Stress-Protected Effect of Torasemide in Acute Immobilization Stress in Rats

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Abstract

The issue of search and selection the novel effective and, most importantly, safe methods of prevention and treatment of stress disorders remains one of the priorities for contemporary medical science. The paper was aimed at the morphological study of the rational use of torasemide as a stress protector. Based on bioethics regulations, 25 albino mature male rats aged were involved into morphological study. Group I involved intact animals; Group II involved animals that were exposed to acute immobilization stress without correction; Group III involved animals, exposed to stress after intraperitoneal administration of sodium chloride; Group IV involved animals, exposed to stress and corrected with mexidol; Group V involved rodents, exposed to stress and corrected with torasemide. After rats' sacrifice, macroscopic and microscopic study of the lungs, liver, kidneys and spleen was performed. Histological preparations were stained with hematoxylin and eosin. Studies of the stress-protective properties of torasemide indicate its positive corrective effect on the structure of the lungs, liver and spleen in acute immobilization stress. However, morphologically, correction with torasemide in acute immobilization stress caused enhancement of stress-related renal alterations. Due to the adverse effects of torasemide on the histological structure of the kidneys under stress, its use as a stress protector is not considered rational.

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Introduction

Notwithstanding a long-lasting study, the problem of stress remains relevant to date. In particular, numerous recent morphological studies promote better understanding the essence of stress-related structural changes and search for novel ways to prevent and treat adverse effects caused by stress[1,2]. However, the issue of finding and selection the advanced effective and, most importantly, safe methods of prevention and treatment of stress disorders continues to be one of the priorities for contemporary medical science[3,4]. Our previous studies give evidence of structural changes in the rat lungs induced by the acute immobilization stress as the predecessors for the development of pulmonary edema[5,6,7]. Therefore, torasemide as a potential stress protector was worth our attention[8,9]. Currently, in the cardiology, the use of this long-acting loop diuretic has become widespread in treatment of essential hypertension, as well as in treatment and prevention of edema and effusions caused by heart failure and cardiogenic pulmonary edema[10,11,12].

The paper was aimed at the morphological study of the rational use of torasemide as a stress protector.

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Materials and methods

25 albino mature male rats aged 8-10 months old of 240-260 g body weight were involved into morphological study.

Group I (controls) (n=5) involved intact animals; Group II (n=5) involved animals that were exposed to acute immobilization stress

without correction; Group III (n=5) involved animals, exposed to stress after intraperitoneal administration of 0.5 ml sodium chloride (NaCl) ; Group IV (n=5) involved animals, exposed to stress and corrected with mexidol, well known for its stress-protective effect; Group V (n=5) involved animals, exposed to stress and corrected with torasemide. Acute immobilization stress was simulated by retention of rats, lying supine for 6 hours. For the purpose of correction, 100 mg/kg body weight mexidol was single-time administered intraperitoneally 20 minutes prior the period of immobilization (Group IV) or with 0.1 mg torasemide (Group V).

The rats were sacrificed within two hours after the period of immobilization by decapitation under intraperitoneal thiopentone anesthesia. After dissection of rats' chest and abdominal cavity, a macroscopic study of its organs was carried out with subsequent collection of the specimens of the lungs, liver, kidneys and spleen for microscopic study. Pieces of investigated organs were fixed in 10% neutral formalin and after dehydration in spirits of the ascending densities they were embedded into paraffin according to conventional technique. The microtome slices were stained with hematoxylin and eosin.

The experiment was performed in compliance with the requirements of international principals of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1985) and corresponding Law of Ukraine "For the Protection of Pet Animals" (No.3 446-IV, 21.02.2006, Kyiv).

Results

The macroscopic analysis showed that in acute immobilization stress without pharmacocorrection, the liver, spleen and kidneys of rodents of the experimental groups did not differ from the organs of intact rats. However, the acute immobilization stress caused marked changes in the lungs, which were plethoric, with numerous areas of hemorrhage beneath the pleura and lung tissue. During the dissection of the lungs, specific hemorrhagic exudate was noted. Macroscopically, in both groups of rats, which were previously administered with the investigated drugs, stress-related pulmonary alterations were less pronounced.

Microscopic study shows that acute immobilization stress causes significant morphological changes in the lungs, liver and kidneys.

No significant structural changes were detected in the spleen; minor perivascular edema of the central arteries in large- and medium-sized lymphoid nodules of the white pulp and single perivascular hemorrhages were noted. Correction, performed with both mexidol and torasemide, eliminated the effect of stress on the spleen at the morphological level: its histological structure did not differ from intact animals.

In the lungs of rats, exposed to stress without pharmacocorrection, histological study showed significant dilation of the alveoli; local destruction and thinning of the interalveolar septa; thickening and destructive changes of the epithelial layer of the mucous membrane of the intrapulmonary bronchi with accumulation of cellular detritus and red blood cells in their lumen; peribronchial leukocyte infiltration; hemomicrocirculation disorders with the development of blood stasis and the appearance of numerous hemorrhages in the interstitial connective tissue and the lumina of the alveoli (Fig. 1). Morphometric study confirmed that in acute immobilization stress in rats of Group II, the average diameter of the alveoli increased significantly ($p < 0,01$) by 67.16%, from (38.83 ± 1.15) μm to (64.91 ± 2.35) μm. Thinning of the interalveolar septa was by 41.3%, from (13.05 ± 0.86) μm to (7.66 ± 0.46) μm, $p < 0.01$. The mucous membrane of the smaller bronchi also significantly thickened ($p < 0.01$) by 86.72%, from (8.81 ± 0.40) μm to (16.45 ± 0.52) μm. The diameter of the internal lumen of the capillaries and venules significantly increased by 104.93%, from (3.65 ± 0.25) μm to (7.48 ± 0.71) μm, $p < 0.01$, and by 37.04%, from (18.87 ± 1.14) μm to (25.86 ± 1.76) μm, $p < 0.01$, respectively.

The use of mexidol and torasemide to correct stress-related pulmonary disorders has shown the effectiveness of these drugs. The stress-protective effect of torasemide was particularly positive. The correction promotes insignificant peribronchial infiltration and edema of the bronchial mucosa, especially with torasemide; detrital masses were not observed in the bronchial lumen; dilation of alveoli and thinning of interalveolar septa was less pronounced; hemomicrocirculation disorders were not so significant (Fig. 2).

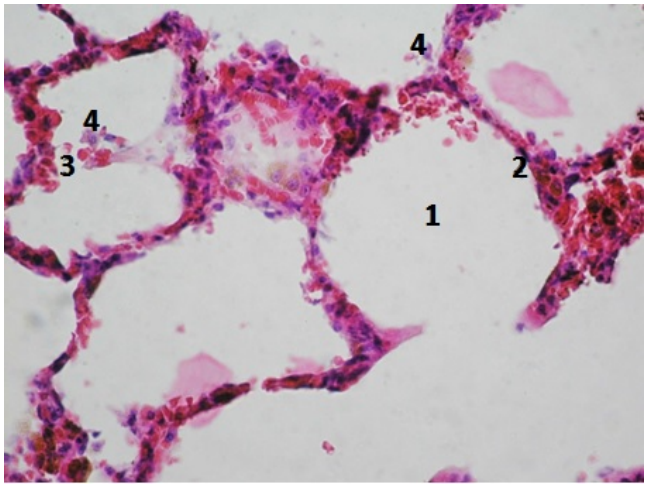

Figure 1. Rat lungs after simulation of the acute immobilization stress. Hematoxylin and eosin stain: 100×magnification: 1 – lumen of the alveoli; 2 – alveolocyte; 3 – destruction of the alveolar wall; 4 – cellular detritus.

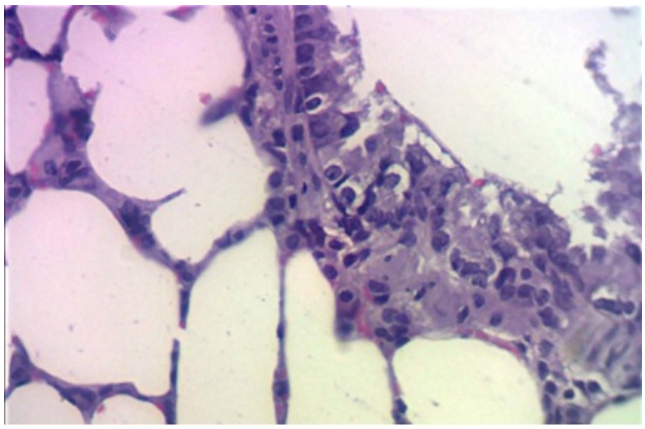

Figure 2. Rat lungs after simulation of the acute immobilization stress with torasemide correction. Hematoxylin and eosin stain: 100×magnification.

The findings of morphometric and statistical studies prove by evidence the positive stress-protective effect of mexidol and torasemide.

In the group of animals administered with mexidol in simulated acute immobilization stress, the average diameter of the alveoli increased by only 34.48%, from (38.83 ± 1.15) μm to (52.22 ± 4.41) μm, p <0 , 01. Thinning of the interalveolar septa was by 11.03%, from (13.05 ± 0.86) μm to (11.61 ± 0.41) μm, p <0.01. The mucous membrane of the smaller bronchi thickened by 48.13%, from (8.81 ± 0.40) μm to (13.05 ± 0.54) μm, at p <0.01. The diameter of the internal lumen of the capillaries and venules increased by 49.86%, from (3.65 ± 0.25) μm to (5.47 ± 0.82) μm, p <0.0, and by 18.44%, from 18.87 ± 1.12) μm to (22.35 ± 1.85) μm, p <0.01, respectively.

Correction with torasemide showed that the average diameter of the alveoli increased by 20.06%, from (38.83 ± 1.15) μm to (46.62 ± 5.63) μm, p <0.01. Thinning of interalveolar septa occurred by 4.67%, from (13.05 ± 0.86) μm to (12.44 ± 0.44) μm, p <0.01. The mucous membrane of the smaller bronchi thickened by 27.36%, from (8.81 ± 0.40) μm to (11.22 ± 0.52) μm, at p <0.01. The diameter of the internal lumen of the capillaries and venules increased by 27.36%, from (3.65 ± 0.25) μm to (4.78 ± 0.58) μm, p <0.01) and by 11.02%, with 18.87 ± 1.12) μm to (20.95 ± 2.05) μm, p <0.01), respectively.

In the liver of rats of Groups II-III, swelling of hepatocytes with smoothing of intercellular borders and extension of perisinusoidal spaces was detected. The central vein and interlobular vessels were plethoric, in most sinusoidal capillaries the sludge phenomena were noted (Fig. 3). Moderate plethora of sinusoidal capillaries and marked perivascular edema was also noted. Dystrophic changes (hydropic and hyaline-drop dystrophy) were observed in the center of the lobules, as well as subcapsular focal colliquative necrosis of individual hepatocytes. Morphometric study showed that stress in rats of Group II promotes in the liver enlargement of the lumina of the interlobular veins by 16.9% (from (6.88 ± 1.06) μm to (8.28 ± 1.20) μm, p < 0.01), spasm of the arteries by 30.13% (from (3.93 ± 0.16) μm to (3.02 ± 0.21) μm, p <0.01) and enlargement of the lumina of the bile ducts by 31, 82% (from (5.72 ± 0.26) μm to (7.54 ± 0.25) μm, p <0.01). In the hepatic lobuli, the central veins were also dilated by 38.22% (from (25.93 ± 2.89) μm to (35.84 ± 2.87) μm, p <0.01.

The correction with mexidol had a positive effect on the structure of the hepatic lobuli, which were preserved; the triads were unchanged; insignificant plethora of interlobular vessels was noted. Hyaline-drop dystrophy was observed in individual hepatocytes around the central veins, and hydropic dystrophy on the periphery of the lobuli was detected in single hepatocytes. Nuclear structures were unchanged. Morphometric study confirmed that administration of mexidol significantly eliminated the adverse effects of stress on the liver: the lumens of the interlobular veins enlarged by only by 11.77% (from (6.88 ± 1.06) μm to (7.69 ± 1.12) μm, p

<0.05, and constriction of the arteries by 14.24% (from (3.93 ± 0.16) μm to (3.44 ± 0.15) μm, p <0.05; enlargement of the lumina of the bile ducts by 10.34% (from (5.72 ± 0.26) μm to (6.38 ± 0.34) μm, p <0.05; enlargement of the inner diameter of the central veins by 15.32% (from (25, 93 ± 2.89) μm to (30.62 ± 2.45) μm, p <0.05, was noted.

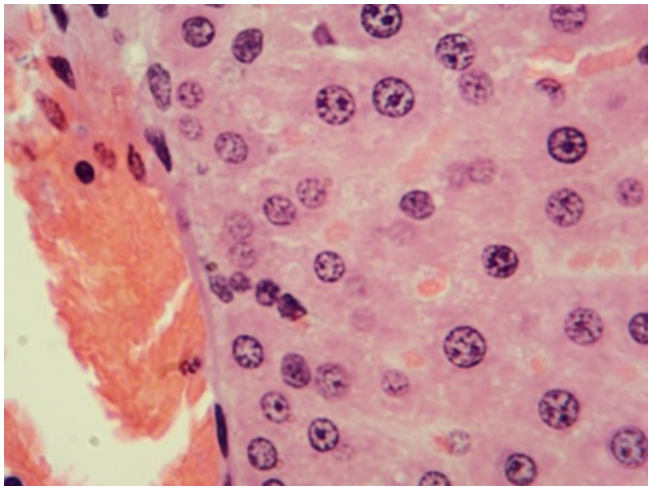

Figure 3. Plethora of interlobular veins with the phenomena of thrombosis in rat liver after simulated acute immobilization stress. Hematoxylin and eosin stain. 400×magnification.

The correction with torasemide had the same positive effect: the structure of hepatic lobules was preserved, the triads were unchanged, sinusoids were not dilated, moderate plethora of central veins was noted, and hydropic dystrophy was observed in some hepatocytes on the periphery of the lobules (Fig. 4). The findings of the morphometric study on correction with torasemide showed that the lumina of the interlobular veins dilated by 12.69% (from (6.88 ± 1.06) μm to (7.88 ± 1.71) μm, p <0.05; constriction of the arteries by 15.93% (from (3.93 ± 0.16) μm to (3.39 ± 0.18) μm, p <0.05; enlargement of the lumina n of the bile ducts by 16.86% (from (5, 72 ± 0.26) μm to (6.88 ± 0.31) μm, p <0.05); enlargement of the inner diameter of the central veins by 21.47% (from (25.93 ± 2.89) μm to (33.02 ± 2.34) μm, p <0.05, was detected.

Histologically, stress-related renal changes promoted enlargement of the lumina of the convoluted tubules with the accumulation in their lumina of cellular detritus due to desquamation of the epithelium. Vacuoles filled with translucent liquid were found in the cytoplasm of epitheliocytes; necrosis of single epithelial cells was noted. The peritubular vascular system was characterized by focal hemorrhages. Morphological changes of the glomerular apparatus were manifested by the significant enlargement of the Shumanski-Bowman's capsule, dramatic plethora of capillaries, edema of the mesangial matrix (Fig. 5). In some renal corpuscles, collapsed cells of the inner leaf of the capsule were detected, which indicated the exclusion of this nephron from the filtration.

Morphometric study of rats of Group II proved the enlargement of lumina of the proximal and distal convoluted tubules by 17.32% (from (18.24 ± 0.32) μm to (22.06 ± 0.56) μm, p <0.05, and by 18.71% (from (18.68 ± 0.31) μm to (22.98 ± 0.75) μm, p <0.05, respectively; thickening of the Shumanski-Bowman's capsule by 11.64% (from (37.98 ± 0.44) μm to (42.95 ± 0.71) μm, p <0.05, and enlargement of the lumen of the capillaries of the glomerular apparatus by 14.67% (from (2.21 ± 0.15) μm to (2.59 ± 0.25) μm, p <0.05.

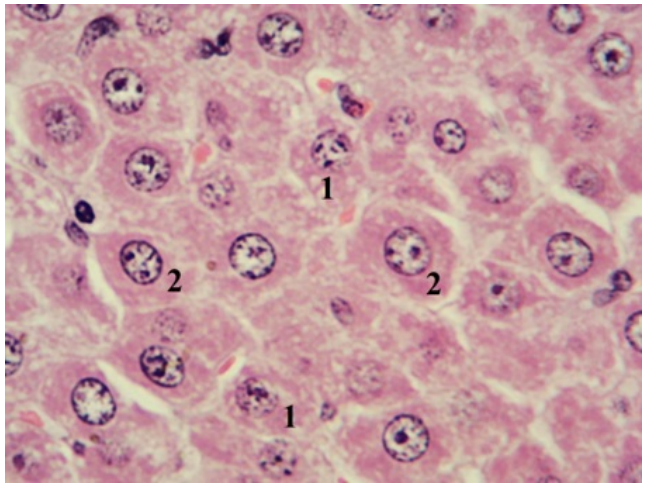

Figure 4. Rat liver after simulation of the acute immobilization stress. Hematoxylin and eosin stain: 200×magnification: 1 – hydropic dystrophy in single hepatocytes; 2 – hepatocytes with preserved structure.

Correction with mexidol alleviated the effects of stress on the kidneys. Sporadic focal changes in convoluted tubules, characterized by swelling of epitheliocytes were noted; in the lumen of individual tubules, minor homogeneous masses were detected. In some glomeruli, insignificant plethora of the capillaries and edematous changes in the mesangium were

detected. The findings of the morphometric study on correction with mexidol showed that enlargement of the lumina of the proximal convoluted tubules was less pronounced, only by 6.96% (from (18.24 ± 0.32) μm to (19.51 ± 0.52) μm, p <0 , 05; distal convoluted tubules by 15.44% (from (18.68 ± 0.31) μm to (22.09) μm ± 0.45, p <0.05; enlargement of the Shumanski-Bowman's capsule by 3.92% (from (37.98 ± 0.44) μm to (39.47 ± 1.44) μm, p <0.05; enlargement of the lumen of the capillaries of the glomerular apparatus by 5.43% (from (2.21 ± 0.15) μm to (2.33 ± 0.15) μm, p <0.05.

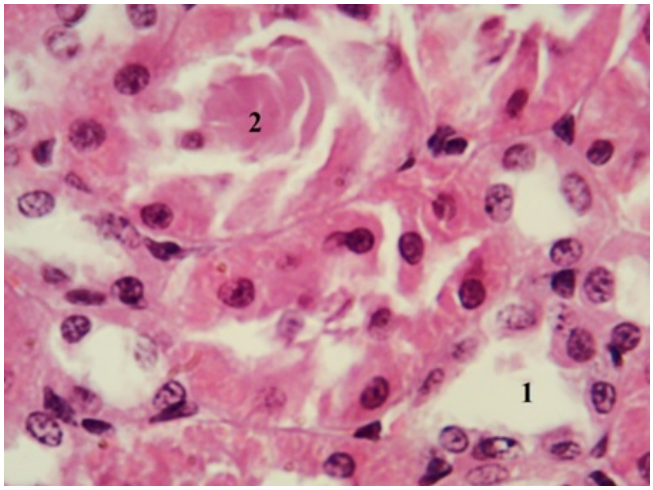

Figure 5. Rat kidney after simulated acute immobilization stress. H&E stain: 400×magnification: 1 – enlargement of the lumen of the convoluted tubules; 2 –eosinophilic masses in the rat kidney associated with stress reaction.

Administration of torasemide had no stress-protective effect on the kidneys of experimental rats. During the correction with torasemide, the lumina of the convoluted tubules remained constricted due to significant swelling of epitheliocytes, and numerous eosinophilic homogeneous masses were noted in their lumina. Necrosis of individual epitheliocytes was detected, and the phenomena of hyaline-drop dystrophy were observed in the cytoplasm of most cells.

In the vessels of the glomeruli a pronounced plethora, edema of the mesangium was noted. The interstitium was characterized by severe edema and significant vascular plethora (Fig. 6).

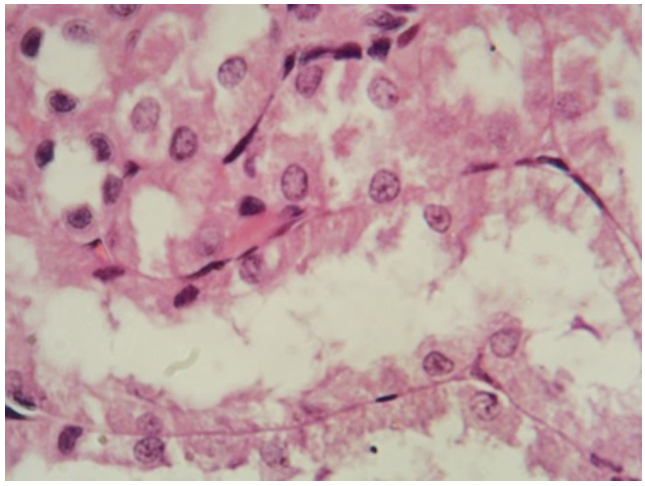

Figure 6. Necrosis of epithelial cells of rat kidney after exposure to stress and correction with torasemide. H&E stain: 400×magnification.

Discussion

Thus, the study confirms the need for a comprehensive study of the effect of the drugs that could potentially be used for the prevention or treatment of stress disorders. Our studies on the stress-protective properties of torasemide indicate its positive corrective effect on the structure of the liver, spleen and, especially, the lungs in acute immobilization stress. However, morphologically, administration of torasemide in acute immobilization stress did not show a positive stress-protective effect in the kidneys of rats: the lumina of the convoluted tubules remained constricted due to significant swelling of epitheliocytes, filled with eosinophilic homogeneous masses; necrosis of individual epitheliocytes was detected; hyaline-drop dystrophy was observed in the cytoplasm of most cells; in the vessels of the glomeruli pronounced plethora, edema of the mesangium was detected; interstitium was swollen with significant vascular plethora. Unlike torasemide, mexidol, the drug of comparison, with its well-studied stress-protective properties[13,14,15] had a positive effect on the structure of the kidneys. We believe that the use of torasemide as a stress protector in acute immobilization stress is not rational. However, this issue needs to be studied more deeply, taking into account a large number of factors.

Conclusions

1. The use of torasemide in acute immobilization stress has a positive effect on the structure of the spleen, liver and lungs, reducing stress-related changes in these organs.

2. Administration of torasemide in acute immobilization stress increases the effect of the stress response on the structure of the rat kidneys.

3. Based on the findings of the study the use of torasemide as a stress protector is not considered rational.

Declaration of Interest

The authors report no conflict of interest.

Compliance with Ethics Requirements

"The authors declare no conflict of interest regarding this article"

„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. "No funding for this study".

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