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MORPHOMETRIC INDEXES OF PLACENTA IN CASE OF NNLA- GESTOSIS OF PREGNANT FEMALE RATS

This article presents the results of morphological studies, which were conducted in rats after NNLA- injections. It was established that NNLA- injection to rats leads to morphostructural changes in uterus with prevailing circulation disorders. Microcirculatory changes can be considered as a manifestation of vascular decompensation, which is accompanied by disorders of blood rheology, endothelial dysfunction, increased vascular permeability, sharp disorders of the circulation in the maternal and fetal zones of the placenta. It was proven that the use of enalapril in treatment of experimental NNLA- gestosis helps to normalize the state of hemodynamics and utero-placental tissue, and significantly reduces the postimplantational embryo death.

Key words: NNLA- gestosis, morphostructural changes, enalapril.

These days, perinatal medicine has been considerably enriched in numerous data disclosing the relationships between mother, placenta and fetus, both during normal pregnancy and in case of abnormal pregnancy. It became possible thanks to the P.K. Anokhin's theory of systems genesis widely applied by morphologists, physiologists and obstetricians that developed the concept of "a functional system – mother-placenta-fetus". The knowledge of fetus system genesis is required to conceive the etiology and pathogenesis of fetal deterioration. It is morphologically certified that endocrine glands pathology in mothers lead to the changes of fetus endocrine glands. Besides, due to the progress of perinatology – the study of the care of the developing fetus and the new-born baby both in normal pregnancy and in cases of pathology, – nowadays, a fetus is considered a competent person with certain rights and to whom special methods of diagnosis, treatment and precautions may be applied. With the interest of the researchers focused on the regularities of fetal development, essential details of morphofunctional development of nervous, endocrine and immune systems have been revealed thus providing homeostasis of the developing organism. Much attention is centered on the study of the influence of various harmful factors on the growing fetus both from the environmental side and mother's organism. Such pregnancy complication as arterial hypertension is a major problem of the present obstetrics. Its frequency varies within 2.5 and 16%. According to the WHO (World Health Organization) data, the arterial hypertension is one of the main reasons of the perinatal diseases and mortality. From accessible resources no data revealing the pathologic influence of mother's arterial hypertension on the deterioration of the reproductive health of the nation and with high indexes of reproductive losses are known. However, the speed of the population decline in Ukraine is the highest in Europe and makes up 0.9 – 1.1% per year. The natural increase of the population is also the lowest and accounts for 1.2 per one woman, while for the simple reproduction it should be at least 2.2 [1, 3]. We couldn't find any experimental studies of the morphological indexes of the placenta of pregnant female rats in cases of gestosis in the accessible resources, and it urged us to work on this matter.

Objective of the research. The purpose of the research is to study the morphometric indexes of placenta in case of NNLA-gestosis of pregnant female rats.

Material and methods. The experiments were conducted on non-line pregnant female rats weighing 180,0–200,0 g. Three groups of animals were arranged, wherein group 1 - intact test; group 2 - pregnant female rats which were provoked with gestosis by hypodermic injection - synthesis inhibitor NO-nitro-L-arginine (NNLA) ('Sigma' series 1096975) in a dose 50 mg/kg from 13th till 19th pregnancy day; group 3-pregnant female rats with gestosis. It was discovered earlier that NNLA injections in period cause symptoms of preeclampsia – hypertension, proteinuria and poor growth of a baby [6, 7, 8]. Taking into account the above, this type of gestosis has chosen for our experiment. Female rats with established estrous cycle phase were placed next to males (in relation 3:1). The date of the fixed pregnancy was defined after studying the uterine smears. The first day of pregnancy was considered the presence of spermatozoid in the smears of female rats [5]. The uterus morphostructure and placenta of pregnant female rats were investigated on the 20th day of gestosis period. The experimental materials were fixed in 10% formalin solution, dehydrated in spirit with increasing concentration, and embedded in celoidin-paraffin. Sections in thickness 3–4 mcm were stained in haematoxylin- eosin. All the results obtained were statistically processed by Student's coefficient, the nonparametric Wilcoxon-Mann-Whitney test [2]. The differences between separate samples were estimated by 95% level of significance. All the investigations with animals were carried out in strict conformity with the conditions set out in the 'European Convention for the protection of vertebrate animals used for experimental and other scientific purposes', Strasburg, 1986 with further amendments made in 1998, and were approved at the First National Congress on Bioethics (Kiev, 2001).

Results and its discussions. The development of gestosis in group 2 of animals was accompanied by major clinical symptoms of pathology (with hypertension and proteinuria) and significant changes in uterus and placental complex that urged on the morphological study of uterus and placenta [4]. NNLA injection to female rats produced morphostructural changes in the uterus with prevailing hemodynamics disorder of the organ.

The content of PAS-positive substances in the mucous membrane stroma has not been changed compared with the intact test. The collagen structure of the mucosa stroma has been changed slightly. Biometrics also revealed packed veins and hemorrhage (both among separate small bundles of muscle fibers and in enlarged connective tissue areas between different layers of biometry), disclosed vasodilatation and plasma soaking, connective tissue growth, and round cell infiltration. In some cases, muscular fiber thinning and vacuolation (signs of tissue hypoxia), weak saturated PAS-positive substances biometry were revealed. Typical disorders have been also found in the womb placental site in viable fetuses, in the upper layers of spongy tissue, there were visible detritus cells. In the deep layers, partial necrosis with fragmented cells, accumulation of cells similar to macrophages, damaged blood vessels with a coarse-collagen-fiber mesh in gaps were discovered. The long resistant erythrocyte stasis led to the disruption in blood circulation in the mentioned placental sites that caused necrosis and tissue lysis. Muscle fibers in the affected zone were vacuolated that manifested themselves as the hypoxia state of biometrics. The tissue saturation with PAS-positive material was reduced.

The enalapril injection has considerably enhanced offspring viability. In 50% females, there was 100% survival of fetuses, while in the rest – the average fetal death in off springs did not exceed 18%. In all female rats, geodynamic disorders in the mucous wall of uterus horn was substantially reduced. Even in cases of some fetal resorption, no blood clots were found on the mucosa surface and in gaps of the most of uterine glands. Proliferative processes in the surface epithelium have not been registered at all or were slightly visible. Among the morphostructure changes in the areas of fetal death: epithelial tissues were depleted in glycogen, endometrial stromal cells were increased, collagen component of uterus mucous membrane became coarse. With viable fetuses, the mucosa state in general was up to the standards.

Epithelial tissues are rich in glycogen. Mucosal stroma was rather swollen, however, it had histochemical characteristics, and properties of intact test group. In the restricted uterus areas of some females, there were some local, relatively small in size hemorrhages in the mucosa. Blood filling of mucosa in most pregnant rats was very close to the intact test. Biometry remained practically unchanged, only in some areas there were small cell clusters. In the placental bed of uterine horn wall there were not any necrotic or proteolytic changes. Though blood vessels were somewhat expanded, they kept the blood flow. Muscle fibers in the sponge area were slightly vacuolated. Biometry under the placental area was also partially vacuolated. The content of PAS-positive substances in the sponge tissue, placental bed and biometry matched the animal intact test.

In 90% cases, NNLA injection to pregnant females resulted in the placenta destructive changes, wherein in 83% cases – it caused significant damage. Architectonics was changed: decidual membranes were narrowed, while trophospongium was on the contrary much-enlarged. Decidual cell cytoplasm was partially vacuolated, and gaps in large maternal vessels were often enriched in leukocytic masses. Glycogenic spots in trophospongium were filled with eosinophilic homogeneous masses instead of typical cells. Periplacentitis was observed in 13% cases. The giant cells between decidual membranes and trophospongium were often vacuolated: the layer of these cells according to the norm – was enlarged.

Despite the fact that the labyrinth cavity as per the norm was slightly narrowed, it had considerable pathological changes. Almost all studies showed that fetal-maternal circulations were disrupted. Maternal vessels were usually widened and filled with aggregated erythrocytes. Sometimes gaps in maternal vessels contained closely spaced erythrocytes.

Fetal capillaries were not only devastated, in their gaps there was proliferation of fibroblast cell line, excessive connective tissue growth, causing abrupt decrease in metabolism. Perivascular connective tissues were also formed around the larger vessels. There were portions of placenta with stagnation of blood circulation. In these ischemic spaces there were only empty vessels. There was such an irreversible form of stromal-vascular dystrophy, as fibrinoid swelling and swelling of the epithelium and stroma. Fibrinoid masses were on the border between trophospongium and labyrinth and filled maternal vessels gaps. An exudative component occurred within the entire area. In 13% cases, these degenerative changes led to focal or widely spread necrosis with homogenized structure and loss of color differentiation. In some cases, it caused only fused cell masses. Glycogen in trophospongium and labyrinth was significantly reduced in comparison with the intact test.

Morphometric indexes of placenta of pregnant female rats in case of experimental gestosis:

Index	Intact control	Pregnant rats with gestosis	Pregnant rats with gestose + enalapril 5mh/kh
Cases of pathological changes detected, %	0	90	26.3
Width decidual shell, mm	0.27	0,14*	0,16*
The width of the intermediate layer, mm	0,48	0,63	0,46*
Width labyrinth layer, mm	2.44	2.20	2,33
Glycogen content in the intermediate zone, ball	3,50	1.64	3,45*
Glycogen content in the maze, ball	3.75	1,86*	3,54*

Notes: * p <0,05 – difference significant with reference to intact test, ** p <0,05 – difference significant with reference to the case of gestosis.

Enalapril injection considerably influenced the destructive processes and hemorheologic disorders in the placenta that were caused by the NNLA injection. The number of experiments with pathological disorders was decreased down to 26%. However, the complete absence of drastic changes in metabolism and body morphostructure should be mentioned. There were only some separate cases of hydropic degeneration of decidual cells, choral structure narrowing with slight connective tissue growth. Maternal blood flow was not disrupted, cases of periplacentitis were not detected. Histoarchitectonics was nearly close to normal. The content of glycogen in trophospongium and labyrinth reached the intact indexes and it manifested as the complete restoration of placental metabolism.

Conclusions

1. The results of morphological studies show that NNLA injection to female rats produced morphostructural changes in the uterus with prevailing hemodynamics disorder of the organ. Microcircular changes can be considered as the manifestations of vascular decompensation (paresis, blood stagnation), accompanied by disorders in the rheological properties of blood, endothelial dysfunction, abnormal vascular permeability.
2. The other disorders of the endometrium and myometrium both in non-placental and placental sites were a secondary factor. NNLA injection to pregnant females resulted in the placenta destructure and inflammation (maternal and fetal zones), accompanied by abrupt disruption in maternal and fetal circulation. The main pathology was excessive proliferation of connective tissue in fetal vessel gaps.
3. It was first proven that enalapril applied in treatment and prevention can normalize gestation process in experimental gestosis: the state of uterine-placental tissue allows to "save" pregnancy of the most females and considerably reduce the postimplantational embryo death.

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Реферати

МОРФОМЕТРИЧНІ ПОКАЗНИКИ ПЛАЦЕНТИ ПРИ NNLA-ГЕСТОЗІ У ВАГІТНИХ ЩУРИВ

Торянік Е. Л.

В роботі наведені результати морфологічних досліджень, які були проведені після введення щурам розчину NNLA. Виявлено, що NNLA-ін'єкції щурам призводять до морфоструктурних змін у матці з переважаючими розладами гемодинаміки органу. Мікроциркуляторні зміни можна розглядати як прояви судинної декомпенсації, які супроводжуються порушеннями реологічних властивостей крові, ендотеліальною дисфункцією, підвищеною судинною проникністю, різким порушенням циркуляції у материнській та фетальній частині плаценти. Було доведено, що застосування еналапрілу в лікуванні експериментального NNLA-гестозу може нормалізувати стан гемодинаміки та матково-плацентарної тканини, значно скоротити постімплантаційну смертність ембріону.

Ключові слова: NNLA-гестоз, морфометричні зміни, еналапріл.

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МОРФОМЕТРИЧЕСКИЕ ПОКАЗАТЕЛИ ПЛАЦЕНТЫ ПРИ NNLA-ГЕСТОЗЕ У БЕРЕМЕННЫХ КРЫС

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В работе приведены результаты морфологических исследований, которые были проведены после введения крысам раствора NNLA. Установлено, что NNLA-инъекции крысам приводят к морфоструктурным изменениям в матке с преобладающими расстройствами гемодинамики органа. Микроциркуляторные изменения можно рассматривать как проявления сосудистой декомпенсации, которые сопровождаются нарушениями реологических свойств крови, эндотелиальной дисфункцией, повышенной сосудистой проницаемостью, резким нарушением циркуляции в материнской и фетальной части плаценты. Было доказано, что применение эналаприла в лечении экспериментального NNLA-гестоза позволяет нормализовать состояние гемодинамики и маточно-плацентарную ткань, значительно сократить постимплантационную гибель эмбриона.

Ключевые слова: NNLA-гестоз, морфометрические изменения, эналаприл.

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