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Morphological features of endothelial cover of pulmonary artery and aorta at fetuses and newborns after chronic intrauterine hypoxia (an experimental study)

ABSTRACT

Morphological features of pulmonary artery and aorta in fetuses and newborns have been studied in order to determine morphological changes in endothelial cover of the pulmonary artery and the aorta in fetuses and newborns affected by chronic fetal hypoxia (CFH) as a result of the experiment on laboratory WAG rats on modeling fetal hypoxia of newborns with asphyxia in delivery. According to results of the research in the artery and the aorta of fetuses and newborns affected by CFH deterioration of trophic processes in the endotheliocyte has been found due to thickening of basal membranes. It causes cells flattening, decrease of adhesive properties which is evidenced by decreased expression of receptors CD 34 by endothelium, and their desquamation amplification. Also sclerotic changes in the basal membranes of both vessels have been also determined due to interstitial collagen type III appearance against type IV collagen deficiency.

Keywords: endothelium, pulmonary artery, aorta, chronic intrauterine hypoxia, experiment.

INTRODUCTION

Chronic intrauterine hypoxia is severe stressor that implements its negative influence on not only mother's but the child' body [2]. It determines development of many diseases of organs and systems in such offspring with leading position of cardiovascular diseases [6]. There is data about chronic oxygen insufficiency influence on morphological status of fetal venosus ductus [7], aorta of chick [11] and rats embryos [10], development of pulmonary hypertension with pulmonary vascular remodeling in the experiment [13] in modern literature. However, in our opinion these data are at times contradictory and does not describe comparison of morphological changes in the pulmonary artery and the aorta in fetuses and infants under the influence of chronic intrauterine hypoxia.

The aim of research is to compare morphological changes of endothelial cover of pulmonary artery and aorta in fetuses and neonates undergoing chronic intrauterine hypoxia.

MATERIALS AND METHODS

The experiment modeling intrauterine hypoxia of newborn with asphyxia in labor was

made on laboratory WAG rats. Pregnant female rats have been affected high-altitude hypoxic influence, which corresponded to 7500 m for 20 minutes each day at the same time, since pregnancy registration to delivery. Rats were divided into two groups: control group 1 – fetuses and newborns from mothers who were not exposed to high- altitude hypoxia (18 cases); *experimental* group 2 – fetuses and newborns from mothers who suffered from high-altitude hypoxic exposure (16 cases). Female and offspring were euthanized. The autopsies of animals were made, the tissue pieces of pulmonary artery and aorta were cut for morphological investigation. They were fixed in 10% neutral formalin solution, then subjected to standard paraffin preparation through increasing alcohol concentration, Nikiforov solution (96% alcohol and diethyl ether 1:1), chloroform, then followed paraffin filling. Prepared blocks were sliced on microtome Microm HM-340 into serial sections $4–5\times10^{-6}$ m. The complex of histological, histochemical, immunohistochemical, morphometric methods was morphological processing. Morphological and morphometric studies were performed on microscope Olympus BX-41 (Japan) using Olympus DP-Soft (Version 3:1) and Microsoft Excel 2010 programs, and fluorescent microscope «Axioskor 40» (Carl Zeiss, Germany). The slides were stained with hematoxylin and eosin, van Gieson's Picric Acid Fuchsin, and according to Mallory. Immunohistochemical study was done on paraffin sections ($5–6\times10^{-6}$ m thickness) with direct Koons' method by Brosman's methodology [9]. Collagens III, IV type were defined by monoclonal antibodies (mAbs) to the respective collagens (Novocastra Laboratories Ltd.). Adhesive properties of the cells were defined by mAbs to CD34 (Novocastra Laboratories Ltd.). Optical density of endothelial and collagen immunofluorescence was measured by method of Gubina-Vaculik G.I. and others [5] with a microscope "Axioskor 40" and software Biostat.exe and was represented in conditional units of luminescence (cond. un. lum.). The findings were worked up statistically with the license application package «Statistica 6.0» («Statsoft, Inc») on the PC. The methods of variation statistics have been used, veracity was determined by the Student t-test [3]. All manipulations with animals were carried out according to the rules of the European Convention for the Protection of Vertebrate Animals (Strasbourg, 18.03.1986), Directive Council of the European Society for Protection of Vertebrate Animals (Strasbourg, 24.11.1986).

RESULTS AND DISCUSSION

Macroscopic examination with a magnifying glass (×3, 8 diopters) showed that the intima of the pulmonary artery and the aorta was smooth and shiny without noticeable differences in

both groups. Some microscopicall differences were revealed in blood vessels of the control group, though in the literature they described identical structure [8]. Endothelial cover both in the pulmonary artery and the aorta was represented by mononuclear cells layer, which are located on the basal membrane closely to each other. The average height of the cells in the first vessel was $3,20\pm0,04\times10^{-6}$ m, and in the second – $3,44\pm0,06\times10^{-6}$ m, which was reliably to each other ($p<0,05$). The average width of endotheliocytes reached $7,12\pm0,15\times10^{-6}$ m for the pulmonary artery and $7,25\pm0,18\times10^{-6}$ m for the aorta, that had no significant difference between these values. The nucleus was stained evenly with hematoxylin and was located centrally, where there was a slight protrusion of cells into the vessel lumen. The cytoplasm was uniformly stained with eosin. There were determined 2-3 desquamated cells (2,40±0,09) in one field of view (×1000) in the pulmonary artery, and 1-3 cells (2,15±0,15) – in aorta, it had no significant difference between each other. Optical fluorescence density of endothelial cells was shown by CD 34 marker, which values were 0,495±0,01 cond. un. lum. for pulmonary artery and 0,476±0,01 cond. un. lum. for the aorta, which were not significant comparatively with each other. Well-defined basal membranes, on which the endothelial cells were localized, were stained evenly with eosin and accumulated evenly type IV collagen in the form of immunofluorescence moderate intensity (in the pulmonary artery – 0,526±0,02 cond. un. lum., in the aorta – 0,531±0,02 cond. un. lum.).

Thus, above-mentioned state of the vessels corresponded to the universally recognized notion of the norm and could be used as a control [1].

Microscopic examination of the same name vessels of *research* groups revealed the following differences. Endothelial cells were located tightly to each other on the basement membrane. Change of cells' width and height in both vessels indicated their flattening (Table 1).

The elongated oval nucleus of endothelial cells was located centrally in both vessels. The cytoplasm was evenly stained with eosin.

In one field of view (×1000) both in the pulmonary artery and in the aorta was detected size increase of desquamation fields at the analysis. Thus, this index is slightly higher (4,87±0,15 cells) in the first vessel than in the second (4,60±0,16 cells) one. That is significantly different from control values of corresponding vessels ($p<0,001$) and are not reliable between each other.

Endothelial cells of both vessels in groups with hypoxia accumulated marker CD 34 worse, which is proved by optical density decreasing. Thus, it was 0,397±0,02 cond. un. lum. in the pulmonary artery, and 0,379±0,02 cond. un. lum. in the aorta, which was significantly

different from the control group values ($p<0,001$ and $p<0,01$, respectively).

Comparative analysis of data of optical fluorescence density CD 34 between the pulmonary artery and the aorta in groups with chronic intrauterine hypoxia were not found significantly different.

Basement membranes were slightly thickened in groups with oxygen deficiency in both vessels. A downward tendency of type IV collagen volume in the study group compared with the control one has been established. The relative fluorescence density of the emission of collagen for the pulmonary artery was 0,497±0,02 cond. un. lum., and for the aorta – 0,495±0,03 cond. un. lum. Significant differences between the values of the optical density of the emission of type IV collagen in the vessels of the groups with chronic intrauterine hypoxia has not been established. Interstitial collagen type III was also determined in the structure of the vessels basement membranes, where as it is known, collagen type IV must be present. It may indicate presence of sclerotic changes [4]. These features can cause violations of metabolic processes in endothelial cover with development of degenerative changes with subsequent desquamation of cells [12]. This is confirmed by the significant increase in the area of desquamation fields in the vessels of the study group.

CONCLUSIONS:

1. Chronic intrauterine hypoxia causes the formation of significant morphological changes in endothelial cover of the pulmonary artery and the aorta in fetuses and newborns. It is manifested by trophic processes deterioration in endotheliocytes due to endothelial basement membrane thickening, which in turn results in cells flattening, reduction of adhesive properties, as evidenced by decreased expression of receptor CD 34 of endothelium, and increased desquamation. These changes are more significant in the pulmonary artery.

2. Basal membranes of the pulmonary artery and aorta have sclerotic changes as a result of interstitial collagen type III presence against type IV collagen deficiency.

3. The above-mentioned morphological changes reflect negative influence of chronic intrauterine hypoxia on the morphological status of the pulmonary artery and the aorta, which may be regarded as substrate for endothelial dysfunction formation in these people.

References :

1. Gambarian P.P., Dukelskaya N.M. Krysa [Rat]. Moskva: Sovetskaya nauka [Moscow: Sovetskaya nauka (Soviet science)], 1955, 254 p.

2. Karpova I.Yu., Parshikov V.V., Mironov A.A. et al. Izuchenie vliyaniya khronicheskoy gipoksii na techenie beremennosti i razvitie potomstva v eksperimente [The study of chronic intrauterine hypoxia effect on pregnancy course and development of the offspring in the experiment]. Meditsinskiy almanakh [Medical almanac], 2011, № 6 (19), pp. 55-57.

3. Sergienko V.I., Bondarev I.B. Matematicheskaya statistika v klinicheskikh issledovaniyakh [Mathematical statistics in clinical trials]. Moscow: GEOTAR MEDITSINA [GEOTAR MEDICINE], 2000, 256 p.

4. Serov V.V., Shekhter A.B. Soedinitelnaya tkan (funktsionalnaya morfologiya i obshchhaya patologiya) [Connective tissue (functional morphology and general pathology)]. Moscow: Meditsina [Medicine], 1984, 312 p.

5. Gubina-Vakulik G.I., Sorokina I.V., Markovsky V.D. et al. Sposib kilkisnogo vyznachennia vmistu antigenu v biologichnyh tkanynakh [Method of quantitative determination of antigen content in biological tissues]. Patent № 46489, 2009, biuleten [Bulletin] № 4.

6. Galyant O.I., Senkevich O.A., Satskaya L.V. et al. Poliorgannye narusheniya u novorozhdennykh detey s gipoksicheski-ishemicheskimi porazheniyami mozga [Multiorgan disorder in newborn infants with hypoxic-ischemic brain damage]. Dalnevostochnyy meditsinsiy zhurnal [Far East Medical Journal], 2013, №3, pp. 58-60.

7. Tyantereva S.G. Morfogenez i patologicheskaya anatomiya venoznogo protoka v norme i v usloviyakh razvitiya vnutriutrobnoy gipoksii ploda [Morphogenesis and pathological anatomy of the venous flow in normal conditions and the development of fetal hypoxia]: Avtoreferat dissertatsii na soiskanie uchenoy stepeni kandidata meditsinskikh nauk. [Thesis abstract on competition of the degree of candidate of medical sciences]. Saratov, 2007, 28 p.

8. Ham A., Cormack D. Gistologiya: v 5 tomakh: Perevod s angliyskogo [Histology: in five volumes: Translation from English]. Moskva: Mir [Moscow: Mir (World)], 1983, V. 4, 245 p.

9. Brosman M. Immunofluorescencne vysetrovanie formal-parafinovego material. Cs. Patol. 1979, Vol. 15, № 4, pp. 215–220.

10. Giussani D.A., Camm E.J., Niu Y. et al. Developmental programming of cardiovascular dysfunction by prenatal hypoxia and oxidative stress. PLoS ONE, 2012, № 7(2): e31017. doi:10.1371/journal.pone.0031017.

11. Rouvet E.V., Tintu A.N., Schellings M.W.M. et al. Hypoxia induces aortic hypertrophic growth, left ventricular dysfunction, and sympathetic hyperinnervation of peripheral arteries in the chick embryo. Circulation, 2002, № 105, pp. 2791-2796.

12. Liliensiek S.J., Nealey P., Murphy C.J. Characterization of endothelial basement membrane nanotopography in rhesus macaque as a guide for vessel tissue engineering. Tissue engineering, 2009, № 9 (15), pp. 2643-2651.

13. Pak O., Aldashev A., Welsh D. et al. The effect of hypoxia on the cells of the pulmonary vasculature. European respiratory journal, 2007, № 2, Vol. 30, pp. 364-372.

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Table 1

Sizes of pulmonary artery and aortic endothelial cells of fetuses and newborns (M±m)

	Width (10^{-6}м)	Height (10^{-6}м)	Width (10^{-6}м)	Height (10^{-6}м)
Control	7,12±0,15	3,20±0,04	7,25±0,18	3,44±0,06#
Chronic intrauterine hypoxia	7,94±0,11*##	2,96±0,03*	5,95±0,10*	3,06±0,06*

* – P<0,001 – the probability of the difference of two medium is reliable between the control and study groups;

– P≤0,05 – the probability of the difference of two medium is reliable between the corresponding values of the pulmonary artery and the aorta;

– P<0,001 – the probability of the difference of two medium is reliable between the corresponding values of the pulmonary artery and the aorta.