Праці НТШ
Мед. науки 2015. Т. XLIII. С. 75–87
Оригінальні дослідження:
Фундаментальні науки

Proc. Shevchenko Sci. Soc.
Medicine 2015. Vol. XLIII. P. 75–87
Research:
Fundamental sciences

УДК: UDK 617.723-018.1:615.212.7] – 08 – 092.4/.9

Lesya MATESHUK-VATSEBA, Uliana PIDVALNA, Anna BEKESEVYCH, Aksiniya ZINKO, Petro POPYK, Ivan DISKOVSKYI

THE EFFECT OF THE PROLONGED INJECTION OF NALBUPHINE ON THE STRUCTURAL ORGANIZATION OF ANGIOARCHITECTURE OF ORGANS

Danylo Halytsky Lviv National Medical University, Lviv, Ukraine, Ukraine, rombek75@gmail.com

Aims: *To establish peculiarities of structural organization of angioarchitecture of pancreas, cerebellum, telencephalon, eyeball and skin in the dynamics of the long-term effect of Nalbuphine.*

Materials and methods: *The study was carried out on 48 mature white male rats divided into 3 groups. Nalbuphine was intramuscularly injected to the experimental animals during six weeks in doses: 8, 15, 20, 25, 30, 35 mg/kg of body weight. 18 white rats to which saline solution was injected served as the control group. The research materials were presented by specimens of the white rats' cerebellum, telencephalon, eyeball and skin with the injected vascular bed, histological specimens and ultrathin sections of the organs. The studies were conducted with application of histological, electron microscopy, injection and morphometric methods of investigation.*

Results: *The first signs of impairment of angioarchitecture have been detected already after 2 weeks of injecting Nalbuphine. Pathological changes keep growing throughout the entire subsequent period of the experiment manifested by obliteration of capillaries, rarefaction of vaculature, tortuosity of the preserved vessels. After 6 weeks of injecting Nalbuphine microcirculatory bed of the cerebellar cortex, white substance of the telencephalon, eyeball vascular tunic, pancreas and skin appear to be at the stage of decompensation, when the capillary component is ruined, arterioles are sharply twisted, deformed, their lumen uneven, venules dilated and deformed.*

Conclusion: *injection of Nalbuphine inflicts a considerable damage on the micro- and ultrastructural organization of the components of microcirculatory bed of the experimental animals' organs.*

Key words: *microvessels, morphology, narcotic drug, white rat.*

Леся МАТЕШУК-ВАЦЕБА, Уляна ПІДВАЛЬНА, Анна БЕКЕСЕВИЧ, Аксінія ЗІНЬКО, Петро ПОПИК, Іван ДІСКОВСЬКИЙ

ВПЛИВ ТРИВАЛОГО ВВЕДЕННЯ НАЛБУФІНУ НА СТРУКТУРНУ ОРГАНІЗАЦІЮ АНГІОАРХІТЕКТОНІКИ ОРГАНІВ

Львівський національний медичний університет імені Данила Галицького, Львів, Україна, rombek75@gmail.com

Мета. *Визначити особливості структурної організації ангіоархітектоніки підшлункової залози, мозочка, кінцевого мозку, очного яблука та шкіри в динаміці тривалого впливу налбуфіну.*

Матеріали і методи. *Дослідження виконані на 48 статевозрілих білих щурах-самцях. Експериментальним тваринам вводили внутрішньом'язево налбуфін впродовж шести тижнів. Контролем слугували 18 білих щурів, яким вводили фізіологічний розчин. Матеріали дослідження представлені препаратами підшлункової залози, мозочка, кінцевого мозку, очного яблука та шкіри щурів з ін'єкованим судинним руслом, гістологічними препаратами та ультратонкими зрізами органів. Застосовували гістологічний, електронномікроскопічний, ін'єкційний та морфометричний методи дослідження.*

Результати. *Виявлено перші ознаки порушення ангіоархітектоніки вже через два тижні введення налбуфіну. Впродовж наступного терміну експерименту патологічні зміни збільшуються, що проявляється облітерацією капілярів, нерівномірністю калібру судин, розрідженням судинної сітки, звивистістю збережених судин. Через шість тижнів введення налбуфіну гемомікроциркуляторне русло всіх органів перебувало на стадії декомпенсації, коли капілярний компонент зруйнований, артеріоли різко покручені, деформовані, їхній просвіт нерівномірний, венули розширені та деформовані.*

Висновки. *Введення налбуфіну значно порушує мікро- та ультраструктурну організацію ланок гемомікроциркуляторного русла органів експериментальної тварини. Найшвидше на негативний вплив опіоїду реагує ендотелій капілярів мозку та очного яблука.*

Ключові слова: *мікросудини, морфологія, наркотичний середник, білий щур.*

INTRODUCTION

The problem of drug addiction in the last decades has become not only a social but, primarily, a medical problem as well (Pidvalna, 2014). According to the recent data prevalence of the diseases of narcologic nature in our country attains in absolute figures 960,000 individuals, or 2,500 cases per 100,000 of population (Vijevs'kyj et al, 2012). Taking into consideration the fact, that sensation of pain attains up to 40% of all complaints of those applying for the initial medical care the problem of pharmacotherapy and an effective pain control remains to be one of the most urgent tasks of modern pharmacology and medical science in general (Buhtiarova et al , 2011; Dachydovych et al; 2011, Maremmani et al, 2009). It is known, that protracted pain is accompanied by the system pathophysiological reaction of the entire organism with the development of oxidative stress syndrome and a multitude of disorders of biochemical reactions in separate cells (Altarifi et al, 2015; Nef'odov et al, 2011). Extensive use of narcotic agents and proliferation of drug addiction predetermine the need for the study of the effect of opioid on the structural organization of the organs and systems, for it is generally recognized, that each function is based on their adequate structure (Popyk, 2014). The use of opioids predetermines development of structural changes in various organs and systems, which reduces expectancy and quality of life of the individuals, using narcotic analgetic agents (Anselmi et al, 2013; Bailey, 2005). It is known, that the risk of cardiovascular diseases, development of hepatic and renal insufficiency in such patients increases (Ogurcov & Mazurchik, 2007). In professional literature there are occasional data on the peculiarities of microcirculation in the vessels of ischemic brain of the rat in case of opioid lymph stimulation (Fomina, 2012) and on the results of biomicroscopic studies of the reaction of microvessels to the intraperitoneal injection of opioid (O'Connor & McMahon, 2008; Voronkov et al, 2008). It is exactly angiopathy that remains to be one of the gravest manifestations of the effect of endo- and exopathogenic factors on the organism (Mateshuk-Vatseba & Diskovs'kyj, 2014). The study of morphology of the effect of narcotic substances on the organism is topical and requires further studies (Borys, 2011). That is why the task of this investigation was to establish peculiarities of structural organization of the components of microcirculatory bed of pancreas, cerebellum, telencephalon, eyeball and skin in the dynamics of the prolonged effect of Nalbuphine.

MATERIALS AND METHODS

The study was carried out on 48 mature white male rats aged 3.0 – 3.5 months and body weight 160-180 g. Nalbuphine was injected intramuscularly daily to the experimental animals of the first group (10 rats) during 2 weeks (1st week – 8 mg/kg, 2nd week - 15 mg/kg); Nalbuphine was injected intramuscularly daily to the experimental animals of the second group (10 rats) during 4 weeks (1st week – 8 mg/kg, 2nd week - 15 mg/kg, 3rd week - 20 mg/kg, 4th week - 25 mg/kg; Nalbuphine was injected intramuscularly daily to the experimental animals of the third group (10 rats) during 6 weeks (1st week – 8 mg/kg, 2nd week - 15 mg/kg, 3rd week - 20 mg/kg, 4th week - 25 mg/kg, 5th week - 30 mg/kg, 6th week - 35 mg/kg (Onys'ko et al, 2013). 18 white rats to which saline solution was injected served as the control group. All animals were kept in the vivarium of Danylo Halytsky National Medical University of Lviv, and the experiments were conducted in compliance with the provisions of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986), European Council Directive 86/609/EEC (1986), the Law of Ukraine #3447-IV «On protection of animals from cruel treatment», general ethical principles of experiments on animals approved by the first National Congress of Ukraine on Bioethics (2001).

Sampling of the material was made after 2, 4, 6 weeks of the experiment. The animals were withdrawn from the experiment by the use of thiopental sodium at the rate of 25 mg/kg of body weight.

The research materials were presented by specimens of the rats' pancreas, cerebellum, telencephalon, eyeball and skin with the injected vascular bed, histological specimens and ultramicroscopic sections of the organs. The sections of the organs for histological examination were stained with hematoxylin and eosin. These specimens were studied and photographed under microscope МБИ-1 and a digital camera Olympus FE210 with microscope magnification ×400.

Indian ink was used as the injection mass for injection in the bloodstream. Clarification of the sections was made in glycerin with 96% ethanol in a 1:1 ratio during 3 days and in pure glycerin afterwards. These specimens were studied and photographed under microscope MBI-1 and a digital camera Olympus FE210 with microscope magnification ×160.

The following quantitative criteria were used for the morphometric analysis of the condition of microcirculatory bed of the organs: diameter of microvessels, arteriolovenular factor, tortuosity coefficient, density of the network of exchange vessels (the number of capillaries per area unit), index of tissue trophic activity (radial diffusion). Statistical study of the results was made using computer with the aid of application package for medico-biological and epidemiological investigations "InStat". Ultrastructural study of the rat's organs was conducted with the aid of electron microscope UEMB-100K (Ukraine) at acceleration speed 75 kV and magnification on the microscope screen ×4000, ×8000. Ultrathin sections were prepared with the aid of ultramicrotome UMTP-3M with the help of glass knives produced on CCH-1 device.

RESULTS

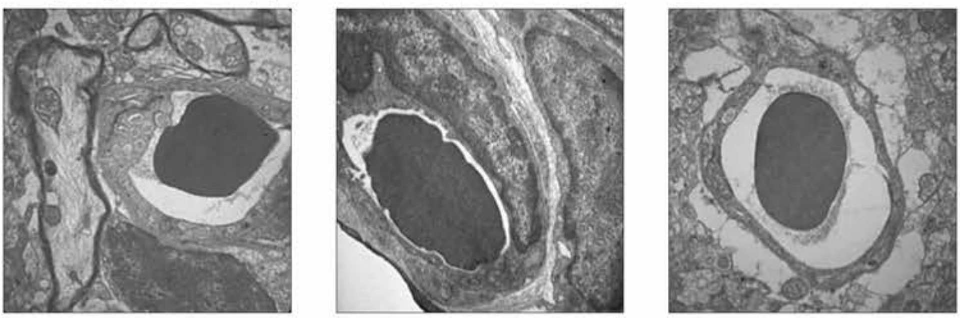
lAfter 6 weeks of injecting Nalbuphine the injected and clarified specimens of the organs showed dilatation of arterioles up to 29.12±1.4 μm (in control group — 27.80±2.29 μm). Lumen of venules at this stage of the experiment, probably, does not change, diameter of the venules attains 29.98±0.85 μm (in control group — 27.09±1.44 μm), arteriolovenular coefficient increases up to 0.98±0.03 (in control group — 0.80±0.07) respectively. The said above is confirmed in Fig. 1.

Ultrastructural organization of capillaries of the rat's cerebellar cortex, eyeball vascular tunic and white substance of telencephalon is impaired considerably after two

A B

Fig. 1. Microcirculatory bed of the white substance of the control group rat's telencephalon (A) and after 2 weeks of Nalbuphine injections (B). Microphotograph. Injection of vessels. Magnification: ×160

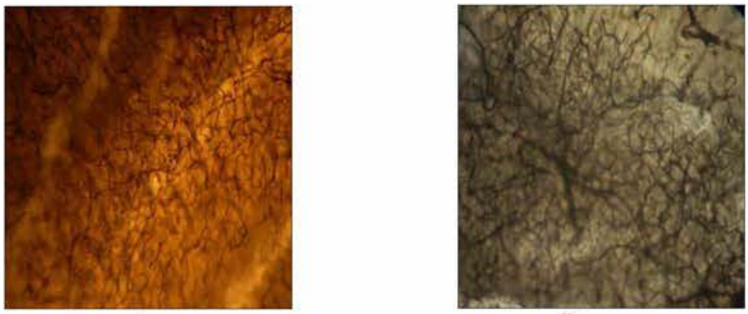
weeks of injecting Nalbuphine (Fig. 2). Edema of endotheliocytes was observed in the capillaries, their lumens acquiring an irregular form. There was found edema and, as a consequence, a thickening of the basal membrane, though its integrity was preserved. Pericytes predominantly preserve connection with the membrane.



A B C

Fig. 2. Ultrastructure of the capillary of the rat's cerebellar cortex (A), eyeball vascular tunic (B) and white substance of telencephalon (C) after two weeks of injecting Nalbuphine. Electron micrograph. Magnification: ×8000

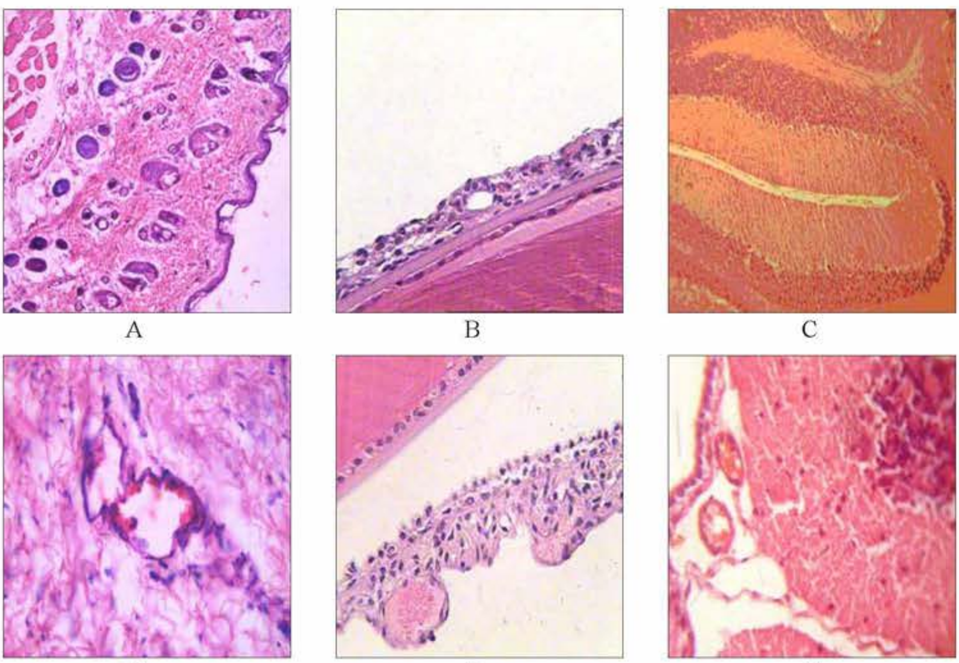
After 4 weeks of injecting Nalbuphine arterioles are sharply twisted, their caliber uneven (Fig. 3). Arterioles tortuosity coefficient is 0.50±0.02 (in control group — 0.29±0.01). Capillary networks are characterized by the following morphometric indices: diameter of the capillaries attains 6.20±0.27 μm (in control group — 5.81±0.21), density of the exchange vessels network — 54.0±0.7 (in control group — 60.8±5.4 μm), tissue trophic activity index — 44.1±3.5 μm (in control group — 46.3±3.4 μm). There is observed obliteration of capillaries, hemorrhages, irregular caliber of the vessels, rarefaction of vasculature, tortuosity of the preserved vessels. Venular component of microcirculatory bed is dilated, diameter of the venules attains 43.63±2.74 μm



A B

Fig. 3. Microcirculatory bed of control rat's cerebellum (A) and after 4 weeks of injecting Nalbuphine (B). Microphotograph. Injection of vessels. Magnification: ×200

Changes have been detected on level of light microscopy in the organs' bloodstream components. Lumens of the vessels are dilated, filled with blood cells. Some arterioles' walls are thinned, there is edema of endothelium. However, other arteriolar walls are thickened, endotheliocytes are distributed unevenly on the internal surface of blood vessels. There are also present «varicose» dilated venules, perivascular polymorphonuclear infiltrates (Fig. 4).

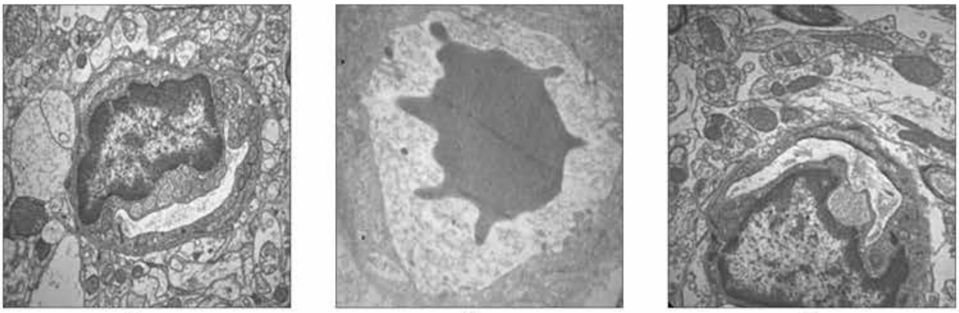


A B C

D E F

Fig. 4. Skin of external femur surface (A), iris of the eyeball (B), cerebellar cortex of the white rat (C) in control group.Skin of external femur surface (D), iris of the eyeball (E), cerebellar cortex of the white rat (F) after 4 weeks of injecting Nalbuphine. Microphotograph. Staining with hematoxylin and eosin. Magnification: ×600 (A, B, D, E, F), ×200 (C).

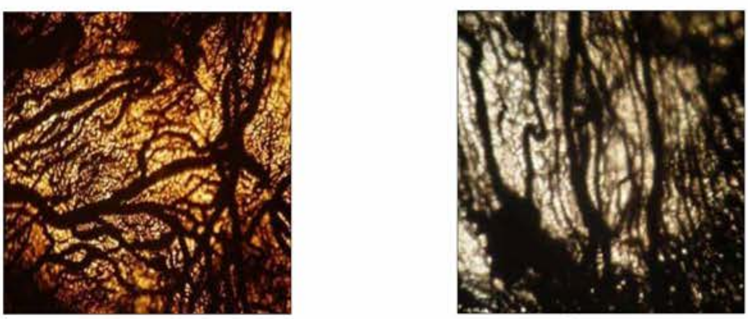
After 4 weeks of the experiment angiopathy develops also in the white substance of telencephalon, in pancreas and in the skin (Fig. 5). Electron-dense nuclei of endotheliocytes protrude into the capillaries' lumen, acquiring an excessively elongated form. Anuclear areas of endotheliocytes are thinned. Basal membrane loses its sharp contour. Pericytes occasionally delaminate from it. Plasmolemma forms protrusions into the capillaries' lumen.



A B C

Fig. 5. Ultrastructure of the white substance of telencephalon capillary (A), pancreas (B) and cerebellar cortex (C) of the rat after 4 weeks of influence of opioid. Electron micrograph. Magnification: ×4000 (A, C), ×8000 (B).

Deep destructive changes in the microcirculatory bed are observed after 6 weeks of injecting Nalbuphine . Vascular loops lose their delicate, tortuous pattern and often break off. Arteriolovenular anastomoses widen and blood from the arterioles discharges into venous bed bypassing the ruined capillaries. Hemorrhages, microaneurysms have been observed (Fig. 6). A change of caliber and density of the vessels, breakdown of their integrity are observed and confirmed by morphometric indices. Diameter of the capillaries drops to 5.19±0.04 μm ($p<0.05$), that of arterioles drops to 18.3±0.1 μm ($p<0.05$), diameter of venules attains 30.8±0.3 μm, arteriolovenular coefficient drops to 0.610±0.004

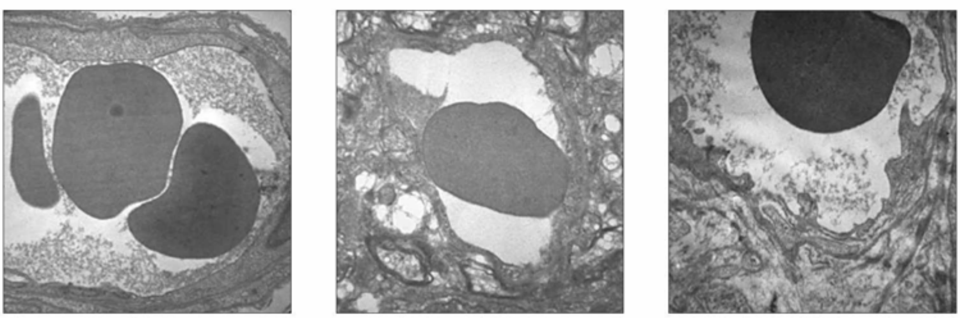


A B

Fig. 6. Microcirculatory bed of the eyeball vascular tunic of control rat (A) and after 6 weeks of injecting Nalbuphine (B). Microphotograph. Injection of vessels. Magnification: ×200

($p<0.05$), arterioles tortuosity coefficient increases up to 0.62±0.02 ($p<0.05$). Density of the exchange vessels network drops abruptly and attains 49.6±0.4 ($p<0,05$) and tissue trophic activity index increases up to 50.1±2.3 μm ($p<0,05$).

At this stage of the experiment we observe a pronounced arteriolar smooth muscle hyperplasia, perivascular infiltrates. Arterioles' walls are thickened due to plasma extravasation, sclerosis and hyalinosis. Lumens of microcirculatory bed elements lose their regular form. The walls of capillaries and venules are deformed. After 6 weeks of the experiment we observe induration of cytoplasm, decrease of pinocytic folds and vesicles, reduction of organelles. An increased number of lysosomes, hypertrophic mitochondrions and Golgi complex are found in some endotheliocytes. Cytoplasm of endotheliocytes contains numerous precipitates and coagulates, nucleolemma becomes loose and disintegrates, karyolysis begins. Basal membrane is blurred, multi-layered with wide gaps between the layers. Basal membrane shows fragmentation. Adhesion of blood cells to endothelium, formation of leukocyte, thrombocyte, erythrocyte aggregates is observed in lumens of the vessels. The form of erythrocytes changes. Fragments of cytoplasm were found in lumens of the vessels. Pericapillary edema, plasma extravasation, mucoid swelling of connective tissue (this is especially pronounced in the pancreas), fibrinoid necrosis (skin) and hemorrhagic infiltration are observed (Fig. 7). Numerous rough fibrous structures appear in the periphery from the basal membrane which testifies to paracapillary sclerosis.



A B C

Fig. 7. Ultrastructure of the capillary of endocrine part of the pancreas (A), cerebellar cortex (B), eyeball vascular tunic (C) of the rat after 6 weeks of injecting opioid.
Electron micrograph. Magnification: ×4000.

The depth of structural transformations of microcirculatory bed of the organs in the dynamics of injecting opioid correlates with the morphometric indices (*Table 1*, Fig. 8–11).

Table 1.

Morphometric analysis of the diameter of arterioles (µm) of the control white rat's eyeball vascular tunic proper and after injecting Nalbuphine during 2, 4 and 6 weeks

Index	*Control*	*1st group*	*2nd group*	*3rd group*
Arteriole diameter, µm	*21,798 ±2,29*	*29,424 ±1,402*	*31,972 ±1,697*	*28,424 ±0,405*
Venule diameter, µm	*27,092 ±1,438*	*29,984 ±0,845*	*43,628 ±2,743*	*48,744 ±2,585*
Capillaries' diameter	*5,644 ±0,419*	*7,074 ±0,232*	*8,142 ±0,122*	*15,132 ±3,547*
Density of exchange vessels network	*120,40 ±7,53*	*115,2 ±5,15*	*96,00 ±3,93*	*64,00 ±5,82*
Index of trophic activity	*18,14 ±0,738*	*12,198 ±0,515*	*13,824 ±0,398*	*31,944 ±2,007*

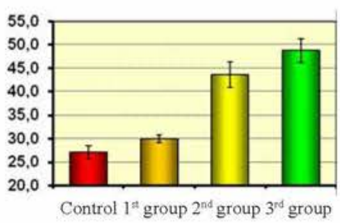


Fig. 8. Morphometric analysis of the diameter of arterioles (µm) of the white rat's eyeball vascular tunic proper after injecting Nalbuphine during 2, 4 and 6 weeks.

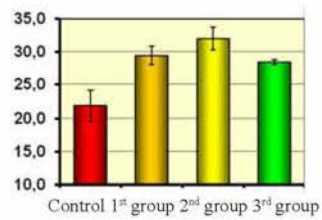


Fig. 9. Morphometric analysis of the diameter of venules (µm) of the white rat's eyeball vascular tunic proper after injecting Nalbuphine during 2, 4 and 6 weeks.

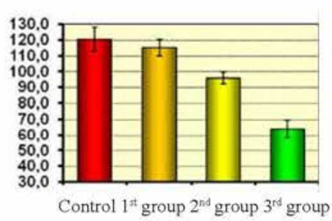


Fig. 10. Morphometric analysis of the index of density of the network of exchange vessels of the white rat's eyeball vascular tunic proper after injecting Nalbuphine during 2, 4 and 6 weeks.

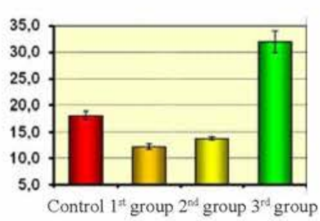


Fig. 11. Morphometric analysis of the index of trophic acivity of tissue of the white rat's eyeball vascular tunic proper after injecting Nalbuphine during 2, 4 and 6 weeks.

DISCUSSION

Despite the wide range of modern methods of studies of the mechanisms of lesions of various organs and systems on the morphological level that appear under the effect of opioids, the results of morphometric, ultramicroscopic studies that could reveal this problem still remain practically undocumented. That is why the study of microcirculatory bed in the dynamics of the prolonged effect of opioid is undoubtedly of essential practical importance both, in medical and in social aspects. Our studies have shown, that already after 2 weeks of injecting Nalbuphine to the white rats there appear the first sings of impairment of angioarchitecture of all organs. There have been found intra- and extravascular changes in the components of microcirculatory bed of the eyeball and the brain. This confirms the idea of a number of the authors, that the vessels of microcirculatory bed of the eyeball and the brain are among the first to react to the pathogenic factors by their structural changes that appear to be the grounds for the development of a pathologic process and determine its character and the peculiarities of clinical manifestations (Ovcharenko et al, 2012). In case of experimental diabetes mellitus reduction of density of the capillaries network, an increased index of tissue trophic activity and diameter of the capillaries, rarefaction of the eyeball vascular tunic have been observed, which testifies to the presence of decompensatory processes (Kyryk, 2013). Already after 2 weeks of the course of streptozotocin-induced diabetes mellitus there have been observed the first changes in ultrastructural organization of the elements of microcirculatory bed of the optic nerve of the rat that progressed in the following periods of the experiment. In the authors' opinion angiopathy is a trigger mechanism for the development of the optic nerve neuropathy (Dac, 2011). Literature describes changes in the elements of microcirculatory bed of skin in the dynamics of experimental diabetes mellitus. The changes in angioarchitecture were irreversible after 8 weeks of streptozotocin-induced diabetes mellitus: far-going generalized impairment of the skin vessels developed up to complete atrophy and decompensation of the capillary component (Borys, 2011). Edema of the tissues of all regions of the eyeball vascular tunic, cerebellar cortex, white substance of telencephalon that we have observed after 6 weeks of injecting Nalbuphine squares with the reports containe in professional literature on the effect of narcotic substances (Bekesevych, 2015; Pidvalna, 2014). It is pointed out, in particular, that the signs of edema and swelling of the brain were found under their effect. There are present perivascular and pericellular edema, иvarious disorders of microcirculation in the form of stasis of erythrocytes in the capillaries, general venous hyperemia, paresis of the resistant component of microcirculation, sludge of erythrocytes, sometimes formation of fibrinoerythrocytic thrombus.

CONCLUSIONS

There is an obvious connection between the depth of structural transformations of microcirculatory bed of the organs and morphometric indices in case of the protracted influence of opioid. The changes, compared with the control specimens, in the diameter of arterioles, venules, capillary loops, density of the network of exchange vessels, arteriolovenular coefficient, index of tissue trophic activity testify to the destructive changes in the components of microcirculatory bed under the effect of Nalbuphine. Injection of Nalbuphine impairs micro- and ultrastructural organization of the components of microcirculatory bed of the organs. Endothelium of capillaries of the brain and the eyeball reacts to the opioid most rapidly.

The obtained results is the basis for further studies to be conducted by morphologists and clinicians with the objective of elaboration in the future of the new methods of prevention and treatment of pathology caused by prolonged application of Nalbuphine

REFERENCES

1. Altarifi A. A., Kenner C. R., Negus S. S., 2015. Effects of μ-Opioid Receptor Agonists in Assays of Acute Pain-Stimulated and Pain-Depressed Behavior in Male Rats: Role of μ-Agonist Efficacy and Noxious Stimulus. Journal of Pharmacology and Experimental Therapeutics 35 (2) 208–217.
2. Anselmi L., Jaramillo I., Palacios M., Huynh J., Sternini C., 2013. Ligand-induced μ opioid receptor internalization in enteric neurons following chronic treatment with the opiate fentanyl. J. Neurosci Res. 91 (6), 854–860.
3. Australian / New Zealand Standard. Procedures for specimen collection and the detection and quantitation of drugs of abuse in urine, 2008. AS/NZS 4308.
4. Bailey C.P., 2005. Opioids: cellular mechanisms of tolerance and physical dependence. Curr. Opin. Pharmacol. 5 (1), 60–68.
5. Bekesevych A.M., 2015. Morfologichni osoblyvosti struktury kory mozochka shhura v normi ta za umov tryvalogo vplyvu opioi'du [Morphological features of the structure of the cerebellar cortex of rats in the norm and under prolonged exposure to the opioid]. Zaporozhskij Medicinskij Zhurnal. 3 (90), 82–85 (in Ukranian).
6. Borys R.Ja., 2011. Jakisno-kil'kisni zminy gemomikrocyrkuljatornogo rusla shkiry pry eksperymental'nomu streptozotocynindukovanomu cukrovomu diabeti [Qualitative – quantative changes of the hemomicrocirculatory bed of the skin in experimental streptozotocin-inducted diabetes mellitus]. Klinichna anatomija ta operatyvna hirurgija. 10 (3), 11–17 (in Ukranian).
7. Buhtiarova T.A., Gubs'kyj Ju.I, Litvinova N.V., 2011. Procesy perekysnogo okyslennja lipidiv ta obmezhenogo proteolizu za modeljuvannja bol'ovogo syndromu zapal'nogo g'enezu ta dii' anal'getykiv [Lipid peroxidation and limited proteolysis

by modeling genesis of inflammatory pain and analgesic action]. Materialy IV Nacional'nogo Z'i'zdu farmakologiv Ukrai'ny : tezy dop. (in Ukranian).

8. Dac R.I., 2011. Ul'trastruktura gemomikrocyrkuljatornogo rusla zorovogo nerva pry eksperymental'nomu cukrovomu diabeti [The ultrastructure of hemomicrocirculatory bed of the optic nerve in experimental diabetes mellitus]. Klinichna anatomija ta operatyvna hirurgija. 10 (3), 30–33 (in Ukranian).
9. Dachydovych O.V., Kopcha V.S., Maslij K.O., 2011. Farmakoterapija bol'ovogo syndromu [Pharmacotherapy of pain syndrom]. Racyonal'naja farmakoterapija. 4 (21), 66–68 (in Ukranian).
10. Fomina K.A., 2012. Anatomo-morfofunkcional'naja harakteristika golovnogo mozga krys razlichnogo vozrasta posle ingaljacionnogo vozdejstvija epihlorgidrina [The anatomic and morphofunctional characteristic of the brain of different aged rats after inhalation exposure of epichlorhydrinum]. Ukr. zhurn. klin. lab. Med. 7(2), 153–156 (in Russian).
11. Kyryk H.A., 2013. Stereometrychna harakterystyka gemomikrocyrkuljatornogo rusla sudynnoi' obolonky ochnogo jabluka v normi ta na rannih etapah perebigu cukrovogo diabetu (eksperymental'ne doslidzhennja) [Stereometrical character of hemomicrocirculation stream of the vascular layer of eyeball in normal conditions and on early stage of diabetes mellitus (experimental investigation)]. Teoretychna ta eksperymental'na medycyna 2 (59), 74 – 76 (in Ukranian).
12. Maremmani I., Pacini M., Popovic D., 2009. Affective temperaments in heroin addiction. J. Affect. Disord. 117 (3), 186–192.
13. Mateshuk-Vatseba L. R., Diskovs'kyj I.S., 2014. Ul'trastrukturni zminy shkiry schuriv pry dovgotryvalomu vplyvi opioi'du [Ultrastructural changes in the scin of rats under opioids exposure]. Aktual'ni problemy suchasnoi' medycyny: Visnyk Ukrai'ns'koi' medychnoi' stomatologichnoi'. 4 (48), 205–209 (in Ukranian).
14. Nef'odov O.O., Mamchur V.J, Opryshko V.I., 2011. Misce neopioi'dnyh anal'getykiv ta rol' oksydu azotu v mehanizmah bolju ta spazmu [The place of opioid analgesics and the role of nitric oxide in the mechanisms of pain and spasm]. Ukrai'ns'kyj medychnyj al'manah. 14 (2), 46–51 (in Ukranian).
15. O'Connor G., McMahon G., 2008. Complications of heroin abuse. Eur. J. Emerg. Med. 15(2), 104–106.
16. Ogurcov P.P., Mazurchik N.V., 2007. Lechenie hronicheskogo gepatita C u lic s narkoticheskoj zavisimost'ju [Treatment of chronic hepatitis C in persons with drug dependence]. Gepatologicheskij forum. 3, 16–20 (in Ukranian).
17. Ovcharenko N.A., Pinskij L.L., Radchenko T.N., Offor Modestus Ikechukvu, 2012. Dinamicheskie izmenenija pokazatelej citoliza, holestaza i lipidogrammy u narkozavisimyh bol'nyh [Dynamic changes of cytolysis, cholestasis and in

lipidograme in addicts patients]. Ukrai'ns'kyj zhurnal klinichnoi' ta laboratornoi' medycyny. 7(4), 116–119 (in Russian).

18. Pat. №76564 U Ukrai'na, MPK A 61 K 31/00 Sposib modeljuvannja fizychnoi' opioi'dnoi' zalezhnosti u shhuriv/ zajavnyky: Onys'ko R.M., Pal'tov Je.V., Fik V.B., Vil'hova I.V., Kryvko Ju.Ja , Jakymiv N.Ja., Fit'kalo O.S.; patentovlasnyk: L'vivs'kyj nacional'nyj medychnyj universytet imeni Danyla Galyc'kogo. – №u201207124; zajavl. 12.06.2012; opubl. 10.01.2013, Bjul. № 1.
19. Pidvalna U.Ye., 2014. Strukturna organizaciya organiv i system pid vplyvom opioyidiv [Structural organization of organs and systems under the influence of opioids]. Eksperymentalna ta klinichna fiziologiya i bioximiya. 1 (65), 71–78 (in Ukranian).
20. Popyk P. M., 2014. Morfologichni zminy sudyn pidshlunkovoi' zalozy pry perytoniti sprychynenomu vvedennjam nalbufinu v eksperymenti [Morphological changes in blood vessels of pancreatic gland in experimental peritonitis caused by the Nalbuphine injection]. Visnyk morfologii. 1, 170–172 (in Ukranian).
21. Vijevs'kyj A.M., Zhdanova M.P., Sydjak S.V., 2012. Zriz narkotychnoi' sytuacii' v Ukrai'ni (dani za 2011r.) [Cut addiction in Ukraine]. (in Ukranian).
22. Voronkov M., Ocheret D., Bondarenko S., 2008. Administration of Nalbuphine to heroin addicts. Feasibility and short-term effects. Heroin Addict Relat Clin Probl. 10 (1), 19–24.

Стаття надійшла 15. 10. 2015
Після доопрацювання 20. 11. 2015
Прийнята до друку 15. 12. 2015