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CHANGES OF COLON ANGIOARCHITECTONICS UNDER CONDITIONS OF 2-4-WEEK OPIOID EFFECT IN THE EXPERIMENT

Abstract. *Opioids are pain-killers widely administered in the treatment of chronic pain syndrome. Although, misuse of opioid agent results in drug abuse which is one of the biggest medical and social problem of the whole society. It is the vessels of microcirculation that first respond to pathogenic factors in the form of pathological process manifested by morphofunctional disorders. Therefore, the objective of our study was investigation of opioid effect on the colon architectonics state on the micro- and ultrascopic levels under conditions of 2 and 4-week nalbuphine administration. The study was conducted on 24 albino male rats of a reproductive age (3 months) with the body weight of 160-180 g. During 4 weeks experimental animals were administered to daily intramuscular injections of nalbuphine in growing doses: I week – 8 mg/kg, II week – 15 mg/kg, III week – 20 mg/kg, IV week – 25 mg/kg. 9 albino rats who were injected with 0,9% sodium chloride solution constituted the control group. The material of the study was presented by the samples of the colon taken from rats with injected vascular bed, histological specimens and ultramicroscopic sections of the colon wall of experimental animals. The method of the colon vascular bed injection of rats with ink-gelatin mass was used in the experimental study. The following quantitative criteria were applied to perform morphometric analysis: diameter of the arterioles, capillaries and venules. The indices of morphometric examination of the colon architectonics of rats under 2-4-week effect of nalbuphine were statistically processed by means of a package of applied computer programs and variation-statistical analysis «GraphPad InStat». Sections of the colon wall were stained with hematoxylin and eosin for histological examinations. The specimens were examined under the microscope Leica DM 2500 and pictures were taken by means of the camera Leica DFC 450 C with microscope magnification: x 200, x 400, x 1000. Electron microscopic method was applied in the study. The material was examined and photographed by means of the microscope YEMB-100 K with acceleration voltage 75 kV and magnification on the microscope screen x1000–8000. The results are indicative of the fact that against the ground of 4-week opioid injection structural changes are found in the blood circulation of the colon manifested by haemostasia, the blood vessels overfilled with erythrocytes with their aggregation and adhesion. The signs of dyscirculatory hypoxia are detected on the ultramicroscopic level. The findings of ultra- and microscopic examination are evidenced by the results of morphometric analysis: dilation of arterioles, irregularity of their caliber, thinning of the capillary network, dilation and deformity of the venules, which is indicative of the development of destructive processes. Thus, even in case of 2-week opioid injection the portions of the microcirculatory bed of the colon are altered resulting in considerable changes of the structural organization in the architectonics of the organ during the following 2 weeks of the experiment.*

Key words: *microcirculatory bed, nalbuphine, colon, rat.*

Introduction. In recent years very fast increase of chronic pain syndromes in general structure of sickness has been found which according to the assessment of different authors is from 15 to 70% [7, 11]. Opioids are used among analgesic agents widely indicated in the treatment of chronic pain syndrome [13]. Opioids include alkaloids of opium poppy (morphine, codeine etc.), a number of

semi-synthetic agents (heroin, nalbuphine etc.) and synthetic ones (promedol, methadone etc.) [12]. Although, misuse of opioid agent results in drug abuse which is one of the biggest medical and social problem of the whole society [5, 9]. One of the mechanisms promoting the development of various somatic pathology caused by drugs is their destructive effect on different organs and systems

[1, 10]. The large intestine is functionally active organ, and therefore it undergoes the effect of harmful factors [3]. It is the vessels of the microcirculation that one of the first respond to pathogenic factors in the form of pathological process manifested by morphofunctional disorders [4]. It should be noted that regularities of arterial remodeling has been examined in case of cardiovascular diseases, pulmonary hypertension, microcirculation bed of the cerebellum and pancreas has been also studied under opioid effect [2, 6, 7, 10]. Although investigations dealing with angioarchitectonics of the colon under conditions of opioid effect are practically absent. Therefore, investigation of structural peculiarities of the colon micro vessels is topical, and it is of a practical value both in medical and social aspects.

Objective: to determine peculiarities of microcirculation bed of the colon under conditions of 2-4-week opioid (nalbuphine) injection in the experiment.

Materials and methods. The study was conducted on 24 albino male rats of a reproductive age (3 months) with the body weight of 160-180 g. During 4 weeks experimental animals were administered to daily intramuscular injections of nalbuphine in growing doses: I week – 8 mg/kg, II week – 15 mg/kg, III week – 20 mg/kg, IV week – 25 mg/kg [8]. 9 albino rats who were injected with 0,9% sodium chloride solution constituted the control group. The material of the study was presented by the samples of the colon taken from rats with injected vascular bed, histological specimens and ultramicroscopic sections of the colon wall of experimental animals.

The method of the colon vascular bed injection of rats with ink-gelatin mass was used in the experimental study. Sections of the specimens were clarified in glycerine with 96% ethyl alcohol in the ratio 1:1 during 3 days, followed by glycerine. The following quantitative criteria were applied to perform morphometric analysis: diameter of the arterioles, capillaries and venules. The indices of morphometric examination of the colon architectonics of rats under 2-4-week effect of nalbuphine were statistically processed by means of a package of applied computer programs and variation-statistical analysis «GraphPad InStat». Sections of the colon wall

were stained with hematoxylin and eosin for histological examinations. The specimens were examined under the microscope Leica DM 2500 and pictures were taken by means of the camera Leica DFC 450 C with microscope magnification: x 200, x 400, x 1000.

Electron microscopic method was applied in the study. Immediately after euthanasia of animals the material was taken by means of standard procedures and prepared for electron microscopy. Ultrathin sections were prepared on the ultramicrotome УЖТП–3 by means of glass knives. The specimens were examined under the microscope Leica DM 2500 and pictures were taken by means of the camera Leica DFC 450 C with microscope magnification: x 200, x 400, x 1000.

The animals were kept in the vivarium of Danylo Halytskyi Lviv National Medical University, the experiments were conducted according to the regularities of the European Convention on Protection of Vertebrate Animals Used in Experimental and Other Scientific Purposes (Strasbourg, 1986), the Directives of the Council of Europe 86/609/EEC(1986), the Law of Ukraine № 3447 – IV «On Protection of Animals Against Cruelty», general ethical principles of the experiments on animals approved by the First National Congress of Ukraine on Bioethics (2001).

Results and discussion. Even after 2 weeks of the experiment architectonic changes in the colon wall are found manifested by dilation and overfilling of vessels with blood (Fig. 1). Sometimes moderately expressed perivascular swellings are registered round hyperemic vessels. Erythrocytes agglutinate in the vascular lumen. Less marked changes are found in the vessels of the muscular layer and submucous layer, in particular the arterioles are inconsiderably dilated.

Histological examination made 4 weeks after the beginning of the experiment found increasing dyscirculatory changes in the vessels of the lamina propria of the mucous layer, submucous layer, muscular and serous layers. It is the vessels of the lamina propria of the mucous layer and submucous layer that are dilated and overfilled with erythrocytes, sometimes they contain neutrophil granulocytes and lymphocytes, signs of haemostasia are found. Disorders of the vascular

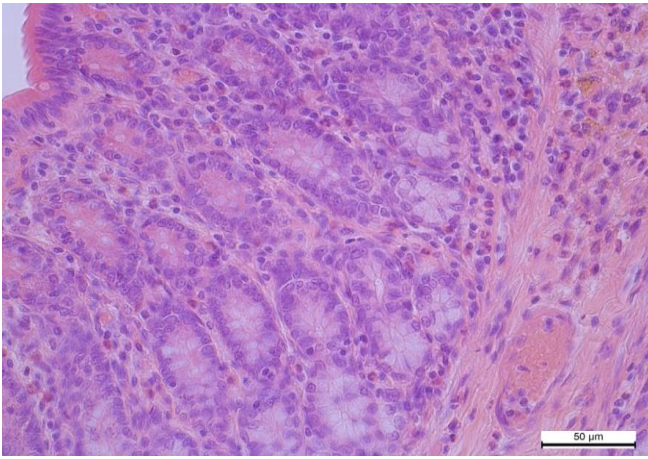


Fig. 1 The colon wall of an albino rat after 2 weeks of nalbuphine injection. Microphoto. Staining with hematoxylin and eosin. Magnification: × 400. Dilation of the vessels of the submucous base and their overfilling with blood.

integrity are found (Fig. 2) as compared to the control group of animals (Fig. 3). In certain capillaries erythrocytes are located in several layers in the form of rouleau, aggregation and adhesion of erythrocytes is found. In certain vessels erythrocyte hemolysis develops. The vessels of the muscular and serous layers are dilated. Certain vessels are hyperemic with moderately expressed perivascular swelling. Dilation and overfilling of the mesenteric veins with blood are determined. The determined changes are not specific, as nalbuphine effect on the kidney body is known to provoke the following changes: capillary loops of the renal glomeruli are dilated and overfilled with erythrocytes [1]

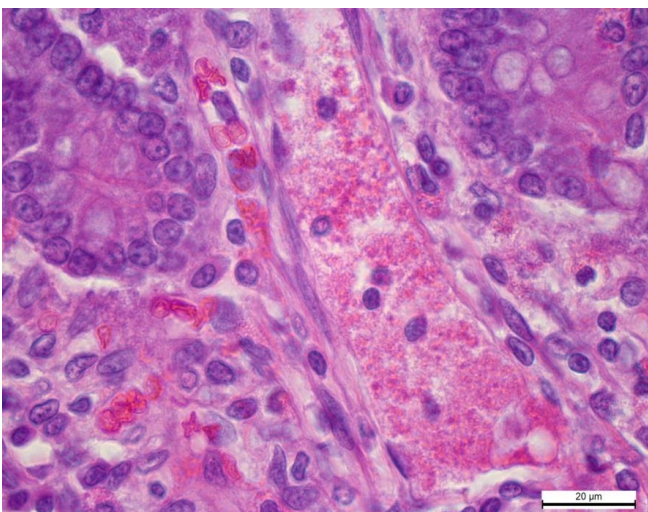


Fig. 2. The colon wall of an albino rat after 4 weeks of nalbuphine injection. Microphoto. Staining with hematoxylin and eosin. Magnification: × 1000. Dilation of vessels of the lamina propria of the mucous layer and overfilling with blood. Erythrocyte hemolysis. Lymphocytes in the vessel lumen.

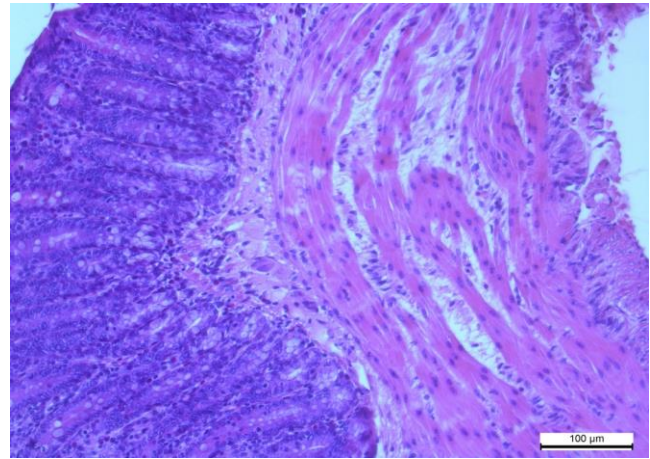


Fig. 3. The colon wall of a control rat. Microphoto. Staining with hematoxylin and eosin. Magnification: × 200.

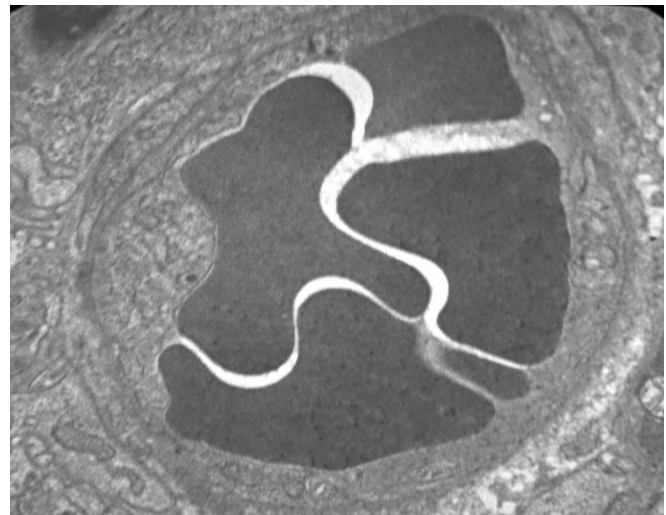


Fig. 4. Ultrastructure of the colon vessels of a rat 2 weeks after nalbuphine injection. Electron microphoto. Magnification: ×4000.

Electron microscopic examination 2 weeks after nalbuphine injection proves morphological changes from the side of microcirculation bed of the colon wall.

Submicroscopic examination determined that lumens of blood capillaries are dilated and filled with erythrocytes of irregular shape, being in a close contact with lumen surface of the endothelial cells (Fig. 4). Swelling of endotheliocytes is found in the capillaries, their nuclear zones with electron-dense nuclei protrude into the lumen of vessels. The basal membrane is preserved, though its swelling is seen, and as a result its thickening.

4 weeks after the beginning of the experiment disruption of the microcirculation bed is manifested by changes of the lumen of a great number of blood capillaries which become of irregular shape, filled with sludges of erythrocytes; they contact closely with each other

and lumen surface of endothelial cells (Fig. 5). Big masses are available on electron microphotos in the lumen of the blood vessels. Microclasmotosis is found that is characteristic for dyscirculatory hypoxia which is described in professional literature [9,10]. The nuclei of endothelial cells are of big sizes as compared to the cytoplasm, sometimes invagination of the nuclear membrane and concentration of heterochromatin along the periphery of the nuclear membrane are seen. Luminal surface of the cellular membrane is irregular, with unclear outlines caused by its protrusions. Structurally changed organelles are found together with preserved organelles. There are rather enlarged round sometimes polygonal in shape mitochondria with destructed crista which is indicative of the development of their swelling. Development of paravasal swelling is also characteristic.

By means of an injection with ink-gelatin mass the portions of the colon microcirculation bed of a rat are examined which are classically presented by arterioles, pre-capillary arterioles, capillaries, post-capillary venules and venules. Certain regularities of changes in the spatial location of micro vessels in the colon are detected. As compared to the colon capillaries of the control group of animals forming a compact network in the shape of regular polygons or "honeycombs" (Fig. 6), even 2 weeks after nalbuphine injection certain changes from the side of the microcirculation bed are found. Blood micro vessels become winding with irregular caliber, sometimes they are broken (Fig. 7). These findings prove morphometric indices found. Thus, the diameter of capillaries becomes longer to $(8,32 \pm 0,32)$ mcm, the control – $(4,8 \pm 0,4)$ mcm, arteriole diameter becomes $(26,72 \pm 2,08)$ mcm, the control – $(15,49 \pm 0,45)$ mcm, venule diameter – $(28,96 \pm 2,08)$ mcm, the control – $(23,86 \pm 0,58)$ mcm.

4 weeks after the beginning of the experimental study more pronounced changes of the angiographic picture are determined on the injected specimens of the vascular bed in the colon wall. The caliber and integrity of capillaries change, they become more winding with irregular outline, sometimes broken (Fig. 8), at this term their diameter is $(6,4 \pm 0,32)$ mcm, the control – $(4,7 \pm 0,7)$ mcm. Arteriole diameter in comparison

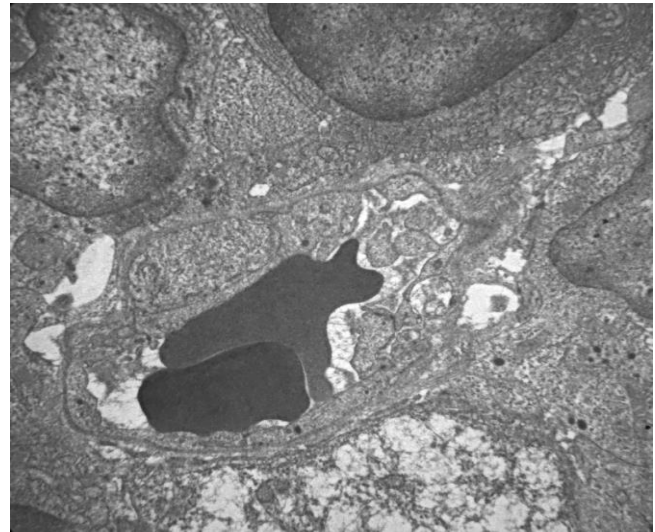


Fig. 5. Ultrastructure of the colon vessel of a rat after 4 weeks of nalbuphine injection. Electron microphoto. Magnification: x4000.

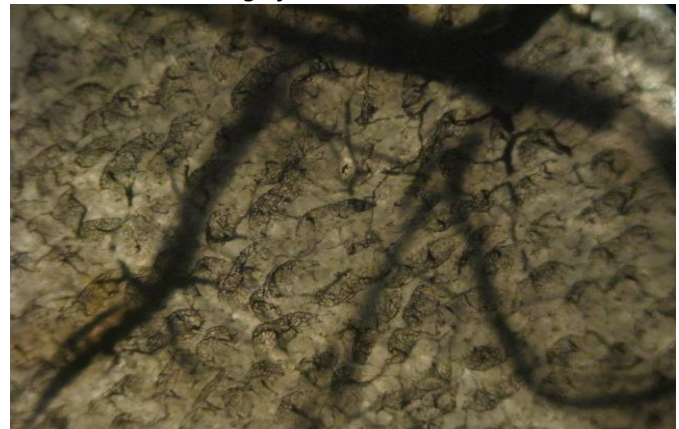


Fig. 6. Microcirculation bed of the colon wall of a control rat. Microphoto. Vascular injection. Magnification: x80

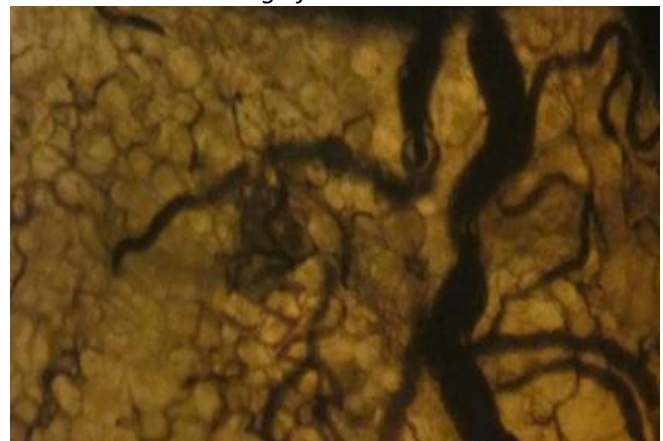


Fig. 7. Microcirculation bed of the colon wall of a rat under conditions of 2-week nalbuphine injection. Microphotot. Vascular injection. Magnification: x 160.

with the 2nd week decreases and is $(25,6 \pm 0,32)$ mcm, venule diameter continues to increase and becomes $(32 \pm 0,64)$ mcm. Arteriole-venule anastomosis appear and dilate that is a certain compensatory mechanism.

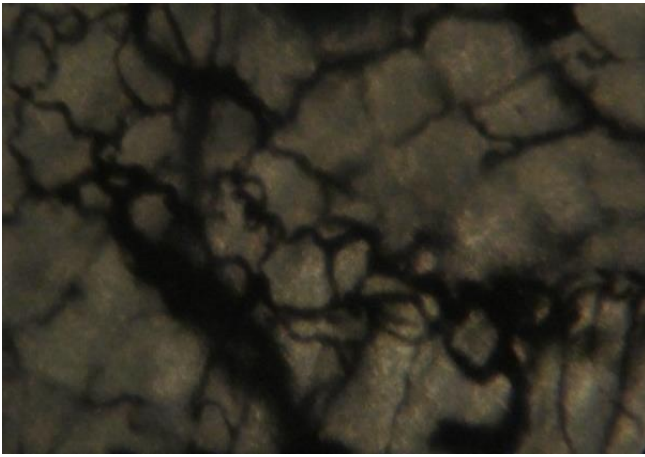


Fig. 8. Microcirculation bed of the colon wall of a rat under conditions of 2-week nalbuphine injection. Microphoto. Vascular injection. Magnification: x 320.

The changes in the portions of the microcirculation bed determined in our study are similar to those found by other researchers while investigating a long effect of nalbuphine on the angioarchitectonics [5, 10], which is the evidence suggested by a number of authors that blood vessels are one of the first structures responding to pathogenic factors [4].

Conclusions. Morphometric analysis of the portions of the colon microcirculation bed enabled to determine the degree of its vascularization under conditions of 2-4-week opioid effect.

A clear relation is seen between the changes in angioarchitectonics on the micro- and ultramicroscopic levels and morphometric signs under conditions of 2 and 4-week nalbuphine injections. Dilation of arterioles, sometimes irregularity of their caliber, thinning of the capillary network, dilation and deformity of venules are indicative of the development of destructive processes.

Prospects of further studies. Structural rebuilding of the microcirculation bed components forms the basis for different diseases of the digestive system. Further investigation of morphological peculiarities of the colon vessels under conditions of a long opioid effect could be applied in practical medicine for prevention, diagnostics and treatment of colon diseases among drug abused.

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