Structural changes of pancreatic components under the conditions of long-time exposure to opioid in the experiment

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Use of narcotic drugs in clinical practice for the purpose of obtaining analgesic and anti-inflammatory effects requires a comprehensive morphological study of the peculiarities of structural arrangement of organs under the conditions of exposure to opioids. The aim of our study was to establish the peculiarities of restructuring of the structural components of the pancreas under the conditions of long-time exposure to opioids in the experiment. The study included 24 adult laboratory white male rats. The test animals were divided into 2 groups, the experimental and control ones. The experimental animals were daily administered narcotic analgesic nalbuphine intramuscularly (once a day in the same interval) for four weeks, and the control animals were administered saline solution. The following research methods were used: bloodstream injection followed by translucence of sections of the pancreas and their photographing, morphometry of the vessels of the pancreatic hemomicrocirculatory bed, histological, histochemical studies and electron microscopy of the pancreas, blood biochemistry test; statistical processing of the study results using a software package. After four weeks of opioid exposure, lesion of the pancreatic parenchyma microstructure was observed, manifested by swelling and infiltration by lymphocytes and macrophages of the pancreatic connective tissue stroma, disorganization of the exo- and endocrine parts of the parenchyma, deep destructive changes in the excretory ducts, as well as in the vessels of the hemo- and lympho-microcirculatory bed of the pancreas. At the ultrastructural level, deep dystrophic changes of exo- and endocrinocytes of the pancreas were identified, in particular, loss of regular shape, karyopyknosis and karyorrhexis of the nuclei, swelling and clearing of cytoplasm, development of microcystic degeneration of cells, loosening and disorganization of the basement membrane, which can result in impairment of the exocrine function of the pancreas and complication of the process of secretory granules excretion into the lumen of the intercalated ducts. A significant decrease, compared to the control group, in the diameter of arterioles, density of exchange vessels network, as well as increase in the diameter of venules, the indicator of trophic activity of the tissue, are the evidence of destructive changes in the hemomicrocirculatory bed of the pancreas under the effects of nalbuphine. Significant changes in blood biochemistry parameters (alanine aminotransferase, aspartate aminotransferase) after a four-week administration of nalbuphine are illustrative of the process of pancreatic tissue destruction. Therefore, four-week administration of opioid leads to profound changes in the micro- and ultrastructure of the pancreas, vessels of its hemomicrocirculatory bed, and blood biochemistry parameters in experimental white rats.

Keywords: pancreas, structural changes, opioid, experiment.

Introduction

The problem of structural changes of organs and systems under the influence of opioids is relevant and important, since the use of narcotic substances is considered in the world not only as a social problem, but also as a general medical problem [14, 26, 30]. The widespread use of narcotic agents in clinical practice with the aim of obtaining analgesic and anti-inflammatory effects requires a complex morphological study of the peculiarities
of the structural organization of organs under the conditions of exposure to opioids [5, 7, 9, 18, 22].

It is possible to predict the negative effect of drugs on the pancreas, but only a few works have been devoted to the study of the structural organization of the pancreas under conditions of harmful effects [29]. In chronic drug intoxication, along with damage to other vital organs and systems [23, 24], the pancreas, which is an important part of the digestive and endocrine systems, is also involved in the pathological process. The pancreas plays a key role in the body's metabolic processes and is particularly sensitive to exo- and endopathogenic factors [6, 25].

The development of pharmacotherapy with narcotic substances requires the development of measures to prevent and correct side effects and complications caused by them, including from the pancreas, which is sensitive to drug effects due to the peculiarities of its structure and function [10]. Morphological reconstruction of the pancreas undoubtedly plays an important role in the pathogenesis of a number of gastroenterological diseases [8, 21]. There remain a number of unsolved questions regarding the problems of structural changes of the pancreas during the use of narcotic agents, as well as the successful selection of the most effective methods of treatment of gastroenterological pathology caused by the influence of opioids. This especially concerns the macro-, micro-, and ultrastructure of the pancreas under the influence of opioids; there are no data on the qualitative and quantitative changes in the angioarchitectonics of the pancreas when using narcotic drugs.

The above-mentioned led to the conduct of a study with the aim of establishing the peculiarities of the restructuring of the structural components of the pancreas under the conditions of long-term exposure to opioids in the experiment.

Material and methods

The studies were performed on 24 sexually mature white male rats with an initial weight of 160-180 g, aged 3 months. Experimental animals are divided into 2 groups - experimental and control. The experimental animals were administered intramuscularly the narcotic analgesic nalbuphine daily (once a day at the same time interval) for 4 weeks, and the control animals were administered a saline solution. Nalbuphine was administered intramuscularly according to the following scheme: 1st week - 8 mg/kg, 2nd week - 15 mg/kg, 3rd week - 20 mg/kg, 4th week - 25 mg/kg. The research material is represented by preparations of the pancreas of white rats with an injected vascular bed, histological preparations and ultramicroscopic sections of the pancreas, as well as blood samples of experimental animals. All animals were kept in the conditions of the vivarium of the Danylo Halytsky Lviv National Medical University. The experiments were carried out in accordance with the provisions of the European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Council of Europe Directive 86/609/EEC (1986) (protocol No. 5 of the meeting of the commission on the ethics of scientific research, experimental of developments and scientific works of Danylo Halytsky Lviv National Medical University from June 22, 2020).

The following research methods were used in the performance of the work: injection of the vascular bed of the rat pancreas with a carcass-gelatin injection mass, followed by illumination of the pancreas sections in glycerol and alcohol and their photography under the MBY-1 microscope, morphometry of the blood vessels of the hemomicrocirculatory bed of the pancreas, histological, histochemical and electron microscopic examination of the pancreas, biochemical blood examination, statistical processing of the research results.

Histological sections (5-7 μm thick) were made using a microtome, the sections were stained with hematoxylin and eosin. For the histochemical study of the pancreas, the PAS reaction according to McManus was used. The preparations were studied and photographed at magnifications of the microscope: x400, x1000. The "AverMedia" computer system was used to photograph micropreparations.

To carry out a morphometric analysis of the angioarchitectonics of the pancreas of a white rat, measurements were made of the diameter of arterioles, capillaries and venules, the density of the network of exchange vessels (namely, capillaries), which was determined by counting the number of vessels per unit area (the area of the field of view of the microscope was chosen as the unit of area), the indicator of trophic tissue activity, or diffusion radius (the distance between two adjacent vessels, divided in half). The preparations were studied on a MBI-1 light microscope at magnifications of the microscope: x120 (objective x8, eyepiece x15); x400 (objective x40, eyepiece x10) and x800 (objective x40, eyepiece x20).

Ultrathin sections of the pancreas were prepared on a UZHTP-3 ultramicrotome using glass knives. Ribbons of sections of silver or delicate citrine color were selected for the study. Sections were contrasted first in a 2% solution of uranyl acetate, and then in lead citrate. Studying and photographing the structures of the pancreas was carried out with the help of a UEMV-100 K microscope at an accelerating voltage of 75 kV and magnifications on the microscope screen of x4000 - x8000.

Standard laboratory methods were used to conduct a biochemical study of blood, determining: the activity of transaminases in blood serum by the Reitman-Frankel method; α-amylase - by the aminoclastic method with a stable starch substrate (Karavey's method); glucose - by the glucose oxidase method ("Filiciti"-Diagnostics kit).

The statistical analysis of the research results was
carried out on a computer using the "InStat" application program package for statistical processing of data from medical, biological and epidemiological studies.

**Results**

After 4 weeks of administration of nalbuphine to experimental animals, the phenomenon of destructuring of the angiographic topography of the pancreas was revealed on pancreas preparations with injected vascular beds. The clear arrangement of the vessels of the hemomicrocirculatory channel is lost (Fig. 1).

In the duodenal part of the pancreas, obliteration of capillaries, hemorrhages, uneven diameter of vessels, rarefaction of the vascular network, tortuosity of preserved vessels are observed in some places (Fig. 2). The diameter of the detected capillaries is 6.002±0.020 μm (control - 6.409±0.210 μm), the density of the network of exchange vessels is 100.0±10.0 (control - 134.0±16.0), the indicator of tissue trophic activity is 34.42±0.86 μm (control - 24.21±0.64 μm).

A reliable expansion of the capillaries of the perilobular network of the biliary part of the pancreas up to 6.230±0.392 μm (control - 4.264±0.442 μm), a decrease in the density of the network of exchange vessels - 96.00±4.00 (control - 128.0±6.0), an increase in the index of trophic activity of the tissue - 24.23±1.62 μm (control - 27.60±0.41 μm). The venular component of the hemomicrocirculatory channel of the pancreas is also expanded.

In the gastro-splenic part of the pancreas, there is expansion of venules, twisting and unevenness of the caliber of arterioles, deformation of capillaries. The diameter of capillaries is 6.232±0.112 μm (control - 6.410±0.211 μm), the density of the network of exchange vessels is 96.50±4.00 (control - 120.0±8.0), the index of trophic activity of the tissue is 23.53±0.44 μm (control - 28.10±0.82 μm).

The capillary component of the vascular glomeruli of the pancreatic islets is partially destroyed. The diameter of preserved capillaries of vascular glomeruli of pancreatic islets increases to 8.492±0.372 μm (p<0.05).

After 4 weeks of the experiment, destructive changes in the microstructure of the pancreas were revealed. The cells of interstitial ducts are thinned, mostly lose their shape, the basement membrane is thickened. Capsules of pancreatic islets are intermittent, loose, swollen, capillaries between islets are dilated, hyperemic. The epithelium of the intralobular and interlobular ducts is thinned, protrusions of the epithelium into the lumen of the ducts are revealed, in the lumen there are single desquamated structures, stagnation of secretion, the own connective tissue plate of the ducts is thickened and swollen (Fig. 3). The shape of most of the acini has changed, their basement membrane is swollen, loose, sometimes stratified, there is an expansion of the zymogenous and a narrowing of the homogeneous zones of exocrine pancreatic islets (Fig. 4).

After 4 weeks of nalbuphine administration, morphological changes characteristic of micro- and

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Fig. 1. Violation of the clear arrangement of the links of the hemomicrocirculatory bed of a white rat pancreas after 4 weeks of nalbuphine administration. Photomicrograph. 1 - intralobular arteriole; 2 - capillary. Injection of blood vessels with mascara-gelatin mass. x160.

Fig. 2. Capillary obliteration, non-uniformity of vessel diameter in white rat pancreas after 4 weeks of nalbuphine administration. Photomicrograph. 1 - interlobular arteriole; 2 - intralobular arteriole; 3 - capillary. Injection of blood vessels with mascara-gelatin mass. x160.

Fig. 3. Pancreas of a white rat after 4 weeks of nalbuphine administration. Photomicrograph. 1 - pancreatic acinus; 2 - stagnation of secretion in the interlobular duct. Staining with hematoxylin and eosin. x400.
fig. 4. pancreas of a white rat after 4 weeks of nalbuphine administration. Photomicrograph. 1 - narrowing of the zymogenic zone of pancreatic exocrinocytes; 2 - expansion of the homogeneous zone of exocrinocytes of the pancreas. Staining with hematoxylin and eosin. x800.

fig. 5. Rat pancreas after 4 weeks of nalbuphine administration. Photomicrograph. 1 - full blood in a vein of medium diameter; 2 - smooth muscle hyperplasia in an organ artery; 3 - lympho- and leukostasis in the lymphatic vessel. Staining with hematoxylin and eosin. x120.

fig. 6. Irregularly shaped nucleus, intussusception of the nuclear envelope, dilated tubules of the granular endoplasmic reticulum of a white rat exocrinocyte after 4 weeks of nalbuphine administration. Electronogram. 1 - nucleus; 2 - nuclear envelope; 3 - mitochondrion; 4 - granular endoplasmic reticulum. x8000.

macroangiopathy occur, hyalinosis of arterioles, small inflammatory infiltrates represented by macrophages and lymphocytes are revealed. The walls of the links of the hemomicrocirculatory channel of the pancreas are thickened, eosinophilic. Microaneurysms of arterioles, hyperemia of capillaries are revealed. In medium-sized veins there is full blood, in the wall of organ arteries smooth muscle hyperplasia is observed, in lymphatic vessels there is lymphostasis and leukostasis (fig. 5). In capillaries, erythrocytes are placed in several rows, often in the form of a coin column, stick together (stasis). In addition to erythrocytes, neutrophilic granulocytes and lymphocytes are visualized in the blood vessels, and a marginal position of neutrophilic granulocytes is observed. In the lumen of the capillaries - segmented neutrophilic granulocytes. Sometimes there are isolated hemorrhages in the loose fibrous connective tissue of the interstitium or in the lumen of the excretory ducts, which are located near the hyperemic vessels.

After 4 weeks of administration of nalbuphine, the

fig. 7. Karyopyknosis in the B-endocrinocyte of the white rat pancreas after 4 weeks of nalbuphine administration. Electronogram. 1 - nucleus; 2 - nuclear envelope; 3 - mitochondria; 4 - granular endoplasmic reticulum. x8000.

fig. 8. Secretion-filled pancreatic duct of a white rat after 4 weeks of nalbuphine administration. Electronogram. 1 - intercalated duct; 2 - secretory granule. x4000.
plasmalemma of some exocrinocytes of the pancreas of experimental animals is destroyed, desquamation of the cytoplasm in the interstitium is observed, vacuoles and lipid inclusions are found in the cytoplasm of many cells, the nuclei are enlarged, irregular in shape, the nuclear envelope has uneven contours, forms numerous intussusceptions, heterochromatin is located on the periphery, many compact electron-dense small blocks of heterochromatin (Fig. 6). Mitochondria are swollen, the membranes of some mitochondria are destroyed, discontinuous, cristae are destructured, the matrix is electron transparent. Tubules of agranular and granular endoplasmic reticulum are expanded. In some acinocytes, the number of ribosomes concentrated on the membranes of the endoplasmic reticulum increases, which indicates the development of compensatory processes.

During this period of the experiment, the cytoplasm of some endocrinocytes contains small structureless areas with low electron density, the nuclei are in a state of karyopyknosis (Fig. 7).

Intercalated and intralobular ducts have an uneven lumen, filled with secretion (Fig. 8).

The lumens of arterioles are filled with irregularly shaped erythrocytes, their walls are destructured, myocytes are thinned, the basal membrane is loose, endothelial cells protrude into the vessel lumen, the subendothelial layer is disorganized (Fig. 9). Significant paravasal edema was found around the microvessels.

Capillaries are dilated, filled with deformed erythrocytes and erythrocytes in various stages of hemolysis, the fenestrae are dilated (Fig. 10). The electron density of capillary endotheliocytes is reduced, pinocytotic vesicles are found near the basal surface of the endotheliocyte cytolemma, some endotheliocytes protrude significantly, and sometimes exfoliate into the lumen of the vessel, as a result of which the basement membrane of the hemocapillary is exposed in some places. The basement membrane mostly retains its integrity, but in some places it is expanded, loosened, fibrous and thickened, its outline is unclear. In the nuclei of pericytes, chromatin is marginally located, mitochondrial cristae are expanded and fragmented.

On the basis of a biochemical study of the blood of a white rat under the conditions of long-term exposure to nalbuphine, the indicators of the activity of transaminases, α-amylase, and glucose in the blood serum were determined. The content of the above indicators in the blood of a control white rat and under the experimental influence of nalbuphine is presented in Table 1.

**Discussion**

Administering nalbuphine to rats for 4 weeks leads to the development of pathological changes that are characteristic of vascular sclerosis, transendothelial transport disorders, disturbances in the coagulation and fibrinolytic systems, circulatory hypoxia, the development of pericapillary edema and the growth of connective tissue in the interstitium of the pancreas, which is characteristic of pancreatic fibrosis.

| Table 1. Indicators of activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), α-amylase, glucose in blood serum. |
|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| Control                                    | 0.882±0.022                                | 1.174±0.062                                | 20.70±0.24                                | 6.211±0.414                                |
| After 4 weeks of nalbuphine administration  | 1.922±0.040*                               | 2.120±0.044*                               | 20.60±0.28                                | 6.133±0.140                                |

**Notes:** * - probable difference in biochemical blood parameters of control and experimental white rats (p<0.05).
[1]. Our results allow us to state that long-term opioid administration leads to the development of productive vasculitis with obliteration of the lumen of arterioles, blood separation, leukostasis, and leukodiapedesis in the venous component of the bloodstream, which is a sign of inflammation. The above confirms the opinion of researchers regarding the primary development of angiopathy in opioid abuse, which, in turn, causes destructive changes in organs [11, 12, 19, 27]. The pancreas is particularly sensitive to a lack of blood supply and this leads to a deep restructuring of the exo- and endocrine part of the pancreas [13, 17]. A four-week exposure to an opioid lead to the loss of the correct shape of exo- and endocrinocytes, karyopyknosis and karyorrhexis, swelling and lightening of the cytoplasm, the development of microcystic degeneration of cells, which can lead to a violation of the endo- and exocrine function of the pancreas and difficulty in the process of removing secretory granules into the lumen of the interstitial ducts. Similar changes in the structural components of the pancreas were observed by researchers in other pathological conditions of this organ [2, 3, 15, 16].

The conducted statistical analysis of the indicators of the activity of alanine aminotransferase, aspartate aminotransferase, α-amyrase and glucose in the blood serum of a white rat makes it possible to make an objective comparative assessment of the main studied biochemical parameters of the blood of experimental and control groups of animals in accordance with the research objectives. Alanine aminotransferase is an endogenous enzyme from the group of transferases, a subgroup of aminotransferases, synthesized intracellularly, mainly by liver cells, but also found in pancreatic cells. The degree of increase in alanine aminotransferase activity indicates the severity of the cytolytic syndrome [28]. A significant increase in the activity of alanine aminotransferase in the blood serum of a white rat after 4 weeks of nalbuphine administration indicates the toxic effect of this drug, which apparently damages or destroys cells, including those of the pancreas. Aspartate aminotransferase is found in the tissues of the pancreas. Since a significant increase in aspartate aminotransferase was established after a 4-week administration of nalbuphine to white rats, damage to the pancreatic tissue can be assumed [4, 20].

The novelty of the research results obtained by us consists in the establishment of morphological changes in the structural components of the pancreas of laboratory white rats under conditions of four-week exposure to the opioid nalbuphine at the level of light and electron microscopy. The relationship between the depth of changes in the structural organization of the hemomicrocirculatory bed of the pancreas under the conditions of nalbuphine administration and the reorganization of exo- and endocrinocytes of the pancreas is proven by reliable changes in morphometric indicators and biochemical indicators of the blood of experimental animals.

The results of the work are of practical importance for morphologists, gastroenterologists and surgeons, as they can serve as a morphological basis for the development of new methods of diagnosis, prevention and treatment of pancreatic pathology in patients forced to use opioids for a long time, as well as in drug addicts.

Further research into the possibilities of correction of micro- and ultrastructural changes of the pancreas under the conditions of exposure to opioids is promising. The obtained results of the study of the structural features of the pancreas under the experimental influence of an opioid are fundamental data that researchers can rely on when studying the problems of experimental and clinical medicine.

**Conclusions**

1. A decrease, compared to the control, in the diameter of arterioles, the density of the network of exchange vessels, as well as an increase in the diameter of venules and the indicator of tissue trophic activity indicate destructive changes in the hemomicrocirculatory bed of the pancreas under the influence of nalbuphine, when the capillary component is destroyed, arterioles are sharply twisted, and their lumen is uneven, venules are expanded and deformed.

2. Violations of the microstructure of the pancreatic parenchyma and its blood stream after 4 weeks of nalbuphine administration are manifested by swelling and infiltration by lymphocytes and macrophages of the connective tissue stroma of the pancreas, disorganization of the exo- and endocrine parts of the parenchyma, deep destructive changes in the excretory ducts, as well as blood and lymph microcirculatory vessels pancreas.

3. Significant changes in biochemical blood parameters (alanine aminotransferase, aspartate aminotransferase) after 4-week administration of nalbuphine confirm the process of pancreatic tissue destruction.

4. A four-week exposure to an opioid lead to deep dystrophic changes in exocrinocytes and endocrinocytes of the pancreas, in particular, loss of the correct shape, karyopyknosis and karyorrhexis of the nuclei, swelling and lightening of the cytoplasm, the development of microcystic degeneration of cells, which can lead to a violation of the exocrine function of the pancreas and difficulty in the process of excretion of secretory granules into the lumen of interstitial ducts.

**References**


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