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MORPHOFUNCTIONAL MANIFESTATIONS OF THE ACUTE EFFECTS OF LEAD SULFIDE (PBS) NANOPARTICLES UPON DIFFERENT METHODS OF THEIR ADMINISTRATION INTO THE ORGANISM OF LABORATORY ANIMALS (RATS)

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Introduction. Lead compounds in the nanometer range (1–100 nm) pose a particular danger when entering the human and animal body through various routes (via respiratory organs, food and water intake, skin penetration, etc.). One of the most hazardous manifestations of the overall toxic effects of lead sulfide nanoparticles (PbS NPs) is their ability to translocate into cells, leading to subsequent distribution and accumulation in target organs. However, the literature lacks a comprehensive understanding of their reactive potential and the impact on the body through different routes of penetration and observation periods. This underscores the relevance and necessity of conducting experimental studies to morphologically assess the manifestations of acute toxic effects of different-sized PbS NPs (12.5 nm, 100 nm) when introduced into the abdominal cavity and through the skin, taking into account their accumulation and distribution in cells and organ tissues.

The aim of the research – the morphofunctional changes in the liver and spleen were determined following a single intraperitoneal administration of PbS nanoparticles of low size (12.5 nm) and large size (100 nm), as well as the dermal changes following a single administration to the skin, taking into account their accumulation in organ and tissue structures.

Materials and methods of the research. The research object was low-sized PbS nanoparticles (NPs) and the Wistar rat strain (n = 18), which were divided into three groups (one control and two experimental groups). The rats were administered 1.0 ml of a colloidal solution of PbS, which contained nanoparticles of different sizes (12.5 nm and 100 nm). The effects were studied after a single intraperitoneal administration and a single dermal application (with a 1-day exposure). Morphological studies were conducted using conventional and specialized histological and histochemical methods.

Results. The morphological studies of the liver revealed significant dilation and congestion of sinusoidal capillaries and pronounced hypertrophy of stellate reticuloendothelial cells (SRECs), associated with the accumulation of small crystalline inclusions in their cytoplasm. Hepatocytes exhibited cytoplasmic clearing and nuclear hypertrophy. In the spleen, the histochemical Perl's stain revealed iron accumulation upon exposure to different-sized PbS nanoparticles (12.5 nm and 100 nm), indicating accelerated erythrocyte breakdown accompanied by the release of iron from hemoglobin, which is phagocytosed by active macrophages in the red pulp. The skin dermis exhibited a significant presence of muscle fibers with accumulation of acidic proteins, transforming them into «hyaline layers» and leading to the development of hyalinosis.

Conclusions. The presence of small crystalline inclusions in the cytoplasm of endothelial cells and stellate reticuloendothelial cells (SRECs) in the liver is associated with their interaction with proteins in the peritoneal fluid and serum, which facilitates the transport of low-sized nanoparticles (NPs) in the body and their interaction with phagocytic cell membranes that engulf the PbS NPs. In the spleen, the 12.5 nm PbS NPs cause significant damage to erythrocytes, resulting in the release of iron from hemoglobin, which is then absorbed by siderophages in the red pulp. In the skin dermis, the deposition of NPs leads to an inflammatory reaction in muscle fibers, characterized by the presence of acidic proteins, further contributing to the process of hyalinosis.

Key words: PbS NPs spleen, liver, skin dermis, stellate reticuloendothelial cells (SRECs), hyalinosis

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Introduction

The rapid integration of nanomaterials into industrial processes has brought about increased interactions between these materials and living organisms, particularly humans. However, our current understanding of their potential toxic effects remains incomplete. In recent years, nanoparticles of lead and its inorganic compounds, such as PbS, PbSe, and PbTe, with dimensions ranging from 1 to 100 nm, have gained widespread popularity in global applications. Their small size and expansive surface area enhance their biological activity and toxicity, thereby posing substantial health risks.

Consequently, this scenario raises concerns for the well-being of workers and populations residing in proximity to lead-related industries or areas contaminated with lead. While fragments of information exist in contemporary scientific literature, they fall short of delivering a comprehensive grasp of lead nanoparticles' toxicity and the diverse effects of their compounds [1–5]. However, it is evident that even a single inhalation of lead nanoparticles can trigger an inflammatory process in the lungs, culminating in fibrosis and cellular necrosis [6]. The pronounced affinity of lead for neural structures determines the neurotoxic consequences of metal nanoparticles and their compounds, facilitated by their capacity to traverse the blood-brain barrier. Within brain cells, lead nanoparticles typically lead to the development of oxidative stress. A comparable mechanism, coupled with inflammatory alterations, lays the groundwork for apoptosis and cellular necrosis, ultimately defining the hepatotoxic and cardiotoxic outcomes linked with lead nanoparticles [7].

Considering the existing literature on the toxicity of nanomaterials, it is important to note that most aspects of addressing the risks associated with nanoparticles are still not fully understood. Specifically, comprehensive data regarding the absorption, metabolism, accumulation, toxic mechanisms of various lead nanoparticle compounds, as well as the critical organs and systems affected by different routes of administration (both acute and chronic), are currently lacking in scientific literature. This highlights the relevance and necessity of conducting experimental studies to morphologically evaluate the manifestations of acute effects of PbS nanoparticles when administered into the peritoneal cavity and skin. This represents an important and understudied medical-biological issue.

The aim of the research – the morphofunctional changes in the liver and spleen were determined following a single intraperitoneal administration of PbS nanoparticles of low size (12.5 nm) and large size (100 nm). Similarly, dermal modifications were investigated after a singular application to the skin, considering the nanoparticles' propensity for accumulation within various organ and tissue structures.

Materials and methods of the research

The study utilized colloidal solutions of PbS nanoparticles obtained through chemical synthesis at the L. V. Pisarzhevsky Institute of Physical Chemistry of the National Academy of Sciences of Ukraine. These solutions were stabilized using 0.1 % and 0.5 % gelatin solutions for PbS nanoparticles. With PbS nanoparticle sizes of 12.5 nm and 100 nm, their nanoparticle concentrations in 1 ml of colloidal solutions were $0{,}95 \cdot 10^{13}$ particles and $0{,}62 \cdot 10^{12}$ particles, respectively.

The experiments were conducted on 18 sexually mature male Wistar rats weighing 150–180 g,

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which were kept under standard conditions in a vivarium at an air temperature of 21–24 °C and a relative humidity of at least 45 %, on a standard diet (pelletized feed). All animal manipulations were performed in accordance with the provisions of the «European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes» (Strasbourg, 1986) [8], approved by the bioethics committee of the National Academy of Sciences of Ukraine.

The rats were divided into three groups: one control group and two experimental groups. The experimental rats were administered a 1.0 ml colloidal solution of PbS nanoparticles with different particle sizes (12.5 nm and 100 nm). The colloidal solutions of nanoparticles were administered through intraperitoneal injection and skin application (with a 1-day exposure).

After decapitation, small samples (10 × 10 mm) of the liver, spleen, and skin were fixed in a neutral formalin solution for 72 hours, rinsed in water, dehydrated in a series of ethanol solutions (70 %, 80 %, 96 %, and 100 %), cleared in xylene, and embedded in paraffin following standard protocols [9]. Paraffin sections, with a thickness of 5–7 μm, were prepared using a Thermo IM 325 microtome, mounted on glass slides (at least 3 sections), and stained with hematoxylin and eosin using the modified Mallory method by Slinchenko and the histochemical Perl's method [10].

Histological preparations were examined using an Olympus DX 54 light microscope with a polarization filter system. The observed changes were documented using an 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 research and their discussion

The results of the histological examinations showed that upon a single intraperitoneal administration of PbS nanoparticles (sizes of 12.5 nm and 100 nm) in rats, significant dilation and congestion of sinusoidal capillaries were observed in the liver. These capillaries often exhibited clusters of lymphocytes, monocytes, and neutrophilic leukocytes alongside erythrocytes. Additionally, a pronounced hypertrophy of vascular endothelial cells and stellate reticuloendothelial cells was observed. The cytoplasm of these cells contained small crystal-like inclusions, which were associated with the interaction of PbS nanoparticles with proteins in the peritoneal fluid and serum proteins. This facilitated their transport in the body and their interaction with the cytoplasmic membranes of phagocytes that engulf the nanoparticles (Figure 1). In contrast to the effects of smaller-sized PbS nanoparticles (12.5 nm), the larger-sized nanoparticles (100 nm) led to a greater presence of small crystal-like inclusions in the cytoplasm of hepatocytes. In these cases, hepatocytes exhibited cytoplasmic swelling, while the action of 12.5 nm PbS nanoparticles resulted in cytoplasmic clearing. Both scenarios of cytoplasmic changes in hepatocytes were accompanied by nuclear hypertrophy. Additionally, it was observed that the acute effects of PbS nanoparticles of different sizes resulted in edema and moderate infiltration of the organ's connective tissue stroma by neutrophilic leukocytes, lymphocytes, and macrophages.

The spleen, being an organ implicated in sequestering iron that is typically released during physiological or heightened erythrocyte breakdown (e.g., induced by diverse hemolytic factors), serves as a convenient subject for the histochemical evaluation of iron accumulation in response to PbS nanoparticles' influence. The findings of our conducted histochemical analyses reveal a notable increase in

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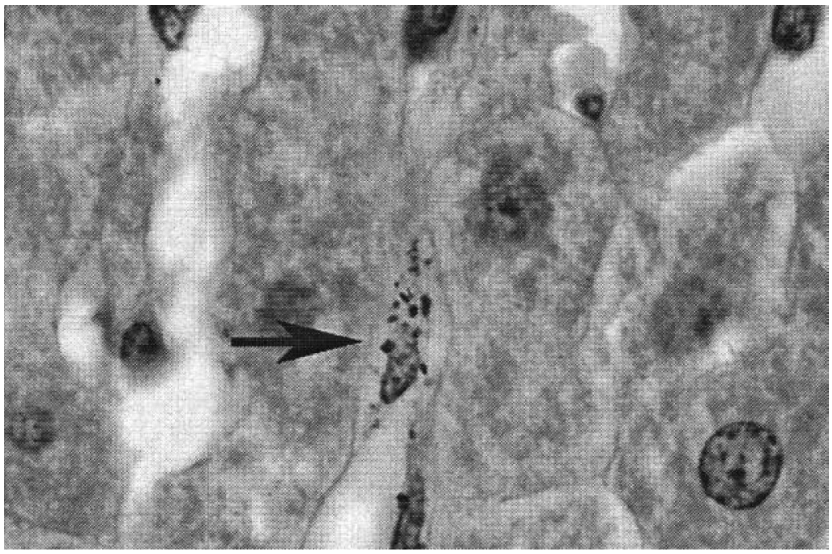

Figure 1. Histological changes in the liver: clustering of lymphocytes, monocytes, and neutrophilic leukocytes in the sinusoidal capillaries (PbS NPs with a size of 100 nm). Hematoxylin and eosin staining

the count of siderophages within the red pulp of rat spleens under the effect of 12.5 nm PbS nanoparticles. Notably, the cytoplasm of these cells showcases the presence of amorphous inclusions generated from the product of the Berlin blue histological reaction (Figure 2). This indicates a substantial elevation in the spleen's iron content, attributed to its active assimilation by macrophages located in the red pulp. In contrast, exposure to 100 nm PbS nanoparticles results in a notably diminished iron presence, as discerned through the Perl's histochemical method, within the spleen. This can be explained by the notion that smaller nanoparticles possess heightened potential to provoke erythrocyte impairment, triggering the release of iron from hemoglobin which is subsequently engulfed by phagocytes.

To assess the absorption of PbS nanoparticles (NPs) in the experiments on rats, we recreated an artificial tissue deposition model of NPs. To accomplish this, we administered colloidal solutions containing PbS NPs of different sizes (12.5 nm and 100 nm) directly into the dermal layer of the skin utilizing a syringe.

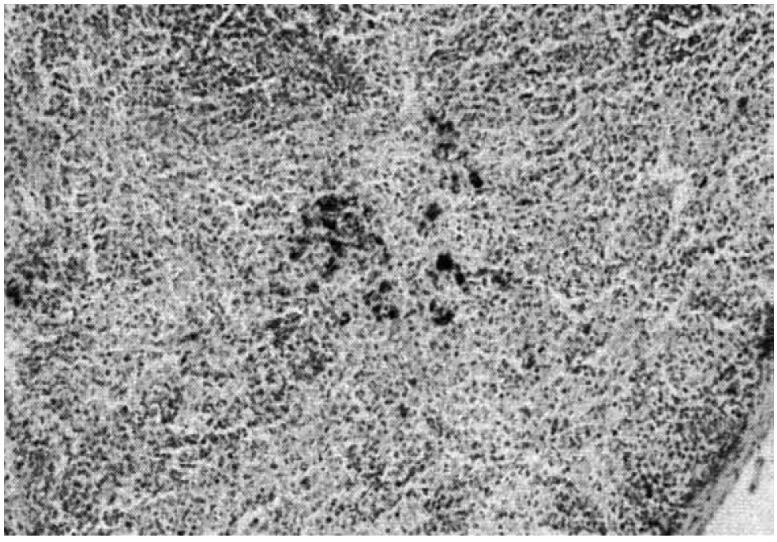

Figure 2. Histological changes in the liver: small crystal-like inclusions in the cytoplasm of endothelial cells and stellate reticuloendothelial cells (PbS NPs with a size of 12.5 nm). Hematoxylin and eosin staining

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Histological examinations of the skin from our experimental rats unveiled a localized inflammatory response situated beneath the epidermis in the dermal layer. This reaction manifested through the dilation of blood vessels and lymphatic capillaries, alongside edematous alterations within fibrous structures. The focal points of inflammation showcased the presence of neutrophilic leukocytes, lymphocytes, and macrophages. Furthermore, a distinctive outcome surfaced under the influence of small-sized PbS nanoparticles (12.5 nm), in contrast to their larger counterparts (100 nm): the dermis exhibited plasmatic impregnation of muscle fibers with acid proteins, leading to hyalinosis. Additionally, a pronounced degranulation of tissue basophils was evident, an effect not observed in the context of 100 nm PbS nanoparticles applied to intact skin.

Using the histochemical reaction with silver nitrate, we determined that the crystal-like cytoplasmic inclusions observed in the muscle fibers of the skin dermis have the ability to interact with silver nitrate and form dense granules of silver sulfide. This finding allows us to confidently conclude that there is an accumulation of lead sulfide deposits in the cytoplasm of myocytes (Figure 3) with a high probability. However, these accumulations were not observed in all myocytes, but only in those exhibiting signs of contracture damage. This suggests that substances capable of interacting with PbS NPs and forming dense crystal-like complexes appear in the areas of contracture damage in myocytes. These complexes can be visualized as cytoplasmic inclusions under a light microscope.

During the histochemical examination using the Mallory-Slincenko method, we observed the accumulation of acidic proteins and the subsequent transformation of cells into «hyaline layers» in the muscle fibers alongside contracture damage of myocytes. These changes were particularly pronounced in the presence of significant edematous alterations in the dermal layer (Figure 4). Such findings indicate a direct impact of PbS NPs on the structure of the myosin protein molecule. This interaction alters the physicochemical properties of myosin, leading to the development of myocyte necrosis and subsequent progression to hyalinosis.

To conclude, our acute experiments on rats employing distinct administration routes (intraperitoneal and intact skin) underscore the pivotal role of PbS NPs size in their absorption, distribution, and

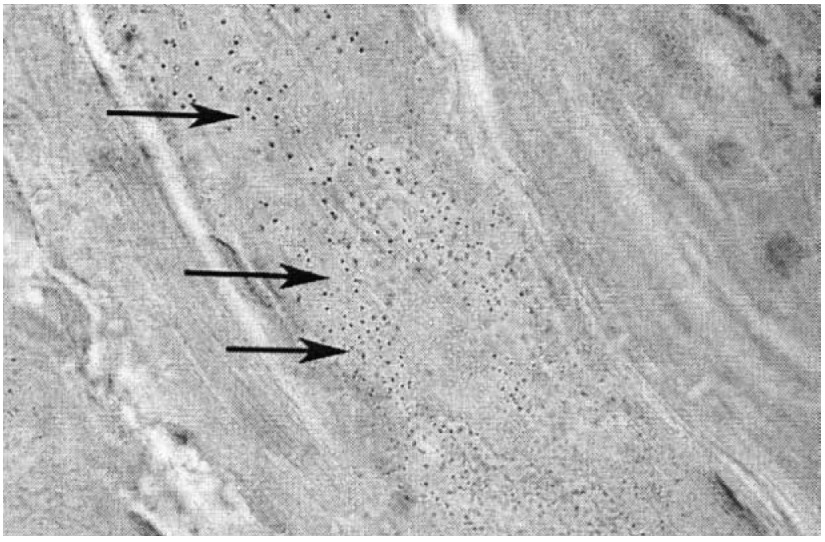

Figure 3. Histochemical characterization of iron accumulation in the spleen: increased number of siderophages with inclusions of the Berlin blue histochemical reaction product (PbS NPs with a size of 12.5 nm). Perl's reaction

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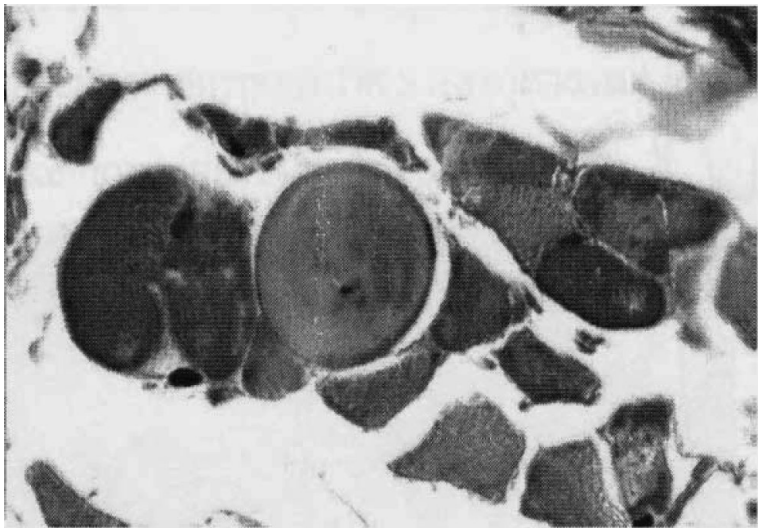

Figure 4. Histochemical changes in the muscle fibers of the skin dermis: accumulation of acid proteins leading to the transformation of cells into «hyaline layers» (PbS NPs with a size of 100 nm). Mallory-Slincenko histochemical method

the emergence of toxic effects. A crucial aspect in the intracellular penetration and dispersion of nanoparticles across diverse tissue types involves their interaction with proteins. Consequently, the complexes thus formed easily interact with cell membranes, thereby being internalized via diverse pathways and initiating structural transformations. Small-sized PbS NPs (12.5 nm) demonstrate a notable capacity for inflicting significant damage upon erythrocytes, triggering the liberation of iron from hemoglobin. This liberated iron is subsequently taken up by tissue macrophages in the red pulp, leading to the formation of siderophages. Within the dermis, our replication of a tissue depot model employing PbS NPs of varying sizes elicits a localized inflammatory response. This response encompasses muscular fiber swelling and, particularly in the case of small-sized PbS NPs (12.5 nm), introduces contracture changes within myocytes, characterized by the presence of acidic proteins, thereby initiating the progression of hyalinosis.

In summary, our study underscores the intricate interplay between PbS nanoparticle size, absorption dynamics, and subsequent physiological responses, contributing to a deeper understanding of their potential toxicological implications.

Conclusions

1. Single intraperitoneal administration of different-sized PbS NPs (12.5 nm and 100 nm) in rats leads to hypertrophy of vessel endotheliocytes and star-shaped reticuloendothelial cells in the liver. The number of small crystalline inclusions in their cytoplasm is significantly higher with the action of 100 nm-sized NPs. Hepatocytes exhibit more pronounced dystrophic changes in the cytoplasm with the action of 12.5 nm-sized PbS NPs.
2. In the spleen, the action of 12.5 nm-sized PbS NPs leads to significant deposition of iron due to erythroclasis of red blood cells, from which iron is released and subsequently engulfed by phagocytes of the red pulp (siderophages).
3. Reproducing the tissue depot model for different-sized PbS NPs results in the accumulation of lead sulfide in the muscle fibers of the skin, forming dense crystalline complexes, which are observed only in cells (myocytes) showing signs of contractile damage.
4. The local inflammatory reaction of the muscle fibers in the dermis is characterized by the presence of acidic proteins, leading to the formation of «hyaline layers» and subsequent development of hyalinosis.

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