ORIGINAL ARTICLE

CONTENTS

Morphological condition of the skin following a 4-week opioid exposure in an experimental study

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ABSTRACT

Aim: The study aimed to determine the peculiarities of the micro- and ultrastructural organization of the skin under conditions of a four-week administration of an opioid to experimental animals.

Materials and Methods: The study material included skin samples of white rats with injected vascular beds, histological preparations, and ultrathin skin sections. The research methods involved injection techniques, histological analysis, electron microscopy, morphometric measurements, and statistical analysis.

Results: The results of the study revealed that after four weeks of nalbuphine administration to experimental animals, blood stasis was observed in the lumen of the capillaries and venules, along with perivascular edema and perivascular infiltrates consisting of neutrophils, lymphocytes, macrophages, and tissue basophils. The electron density of the nuclei and cytoplasm of the granular layer keratinocytes was reduced, keratinocytes in the stratum spinosum acquired a rounded shape, with some nuclei appearing shrunken and hyperchromatic, and their cytoplasm exhibiting vacuolization. In the reticular layer, thickened bundles of collagen fibers were observed, with localized swelling and fragmentation of the collagen fibers. Excessive formation of scales was noticed in the stratum corneum. The papillary layer of the dermis contained numerous mast cells and lymphocytes near blood vessels. The shape of sebaceous and sweat gland cells was altered, with swollen cytoplasm, and lymphohistiocytic infiltration was observed around them. A decrease ($p<0.05$) in the density of capillary loops in the subpapillary vascular plexus of the skin in the gluteal region of white rats after four weeks of nalbuphine administration, along with an increase ($p>0.5$) in the trophic activity index of the skin, confirms profound destructive changes in the vascular architecture of the skin.

Conclusions: Four weeks of nalbuphine administration induces irreversible pathological processes in all skin components.

KEY WORDS: skin, nalbuphine, opioids, experiment, morphology

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INTRODUCTION

In modern medical practice, opioids are widely used to manage pain in the postoperative period and to alleviate suffering of patients with oncological diseases [1-5]. However, the safety of opioid use and the impact on the structural organization of organs remains a controversial issue. Scientific literature includes research papers emphasizing the positive effectiveness of opioids, particularly nalbuphine [6]. Nevertheless, most studies highlight the negative impact of opioids on the body [7-10]. Moreover, it is essential to consider not only the medical but also the social problem. The number of individuals with opioid addiction, whose lives are persistently tied to opioid consumption, continues to grow [11-13]. Studies focusing on the effects of opioids on the immune system are of particular interest. Research findings indicate suppression of immune responses under opioid use [14], while some authors describe the development of an inflammatory reaction in response to opioid exposure [15]. Opioids have also been shown to cause destructive changes in skeletal tissue [16]. Only comprehensive experimental studies can elucidate the mechanisms of opioid action at the organ, tissue, and cellular levels [17]. A significant number of experimental and clinical studies are dedicated to examining the skin layers under various factors in both experimental and clinical settings [18, 19], as the skin is a complex organ that directly interacts with the external environment, performing barrier and protective functions, as well as helping maintain the internal equilibrium of the body. The skin is the largest human organ, continuously affected by exogenous and endogenous factors. Thus, the morphofunctional organization of the skin during prolonged opioid use is currently of a particular interest.

AIM

To determine the microstructural and ultrastructural features of skin organization under conditions of opioid administration to experimental animals for four weeks.

MATERIALS AND METHODS

The study was conducted on 25 sexually mature male white rats, aged 3 months, with an initial body weight of 160–180 g.

All animals were kept in the vivarium of Danylo Halytsky Lviv National Medical University. The experiments were conducted in compliance with the humane treatment requirements for laboratory animals as regulated by the Law of Ukraine "On the Protection of Animals from Cruelty" (No. 3447-IV, dated 21.02.2006) and the treaty of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 18.03.1986).

The research material included skin samples from white rats with injected vascular networks, histological preparations, and ultramicroscopic sections of the skin.

The modeling of prolonged opioid exposure in white rats was performed through daily intramuscular administration (once per day) of the narcotic analgesic nalbuphine into the right gluteal region. The administration followed this schedule: week 1: 8 mg/kg, week 2: 15 mg/kg, week 3: 20 mg/kg, week 4: 25 mg/kg. The impact of the opioid was studied in four weeks from the start of the experiment. The control group consisted of 10 rats that received a 0.9% sodium chloride solution.

Euthanasia was performed by overdose of intraperitoneal anesthesia with thiopental sodium (at a dose of 25 mg/kg of an animal's body weight).

For the injection of the vascular network in a rat's skin, an injectable gelatin microgel-based composite ink was used. A volume of 10–15 ml of the mixture was sufficient to fill the arterial network. After clearing the preparations in glycerin, the vascular segments of the skin were photographed under transmitted light using an MBI-1 microscope at magnifications of ×80 (objective 10, eyepiece 8) and ×160 (objective 20, eyepiece 8). The images were captured with an Olympus FE 210 digital camera.

For morphometric analysis of the vascular architecture of the rat's skin, measurements of the diameter of arterioles, capillaries, and venules were taken. The density of the exchange vessel walls (specifically capillaries) was determined by counting the number of vessels per unit of area (with the area of the microscope field of view chosen as the unit of measurement). Additionally, the trophic activity index of the tissue, or the diffusion radius, was assessed.

For histological examination, skin samples from the white rat's gluteal region were placed in Bouin's solution. The material was embedded in paraffin, and 5-7 μm thick sections were cut using a MC-2 sliding microtome. The histological sections were stained with hematoxylin and eosin, and benzopurpurin (diamino red) according to standard protocols. The preparations were studied under a light microscope (MBI-1) at magnifications of ×120 (objective 8, eyepiece 15), ×160 (objective 8, eyepiece 20), and ×600 (objective 40, eyepiece 15). Micropreparations were photographed using the "AverMedia" computer system.

Electron microscopy of the white rat's skin was performed using the UEMB-100K electron microscope at an accelerating voltage of 75 kV and magnifications of ×4000 to ×8000.

Statistical analysis of the research results was conducted on a computer using the "InStat" statistical software package for medical, biological, and epidemiological data processing.

RESULTS

After four weeks of nalbuphine administration to white rats, the lumen of the arterioles becomes uneven, with areas of both constriction and dilation. The venules expand, deform, and acquire irregular shapes, with some exhibiting aneurysmal bulges. Isolated aneurysmal sacculations in microvessels were observed. These changes result in alterations in the spatial configuration of the subpapillary and dermal vascular networks, as well as the subcutaneous venous plexus in the gluteal region of the white rat's skin following four weeks of nalbuphine administration.

The subpapillary vascular plexus of the white rat's skin at this stage of the experiment is characterized by the following morphometric parameters: the diameter of the intrapapillary capillary loop is 6.26±0.12 μm, the tissue trophic activity index of the skin is 22.47±2.44 μm, the diameter of the venules in the subpapillary vascular plexus is 59.65±0.83 μm, the diameter of the arterioles in the dermal vascular plexus increases to 55.18±4.18 μm, the diameter of the venules in the dermal vascular plexus increases to 142.31±2.46 μm, the diameter of the venules in the subcutaneous venous plexus increases to 158.57±1.98 μm.

After four weeks of the experiment, intensive keratinization processes take place, as evidenced by the thickening of the stratum corneum of the epidermis (Fig. 1).

In the stratum corneum, excessive formation of scales is observed. However, at this stage of the experiment, a reduction in folds and thinning of the epidermis are occasionally noted. The secretory sections and excretory ducts of the sweat glands are dilated. The papillary layer of the dermis is infiltrated with polymorphonuclear leukocytes. Edema of the reticular layer and hypodermis is observed, with loosening of collagen fibers in the reticular layer (Fig. 2).

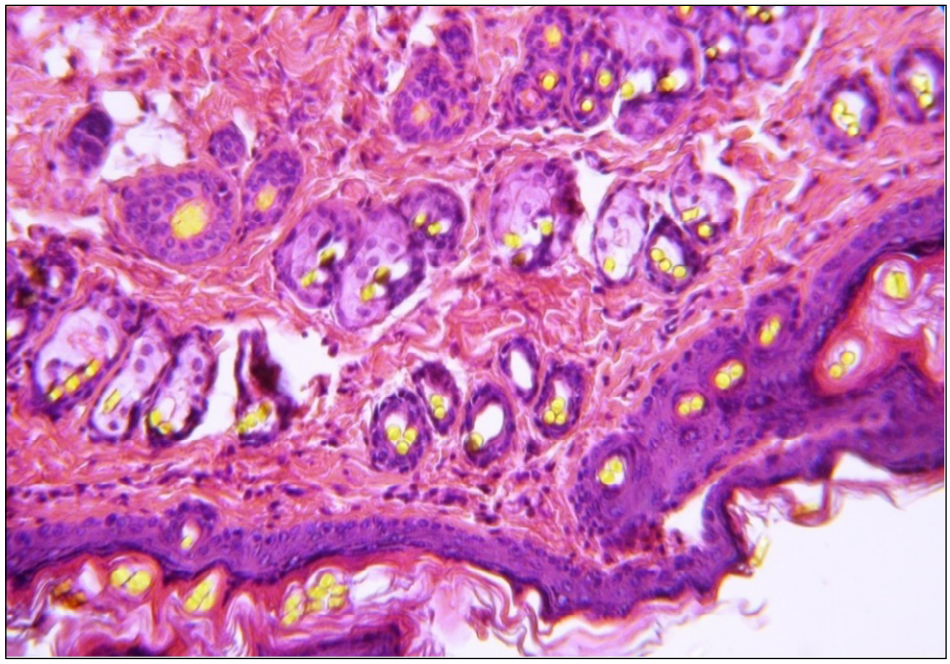

Fig. 1. Skin of the gluteal region of a white rat after 4 weeks of nalbuphine administration. Microphotograph. Staining with hematoxylin and eosin. Magnification: objective 8x, eyepiece 15x.

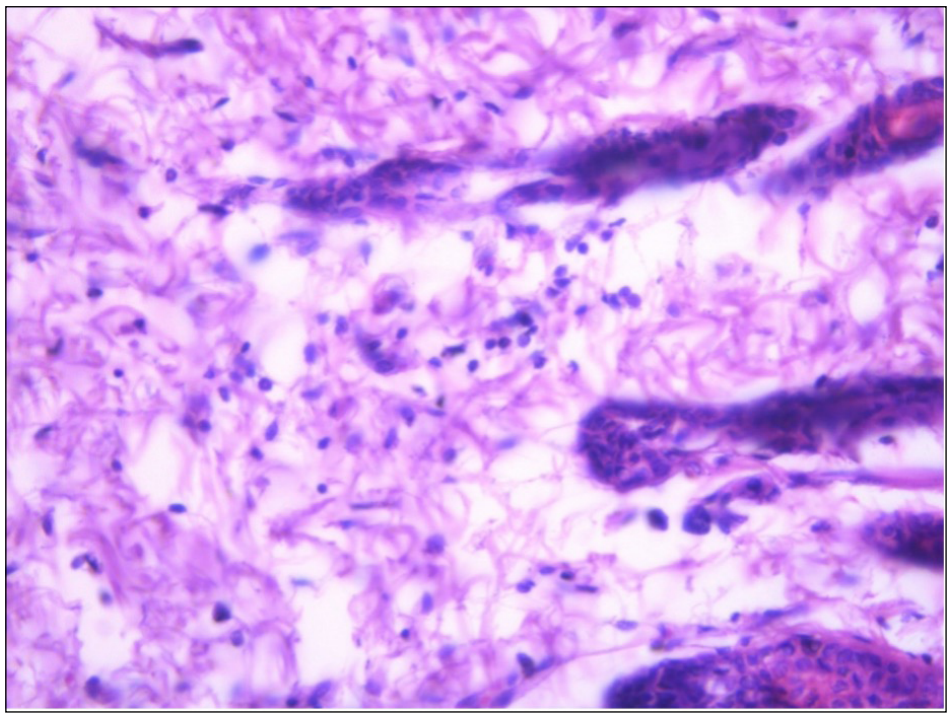

Fig. 2. Skin of the gluteal region of a white rat after 4 weeks of nalbuphine administration. Early atrophic changes in hair follicles and moderately pronounced round-cell infiltration in the hypodermis are observed. Microphotograph. Staining with hematoxylin and eosin. Magnification: objective 20x, eyepiece 15x.

After four weeks of nalbuphine administration to rats, changes in the vascular system of the skin were also observed. The lumens of the dermal blood vessels were dilated and filled with blood cells. The walls of some arterioles were thinned, endothelial swelling was noted. However, the walls of other arterioles were thickened, and the endothelial cells were unevenly distributed along the inner surface of the vessels. "Varicose" dilation of hypodermal venules and perivascular polymorphonuclear infiltrates were also observed.

After four weeks of the experiment, keratinocytes in the stratum corneum form continuous layers, merging with one another. In some areas, vacuole-like structures were detected between the cells. Dystrophic changes in the granular layer cells were predominantly manifested as nuclear pyknosis and cytoplasmic vacuolization (Fig. 3). Cells in the basal layer exhibited altered shapes and sizes, with condensed chromatin in some keratinocyte nuclei, absent nucleoli, invaginated nuclear envelopes, and vacuoles in the cytoplasm. Mast cell cytoplasm also displayed vacuolization, with hyperplastic and hypertrophic mitochondria showing widened spaces between cristae, the matrix is clarified.

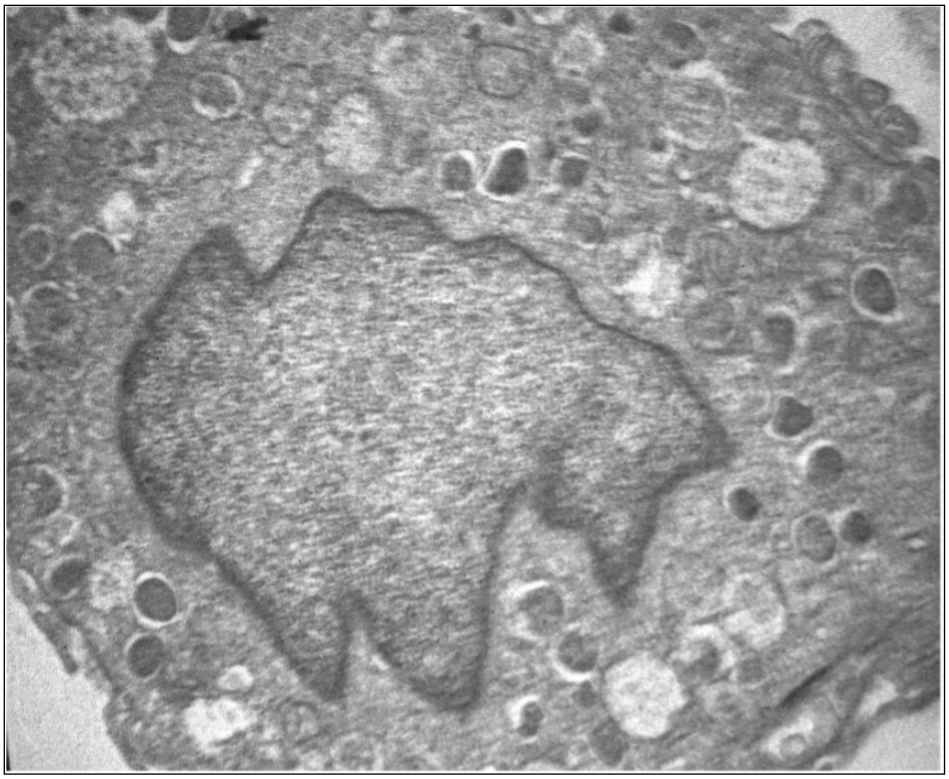

Fig. 3. Ultrastructure of the granular layer of the skin in the gluteal region of a white rat after 4 weeks of opioid administration. Electron micrograph. Magnification: x8000.

In the papillary layer of the dermis, a decrease in the number of connective tissue cells was observed, along with clearing and widening of the intercellular spaces, indicative of edema. Fibroblasts exhibited low electron density in their cytoplasm and nuclei. Peripheral areas of fibroblast cytoplasm showed vacuolization, with loosened membranes of the endoplasmic reticulum tubules, Golgi complex, and mitochondria. Disorganized ribosomes and polysomes were sporadically detected. Venules were congested with blood. The cells of the terminal secretory sections of sebaceous glands were hypertrophied, with enlarged nuclei, invaginated nuclear envelopes, hyperplastic mitochondria, and destroyed mitochondrial cristae.

Thus, profound changes in the epidermis and structural components of the dermis were observed after four weeks of nalbuphine exposure.

DISCUSSION

The results of our study confirmed the primary role of vascular reactions in response to opioid exposure [20]. Several studies in the specialized literature have been dedicated to investigating the mechanisms underlying reduced resistance of skin capillaries under various pathological conditions. The indicator of skin capillary permeability was the number of petechiae that appeared following the examination. Three degrees of skin vascular reactions were distinguished: first degree – on a slightly hyperemic skin, small and medium-sized hemorrhages were evenly scattered across the entire field; second degree – a hyperemic field with numerous small, medium, and large hemorrhages; third degree – confluent hemorrhages of various sizes scattered across the entire test field in unlimited numbers. The second and third degrees were considered a pathological permeability of the skin vessels [21, 22]. Our study confirms that the development of macro- and microangiopathies — such as skin hyperemia, the presence of small, medium, and large hemorrhages, arteriolar and capillary dilation, arteriolar tortuosity, thickening of arteriolar walls, thrombi in venules, enlargement of pericapillary zones, endothelial swelling, proliferation, loosening, and desquamation into the vascular lumen — under prolonged (four-week) nalbuphine administration can be considered the triggering mechanism for all subsequent profound changes in the structural organization of the skin. Changes in the skin at the cellular level identified in our study, combined with comparisons to scientific literature on the effects of nalbuphine on cells of other organs [23, 24], allow us to outline the patterns of cellular reorganization resulting from nalbuphine exposure. These patterns include changes in cell shape, disruption of cytoplasmic electron density, organelle destruction (particularly of mitochondria), apoptosis, karyopyknosis, nucleolar deformation or lysis, and invaginations of the nuclear and cytoplasmic membranes.

CONCLUSIONS

1. After four weeks of nalbuphine administration, a noticeable impact on the morphological condition

of the skin was observed. The arterioles and capillaries of the skin were dilated, with arterioles becoming tortuous. The diameter of the arterioles in the subpapillary arterial plexus increased to 28.62±1.07 μm (control: 22.24±0.73 μm), while the diameter of the intrapapillary capillary loop increased to 6.20±0.11 μm (control: 5.91±0.26 μm). A reduction ($p<0.05$) in the density of capillary loops in the subpapillary vascular plexus of the gluteal skin and an increase ($p>0.5$) in the trophic activity index confirm profound destructive changes in the vascular architecture of the skin. The loops of the vascular plexuses lost their regular delicate pattern, with microaneurysms in the arterioles and saccular dilations of the venules being identified.

2. In the skin micropreparations of experimental animals after four weeks of nalbuphine administration, phenomena of blood stasis in the lumens of capillaries and venules were observed, along with endothelial swelling, perivascular edema, and perivascular infiltrates consisting of neutrophils, lymphocytes, macrophages, and tissue basophils. The epidermal folds were slightly smoothed, while the cells of the stratum corneum appeared flat and elongated, occasionally losing their distinct contours and partially or completely merging with each other.
3. The electron density of the nuclei and cytoplasm of keratinocytes in the stratum granulosum was reduced. Keratinocytes in the stratum spinosum acquired a rounded shape (in contrast to the polygonal shape observed in the control). The nuclei of some keratinocytes were shrunken and hyperchromatic, with cytoplasmic vacuolization noted. In the reticular layer, thickened bundles of collagen fibers were observed, along with localized edema and loosening of collagen fibers. Excessive formation of scales was detected in the stratum corneum. The papillary layer of the dermis showed numerous mast cells and lymphocytes near blood vessels. The shape of cells in sebaceous and sweat glands was altered, with swollen cytoplasm, and lymphohistiocytic infiltration was observed around them.

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CONFLICT OF INTEREST

The Author declare no conflict of interest

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A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

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