Original article

Effect of heavy metals on the readaptive processes in the urinary bladder

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Abstract:

Introduction: Heavy metals (HM) are dangerous elements due to their toxicity and prevalence play an important role in the environmental pollution and hazards to health risk. Impact of HM at high concentrations may lead to the failures of urinary bladder (UB) structure and functions. The aim of our study was to determine the readaptive changes in the UB of the rats and effectiveness of vitamin E using after the long-term intake of the HMS mixtures. ***Materials and Methods:*** We used mature male laboratory rats which were divided into three series with special experimental conditions after the influence of HM. The detailed analysis of the UB state was performed by using morphometric, spectrophotometric, histological and electron microscopy methods. ***Results:*** We had determined that the long-term intake of HM caused the significant increase of their accumulation in the tissue and morphometric changes of UB. Obtained results after the cancelation of the HM effect demonstrate smooth morphological restructuring of the UB wall with the reduction of difference of accumulation and morphometric indicators with control. The adjustment of vitamin E during there adaptation period showed better result recovery and optimization of all parameters, but they haven't completely returned to normal state. ***Conclusions:*** The influence of HMS mixture led to significant negative effect on the UB at all stages of research. However, under the conditions of readaptation the tendency towards a certain stabilization of all indicators was observed, but they didn't completely pass to the end of the study. The vitamin E using has caused the acceleration and improved the development of readaptive change after HM exposure.

Keywords: urinary bladder; heavy metals; readaptation, rats; vitamin E.

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Introduction

Large number of studies both in national and world literature is devoted to the problems of environmental pollution[1,2]. Unfortunately, due to technologic progress the toxic substances in different states get into the environment and lead to the formation of artificial biogeochemical provinces. Heavy metals salts (HMS) are exogenous factors that propagate in the biosphere and can affect the health and ecosystems[3-5]. Furthermore, ions of some heavy metals (HM) circulate in the ecosystem in normal doses and in the background doses affect the regulation of many processes in almost all organisms. However, the damage of physiological level of ions, at least of one element doesn't pass unnoticed and their combined impact may lead to significant disorders in the organism functioning [5-8].

It is important to note that the excessive entry of

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HM into the body results in their accumulation in different organs and systems and partial excretion[6,9]. Unfortunately, the imbalance in chemical elements leads to the homeostasis disruption and the development of the pathologies, caused by the effect of these pollutants[3,6,8]. More recently, new information appears in the world literature that describes the pathogenetic features of the development of environmentally caused diseases due to the residence in contaminated areas[8,10]. Uncontrolled growth of the rate of HMS is mostly accompanied by the failure of the compensatory mechanisms of the body with the development of oxidative stress due to the imbalance between the active oxygen species and antioxidants[11]. Therefore, lately, significant attention is paid to the search of powerful antioxidants in order to understand how to counter HM and other pollutants with similar effects and to reduce the health risks worldwide[11,12].

One of the most powerful antioxidant that can withstand the effects of HM on the body is vitamin E[13]. Numerous publications indicate that vitamin E is located on biological membranes and protect cells from the effects of negative factors. It is also known that this vitamin has many important functions, such as: increases the level of antioxidant defense, binds free radicals, prevents oxidative stress and reduces the impact of pollutants in the organism[12-14].

An important role in the removal of metabolism products and toxins is given to the urinary bladder (UB). This organ has functional peculiarities and provides long and controlled urine store in its cavity[15]. It is proved that wide range of factors can affect the development of its pathological changes and dysfunctions[15-17]. Despite many scientific advances, the data on the mechanisms and dynamics of the development of recovering processes is rarely found in the literature. Consequently, the object of our study was to determine the readaptive changes in the UB of the rats and effectiveness of vitamin E using after the long-term intake of the HMS mixtures.

Materials and Methods:

The UB of the mature male laboratory Wistar albino rats were used as the materials for this study. For the period of experiment the laboratory animals were kept in vivarium with a controlled temperature, humidity, 12 hours light/dark cycle, with free access to food and water. All research on animals was conducted in accordance with the regulations, adopted by "European Convention for the protection of vertebrate animals used for scientific purposes" (Strasbourg, 1986), «General ethical principles of animal experiments», adopted by the First National Congress on Bioethics (Kyiv, 2001); Ethical Principles and Guidelines for Experiments on Animals: 3rd edition (Swiss, 2005).The development of adaptive mechanisms in rats UB was studied after influence by the potentially dangerous HMS combination (zinc ($ZnSO4\times7H2O$) – 5 mg/l, copper ($CuSO4\times5H2O$) – 1 mg/l, iron (FeSO4) – 10 mg/l, manganese ($MnSO4\times5H2O$) – 0.1 mg/l, lead (Pb(NO3)2 – 0.1 mg/l, chromium (K2Cr2O7) – 0.1 mg/l)in excessive concentrations.Also, it was decided to study readaptation against the background of own forces of organism and with the corrective therapy by vitamin E (9.1 mg/kg of 10% oil oral solution taking into account the recount of the dose for animals). Therefore, laboratory rats were divided into three series: I series (control) - animals that received normal drinking water for the whole period; II series - animals that had been consuming ordinary drinking water within 90 day after discontinuation of intake of the solution with combination of HMS; III series - rats that started to use vitamin E correction with drinking water after discontinuation intake of the solution with combination of HMS during 90 days. Rats were taken out on the 180 day of the experiment by decapitation under anesthesia, whereupon UB was removed by the midline laparotomy. The organ was fixed in 10% buffered formalin solution (24 hours), dehydrated in ascending grades of ethanol, embedded in paraffin and 3-5-μm-thick samples were made on a rotary microtome. To study general features of UB, the samples were stained with hematoxylin-eosin. Histological specimen and morphometric study were analyzed and photographed using light-optical microscope «Carl Zeiss Primo Star» (Germany) with a digital camera «Zeiss Axio Cam ERs 5s» (Germany) and software with image output system «ZEN 2 (blue edition)» (Germany) with digital micrometer and calculation output (μm).Digital images of all specimens located on the spectral graphite rods were examined with a scanning electron microscope (SEM–102E) with the software package «VCU» using. The samples for atomic absorption spectrometry were weighed on analytical balance, dried out and burnt in porcelain crucibles in oven at 450° C for 48 hours. Obtained ash was dissolved in 10% hydrochloric and nitric acids. Thereafter, a spectrophotometer C-115M1 with computer recording of the analytical signal and calculated program "AAS SPEKTR" were used to determine the amount of chemical elements considering the length of the wave of each: Zn (213.9 nm),Cu (324.7 nm), Fe (248.3 nm), Mn (279.4 nm), Pl (283.3 nm), Cr (357.9 nm).The scanning electron microscopy of UB was carried out according to the generally accepted technique on the REM-102E microscope and visualization software package «VCU». An assessment of the differences and interconnections between the groups was investigated according to the nonparametric Mann-Whitney test and the Spearman correlation analysis (r). $p<0.05$ was considered statistically significant. The mathematical calculations of the results were performed using Microsoft Excel 2007 with the application Attest at 12.0.5 and Graph Pad® 6.0 software.

Ethical approval: This study was approved by the Ethics Committee of Sumy State University, Ukraine

Results:

On the 90th day of the readaptation, the indicators of morphometric analysis of the thickness of the organ's wall have significantly increased (21.14% ($p<0.01$) and 15.96% ($p<0.05$) vs control) in II and III series. In general, these changes were caused by the changeability of the values of each layers in the respective series: mucosa – (-2.68%), $p>0.05$ and 1.25%, $p>0.05$ (taking into account the decrease of the epithelial height while the thickness of the lamina propria has increased), submucosa – 36.65% ($p<0.05$) and 24.21% ($p>0.05$), muscularis – 20.39% ($p<0.01$) and 16.22% ($p<0.05$), adventitia – 14.67% ($p<0.01$) and 6.88% ($p>0.05$). Thus, when the HMS intoxication was canceled, the difference between parameters of UB thickness in different series was still expressive (compared with control). However, the tendency for normalization of the indicators in the III series was more expressed. The inaccuracy of some results indicates the reduction of their difference with control (Fig. 1 – A).

For a better understanding of readaptation opportunities, the level of exogenous elements accumulation in the UB wall on the 90th day of the readaptive period should be indicated, which has increased and significantly exceeded the control. The level of HM ions in the II series was at a high level (51.28%, $p<0.01$) even after discontinuation of HMS exposure. However, the frequency of the chemical elements removal in series with corrector was slightly higher (34.4%, $p<0.01$). It should be mentioned that the concentration of each chemical element varied at different levels during the recovery period in the II and III series: for zinc – 17.03% ($p<0.01$) and 13.3% ($p>0.01$), for copper – 38.46% ($p<0.01$) and 34.26% ($p<0.01$), for iron – 59.33% ($p<0.01$) and 38.82% ($p<0.01$), for manganese – 31.26% ($p<0.01$) and 22.45% ($p<0.01$), for lead – 67,23% ($p<0.01$) and 54.55% ($p<0.01$), for chromium – 43.95% ($p<0.01$) and 35.05% ($p<0.01$) (Fig. 1 – B).

During the experiment, we found strong correlation between the increase in the rate of chemical elements in the tissue and simultaneous increase in the organ's wall thickness ($r=0.74$, $p<0.01$). The excessive rate of HM had strong impact on the sensitivity of the submucosal ($r=0.51$, $p<0.05$), muscular ($r=0.59$, $p<0.01$) and adventitious ($r=0.66$, $p<0.01$) layers. Correlation dependence of the mucous membrane was not accurate, due to the different direction of the urothelium and lamina propria. Among all HM, iron ($r=0.76$, $p<0.01$) and copper($r=0.7$, $p<0.01$) had the strongest impact on the UB, on the other hand, zinc had the smallest ($r=0.44$, $p<0.05$), correlation, but its importance was not lost (Fig. 1 – C).

In the period when the HMS was cancelled, we noted the difference in the morphological features between the series of readaptation with variety and complexity of regenerative processes. During 90 days of remote readaptation the slow signs of the structural elements recovery of the UB wall were observed in the III and IV series (Fig. 2). It was noted the partial recovery of the epithelium structure. All its layers were better visualized. The frequency and depth of desquamation lesions gradually reduced. However, the areas of enhanced proliferation and flattening of the urothelial height, accompanied by the imbalance of epithelial cells were still present. Moderate swelling of stromal and parenchymal components of the organ with better visualization of the direction of myocytes were observed. The area of the connective tissue gradually decreased due to the established fibrotic changes. Inflammatory infiltration was less observed in the stroma and near the vessels. The frequency of dyscirculatory disorders as well as perivascular edema and extension of the arteriol lumen decreased. However, the vascular congestion and stasis were still singularly observed. It should be mentioned that the adaptive processes were less expressed in the UB of the rats from II experimental series compared to the animals from III corrector series, where the reduction of pathological features had faster rates of histoarchitecture recovery and the tendency to decrease the difference with the control, but was not completely normalized.

Electron and microscopic study of the II series revealed the structural rearrangement in the UB architectonics, the phenomena of swelling, disorganization of connective tissue and muscle fibers. Changes in the height of the epithelium were represented by the desquamation lesions caused by the atrophy and hyperplasia; in urothelial cells the dystrophic changes were observed. These changes worsened the visualization of the parenchymal and stromal components of the organ(Fig. 3 – A).In the period of recovery the following changes in the UB of the animals from III series were observed: injuries of morphomicrostructural homeostasis decreased that was expressed by the reduction of swelling of parenchymal and stromal components with the improvement of the organization of their fibers, regeneration of the state and height of the epithelium was observed as well as the visualization of the its layers was improved. It is important to note that the expressiveness and the rate of readaptive changes depended on the presence of vitamin E in the diet that is demonstrated by certain diversity of the results in both series (Fig. 3 - B).

Discussion

The results of our experiment demonstrated that the model of long-term HM exposure causes the destruction of protective components with the predominance of significant morphological transformations in the wall of the UB, the growth of morphometric indices and an increase of HM accumulation in the tissue with strong correlation interactions. Interestingly, that UB can be attributed to a few organs that fall under the indirect hematogenous and direct contact impact of HM ions that enter the body in the urine and blood[11,19]. Due to this, the metal-microelements can cause various variation of morphological changes in the UB during subacute and chronic periods of intoxication[18,20]. In our previous histological and immunohistochemical researches of UB, we demonstrated that HM impact is the cause of significant morphological transformations in all structural components of the organ with the specific features of each layer. Furthemore, we have shown that the sequences of intensity and depth of pathological lesions were dependent on the duration of the experiment[18]. Nevertheless, more recent research has shown that the that long-term of HMS exposure leads to increases of HM accumulation level in the UB tissue which influence significantly on the morphometric parameters of organ wall thickness (this data has not been published yet).A lot of simulated studies demonstrated that the organ easily exposed to development of inflammatory diseases of various genesis that accompanied by urodynamic disorders[15,17]. A considerable amount of scientific data on associate disruption of the level of physiological concentrations trace elements with the development of oncological pathology of the bladder and other different organshas been published[9,10,19-21]. Taking into account, the technological development of the industry and the use of chemical fertilizers in agriculture led to environment pollution by HM[22]. However, the risk of concentration increasing of HM binds with living on the ecologically dangerous areas with disorders of metabolic processes, abnormal functioning and deepening of the consequences of their actions against the background of pollutants accumulation [19,23,24]. It is known that excessive concentrations of HMS are able to generate the reactive oxygen intermediates and free radicals and suppresses antioxidants that lead to oxidative stress. The last mentioned are involved in many chemical reactions in small amounts, but the excessive increase leads to the damage of cellular components - proteins, lipids and DNA[11,12,25].

Especially interesting fact is that the cancellation of the HM intake is able to develop adaptive rearrangements of the UB wall that leads to an improvement of its structure and morphometric parameters. However, after 90 days of readaptation, the complete regeneration of the UB structural didn't occur, which can be explained by damage to self-regulating systems and still high content of chemical elements. We have also demonstrated improving regenerative properties in the morphological state of UB against the background of faster purification from the pollutants. It is thought that effectiveness of vitamin E immediately after the cancellation of HM impact probably depend from increasing of antioxidant reactions in the organism, a blocking reactive oxygen species and free radicals actions before they can damage cells, which in turn was accompanied by the inhibition of oxidative stress [12,14,22,26,27]. Also, vitamin E benefits include its role in proper function of many organs, promote regeneration of tissues and immune response, stabilizes blood filling and other[12,13,26]. According to the results of our research, in addition to vitamin E properties as an antioxidant, it is involved in counteract the input of HM into a cells, its protections and promote rapid cleansing. In our view, exactly these characteristics are significant factors which contribute to a more rapid regeneration of morphological structure of UB (Fig. 4).

Conclusions

The influence of heavy metal salts mixture leads to ions accumulation and causes to significant negative effect on the bladder even in readaptation period. The consequences of these changes don't completely pass even after 90 days of HM impact discontinuation.

Under the conditions of readaptation the tendency towards a certain stabilization of all indicators is increasing, in parallel with decrease of chemical elements level in the tissue. The assessment of the readaptation results confirms the dependence of the detected changes from the additional using of vitamin E as a corrector.

Conflict of interest: The authors declare that they have no competing interests.

Authors' Contributions:

The work was carried out with the support of the research theme "Development of the diagnosis method of the reproductive system organs tumors using cellular adhesion molecules of cancer-embryonic antigen"(state registration number 62.12.01-01.17/20.3П).

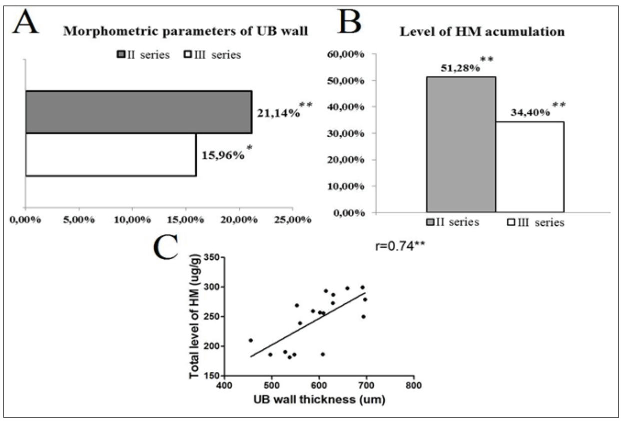

*Fig. 1. Changes of indicators level of UB researches on 90th day of readaptation after HMS influence. All indicators are presented as percent increase over control (0%) in the graph. A - growth of morphometric indices; B - variations of spectrophotometric levels of chemical elements; C - correlation analysis between morphometric (A) and spectrophotometric (B) parameters during the experiment period. Statistical significant: *p<0.05; **p<0.01.*

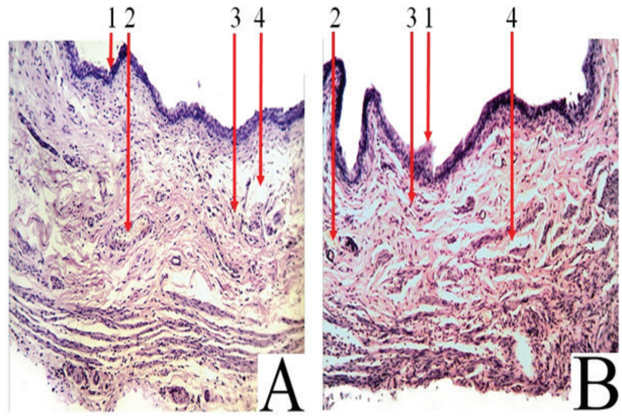

Fig. 2. The microscopical features of the UB state: A - Rats of the II series on the 90th day of readaptation period; B - Rats of the III series ofreadaptation period + Vit. E. 1 - epithelium disorganization;2 - vascular stasis; 3 -inflammatory infiltration;4 - tissue edema.H&E staining.Magnification 200×.

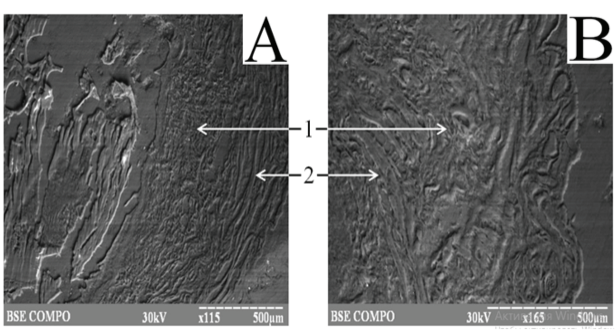

Fig. 3.The scanning images of UB tissue of the II (A) and III (B) animals serieson the 90th day of readaptation period:1 - stromal component of tissue; 2 -parenchymal component tissue. Magnification: A x115; B x165.

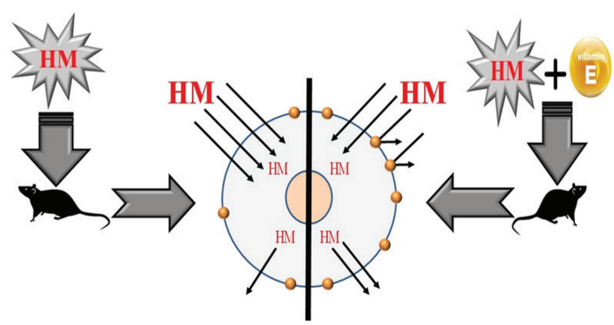

Fig. 4.Scheme of HM entry into the cell and protective properties of vitamin E.

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