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MORPHOLOGICAL CHARACTERISTICS OF LUNGS IN THE DYNAMICS OF DEVELOPMENT OF A NEW EXPERIMENTAL MODEL OF SURGICAL SEPSIS

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ABSTRACT

This is an experimental study of lung morphology in modeling sepsis on a background of severe purulent-inflammatory disease of soft tissues (necrotizing fasciitis) by original authors' method. The study showed that early stages of sepsis (1-3 days) were characterized by manifestations of changes in lung tissue as vascular response, in the second period (the 7th day) by appearance and growth of non-obstructive microatelectases, whereas the third period (the 14th day) was characterized by progression of purulent-necrotic processes in soft tissues, development of surgical sepsis and acute respiratory distress syndrome. These changes in morphological structure of lung tissue are specific for generalized septic process and consequences of necrotizing fasciitis with its septic complication.

UDC CODE & KEYWORDS

616.29-08.30.14 Surgical sepsis Generalization of infection Pulmonary atelectasis Endothelial damage

INTRODUCTION

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Surgical sepsis is one of the most important etiological factors of syndrome of acute lung injury which is transformed in extrapulmonary type of acute respiratory distress syndrome at formation of hyaline membranes in the alveolar lining (Adroge et al., 2008; Bhatia et al., 2008).

Bagdatyev et al. (2006), Kassil et al. (2008) and Lewis et al. (2007) noted that multifactorial nature of pathogenesis, polymorphic clinical symptoms, and absence of clear diagnostic criteria are the features of acute respiratory distress syndrome in surgical sepsis that determining treatment tactics. So, we can assume that despite of intensive studies on the issue of acute respiratory distress syndrome, both clarity of pathogenesis and effectiveness of therapy are far from being resolved. Evidence of this is the highest mortality rates for this complication, reaching for burns and injuries to 18.6% (Adroge et al., 2008), and in severe forms of chronic inflammatory diseases of soft tissues up to 74.6% even in those institutions that specifically deal with this problem. In this regard, studies aimed at identifying the features of ultrastructural structures of lung tissue in the dynamics of development of experimental models of surgical sepsis are relevant issue.

Certainly, infection plays the important role in the pathogenesis of acute respiratory distress syndrome in sepsis. According to Ashbaugh et al. (1967), hematogenous infection of lungs with further development of pulmonary respiratory failure in bacteremia is almost inevitable. However, variety of pathological source and its nature (purulent, necrotic, putrid, etc.) is of considerable importance in the progression of sepsis.

The purpose of our study was to study lung morphology in modeling sepsis on a background of severe purulent-inflammatory disease of soft tissues.

Material and methods

Experiments were done in 36 outbred rabbits of both sexes weighing 1500-2500 g, being fed standard laboratory rations. Each group consisted of 12 rabbits. All animals were divided into two groups:

- Control group 12 intact (uninfluenced) rabbits (without modeling of pathological process);
- Main group 24 rabbits with experimental model of sepsis on a background of severe purulent-inflammatory disease
 of soft tissues (necrotizing fasciitis type I).

Experimental model of sepsis was reproduced on the background of necrotizing fasciitis type I according to our original method. Modeling was performed as follows: rabbits on an empty stomach were injected intraperitoneally with antilympholine-Cr at a dose of 0.03 mg per 100 g of animal weight under ether anesthesia within two days. At the third day, five points of back of animal were injected subcutaneously with 3-4 ml of 30% suspension of animal autoexcrement diluted with 10% solution of calcium chloride.

Slaughtering of animals was performed taking into account the recommendations of the European Committee for the humane treatment of laboratory animals at the 1, 3, 7 and 14 days after introduction of autoexcrement suspension into soft lumbar regions of animal.

Pieces of organ were fixed in formalin and glutaraldehyde using a standard method. Coloration of tissue sections was carried out by hematoxylin-eosin and fuxin-methylene blue.

Results and discussion

After modeling surgical sepsis on the background of necrotizing fasciitis, we macroscopically revealed irregularity of blood supply in lung parenchyma over all periods of observation, and at later period cyanotic acinar and subsegmental sinking parts, and small subpleural hemorrhages.

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Light-optical microscopy found progression of histological changes in tissue and predominant changes in vessels of microvasculature of lungs.

For 24 hours after modeling necrotizing fasciitis in rabbits, in lung tissue were observed changes mainly of vascular nature. In this regard, changes in blood vessels in the form of narrowing of the lumen through a spasm of venules were combined with vasodilatation and increased concentrations of blood cells, mainly leukocytes (Figures 1-2).



Adroge et al. (2008) and Bhatia et al. (2008) noted such antagonistic relationship of vascular response with progressive increase of blood flow volume. Capillaries and small venules were in collapsed state, endothelial cells were bulging into the lumen of microvessels where there was increase in the number of neutrophils and monocytes. Adjacent sections of lung tissue were characterized by a mosaic combination of lightness of the alveoli. In the lumen of the alveoli with reduced lightness was detected a large number of blood cells, mostly erythrocytes, whereas in uncovered area of the alveoli there was a cluster of microaggregates in capillaries (Figures 3-4).



Source: Author

Source: Author

At the 3rd day of development of surgical sepsis on the background of necrotizing fasciitis, macroscopically was observed intensification of hyperemia in lung tissue. Microscopically, in pulmonary capillaries, predominantly in venules, there was formed stasis and erythrocyte aggregation. Plethora in capillaries and sites with intraalveolar edema were increased. Aggregation of erythrocytes in microvascular bed acquired the character of massiveness. In physiological areas prone to the maximum of possible blood stasis, appeared "blood lacunae" which blocked the downstream areas of aerohematological barrier (Figures 5-6).

Blood sludge in subbasal areas of microvascular shunts as evidenced by the same pattern in the straightened and collapsed alveoli is a distinctive feature. These sites of lung microvessels were characterized by blood clots and accumulation of leukocytes.

Edema in interstitial tissue increased possibly due to formation of erythrocyte aggregates in veins, venules and capillaries of the lungs. This led to significant changes in cellular and extracellular components of the alveoli.

Increase in size and change of shape of alveocytes are evidence of their swelling both in alveocytes of I and II types. Multiple ruptures of membranes and strongly deformed Kohn pores were combined with presence of sites of non-obstructive microatelectases (Figures 7-8).

The 7th day of experimental model of surgical sepsis with necrotizing fasciitis was characterized by growth of manifestations of congestion and stasis of morphological structure of lung tissue that manifested by massive accumulation of blood cells in capillaries and venules. This, in turn, has contributed to massiveness of thrombus formation over all large areas of the lung. Interendothelial capillary gaps widened.

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In lung parenchyma were found non-obstructive microatelectases of disseminated nature in which alveocytes of type II had enlightened sites. In the cytoplasm of endothelial cells were observed large vacuoles. There were marked swelling of the interstitium, hemorrhage, and foci of inflammation around microatelectases. In the alveolar lumen were appeared fluid rich for protein, filaments of fibrin, and desquamated alveolocytes (Figures 9-10).

Increase in pulmonary capillary occlusion and endothelial cell swelling was accompanied by increased formation of microatelectases, whereas extracellular fluid in the lungs was increased insignificantly (Figures 11-12).

Signs of expression of vascular permeability were increased. Changes occurred not only in endothelial cells but also in cells of the alveolar epithelium. Edema increased, particularly in larger vessels.



Source: Author

Significant changes in deformation of the alveolar walls, the presence of atelectasis areas of lung tissue and foci of inflammation were observed. There was noted severe destruction and sometimes ruptures of cell membranes, swelling and loosening of the basal membrane. We also observed sites of lamination of the alveolocapillary membrane.

In some areas the cells exfoliated from the basal membrane with penetration and accumulation of a large number of erythrocytes. There was significant swelling of endothelial cells of the vascular wall with their protrusion into the lumen, as

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Source: Author

well as significant swelling of alveolocytes of I and II degrees with disturbance of their shape. We revealed edema and cellular infiltration of the interalveolar septa and interstitial space surrounding the airways, stasis of erythrocytes, platelet and granulocyte aggregates in foci of alveolar and interstitial hemorrhages (Figures 13-14).

Figure 13. Edema in pulmonary vessels at the 7th day of modeling surgical sepsis. Colouring with hematoxylin-eosin. Magnification: ob x 40, oc x 15.	Figure 14. Deformation of the alveoli, gap between its sites, swelling and loosening of the basal membrane of lung cells at the 7th day of experiment. Colouring with hematoxylin-eosin. Magnification: ob x 40, oc x 15.
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Source: Author

Source: Author

Hyperplasia and dysplasia of granular alveocytes were often found. There were formed hyaline membranes in alveolar walls which were accompanied by destruction of interalveolar septa. In the alveolar lumen was marked fibrin. Formation of hyaline membranes was combined with other proliferative changes in the form of fibrosis of lung tissue (Figures 15-16).



Source: Author

Source: Author

At the 14th day of modeling surgical sepsis with necrotizing fasciitis, the intensity of pathological changes decreased despite increase of pathological changes in lung tissue of rabbits. The number of non-obstructive microatelectases of disseminated nature increased. Microatelectases were formed in areas of lung tissue with alveolar decay.

The number of hyaline membrane increased, fibrosis and obliteration of the alveoli developed. Along with strengthening of pathological changes described at the 7th day of experiment, sometimes detachment of alveocytes of type I from the 37 http://health.journals.cz





basal membrane was observed. In peripheral regions of the lungs, alveocytes of II type saved foci of enlightenment with increase in number and size of vacuoles. Dystrophic changes in alveocytes were intensified up to the individual sites of the collapse of these cells. In addition to alveocytar dystrophy, we observed desquamated cells in the alveolar lumen.

In the lumen of capillaries were observed thrombosis formed and aggregates, there were signs of intraalveolar edema. In addition to erythrocytes aggregates or stasis, the number of leukocytes, particularly monocytes, was increased in the lumen of vascular bed of pulmonary basin.

In the lumen of alveoli, along with erythrocytes, were appeared small granular masses that characterized deterioration of quantitative and qualitative morphological states of the lung tissue.

Endothelial cell cytoplasm contained a large number of vacuoles. Endothelial cells themselves in most cases had irregular shape.

The number and sizes of atelectasis, as well as the number of capillaries with stasis with microthrombus increased, and more increased interstitial and intraalveolar edema (Figures 17-18).

Figure 17. The increased number of sites of pulmonary atelectasis with interstitial and alveolar edema, as well as abscess formation at the 14th day of modeling surgical sepsis. Staining with hematoxylin-eosin. Magnification: ob x 40, oc x 15.

Figure 18. Formation of small granular masses, strands of fibrin, and products of alveocytar degradation in the lumen of the pulmonary alveoli with interstitial and alveolar edema, as well as vascular thrombosis at the 14th day of modeling surgical sepsis. Staining with hematoxylin-eosin. Magnification: ob x 40, oc x 15.



Source: Autho

Source: Author

Against the background of intraalveolar edema, erythrodiapedesis, increase in the number of atelectasis sites, we noted the appearance of small foci of pneumonia and abscess formation centers. In particular, we observed that small focal pneumonic areas were located mainly in the peripheral regions of the acini, occupying the region of branching of respiratory bronchiole of III degree and alveolar paths. These foci in the lumen of the alveoli had small granular masses, filaments of fibrin, and products of alveocytar degradation, as well as granulocytes and macrophages. There were preserved aggregation stasis of blood cells and formed blood clots in the vast areas in capillaries and venules.

Thus, in the early stages of modeling surgical sepsis in pulmonary capillaries we noted formation of erythrocytes aggregates. This process is more pronounced in venules where blood flow is significantly reduced as a result of progressively developing dilatation, apparently, due to the products of local metabolism. Then the aggregation is disseminated to other vessels. As results of that, reduction of blood flow velocity, separation of plasma from erythrocytes, multiple microthrombogenesis, as well as the phenomenon of stasis in the venules, capillaries and sinusoids lead to development of tissue hypoxia in many organs. After modeling of surgical sepsis in the early stages, there is a large number of capillaries filled with only plasma, and in some capillaries plasma flow is stopped, while in other ones perfusion of plasma through the capillaries continues.

In the dynamics of pathological process microcirculatory disorders increase, resulting in diffuse erythrocytes aggregation, embolism and thrombosis. Other studies conducted by Lewis et al. (2007) and Veldhuizen et al. (1996) attributed this phenomenon by plasma separation and disclosure of arteriolar-venular anastomoses, especially in lung tissue.

According to Kassil et al. (2008), Rappoport et al. (1990)[3,4,5], shunt blood flow that increasing in pathological conditions, when the capillaries are blocked, indicates high adaptive capacity of the organism as a whole and cardio-vascular system in particular. However, [2,6] considered that full compensation does not occur, and develop such disorders as stasis, thrombophlebitis, and tissue acidosis.

Conclusion

Our study showed that early stages of sepsis (1-3 days) were characterized by manifestations of changes in lung tissue as vascular response, having in most cases functional and compensatory character.

In the second period of experimental surgical sepsis on a background of necrotizing fasciitis (the 7th day) changes in the structure of lung tissue beyond the scope of vascular reaction, and were characterized by appearance at first, and subsequently growth of the number of non-obstructive microatelectases which acquired disseminated nature.

The third period (the 14th day) was characterized by progression of purulent-necrotic processes in soft tissues, development of surgical sepsis and acute respiratory distress syndrome, which was accompanied by appearance of fluid rich for proteins and fibrin strands in the lumen of the alveoli, as well as alveolocytes exfoliation. The alveolar walls acquired irreversible morphologic character of changes and accompanied by formation of hyaline membranes which are known capable to disrupt oxygen diffusion. Interalveolar septum destroyed, sometimes fibrosis and obliteration of the alveoli developed, foci of abscess formation appeared. These changes were not reversible.

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Thus, changes revealed in morphological structure of lung tissue allowed determining the number of changes that specific for generalized septic process. This, in turn, confirms that the changes in lung tissue are the consequences of necrotizing fasciitis with its septic complication.

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