Ultrastructural Changes in the Vessels of Hemomicrocirculatory Bed of the Iliac Lymph Nodes of White Rats in the Durable Action of the Opioid Nalbuphine

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Abstract
This article represents the electronic-microscopic examination of blood vessels of the hemomicrocirculatory bed of the iliac lymph nodes of white rats, males of reproductive age, who, during six weeks, received narcotic opioid analgesic — nalbuphine, increasing the weekly dose of the medicine to create a model of physical opioid dependence according to Ukraine Patent # 76564 U.

It was determined that opioid nalbuphine causes the reactive changes in the blood vessels of the hemomicrocirculatory bed of the iliac lymph nodes in the early stages of drug administration — within 1-2 weeks. A prolonged six-week administration of nalbuphine leads to profound destructive changes in the microvessels: the lumen of the hemocapillaries, which contains mainly the destructively altered erythrocytes, is expanding; the wall of microvessels, accompanied by hemorrhages into the vascular space, is violated; the structure of endotheliocytes nuclei changes, their cytoplasm swells, and organelles are damaged in it; the basement membrane thickens; the perivascular space swells and dilates. One week after the abolition of nalbuphine, the inverse changes in the blood vessels of hemomicrocirculatory bed do not occur.

Keywords
lymph node; hemocapillary; venule; endotheliocyte; nalbuphine; effect; white rat

Problem statement and analysis of the recent research
Lymph node — is a secondary lymphoid organ, in which antigen-dependent proliferation and differentiation of sub-populations of T- and B-lymphocytes, as well as purification of the lymph through which it passes, occur [8]. Scientific literature has a lot of data on the influence of various physical and chemical factors, medicines, antigens on these important organs, since it depends on them the formation of both humoral and cellular immunity [4, 6, 8].

An important social and medical problem in recent decades is drug addiction [9, 12, 16]. Widespread use in the clinic of narcotic analgesics, in particular opioids, has led to the study of the influence of these medicines on various organs and systems of the body [5, 7, 11, 13]. Opioids — are opioid poppy alkaloids (morphine, codeine, etc.), as well as synthetic substances (methadone, tramadol, etc.) and medicines of semi-synthetic origin (nalbuphine, ethylmorphine, heroin, etc.). These substances specifically interact with opiate receptors of the brain, thus realizing the narcotic effect [14, 15].

The influence of opioids, in particular nalbuphine, on the primary lymphoid organ – thymus [1-3] — has been studied. In the scientific literature there are no data on the reaction of the lymph nodes on opioids, which emphasizes the topicality of our study.

The objective of the study. To establish the peculiarities of the reconstruction of blood vessels of the hemomicrocirculatory bed of the parenchyma of the lymph nodes of white rats-males of reproductive age in the dynamics of a prolonged six-week influence of nalbuphine on the organism before and after its abolition.

1. Materials and methods of the research
This study was performed on 52 white, outbred male rats of reproductive age, with an initial body weight of 140-150 g (1.5-2 months old). Opioid - nalbuphine was injected to the test animals daily, at identical intervals, at the right buttock site, - intramuscularly, during 6 weeks. The weekly dose of the medicine for injections was gradually increased, according to the patent number # 76564 U "Method of the modeling of physical opioid dependence in rats” [10].

Experimental animals are divided into 8 groups: group 1 - 5 intact rats; group 2 - 5 rats, who received nalbuphine daily
for one week at a dose of 8 mg/kg; group 3 - 5 individuals who received nalbuphine during the second week at a dose of 15 mg/kg; group 4 - 5 animals, nalbuphine was administered during the third week at a dose of 20 mg/kg; group 5 - 5 individuals who received nalbuphine during the fourth week at a dose of 25 mg/kg; group 6 - 5 animals that received nalbuphine for the fifth week at a dose of 30 mg/kg; group 7 - 5 rats who were given nalbuphine during the sixth week at a dose of 35 mg/kg; group 8 - 5 individuals who did not receive the opioid (weaning off) during the seventh week. The dose of nalbuphine was selected according to the patent # 76564 U "Method of the modeling of physical opioid dependence in rats" [10].

Experimental animals were kept in the vivarium of Lviv National Medical University named after Danylo Halytsky, according to the agreement of 2013.11.18 about the cooperation between the Department of Normal Anatomy of the Lviv National Medical University named after Danilo Halytsky and the Department of Human Anatomy and Histology at the Medical Faculty in Uzhhorod National University. Experiments were performed in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), Council of Europe Directives 86/609/EEC (1986), Law of Ukraine # 3447-I "On the Protection of Animals from cruel treatment", "General ethical principles of the experiments on animals", adopted by the first National Congress of Ukraine on Bioethics (2001).

Histologic preparations, thickness of 5-7 microns, stained with hematoxylin and eosin were made according to generally accepted rules. Images of the histological preparations on the computer monitor were taken from a microscope MICROMed-SEOSCAN and using a VisionCCDCamera.

For an electronic-microscopic examination the pieces of lymph nodes in the volume of 1-1.5 mm³ were fixed by a 1.5% solution of osmium tetroxide in a 0.2 M solution of sodium cacodylate at pH 7.2 during 2-2.5 hours in cold. After this, the organ samples were dehydrated in the increasing concentrations of ethyl alcohol (50°, 70°, 90°, 100°) for 30 minutes in each and propylene oxide for 10 minutes, were poured into a mixture of epoxy resins and polymerized for 24 hours in a thermostat at 60°C. The slices were made on ultramicrotome UMTP-6M using a diamond knife (DIATOM) and double-contrasting was performed according to Reynolds and uranyl acetate. Using the TEM-100 electronic microscope, sections of the lymph nodes were examined and photographically documented using SONY-H9 digital camera.

The half-thickness sections 1-2 microns were made using ultramicrotome LKB-3 (Sweden). They were stained with methylene blue.

2. Results of the research and their discussion

The performed electronic microscopic study showed that in the use of opioid nalbuphine there are changes not only in structural components of the lymph nodes, but also in the blood vessels of the hemomicrocirculatory channel. In the short-term effect of nalbuphine within 1-2 weeks, there are the first submicroscopic reactive changes in the microvessels. Hemocapillaries are significantly enlarged and full-blooded. The nuclei of the endothelial cells are enlarged and have uneven contours - the nucleus forms a shallow invaginations and protrusions, the karyoplasm contains mainly euchromatin, a few organelles are in the cytoplasm of these cells, some of them destructively altered. Basal membrane is somewhere unclearly contoured, thickened and wavy. There is moderate perivascular edema, in the loose connective tissue the content of the amorphous component increases (Fig. 1).

Venules are dilated and full-blooded; there is an edema of the endothelial cells’ cytoplasm and destruction of the organelle. Basal membrane somewhere thickens, it is unclearly contoured, the perivascular spaces are moderately enlarged (Figs. 2, 3).

Administration of the opioid nalbuphine to animals within 3-4 weeks results in destructive submicroscopic changes in the structural components of the blood vessels of the hemomicrocirculatory bed of the iliac lymph nodes of white male rats. Lumen of hemocapillaries is considerably expanded and blood-filled. The nuclei of the endothelial cells are enlarged; heterochromatin clots are present in the karyoplasm. In the cytoplasm of these cells there are many edematous mitochondria with cristae destruction, their matrix is electronically light. Basal membrane of hemocapillaries is uneven, has considerably thickened areas (Fig. 4).

Submicroscopic changes in venules also increase: their diameter is significantly increased, and the extended lumen is full-blooded; endothelial cell cytoplasm is edematous; parts of the organelles are damaged. Basal membrane of venules is uneven, has considerably thickened areas (Figs. 5, 6).

In the long-term administration of opioid nalbuphine during 5 and 6 weeks on electronograms of lymph nodes in the part of blood capillaries, in their expanded lumens, there is a sludge-effect - erythrocytes form dense clusters. The invagination of the nuclear membrane (karyolmma) of the endothelial cells remains, and in the karyoplasm there are concentrations of heterochromatin near the nuclear membrane, which have the form of the osmophilic strip. Cytoplasmic organelles are significantly damaged. There are few pinocytic vesicles in edematous cytoplasmic regions of the endothelium. The basal membrane is thickened, it is not clearly contoured. Perivascular spaces are enlarged due to edematous adventitia (Fig. 7).

Venules' ultrastructure has changes similar to the previous experiment period: their lumen is enlarged and blood-filled; cytoplasm of the endothelial cells is swollen, organelles are damaged, there are few pinocytic vesicles; basal membrane is thickened, unclear (Fig. 8).

One week after the abolition of nalbuphine, submicroscopic structural disorders of the blood vessels of the hemomicrocirculatory bed of the iliac lymph nodes were similar to

Figure 1. Ultrastructure of the hemocapillary of the iliac lymph node of white male rat within one week of the action of nalbuphine: erythrocyte (1) in the lumen of the hemocapillary; increased nucleus with invaginations of the nuclear membrane (2) and cytoplasm (3) of the endothelial cell. Magnification: ×14000.

Figure 2. Ultrastructure of the venule of the iliac lymph node of white male rat after two weeks of the effect of nalbuphine: dilated lumen of the venule (1) with erythrocytes (2) and lymphocytes (3); thickened basal membrane (4). Magnification: ×9000.

changes after six weeks of nalbuphine action and did not recur.

Part of the blood capillaries have a non-wide lumen filled with erythrocytes. The nuclei of endothelial cells are elongated; in their karyoplasm heterochromatin is located perinuclearly. In the paranuclear cytoplasm there are few organelles, they are damaged, there are unstructural areas. The basal membrane of hemocapillaries is unclearly contoured; perivascular spaces are dilated and lumenized, indicating swelling (Fig. 10).

Venules are enlarged, full-blooded one week after the cessation of medicine use. In the part of the endothelial cells there are changed nuclei having an uneven nuclear membrane, and
in the karyoplasm osmiophilic granules of heterochromatin. There is the destruction of the cytoplasm organelles, there are vacuoles of various. The basal membrane is thickened, poorly contoured, perivascular spaces are enlarged.

3. Conclusions

1. It has been established that even in the short-term, within 1-2 weeks, introduction of opioid nalbuphine to test animals, there are reactive changes in the blood vessels of hemomicrocirculatory bed of the iliac lymph nodes, which are manifested by moderate expansion

Figure 3. Fragment of the lymphoid node of the iliac lymph node of white male rat after two weeks of the action of nalbuphine: moderately expanded hemocapillary (1), filled with the blood corpuscles (2); lymphocytes (3). Semifine section. Staining with methylene blue. Magnification: ×400.

Figure 4. Ultrastructure of the hemocapillary of the iliac lymph node of white male rat three weeks after the action of nalbuphine: the lumen of the hemocapillary with destructively altered erythrocytes (1); nucleus (2) and edematous cytoplasm (3) of the endotheliocyte; thickened basal membrane of hemocapillary (4). Magnification: ×10000.
and blood-filling of hemocapillaries; an increase of endotheliocytes’ nuclei and the appearance of destructively altered organelles in their cytoplasm. Basal membrane is somewhere unevenly contoured, thickened and wavy. There is a slight perivascular edema. Such changes are also characteristic for venules.

2. Subsequent administration of narcotic analgesic nalbuphine up to six weeks deepens the destructive changes in blood vessels of the hemomicrocirculatory bed: the lumen of hemocapillaries is expanded, filled mainly with destructively altered erythrocytes, somewhere the integrity of the walls of hemocapillaries is violated with the release of the formed blood elements into the perivascular space; endothelial cells nuclei are in-

Figure 5. Ultrastructure of the venule of the iliac lymph node of white male rat in four weeks of nalbuphine action: deformed erythrocyte (1) in the enlarged lumen of the venule; endothelial cell cytoplasm (2), thickened and edematous basal membrane (3). Magnification: ×9000.

Figure 6. A fragment of the paracortex region of the cortical substance of the iliac lymph node of white male rat in four weeks of nalbuphine action: an enlarged and full-blooded venule with a thickened wall (1); lymphocytes (2); expanded retrocapillary venule (3). Semifine section. Staining with methylene blue. Magnification: ×400.

Figure 7. Ultrastructure of the hemocapillary of the iliac lymph node of white male rat after five weeks of nalbuphine action: nucleus with invaginations and protrusions of the nuclear membrane (1) and cytoplasm (2) of the endotheliocyte; protrusions and invaginations of plasmolemma on the lumen surface of the endotheliocyte (arrows); erythrocyte (3) in the lumen of the hemocapillary. Magnification: ×17000.

Figure 8. Ultrastructure of the venule of the paracortex region of the cortical substance of the iliac lymph node of white male rat in six weeks of nalbuphine action: deformed erythrocytes (1) in the lumen of the venule; endothelial cell cytoplasm with damaged organelles (2); thickened and stratified basal membrane of the endothelial cell (3). Magnification: ×15000.

creased, irregular, cytoplasm is edematous with damaged organelles; basal membrane is thickened; perivascular space is swollen and enlarged.

3. After the abolition of the medicine nalbuphine there are no reversible changes of vessels of the hemomicrocirculatory bed of the iliac lymph nodes.

References

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Figure 9. A fragment of the paracortex region of the cortical substance of the iliac lymph node of white male rat in six weeks of nalbuphine action: an enlarged vein (1) with a damaged wall (2) and the release of the formed blood elements (3) into the parenchyma of the node; lymphocytes (4). Semifine section. Staining with methylene blue. Magnification: ×400.

Figure 10. Submicroscopic state of the hemocapillary of the iliac lymph node of white male rat within one week after the abolition of nalbuphine: deformed erythrocyte in the lumen of the hemocapillary (1); elongated form of nucleus (2) and cytoplasm (3) of endotheliocyte; unclearly contoured basal membrane of hemocapillary (4); edema around the capillary space (5). Magnification: ×9000.


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Ultrastructural Changes in the Vessels of Hemomicrocirculatory Bed of the Iliac Lymph Nodes of White Rats in the Durable Action of the Opioid Nalbuphine — 8/8

(56): 55-59.


Received: 11 Feb 2018
Revised: 25 Feb 2018
Accepted: 22 Mar 2018