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THE MECHANISM OF PENETRATION OF LEAD SULFIDE NANOPARTICLES THROUGH THE PATHOLOGICALLY CHANGED STRUCTURES OF THE BLOOD-TISSUE BARRIER OF THE SKIN DERMIS BY MORPHOLOGICAL FEATURES

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Introduction. Today, nanomaterials have far-reaching application prospects in various fields of industry, agriculture, biology, pharmacology, and medicine. Nanoparticles (NPs) of lead compounds, in particular lead sulfide (PbS), in the nanometer range (12.5 nm – 100 nm) have become widely used. The human and animal body has a large number of different barriers of regulatory and protective functions for the formation and preservation of intra-organ homeostasis. The main function of hemato-tissue barriers (HTB) is their permeability, which consists of two types: physiological, and when the structure of the barrier elements is disturbed, which leads to the penetration of toxic substances into the blood. Damage of the barrier components structure, as a result of the exposure to harmful substances, leads to their permeability and the occurrence of pathological processes. The nature of the latter depends on the functional properties of different types of barriers, where each of them has its own morphological properties of the substrate. Therefore, the acquisition of medical and biological knowledge about the role of HTB in the processes of permeability of PbS NPs through its pathologically changed structures helps in understanding the mechanism of these processes and changes in the functional properties of barrier elements in terms of their maintenance of homeostasis by morphological features.

The aim of the research is to determine the mechanism of penetration of PbS NPs through pathologically changed structures of the blood-tissue barrier of the dermis in relation to their functional properties of maintaining homeostasis by morphological features.

Materials and methods of the research. The object of the study is PbS NPs and male Wistar rats (n = 20), which are divided into 3 groups (1 control, 2 experimental). Experimental rats were treated daily (5 days a week) for 1 month (a 3-month experiment) with a colloidal solution of PbS with NP sizes of 12.5 nm and 100 nm at the rate of 0.001 mol/100 g of body weight on intact (excised) skin with an area of 2 cm^2. Morphological studies were performed using generally accepted and special histological and histochemical methods.

Results. Morphological studies of the structural elements of HTB of the skin dermis revealed swelling of the cytoplasm of the endothelium of capillaries with the presence of small granular crystal-like inclusions in the cytoplasm. Small foci of subendothelial vascular edema were observed, sometimes focal damage to the integrity of the structure of the vascular endothelium (adhesion of blood cells on its surface). Diffuse swelling of the amorphous main substance with a decrease in the activity of glycosaminoglycans and a significant amount of acidic proteins was revealed. The determined morphological changes should be considered as a pathological state of the structure of the HTB elements, which is more significant due to the action of PbS NPs of small sizes (12.5 nm).

Conclusions. The identified histological and histochemical changes in the structures of HTB elements are evidence of a damage of their protective functional properties, which indicates the penetration of PbS NPs through the barrier into the blood and is confirmed by their accumulation in the form of small granular protein inclusions in the target organs (kidneys, liver) and in the endothelium of their blood capillaries.

Key words: blood-tissue barrier, hemato-tissue barrier (HTB), mechanisms of penetration, lead sulfide nanoparticles, dermis, blood vessels, basement membrane, interstitial gel, glycosaminoglycans, acidic proteins

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Introduction

The rapid implementation of various metal and compound nanoparticles requires a comprehensive approach to studying the mechanisms of their absorption through the hematotissue barrier (HTB) structures of the skin dermis and their toxic effects on the human body. Lead compounds, particularly lead sulfide nanoparticles (PbS NPs) of various sizes (12.5 nm and 100 nm), have recently gained widespread use, and their biological activity and toxicity pose a significant risk to human health [1–3]. A review of the scientific literature indicates a lack of comprehensive data on the mechanism of PbS NPs absorption through the HTB under pathological conditions. It is known that the main function of any barrier is to maintain the stability of the internal environment of cells, tissues, organs, and the organism, both under physiological requirements for the delivery of substances to cells and during the penetration of various toxicants, leading to pathological conditions.

The diversity of protective barriers is characterized by varying resistance to the penetration of foreign substances, depending on their physicochemical properties and the physico-biochemical mechanisms of barrier elements. These barriers exist between the blood and all tissues without exception. The main structural elements of the blood-tissue barriers (BTBs) are endothelial cells of vessels, capillaries, and their basal membrane. They are the first line of barrier mechanisms and determine the process of absorption and penetration of nanoparticles into the blood. In different organs, the endothelium of capillaries has its morphological features, which are the basis for selective penetration. On the other hand, the basal membrane is more universal, but also has differences in various organs [4].

The role of skin as a complex organ with its morphological structure and physiological functions is crucial in maintaining the stability of the internal environment of the organism. The permeability of toxic substances through the entire thickness of the skin is a complex and multi-stage process [5]. The epidermal permeability is the first phase of this process, while further penetration into the dermis and entry into the bloodstream constitutes the second final phase, in which the stratum corneum plays a significant role. The activity of the second phase of penetration sometimes depends on the intensity of blood flow, interstitial fluid movement, and the functional state of tissue basophils (synthesizing heparin, histamine) [6].

The main amorphous substance of the connective tissue is an important link in permeability. With the help of electron microscopy, it has been determined that it is present in all without exception extracellular components and the basement membrane of vessels and capillaries. In some pathological processes, the first and fundamental changes begin in the amorphous ECM of the interstitial tissue, where all the components of the extracellular matrix react as a single system. The diffusion processes through the endothelial cell membranes depend on the chemistry of its structure, which, in essence, determines its permeability and can serve as the basis for the development of pathological processes in the ECM [7]. Due to the presence of glycosaminoglycans (GAG) in the substance and their composition, it is very variable, which affects permeability processes. Their binding with proteins changes the properties of the amorphous substance (gel). It is known that the content of GAG in the mixture with albumins of blood potentiates the high osmotic (hydrostatic) effect of the main amorphous substance and causes its hydration and, as a result, swelling, which enhances permeability. Therefore, the leading factor of pathological changes in the amorphous ECM and its permeability

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should be attributed to changes in the content of acidic GAG and acidic proteins in it [8–9].

Thus, the disturbance of the biochemical and structural components of the skin's barrier function is the cause of the penetration of foreign chemical substances into the bloodstream and the development of pathological processes. Their pathogenesis cannot be determined without considering the disturbances in permeability. The mechanisms of the penetration of foreign substances through pathologically altered elements of the skin's stratum corneum and the nature of the development of pathological processes have long been the subject of attention among medical professionals and biologists. However, these issues are unjustifiably given little attention in scientific literature, which emphasizes the relevance and necessity of conducting in-depth morphological studies on the main structural elements of the skin's barrier function, responsible for this process in conditions of their pathological state.

The aim of the research is to determine the mechanism of penetration of PbS NPs through pathologically changed structures of the blood-tissue barrier of the dermis in relation to their functional properties of maintaining homeostasis by morphological features.

Materials and methods of the research

Experiments were carried out on 20 sexually mature male Wistar rats with an initial weight of 150–180 g, which were kept under standard conditions in the vivarium at an air temperature of 21–24 °C and a relative humidity of not less than 45 %, on a standard diet (pelleted feed). All manipulations with the animals were carried out in accordance with the provisions of the «European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes» (Strasbourg, 1986) [10], approved by the Bioethics Committee of the National Academy of Sciences of Ukraine.

The rats were divided into 3 groups (1 control, 2 experimental). The sub-experimental rats were treated daily (5 days per week) with colloidal solutions of lead sulfide (PbS) with particle sizes of 12.5 nm and 100 nm applied to the intact skin area of 2 cm^2 (previously clipped) at a concentration of 0.001 mol/100 g of body weight. The animals were euthanized by decapitation after prior anesthesia with a 2.5 % solution of 2,2,2-tribromoethanol («Aldrish») in 2-methylbutanol (working dilution 1650 in RBS and at a dosage of 300 mg/kg).

After decapitation, small pieces of skin measuring 10×10 mm were fixed in a neutral solution of formalin for 72 hours, washed in water, dehydrated in a series of ethanol solutions (70 %, 80 %, 96 %, 100 %), cleared in xylene, and embedded in paraffin according to standard protocol [11]. Paraffin sections of 5–7 μm thickness were prepared using the Thermo IM 325 microtome, mounted on glass slides (at least 3 sections per slide), and stained with hematoxylin and eosin, toluidine blue, the Brachet method, the aldehyde-fuchsin method by Gomori, and the Mallory modification by Slinchenko [12]. Histological preparations were examined using the Olympus DX 54 light microscope with a polarizing filter system. Changes observed were documented using the Olympus C-5050 ZOOM camera with Olympus DP-Soft software. The crystalline nature of the morphological inclusions present in the cells was determined using a polarizing microscope.

Results of the research and their discussion

Morphological characteristics of the elements of the extracellular matrix (ECM) of the skin dermis in the experiment are considered by us as a patho-

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logical reaction of vessels, their basement membranes, and the main amorphous substance to the influence of different-sized low-frequency PbS particles. It should be noted that the main amorphous substance of the dermis is present in all structures of the ECM. This indicates its significant and primary role in metabolic processes. Therefore, changes in the chemistry of the main substance (interstitial gel) determine the permeability of foreign substances not only through the ECM itself but also through vessels and their basement membranes. The latter constitute a significant portion of the main substance [7]. Morphological studies have shown that long-term exposure to different-sized low-frequency PbS particles on the skin is accompanied by focal and diffuse swelling of the main amorphous substance, which contains water and glycosaminoglycans - the main component of the extracellular matrix. Thionin staining revealed a decrease in the intensity of histochemical reactions and a reduced amount of glycosaminoglycans compared to the control. Overall, these changes indicate an increase in permeability and may be a consequence of the seepage of plasma proteins through the altered structures of the endothelial cells, which stimulates the osmotic effect of the gel and determines the hydration of the main amorphous substance of the dermis. Confirmation of this is the excessive accumulation of acidic proteins, as evidenced by their staining in bright red, which increases tissue swelling and promotes the increased activity of low-frequency PbS particle penetration processes.

Histological studies of blood vessels in the skin under prolonged exposure to low-frequency PbS cause dilation of their lumens and hyperemia against the background of focal perivascular edema. In some vessels and capillaries when applying low-frequency PbS with a size of 12.5 nm, stasis of blood in their lumen was detected, and sometimes sludge of erythrocytes and dystrophic changes in endothelial cells in the form of cytoplasmic edema and small foci of subendothelial edema (basal membrane) were found. The determined morphological manifestations of subendothelial and perivascular edema, foci of plasmatic impregnation of the wall of small-caliber arteries with the accumulation of acidic proteins in them should be characterized as a significant pathohistological manifestation of structural disorders in blood vessels, which stimulates the processes of permeability for foreign substances. Along with the identified changes at the periphery of blood capillaries, a pronounced morphofunctional activity of tissue basophils (hyperplasia, hypertrophy) – regulators of tissue homeostasis, blood supply, and tissue permeability, providing the secretion of heparin and histamine – was observed compared to the control. At the same time, it should be noted that the morphofunctional state of these cells under the influence of low-frequency PbS with a size of 12.5 nm was characterized by a significantly larger number of them in a state of cytoplasmic degranulation, which indicates the intensification of the process of their penetration through the barrier and determines differences in the effects of nanoparticles depending on their size. Special attention should be paid to the fact that in the processes of penetration of low-frequency PbS through blood vessels, their individual properties regarding interaction with serum proteins, cytoplasmic and plasmatic cell membranes, and extracellular matrix proteins may play a role. As a result, complex molecular complexes of low-frequency PbS with proteins are capable of easily interacting with endothelial cell membranes and being absorbed through them by phagocytosis, pinocytosis, and endocytosis, which is confirmed by their accumulation in target organs [13].

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Conclusion

1. Hemato-tissue barriers (HTB) provide for the preservation of the constant composition of tissue substances (homeostasis) while simultaneously preventing the penetration of foreign substances into them. Disruption of the structure of the barrier components due to toxic effects can be the basis for the development of pathologies, depending on the barriers of different systems and their organs.
2. More significant manifestations of the toxic effect on the HTB vessels of the dermis were determined during the action of 12.5 nm NCH PbS. These changes were characterized by swelling of the endotheliocytes and subendothelial layer (basement membrane), which is leading in the mechanism of NCH PbS penetration through the main barrier line of the HTB.
3. In the mechanism of the process of penetration of low-frequency PbS nanoparticles of various sizes (12.5 nm – 100 nm) through the main amorphous substance of the connected tissue of the dermal HTB, the presence of a significant amount of acidic proteins and a decrease in GAG activity compared to the control play a role. No significant differences in histochemical activity between low-frequency nanoparticles of different sizes were observed, indicating the same mechanism of low-frequency PbS nanoparticle effect on the amorphous main substance of the dermal HTB and its permeability through it into the blood.
4. The relatedness and interaction of low-frequency PbS nanoparticles with proteins, depending on their sizes, play an important role in the mechanism of penetration through the cell membrane of endothelial vessels and non-cellular matrix of the dermal HTB, associated with the disruption of their integrity. The mechanism of low-frequency PbS nanoparticle permeability through the HTB into the blood and transport to target organs is confirmed by various cumulative properties of nanoparticles in the cytoplasm of cells and is determined by the more significant presence of small granular crystal-like inclusions with the action of low-frequency PbS nanoparticles with a size of 12.5 nm.

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