the surface of epithelial lining. Third day of observational endobronchit. SEM x 1000

Under the TEM in the Neutrophil Lymphocyte has the place for phagocytosis microorganisms, on the surface NL is defining singular outgrowth, invagination of plasmatic membrane. In the cytoplasm is being noted vacuolization, reduction of grainy endoplasmic cellular and independent ribosome profiles. Phagocytosed microorganisms don’t face the visible ultrastructural changes, showing their lysis.

Microorganisms (MO) in the polymorphonuclear leukocytes. 3rd day of experimental endobronchit. TEM x 7500

During the one week, modelling enlarged degenerated NL, phagocytosis activity seldom seen, raised desquamation epithelial cellular and gathering on the light bronchus conglomerate from desquamated cellular, lymphocyte, neutrophil lymphocyte and glair. Walls of the bronchus and zone around it unfiltered heavily with polymorph-cellular elements.
Edema and infiltration of sept, big amount of cells and mucue in light of alveolar. 7th day of experimental endobronchit. SEM

CEM showed not big gathering of glair, epithelial and intercellular on the light of alveolar and the around the bronchus. At the same time, several parts of the alveolar kept puffiness, despite on their lightrevealed migrating erythrocytes and cellular of connecting webs.

On the 10th day in cytogram noticed several decreases of NL and their phagocytosis activity are not big, increasing the amount of the alveolar macrophages are statistically unreliable. On the bacterial researches of BALF revealed the staphylococcus, mud puppy, lambda where their semination were until 10^5.

During the experiment from this group fell three animals.

In the animals of third group have already once co-acted with ozone, leading to a decrease the number of degenerated neutrophils on endobronchial cytogram, increasing percentage correlation of alveolar macrophages with restoration of their functional activity.

Histological research showed the decrease of inflammation changes in bronchus. Desquamation epithelia is decreasing, tear-producing tissues of bronchus are restoring, but in some cases, at the walls of bronchus are being continued producing significant gathering of polymorph tissues. Weakly formed desquamation tissues of epithelia lining are being kept and after twice co-action with ozone. However, inflammation changes of the bronchus walls in significant level reduced. Preserves only weakly described edema.
Decreasing appeared edema and migration in alveolar. First short co-action with ozone. SEM x 300

Lysis microbus (LO) in polymorphonuclear leukocytes. First short co-action with ozone. TEMx7500

TEM showed up in the neutrophilic leukocytes and epithelia lining of phagocytosed microbus in the condition of intensive phagocytose.

After three-times session of ozone in the endobronchial cytogram slightly decreased the amount of degenerated neutrophilic leukocytes, the number of AM significantly increased and their phagocyte action raised. In the bacterial researches of BALF in singular cases found colibacillus and mud puppy and their semanation were under the $10^3$. 
In histological research, the whole inflammation change in the bronchus wall was not observed. Not big infiltrates were continuously being produced in them. However, lights of the bronchus were independent from desquamating migrated cells and tear. Overall, restoring structure of alveolar and their airiness.

CEM also revealed restoring structure of alveolar. In septum are being defined the cells that are particularly combined for them, seldom is met erythrocytes

IV. CONCLUSION

1. Cytological and microbiological research BALF is informative enough test that characterize activity and dynamics bronchus-lung process in experiment.

2. Endobronchial application ozone-physiological fluid causes positive dynamic to indexes of cellular structure endobronchial cytogram and microbial semination which characterizes reduction of inflammation process.

3. Results of light-optical and electromicroscopical researches certify about high – effective ozonized physiological fluid in the purulent endobronchitis experiment.

REFERENCE LIST


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