ORIGINAL ARTICLE

PECULIARITIES OF ELECTRON MICROSCOPIC HIPPOCAMPAL FORMATION DEVELOPMENT CHARACTERISTICS IN POSTERITY OF RATS AFTER PGE2 INJECTION FOR LABOR INDUCTION

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Olena A. Hryhorieva[1], Iryna Yu. Mamay[1], Serhii Tertyshniy[1], Volodymyr Dariy[1], Yuriy Y. Guminsky[2]
[1]ZAPORIZHZHIA STATE MEDICAL UNIVERSITY, ZAPORIZHZHIA, UKRAINE
[2]NATIONAL PIROGOV MEMORIAL MEDICAL UNIVERSITY, VINNYTSIA, UKRAINE

ABSTRACT

The aim: To determine the peculiarities of electron microscopic hippocampal formation development characteristics in postetry of rats during two first weeks of postnatal life after intravaginal injection of prostaglandin E2 for labor induction.

Materials and methods: The ultrastructural changes of hippocampal formation in posterity of white syngenic rats at the 1st, 7th and 14th days of postnatal life were examined. In this study we used electron-microscopic method. Brain tissue from experimental animals underwent standart stages necessary for electron microscopy and poured into pure Epon. Epon polymerization was carried out in two stages at 36 ° C (12 h) and 56 ° C (24 h). Ultrathin (50-60 nm) sections were obtained on a PowerTome RMC Boeckeler ultratome and then contrasted according to the E. Reynolds method. Ultrathin sections were studied in a PEM-100 electron microscope with an accelerating voltage 60 kV.

Results: Based on the obtained data in the study of the hippocampal formation in postery of rats after induction of labor, analysis of the literature devoted to the electron microscopic study of the brain after ischemic injuries, it can be concluded that on the background of stimulation of labor by PgE2, changes corresponding to ischemic damage take place in the rat brain.

Conclusions: In posterity of rats after receiving PgE2 for labour induction it was revealed microcirculatory changes; edema of the presynaptic endings, synaptic vesicles aggregation in the center of the presynaptic processes, swelling and destruction of mitochondria; oligodendroglia changes; ultrastructural changes in neurons like edema and vacuolization of mitochondria.

KEY WORDS: hippocampal formation, oligodendroglia, synaptic apparatus, neurons, prostaglandin E2

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INTRODUCTION

Labor induction is a widespread obstetric manipulation performed on each fourth or fifth women in the well-developed countries all over the world. According to the data of the World Health Organization on Global Survey on Maternal and Perinatal Health, based on 373 clinics in 24 countries and almost 300,000 births, the medical induction of labor occurs in 9.6% of cases [1]. This index tends to increase. In the USA, the rate of labor induction grows up to 24.5%, in Europe from 6,8 to 33 % [2]. Despite enormous possible complications associated with labor hyperstimulation, universal standards on criteria that should be used to determine unsuccessful induction and methods for accurately determining the time intervals for re-induction are still absent [3].

A meta-analysis conducted by W. Chen et al. (2016), based on 96 studies (17,387 women), showed the efficacy of vaginal misoprostol for inducing labor within 24 hours, but under uterine hyperstimulation it severely changed the heart rate of fetus [4]. Yi-Ran Liu et al. (2018) paid attention at decrease in the arterial blood pH of the newborns umbilical cord <7.1 after dinoprostone injection to pregnant women [5].

Due to increased contractile activity of the uterus labor induction can lead to jatrogenic prematurity, physical injury and hypoxia of fetus. Children who have undergone a hypoxic-ischemic state in the neonatal period show an increased risk of speech and communication impairments [6]. In newborns, pyramidal cells of the hippocampus are still migrating to the corresponding layers CA1, CA2 and CA3, and are vulnerable to hypoxic-ischemic conditions [7,8]. Hippocampal damage leads to cognitive deficits [9] that may replicate some phenotypes of children with neurological disorders [10].

Thus, it is important to study the peculiarities of hippocampal formation development characteristics in postetry of rats during two first weeks of postnatal life after intravaginal injection of prostaglandin E2 for labor induction.

THE AIM

To determine the peculiarities of electron microscopic hippocampal formation development characteristics in postetry of rats during two first weeks of postnatal life after intravaginal injection of prostaglandin E2 for labor induction.

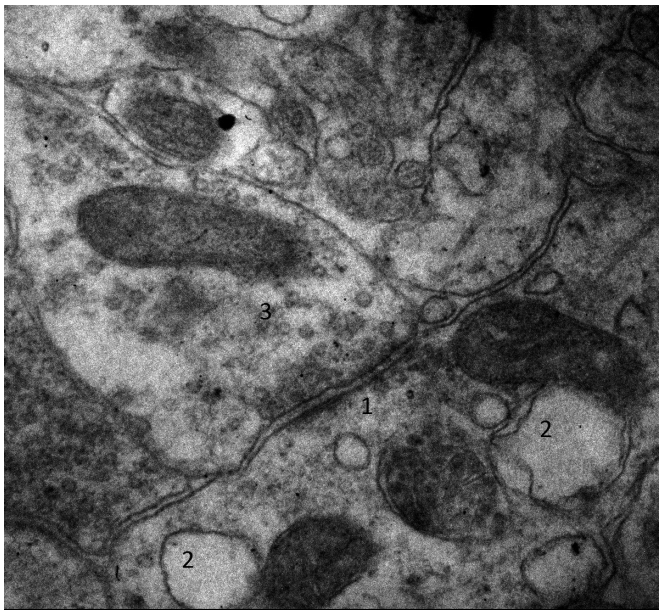

Fig. 1. Axo-dendritic synapse of the hippocampal formation of experimental rat on the 7th day of life, × 19,000.
Note: 1- perforation of synaptic seals; 2- vacuolization of postsynaptic processes; 3- destruction of synaptic vesicles.

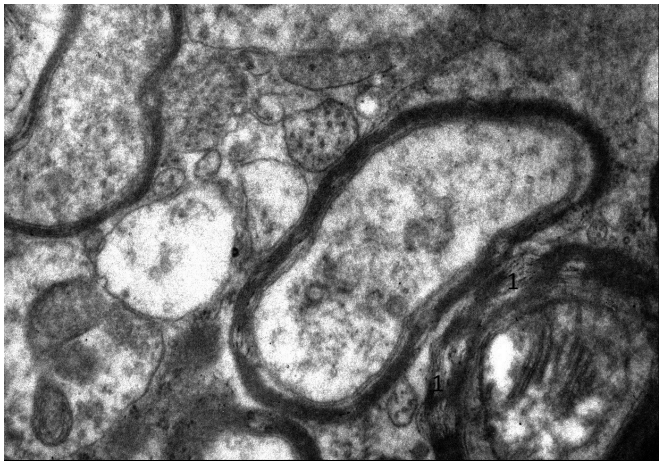

Fig. 3. Hippocampal formation of experimental rats on the 14th day of life. × 11,000.
Note: 1- stratification of the myelin sheath.

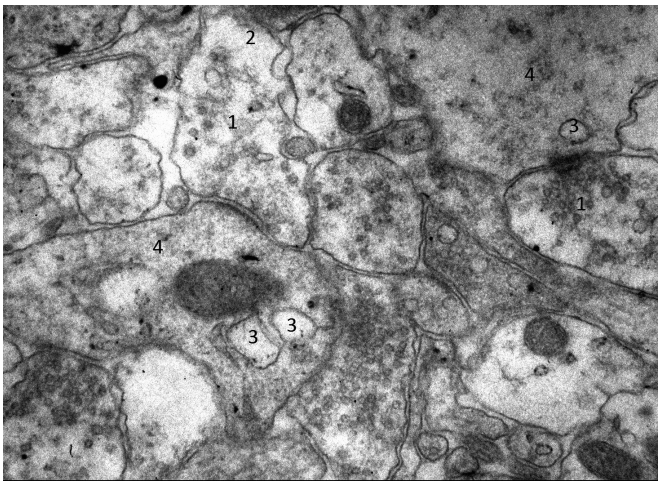

Fig. 2. The synaptic apparatus of the hippocampal formation of the experimental rat on the 14th day of life with ultrastructural changes according to the light type of destruction. × 15,000.
Note: 1- violation of the grouping of synaptic vesicles; 2- edema of presynaptic endings; 3- vacuolization of the postsynaptic process; 4- edema of the postsynaptic process with impaired spatial organization of microtubules.

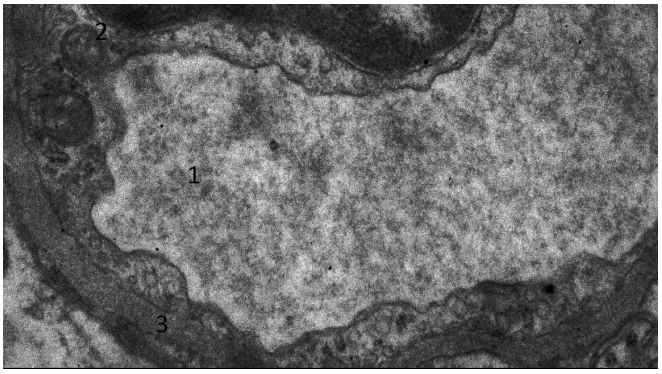

Fig. 4. The ultrastructure of the capillary endothelium of the hippocampal formation in the control group of animals on the 14th day of life. × 11,000.
Note: 1- vessel lumen; 2- endothelial cell; 3- basement membrane.

MATERIALS AND METHODS

There were examined the ultrastructural changes of hippocampal formation in posterity of white syngenic rats in the first two weeks of postnatal life. The first day of pregnancy was defined by the method of vaginal smears stained with methylene blue; the presence of spermatozoa in smears determins the 0 day of pregnancy. On the 22th day of pregnancy for labor stimulation pregnant females of the experimental group were injected intravaginally by PgE2 in the form of a gel. The duration of the experimental rats pregnancy counted up to 23 day, in the intact group - 23-24 day after conceiving. Intact group of animals served as a control one. Animals were contained in standard conditions of vivarium according to "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasbourg, 18.03.86 G.) and the Law of Ukraine № 1759-VI (15.12.2009) On the Protection of Animals from Cruelty. Food and water were available ad libitum. At the 1st, 7th and 14th days after birth brain tissue of control and experimental animals after grinding in a drop of 2.5% glutaraldehyde in 0.1 M phosphate buffer with pH 7.4 were fixed in a similar solution for 2 hours at t + 4C. After washing the fixing solution in phosphate buffer, the material was treated for two hours in 1% OsO4 solution. Subsequently, the pieces were dehydrated in an ascending battery of ethanol up to 100% acetone with additional contrasting for two hours with 2.5% uranyl acetate on 70% ethanol; pouring into the block was carried out by gradually soaking the tissue with a compound of acetone and Epon (2: 1, 1: 1, 1 : 2) and poured into pure Epon. Epon polymerization was carried out in two stages at 36 ° C (12 h) and 56 ° C (24 h).

Ultrathin (50-60 nm) sections were obtained on a PowerTome RMC Boeckeler ultratome. Ultrathin sections were

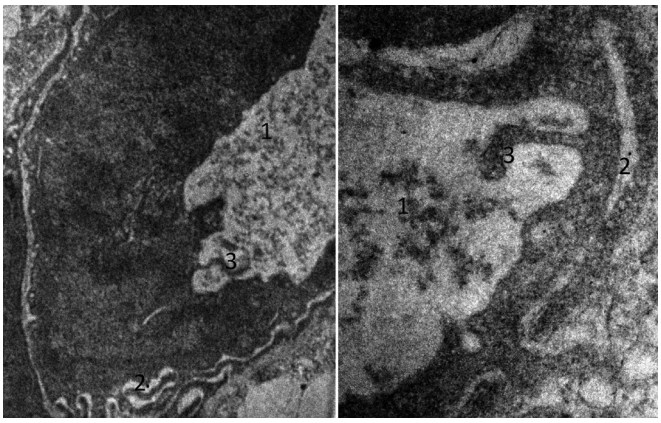

Fig. 5. Ultrastructural changes in the microvasculature of the hippocampal formation in the experimental group on the 7th day of life. A) × 3 800, B) × 15 000.
Note: 1- vessel lumen; 2- broken contacts between endothelial cells; 3- fragmented processes of endothelial cells.

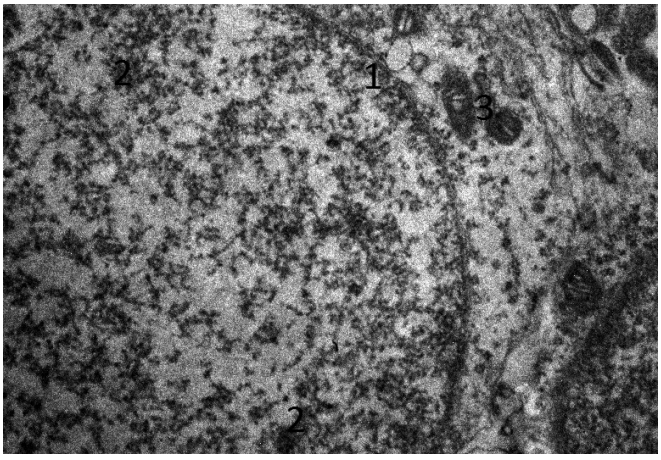

Fig. 7. Ultrastructural disorders of the light neuron CA2 hippocampal zone in experimental animals on the first day of life. × 6,000.
Note: 1- pores in the nuclear envelope; 2- increasing the activity of the protein synthesis apparatus: mass formation of granular vesicles and microtubules; 3- destruction of mitochondrial cristae.

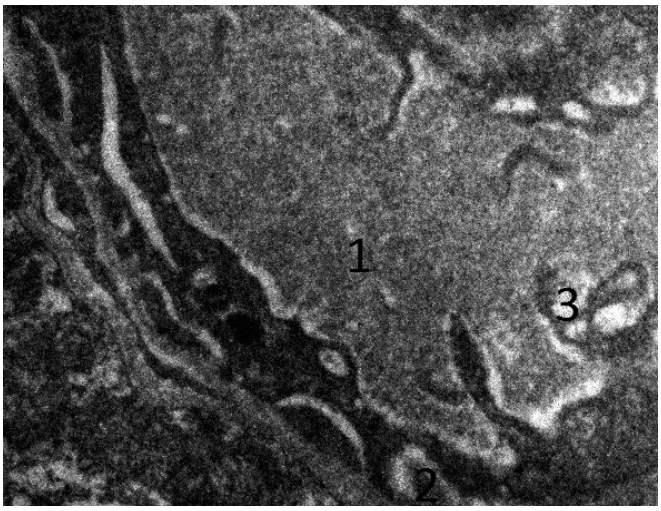

Fig. 6. Ultrastructural changes in the endothelium of the hippocampal formation capillary in the experimental animals on the 14th day of life. × 6,000.
Note: 1 - vessel lumen; 2- broken contacts between endothelial cells; 3- fragmented processes of endothelial cells.

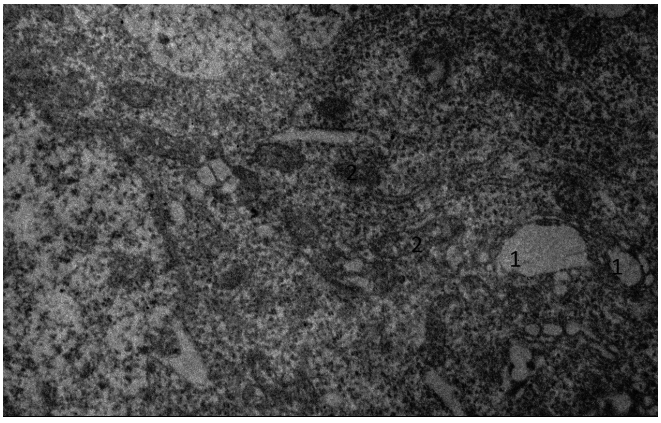

Fig. 8. Subcellular changes in the cytoplasm of the dark neuron CA 2 hippocampus zone in the experimental animals on the 7th day of life. × 6,000.
Note: 1- hypertrophy of the Golgi apparatus; fragmentation of mitochondrial cristae.

contrasted with lead citrate according to the E. Reynolds method for 30 minutes at room temperature. Ultrathin sections were studied in a PEM-100 electron microscope with an accelerating voltage 60 kV.

RESULTS

The most prominent early changes were observed in the synaptic apparatus in posterity of female rats after PgE2 injection for labor induction. In the experimental group at the first day after birth, the neuropil of the hippocampal formation is characterized by edema of astrocytic processes, pre- and postsynaptic processes, impairment of the mitochondrial apparatus, and intercellular edema. In this group of animals in comparison with intact rats, changes in the presynaptic terminals include edema of the presynaptic endings, aggregation of synaptic vesicles in the center of the presynaptic processes with their distancing from the presynaptic membrane, swelling and destruction of mitochondria, the appearance of large vacuoles in the presynaptic processes, perforations at the contact points of pre- and postsynaptic seals (Fig. 1, 2).

Changes in the synaptic apparatus were followed by changes in other structural components of the nervous tissue. Ultrastructural changes in oligodendroglia were revealed at all periods in the experimental group: hypertrophied, fragmented nuclei of oligodendrocytes, stratification of the myelin sheath of the axons of the hippocampal formation (Fig. 3).

It is settled that the severity of ultrastructural impairment in the neuropil is in direct proportion to the severity of microcirculation disorders in the tissue of the hippocampal formation. In the brain of animals of the control group, the lumen of the capillaries and blood vessels is smooth with tight intracellular contacts between endothelial cells (Fig. 4).

On the contrary, in the brain of experimental rats, characteristic microvilli on the endothelial surface, disruption of contacts between endothelial cells and fragmentation of the cytoplasm of endothelial cells with the formation of villi in the lumen of the capillary were found (Fig. 5, 6).

The study revealed changes in the CA1 and CA2 neurons of the hippocampus, dentate gyrus and entorhinal cortex.

The proposed images show that the ultrastructure of mitochondria in the control group had a clear structure of mitochondrial cristae, but the mitochondria of neurons in the hippocampal formation of experimental animals became swollen, vacuolated with rupture and destruction of the cristae. In most cases, mitochondria had an irregular shape, and mitochondria with damaged internal structures and a reduced number of cristae were observed (Fig. 7, 8).

DISCUSSION

According to several authors the effect of PgE2 on laboratory animals during pregnancy, type ischemic injury, disturbances the morphogenesis of the brain in the postery of experimental animals were revealed [11]. We have previously identified changes in the structure of the cerebellar cortex in the form of increasing the distance between the Purkinje cells in posterity of female rats after PgE2 injection for labor induction, which may indicate hypoxia of the brain tissue [12].

The mechanisms of damage are probably caused by hyperstimulation of the uterus with or without changes in fetal heart rate. This refers to an increase of the number of uterine contractions (> 5 contractions in 10 minutes for at least 30 minutes) or uterine hypertonicity (contractions lasting at least two minutes) with a normal fetal heart rate [13]. First of all, under the conditions of these mechanisms, the structures of the brain that are sensitive to hypoxia are damaged. In newborns, pyramidal cells of the hippocampus are still migrating into the corresponding layers CA1, CA2 and CA3 [7,8]. Granular neurons of the dentate gyrus of the hippocampus are forming mainly during the first week after birth [8]. During the 20-30th days after birth, stem cells in the hippocampus are preserved under the granular layer [7].

The changes in the hippocampal formation of rats' offspring after induction of labor include edema of astrocytic processes, pre- and postsynaptic processes, the appearance of large vacuoles in the presynaptic processes, perforation of pre- and postsynaptic seals. Oligodendroglial changes include hypertrophy, oligodendrocytes nuclei fragmentation, stratification of the axons myelin sheaths. We revealed ultrastructural changes in the microvasculature such as a violation of contacts between endothelial cells and the appearance of fragmented sections of the endothelial cells cytoplasm in the lumen of capillaries. Neuronal changes found in experimental animals include disturbances of the mitochondrial apparatus in the form of destruction and fragmentation of cristae, hypertrophy and edema of the Golgi apparatus. Similar changes were revealed in a study of synaptic transmission in the neostriatum after ischemic exposure by Zhi-Ping Pang (2002): vacuolization, edema of astrocytic processes in the neuropil, changes in the presynaptic processes [14]. The revealed changes in the postsynaptic processes are consistent with the results of other researchers describing a decrease in postsynaptic potentials and hypoxia-induced synaptic depression in the hippocampus after hypoxic exposure. [15]. In a study of the reorganization of synaptic structures, Lirui Zhu et al. (2016) observed a significant decrease in the number of synaptic vesicles and impaired spatial organization of postsynaptic structures after total cerebral ischemia [16]. The revealed violations of the integrity of synaptic seals do not contradict the results of a study of the effect of temporary cerebral ischemia, in which M. Martone et al. (1999) revealed a significant increase of perforated synapses [17]. In addition, the unfolding of proteins caused by ischemia can cause aggregation and a change in the organization of postsynaptic compaction [17]. The revealed changes in synaptic compaction may be associated with denaturation of proteins and exposure of their hydrophobic segments during ischemia, causing interprotein aggregation in postsynaptic compaction after ischemia. In accordance with this hypothesis, Martone et al. (1999) in their study describe the ubiquitinization of postsynaptic condensation proteins, which indicates their degradation [17]. Thus, transient ischemia can lead to degradation of synaptic proteins by ubiquitin-dependent proteinases, which leads to dysfunctional synaptic transmission in the altered synapse [18, 19].

The identified ultrastructural disorders of oligodendroglia are consistent with studies by CE Ahearne (2016), Shishkina T et al. (2018) aimed at studying the maturation of oligodendrocyte precursors and their ability to myelinate the developing central nervous system after perinatal asphyxia, which is considered the main cause of long-term neurological complications known as periventricular leukomalacia [20, 21].

Similar disorders were described by Malgorzata Ziemka-Nalecz et al (2018) in a study investigating the effect of total cerebral ischemia on the formation and function of oligodendrocytes. In the brain of experimental rats, they revealed microvilli on the surface of the endothelium and macrophages located in the wall of a blood vessel, indicating a temporary violation of its integrity. In addition, edema and vascular collapse were observed [22]. Global ischemia and subsequent reperfusion of the brain causes neuronal damage in vulnerable areas, which leads to mitochondrial dysfunction and subsequent neuronal death. The induction of neuronal death is mediated by the release of cytochrome c from mitochondria, which is manifested by an increase of the permeability of the outer mitochondrial membrane [23]. The consequences of transient global cerebral ischemia are often quite serious and selectively affect vulnerable areas of the brain, including the pyramidal neurons of the CA1 zone of the hippocampus. The specific molecular mechanisms of CA1 neuronal death are not fully understood, but it is well known that mitochondria play a central role in apoptotic cell death [24]. Haibin Wang's (2016) study of the hippocampus subjected to oxygen-glu-

cose deprivation and reoxygenation as a model of cerebral ischemia found ultrastructural changes in mitochondria using transmission electron microscopy [25].

Thus, based on the data obtained in the study of the hippocampal formation in postery of rats after induction of labor, analysis of the literature devoted to the electron microscopic study of the brain after ischemic injuries, it can be concluded that on the background of stimulation of labor by PgE2 in the rat brain, changes corresponding to ischemic damage.

CONCLUSIONS

1. After labor induction by PgE2 the ultrastructural impairment of the endotheliocytes of blood capillaries of the posterity's brain within two weeks after birth is characterized by fragmentation of the cytoplasm, contacts contravention between neighbor endotheliocytes, villi appearance on the luminal suface of endotheliocytes.
2. In the CA1, CA2 and dentate gyrus regions of experimental rats hippocampus within two weeks of postnatal life a hypertrophy of oligodendroglial cells, oligodendrocyte nuclei fragmentation, stratification of the myelin sheath of axons, mitochondria edema and vacuolization, rupture and destruction of mitochondria cristae, massive granular vesicles and microtubules formation, Golgi apparatus hypertrophy in neurons were established.
3. Throughout two weeks after birth the violation of the synaptic apparatus in the form of presynaptic endings edema, synaptic vesicles aggregation in the center of the presynaptic processes, mitochondria swelling and destruction, large vacuoles formation in presynaptic processes, perforations at the sites of contact of pre- and postsynaptic seals are revealed.

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Supporting and withdrawal of animals from experiment was carried out in accordance with the requirements of the European Commission Directive (86/609/EEC), Law of Ukraine № 1759-VI (15.12.2009) On the Protection of Animals from Cruelty.

ORCID and contributionship:
Olena A. Hryhorieva: 0000-0002-6101-8322 [A,D-F]
Iryna Yu. Mamay: 0000-0002-1437-8106 [B-D]
Serhii Tertyshniy: 0000-0003-3856-4234 [E,F]
Volodymyr Dariy: 0000-0001-9074-6911 [E,F]
Yuriy Y. Guminsky: 0000-0002-8688-9829 [E,F]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Olena A. Hryhorieva
Zaporizhzhia State Medical University
26 Mayakovstiy pr., 69035 Zaporizhzhia, Ukraine
tel: +3800505450471
e-mail: elengrig212@gmail.com

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