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THE ROLE OF ENDOTHELIAL SYNTHASE (eNOS) IN THE PATHOGENESIS OF HYPOXIC-ISCHEMIC INJURIES BRAIN DAMAGE IN PREMATURE INFANTS

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The connection of the publication with planned research works. This work is a fragment of the dissertation for the degree of Doctor of Philosophy in medicine "The role of vasoregulatory mechanisms in the defeat of the central nervous system in premature newborns»

Introduction. Perinatal hypoxic-ischemic lesions of the central nervous system (CNS), which make up 60-80% of cases, occupy a significant place in the structure of pathology of newborns, especially premature infants [1, 2]. Pathology of the perinatal period is more common among premature infants and children with extremely low body weight [2, 3, 4, 5].

To date, the issue of determining the pathogenetic mechanisms of hypoxic-ischemic brain damage remains relevant, and the search for markers that adequately reflect the severity of damage to the nervous system requires further scientific research. Nitric oxide (NO) plays a critical role in maintaining normal endothelial function [3, 6, 7]. At the same time, it has not only a vasorelaxing effect, but also participates in the regulation of intracellular signaling, functionally connecting both presynaptic and postsynaptic neurons. NO is a compound produced by nitric oxide synthases (NOS), which exist in 3 isoforms (endothelial, neuronal, and inducible synthases) expressed in neurons, astrocytes, and endotheliacites and activated only by microglia [3, 7, 8]. The endothelial form of NOS (eNOS) has a neuroprojective function, which is realized through a NO-mediated increase in brain perfusion [7, 8]. Despite the key role of endothelial dysfunction in the pathogenesis of cerebrovascular disorders, there is practically no information about the nature of changes in vasoregulatory systems in premature newborns with hypoxic-ischemic CNS damage, depending on gestational age, the nature of morphological changes in the brain, and the state of cerebral hemodynamics (CH).

The aim of the study was to study the role of endothelial synthase (eNOS) in the pathogenesis of perinatal hypoxic-ischemic CNS damage in premature infants, depending on gestational age.

Object and methods of the research. We examined 102 premature infants with perinatal hypoxic-ischemic CNS lesions from mothers with a burdened obstetric history at the age of 1 day to 1 month of life. Written informed consent was obtained from all mothers whose children participated in the study. The diagnosis was based on the data of the antenatal anamnesis, the clinical symptom complex of the disease in the first 2-3 weeks of the child's life, and the detection of pathognomonic symptoms of central nervous system damage. The assessment of the children's condition was carried out on the Apgar and Silverman scale. The analysis of anamnestic data showed that the majority of children were born to mothers under the age of 35-72 (70.6%), the rest were over 35-30 (29.4%). Premature children of the male sex were 53(52%), female – 49(48%); residents of the city – 70 (68.7%), the village – 32 (31.3%). The examination of premature infants was carried out on days 1-3, 5-7, and again on days 10-14. The following research methods were used: clinical (post-natal assessment of gestational age (Dubowits L. scale, Dementieva G. M. scale and Korotkova E. V.), parameters of morphofunctional immaturity on the Hoepffnes W., Rauntenbach M. scale, the temperature curve, anthropometric indicators, and the functional state of organs and systems were analyzed. The gestational age (g/v) of infants was determined on the basis of the maternal history, the results of ultrasound performed during pregnancy and confirmed on the basis of the Ballard scale. The severity of neonatal encephalopathy was determined based on the results of the Sarnat H. B. assessment during the first 24 hours of the infant's life. The generally accepted routine laboratory and instrumental methods of research were carried out. All children were monitored (heart rate, BH, BP, SpO2, pCO2), hourly and daily diuresis was determined, and neurological status was assessed. The tests included: hematological (hemogram, hemostasis), biochemical tests (blood gases, Na, K, Ca, Mg, protein, albumin, glucose, CRP). Endothelial nitric oxide synthase (eNOS or NOS-3) was determined by the enzyme immunoassay method (ELISA) with a set of reagents "Human eNOS Immunoassay R&D System (cat. no. DEN00, Biochemmak CJSC, Moscow). In addition, instrumental methods of research were also carried out: RN-diagnostics of the chest organs, NSG (the "Microl Mabs" device of the company "Ausonic", Australia); Doppler studies of the vascular system (the "ALOKA SSD-1700" device, with a microconvex sensor with a frequency of 5 MHz and a convexal sensor with a frequency of 3.5 MHz); EchoCG, ECG (electrocardiograph "EKIK-01"; EchoCG studies were carried out using the "Medikor FTS"device-21"). Preterm infants were examined by specialized specialists: a neurologist, an optometrist, a cardiologist, an otolaryngologist, and a surgeon. The mothers were examined by a therapy degree.

Statistical processing was carried out using the software package "STATISTICA-6", "Microsoft Excel 2010", graphs were built using "ORIGN-7".

The results of the study and their discussion. We conducted a comprehensive examination of 102 premature infants with hypoxic-ischemic encephalopathy (HIE). Cerebral ischemia (CI) of the first degree was detected in n=51; CI of the second degree in n=32; CI of the third degree in n=19 premature infants, respectively.

Thus, in premature infants with a gestational age (g/v) of 28-30 weeks in one case, which was 3.1%, moderate CI (II degree) was detected; while severe CI (III degree) – in 5.3% of cases, respectively. It should be noted that in this contingent of children, mild CI (I degree) was not detected in any case. Among premature infants with g/v30-33 weeks, the highest frequency was detected in 14 (73.7%) cases of CI III degree; whereas CI II degree-in 4 (12.5%) cases; CI I degree – in 1.9% of cases, respectively. Among premature infants with g/v 34-35 weeks, the most frequently detected CI of the II degree – in 21 (65.6%) cases; while CI of the I degree and III degree was observed with the same frequency in the percentage ratio – 15.7%, respectively. Analyzing the results obtained, regarding the detection of CI in premature infants with g/v 36-37 weeks, it should be noted that the most often detected CI I degree in 42 (82.4%) cases, while CI of the second degree – in 6 (18.8%); CI of the third degree -in 1 (5.3%) cases, respectively.

To identify the role of vasoregulatory mechanisms in CNS damage, we studied the system of nitric oxide synthases, in particular the role of endothelial synthase (eNOS) in 71 premature infants, depending on g/v and the severity of CI. The studies were conducted on the 1-3 and 5-7 days of the infants ' life. The preterm infants were divided into 3 main groups: Group I consisted of n=20 preterm infants with CI of the first degree; Group II consisted of n=32 preterm infants with CI of the second degree; Group III consisted of n=19 preterm infants with CI of the third degree The control group IV (KG) consisted of n=20 conditionally healthy premature infants. **Figure 1** shows a comparative diagram of the eNOS content in the peripheral blood of infants in accordance with g/v. Thus, in an infant with a g/b of 30-33 weeks on the 1st-3rd day of life, the eNOS level was determined in the range of 8.6 IU/ml, while on the 5th-7th day there was a slight decrease in this indicator, which was 7.5 IU/ml, which significantly exceeded the eNOS levels in KG: 2.11±0.004 IU/ml in 1-3 and 1.98±0.01 IU/ml on the 5th-7th day of life, respectively. In children (n=8) with g/v 34-35 weeks, an increase in the level of eNOS was also noted in comparison with the data of KG: on the 1st-3rd day of life, the level of this indicator was 8.92±0.12 IU/ml, on the 5th-7th day, a slight decrease of 7.77±0.11 IU/ml was registered. Similarly, an increase in eNOS was observed in infants (n=11) with g/v 36-37 weeks in relation to the KG data: 9.79±0.14 IU/ml at 1-3 and 6.36±0.09 IU/ml ($p<0.05$) at 5-7 days of life, respectively. It should be noted that in none of the cases in infants with g/v 28-30 weeks, CI I degree mild form was not detected.

Analyzing the results obtained by eNOS, it can be concluded that in premature infants with CI II degree, with a moderate-severe form of HIE, depending on g/v, fluctuations in the average values (M±m) were detected in certain reliable intervals in relation to KG. In particular, in infants with g/v28-30 weeks, the eNOS level at 1-3 days was determined in the range of 5.82 IU/ml, while on the 5th-7th day the eNOS values were 5.42 IU/ml in relation to KG: 2.11±0.004 IU/ml and 1.98±0.01 IU/ml, respectively **(Fig. 2)**. In infants with g/v30-33 weeks, the eNOS level was also slightly increased in relation to KG. If the level of this parameter varied in the range of 6.52±0.18 IU/ml for 1-3 days, then on 5-7 days the level of eNOS decreased slightly and was determined in the

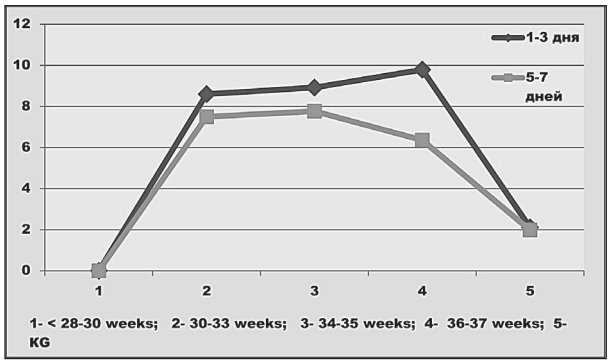

Figure 1 – Comparative content diagram eNOS (IU/ml) in peripheral blood in premature infants with CI, depending on gestational age (mild form).

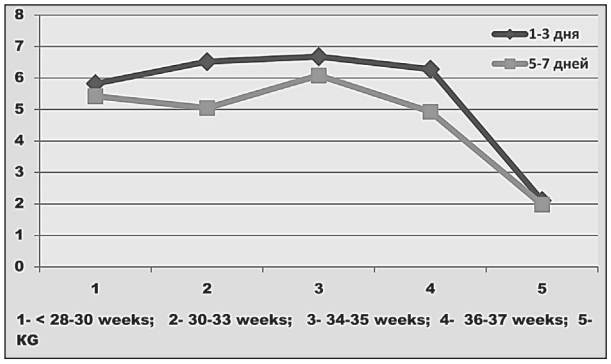

Figure 2 – Comparative content diagram eNOS (IU/ml) in peripheral blood in premature infants with CI, depending on gestational age (medium-heavy form).

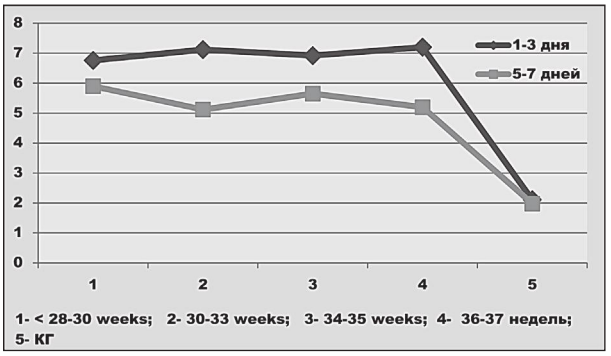

Figure 3 – Comparative content diagram eNOS (IU/ml) in peripheral blood in premature infants with CI, depending on gestational age (severe form).

range of 6.08±0.15 IU/ml in relation to KG: 1.98±0.01 IU/ml, respectively ($p<0.05$). Similarly, an increase in the level of eNOS was observed in children with g/v36-37 weeks. In particular, if the eNOS level was determined in the range of 6.28±0.12 IU/ml in 1-3 days, then on 5-7 days there was a slight decrease of 4.93±0.05 IU / ml compared to KG.

It should be noted that the greatest increased activity of eNOS was observed in infants with g/v34-35 weeks: 6.68±0.009 IU/ml and 2.11±0.004 IU/ml relative to KG at 1-3 days, although at 5-7 days, despite a slight decrease in the expression of this indicator, 6.08±0.15 IU/ml compared to KG-1.98±0.01 IU/ml, respectively ($p<0.05$).

In infants with severe HIE, the nitric oxide synthase system varied in an increased range **(Figure 3)**.Thus, in premature infants with g/v 28-30 weeks, the eNOS level at 1-3 days was determined in the range of 6.76 IU/ml,

whereas on the 5-7 day, the eNOS values were 5.90 IU/ml in relation to KG: 2.11±0.004 IU/ml and 1.98±0.01 IU/ml, respectively (p<0.05). In infants with g/v30-33 weeks, the level of eNOS at 1-3 days was determined in the range of 7.12±0.03 IU/ml. Despite a slight decrease in the level of this indicator on the 5th-7th day, the eNOS values exceeded the values in KG: 5.12±0.03 IU/ml and 1.98±0.01 IU/ml, respectively (p<0.05). In infants g/v 34-35 weeks; 36-37 weeks, eNOS levels were 5 times higher than the values of KG **(Fig. 3).**

Summarizing the results, it should be emphasized that in infants with severe HIE, regardless of their g/v, eNOS levels were both 1-3 and 5-7 days higher than the values of KG, despite a significant decrease in the level of this indicator in relation to the compared group of conditionally healthy infants.

Thus, studies have shown that increased eNOS expression plays a leading role in the development of cerebral ischemia of varying severity. Consequently, the effect of hypoxia and ischemia on the brain serves as a trigger in the violation of physiological vasoregulatory mechanisms, thereby contributing to the activation of endothelial nitric oxide synthase, causing increased expression of eNOS. eNOS synthases are known to be present in brain endothelial cells and actively contribute to the production of NO in these cells. Of course, in the regulation of homeostasis, the state of the vascular endothelium plays an important main role. An increase in eNOS contributes to the activation of NO production in these cells. Namely, in the endothelial cells of the brain, there is an increased regulatory activity of eNOS, which contributes to its accumulation in these cells, leading to their dysfunction. As a result, not only the ability of the endothelial cells to function adequately, but also their structure is disrupted.

Conclusions. In the pathogenesis of hypoxic ischemic encephalopathy, eNOS is the main indicator of the processes of endothelial dysfunction of the cerebral vessels, simultaneously reflecting both its cause and effect.

Prospects for further research. Study of changes in vasoregulatory systems in premature newborns with hypoxic-ischemic CNS lesion.

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РОЛЬ ЕНДОТЕЛІАЛЬНОЇ СИНТАЗИ (eNOS) В ПАТОГЕНЕЗІ ГІПОКСИЧНО-ІШЕМІЧНИХ ПОШКОДЖЕНЬ ГОЛОВНОГО МОЗКУ У НЕДОНОШЕНИХ ДІТЕЙ

Асадова Т. А.

Резюме. Було обстежено 102 недоношені дитини з перинатальним гіпоксично-ішемічним пошкодженням ЦНС, що народилися від матерів, що мали обтяжений акушерський анамнез від 1 дня до 1 місяця життя. Більшість дітей народилися від матерів у віці до 35 років – 72 (70,6%), решта жінок були у віці старше 35 років – 30 (29,4%). Недоношені діти чоловічої статі склали – 53 (52%), жіночої статі – 49 (48%); мешканці міста – 70 (68,7%), села – 32 (31,3%). Обстеження недоношених новонароджених проводили на 1-3, 5-7 добу та повторно на 10-14 добу. Було використано наступні методи дослідження: клінічні, параметри морфофункціональної незрілості, антропометричні показники, функціональний стан органів та систем. Гестаційний вік (г/в) малюків визначався на основі анамнезу матерів, результатів УЗД. Ендотеліальна синтаза оскиду азоту (eNOS або NOS-3) визначалася імуноферментним методом (ІФА). Проводилося дослідження: RH – діагностика органів грудної клітки, НСГ; доплерографічне дослідження судинної системи; Ехо-КГ, ЕКГ.

Результати дослідження. Церебральна ішемія (ЦІ) I ступеня була виявлена у n=51; ЦІ II-ого ст. у n=32; ЦІ III-ого ст. у n=19 недоношених дітей відповідно. Для встановлення ролі вазорегуляторних механізмів у пошкодженні ЦНС було вивчена система синтаз оксиду азоту, зокрема роль ендотеліальної синтази (eNOS) у 71 недоношених дітей в залежності від г/в та ступеня тяжкості ЦІ.

Було показано, що в розвитку церебральної ішемії eNOS відіграє ключову роль. Виявлено, що підвищена експресія та активація eNOS прямо залежить від гестаційного віку малюка. Чим більш виражена дія гіпоксії та ішемії, тим виразніше порушення здатності адекватної функції та структури клітин ендотелію судин головного мозку, що сприяє виникненню церебральної ішемії різного ступеня тяжкості що веде до серйозних наслідків.

Висновки. У патогенезі гіпоксичної ішемічної енцефалопатії, eNOS є основним показником процесів ендотеліальної дисфункції судин головного мозку, одномоментно відображаючи як її причину, так і наслідок.

Ключові слова: недоношеність, патологія центральної нервової системи, гіпоксичне ураження центральної нервової системи, ендотеліальна синтаза eNOS.

THE ROLE OF ENDOTHELIAL SYNTHASE (eNOS) IN THE PATHOGENESIS OF HYPOXIC-ISCHEMIC INJURIES BRAIN DAMAGE IN PREMATURE INFANTS

Asadova T. A.

Abstract. 102 premature infants with perinatal hypoxic-ischemic CNS lesions from mothers with a burdened obstetric history aged from 1 day to 1 month of life were examined. Most of the children were born to mothers under the age of 35-72 (70.6%), the rest were over 35 – 30 (29.4%). Premature children of the male sex were 53 (52%), female – 49 (48%); residents of the city – 70 (68.7%), the village – 32 (31.3%). The examination of premature infants was carried out on days 1-3, 5-7, and again on days 10-14. The following research methods were used: clinical, morphofunctional immaturity parameters, anthropometric indicators, functional state of organs and systems. The gestational age (g/v) of infants was determined based on the mothers ' anamnesis and ultrasound results. Endothelial nitric oxide synthase (eNOS or NOS-3) was determined by enzyme immunoassay (ELISA). Studies were carried out: RN-diagnostics of the chest organs, NSG; Doppler studies of the vascular system ; EchoCG, ECG.

Results. Cerebral ischemia (CI) of the first stage was detected in n=51; CI of the second stage in n=32; CI of the third stage in n=19 premature infants, respectively. To identify the role of vasoregulatory mechanisms in CNS damage, the nitric oxide synthase system was studied, in particular, the role of endothelial synthase (eNOS) in 71 premature infants, depending on g/v and the severity of CI.

eNOS has been shown to play a leading role in the development of cerebral ischemia. It was revealed that the increased expression and activation of eNOS directly depends on the gestational age of the infant. The more pronounced the effect of hypoxia and ischemia, the more pronounced the violation of the ability of adequate function and structure of the endothelial cells of the brain vessels, which contributes to the occurrence of cerebral ischemia of varying severity, leading to serious consequences.

Conclusions. In the pathogenesis of hypoxic ischemic encephalopathy, eNOS is the main indicator of the processes of endothelial dysfunction of the cerebral vessels, simultaneously reflecting both its cause and effect.

Key words: prematurity, pathology of the central nervous system, hypoxic lesion of the central nervous system, endothelial synthase eNOS.

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DETERMINATION OF THE DEGREE OF THE KIDNEY INJURY IN LOW BIRTH WEIGHT INFANTS DEPENDING ON THE CORRESPONDENCE OF THEIR ANTHROPOMETRIC PARAMETERS TO GESTATIONAL AGE

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The connection of the publication with planned research works. This work is fragment of the dissertation for the degree of Doctor of Philosophy in medicine "Early diagnosis and prognosis of ischemic nephropathy in low birth weight infants".

Introduction. Low birth weight newborns' (LBW) pathology accounts for a significant portion of the causes of perinatal morbidity and mortality. According to the American Academy of Pediatrics, Acute Kidney Injury affects 12.5-18% of low-birth-weight infants, with a mortality rate of 42% relative to low-birth-weight infants that do not have kidney damage (5%). SGA (Small for gestational age) newborns hold a unique position among LBW children, since this group of children suffers from chronic intrauterine hypoxia due to a variety of pathological causes. This suggests that the hypoxia factor affects them for a longer period of time, and they have less nephrons at birth, resulting in residual nephron hypertrophy and hyperfiltration. In the context of other pathological conditions, such as chronic hypoxia and asphyxia, a kidney functioning in the hyperfiltration mode is more susceptible to the effects of various pathological factors [1, 2].

There is a high incidence of ischemic nephropathy (IN), a pathology that is a manifestation of kidney damage, in newborns, especially premature SGA infants, who have experienced perinatal asphyxia [3, 4].

The early detection of this disorder in newborns is difficult as the changes in the kidneys are often "masked" as signs of other diseases [5]. The commonly accepted kidney injury criteria, based on the urinary syndrome markers, do not allow for an early diagnosis of the severity and the localization of glomerular or tubular lesions [6].

The discovery of biomarkers associated with the early stages of nephropathy that are independent of the kidneys' filtration capability, is linked to the pursuit for new and improved methods of diagnosing hypoxic kidney damage.

KIM-1 (Kidney Injury Molekule-1) and NGAL (Neutrophil Gelatinase-Associated Lipokalin), as well as Cystatin C, a marker of glomerular function disorder, are currently the most promising biomarkers of ischemic nephropathy.

KIM-1 (Kidney Injury Molecule) is a transmembrane glycoprotein that is not found in the urine in a healthy kidney but is synthesized in high concentration by epithelial cells of the proximal tubules after ischemic damage and persists until tubular function is completely restored [7].