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PATHOMORPHOLOGICAL CHANGES OF AORTA IN FETUSES AND NEWBORNS EXPOSED TO EXPERIMENTAL MATERNAL ESCHERICHIOSIS

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Abstract

Intrauterine infections occupy a leading place in the perinatal mortality structure. The aim of this study was to identify the morphological features of the aorta in progenies born from mothers with subacute prolonged infectious and inflammatory process. Two experimental studies were performed on WAG line laboratory rats. The comparison group consisted of newborns died due to acute postnatal hypoxia. The main group included fetuses and newborns born from Escherichia coli infected mothers. The differences between the vessels of two study groups were not observed in macroscopic examination. In the group of progenies born from infected mothers, the average thickness of the aorta increased in comparison with the group under hypoxia influence, due to the inner and middle membranes, which can be interpreted as the sclerotic changes development. There was tunica adventitia volume increase in the group with hypoxia, which can be explained by edema caused by increased vascular permeability. Morphological signs of endothelial dysfunction were found in both study groups, which were expressed in the endotheliocytes flattening with their subsequent desquamation more pronounced in the group with hypoxia. It is apparently related to the process severity. There is a violation of the elastic and collagen fibers ratio towards the

second in the vessel wall under the hypoxia influence, which reduces the elastic properties of the aorta.

Key words: aorta, fetuses, newborns, acute postnatal hypoxia, intrauterine infection.

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Abstract. The level of infant mortality is one of the most important indices of the development of the world society. Despite the achievements of medicine, this indicator remains high in our country, as well as in the whole world, and according to the WHO statistics in different countries, comprises between 20 and 50 per 1000 newborns [1]. Among the possible causes of childhood disability, 70% are due to perinatal factors [2, 3, 4]. Intrauterine infections (IUI) occupy one of the first places in the structure of perinatal mortality. According to statistics, the incidence of IUI in Ukraine is from 6 to 53%, and in preterm infants reaches 70% [5], 14.9-16.8% die before birth [6]. According to international studies, Escherichia coli is the main causative agent of infectious pathology of the genitourinary tract, which is detected in 75-90% of cases [7]. Infectious-inflammatory diseases caused by conditionally pathogenic microorganisms can lead to reproductive function disorders [8], preterm births, intrauterine infections of the fetus [10], premature abruption of placenta, antenatal death, fetoplacental insufficiency, and as a consequence, to hypoxia. Fetoplacental dysfunction due to placentitis leads to fetal hypoxia and birth of children with low body mass. Intrauterine hypoxia can trigger postnatal asphyxia, increasing the effect of the damaging factor, which leads to a malfunction in the body of the newborn, with the involvement of cardiovascular system in 40-70% of cases [11, 12, 13]. Intrauterine infection results in disability in 50% of children [14, 15]. The literature contains information on morphological features of the aorta in offspring born from mothers with complicated pregnancy. So far there have been numerous experimental studies analyzing the impact of chronic intrauterine hypoxia [16], preeclampsia of different degrees of severity [17, 18] and arterial hypertension on the morphofunctional state of the aorta, and the state of the cardiovascular system in children with intrauterine growth restriction syndrome [19].

However, the impact of maternal infection on pathomorphological structure of the aorta in fetuses and newborns is not sufficiently studied.

The purpose of the study was to reveal morphological features of the aorta in laboratory animals (fetuses and newborn rat pups) born from mothers with subacute prolonged infectious-inflammatory process.

Object and methods of research To accomplish the objectives, two experimental studies were conducted on laboratory WAG rats. The first trial involved generating high altitude hypoxia in a pressure chamber in the conditions of a sharp decrease in atmospheric pressure. For simulation of acute postnatal hypoxia, newborn rat pups in the first days of life were once exposed to high altitude hypoxia (which corresponded to the altitude of 7,500 meters) for 20 minutes, and then withdrawn from the trial, being included in the comparison group. The second trial, the task of which was to study the impact of maternal infection on the state of fetuses and newborns, was carried out in two stages. The first stage implied determination of an infective dose for the development of subacute infectious-inflammatory process. Reference strains of Escherichia coli (ATCC 25922 (F50)) were used as an infectious agent. At the second stage subacute prolonged infectious-inflammatory process was modeled in the abdominal cavity of the female rats, after which the rats fell pregnant, and the offspring (fetuses and newborns) derived from them were withdrawn from the trial for further study. Euthanasia of laboratory animals was carried out by overdosing of sodium thiopental and subsequent decapitation, following ethical standards in accordance with the rules of the "European Convention for the Protection of Vertebrates, Used for Experimental and Other Purposes" (Strasbourg, 1986).

During the autopsy, a macroscopic study of the aforementioned vessel was performed, with further sampling for morphological examination. For the manufacture of micro-preparations, the obtained material was fixed in a 10% solution of neutral formalin, subjected to standard paraffin carrying through spirits of increasing concentrations, Nikiforov solution (96% alcohol and diethyl ether 1:1), chloroform, and then poured with paraffin. The prepared paraffin blocks were used for making slices 4-5×10-6m thick, which were then stained with hematoxylin and eosin, with Mallory staining, and picrofusin with van Gieson staining. The preparations were studied on an "Olympus BX-41" microscope with the Olympus DP-soft version 3.1 software processing. The digital material obtained in the study was processed using mathematical statistics on a computer using the IBM SPSS Statistics 22 software. A nonparametric statistical criterion, Mann-Whitney U-criterion (Mann-Whitney U test), was used to evaluate the differences between the two samples.

Results and discussion. Macroscopic examination of the aorta was performed using a magnifying glass (× 3.8 diopters). In the two groups under study, its wall was elastic, with a shiny and smooth inner sheath, of ivory color. Microscopic examination of the vessel revealed differences between the groups.

The aortic wall in the newborns rat pups exposed to acute postnatal hypoxia consisted of the outer (tunica adventitia), middle (tunica media) and inner (tunica intima) sheaths. Tunica adventitia was made up of elastic and collagen fibers, sometimes full-blooded vasa vasorum, nervi vasorum without signs of dystrophy, lymphatic vessels. Van Gieson picro-fuchsin staining of the aorta showed an increase in fuchsinophilia, indicating the presence of sclerotic processes. Bundles of elastic and collagen fibers formed tunica media. Mallory staining showed relative volumes of elastic and collagen fibers. In rat pups with hypoxia, collagen fibers predominated in the wall of the vessel with a relative volume of 52.0 ± 10.65% (which was significantly higher in relation to the first group) ($p < 0.05$), the relative volume of elastic fibers was 48.14 ± 10.65%. The obtained indices confirm the available literature data on the ability of hypoxia to enhance collagen formation [20]. Tunica intima was represented by endothelium, a subendothelial layer and a plexus of elastic fibers. The relative volumes of the inner and middle sheaths were 48.26 ± 14.42%, the outer sheath comprised 51.89 ± 14.42%. In our opinion, an increase in the volume of the adventitious membrane in comparison with the findings in the main group may be due to the edema triggered by an increase in vascular permeability resulting from acute hypoxia [21]. The endothelium consisted of single-nuclear polygonal cells located on the basement membrane. On average, the width of the endothelial cells was 5.86 ± 0.21 × 10-6 m, the height was 2.37 ± 0.07 × 10-6 m, indicating flattening of the endothelial cells in comparison with the indices of the main group. The average area of the nuclei was 8.87 ± 0.44 × 10-11 m2, the average area of the cytoplasm was -8.33 ± 0.66 × 10-11 m2, the nuclear-cytoplasmic ratio (NCR) was 1.16 ± 0.08. The area of desquamation fields was 4.14 ± 0.51 of a sample (× 1000). The subendothelial layer was represented by a fibrous connective tissue and star-shaped cells.

The aortic wall in the progeny born to mothers with subacute prolonged infectious-inflammatory process caused by Escherichia coli also had three layers (tunica adventitia, tunica media, tunica intima). Tunica adventitia was edematous, formed by a large number of elastic and collagen fibers, lymphatic vessels, nerve fibers without signs of dystrophy, moderately full-blooded blood vessels. Examination showed moderate sclerotic changes, as indicated by enhancement of fuchsinophilia in Van Gieson picro-fuchsin staining.

Tunica media of the aorta was formed by elastic and collagen fibers. In the offspring born to infected mothers, elastic fibers predominated in the wall of the vessel, the relative volume of which was 60.99 ± 8.65%, the relative volume of collagen fibers was 39.80 ± 8.65% (which was significantly different from that of the comparison group , $p < 0.05$). When compared to the group of newborns who suffered from hypoxia, the indices of relative volumes of inner and middle sheaths increased significantly and amounted to 61.68 ± 9.92% ($p < 0.05$), the volume of adventitia decreased to 38.48 ± 9.92% ($p < 0,05$), which can be interpreted as the development of sclerotic changes in these aortic membranes due to the prolonged action of the pathological factor.

The endothelium consisted of cells located on the basal membrane, with an average width of 6.82 ± 0.15 × 10-6 m and a height of 2.77 ± 0.04 × 10-6 m (which was significantly different from those of the first group ($p < 0.05$). The average area of the nuclei was 9.74 ± 0.44 × 10-11 m2, the average area of the cytoplasm was 8.96 ± 0.46 × 10-11 m2, which was significantly higher than in the previous group ($p < 0.05$), the nuclear-cytoplasmic ratio was 1.12 ± 0.06 ($p < 0.05$). The number of desquamated cells decreased and amounted to 3.37 ± 0.26 endothelial cells in the field of vision (× 1000), which significantly differed from the group that underwent acute postnatal hypoxia ($p < 0.05$) These results may be explained by the fact that subacute prolonged infectious-inflammatory process triggered compensatory and adaptive changes, and hypoxia, having an acute nature, exerted a stressful effect on the vessels, which caused an increase in desquamation of endothelial cells. The subendothelial layer was formed by loose connective tissue and star-shaped cells.

Conclusion

1. In the group of offspring born to mothers with subacute prolonged infectious-inflammatory process caused by Escherichia coli, the average thickness of the aorta increased as compared to the group of newborns who suffered from acute postnatal hypoxia, due to inner and middle sheaths, which reduced the nourishment and decreased the elastic properties of the aortic walls. On the contrary, the group with acute postnatal hypoxia was found to have an increase in the volume of the adventitious membrane, which can be attributed to the edema due to an increase in vascular permeability resulting from acute hypoxia.

2. The aorta of the two groups under study was shown to have morphological signs of endothelial dysfunction, which was expressed in flattening of the endothelial layer, in some fields of view, “palisade” location of the endothelial cells, with their subsequent desquamation, more evident in the group undergoing acute postnatal hypoxia, which was probably due to the severity of the process.

3. Exposure to acute postnatal hypoxia in the aortic wall in animals triggered an impairment of the ratio of collagen and elastic fibers towards the former, which reduced the elastic properties of the vessel. The revealed changes can cause a cardiovascular abnormality in future.

Prospects for further research. This research will be followed by a immunohistochemical study.

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