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## **MORPHOLOGICAL CHANGES OF KIDNEY TISSUE WHEN USING GLUTATHIONE AT RHBDOMYOLYTIC ACUTE KIDNEY INJURY**

**Abstract.** *Morphological study of rats kidneys after the simulation of rhabdomyolytic acute kidney injury by the single injection of 50% glycerol revealed the morphological changes in the renal cortex and medulla, which were manifested by the luminal obstruction of the cortical and medullary tubules with myoglobin casts, epithelial dystrophy and signs of coagulative necrosis. Use of glutathione improved the renal histology significantly, which was confirmed by restriction of dystrophy and necrotic processes, indicating its nephroprotective activity.*

**Keywords:** *rhabdomyolytic acute kidney injury, glutathione, morphological changes.*

**Introduction.** Acute kidney injury (AKI) is a polyetiologic syndrome, which is characterized by violation of filtration and concentration, excretory and incretory functions of the kidneys caused by rapid multiple changes of major kidney functions due to intrarenal blood flow disorder and occurrence of acute tubular necrosis [4, 6, 10–12]. This sudden renal damage is caused by prerenal, renal and/or subrenal influences of etiologic factor. Myorenal syndrome and pigment myoglobinurial nephrosis caused by massive rhabdomyolysis are the most common causes of renal AKI. Rhabdomyolysis is a syndrome of skeletal muscle damage leading to lysis of myocytes, resulting in excretion of intracellular contents, including myoglobin (MG) and creatine kinase (CK) into blood plasma. Along with traumatic rhabdomyolysis (crush syndrome, cramps, excessive exercise) nontraumatic rhabdomyolysis often develops as a result of various toxic and physical actions (poisoning with heavy metal compounds, drugs, medicines), muscle ischemia, infections etc. Normally MG freely binds to plasma globulin and just a small amount gets into urine. But at its mass excretion plasma cannot bind all MG in the bloodstream. As a result, it is filtered through the glomerular filter and gets into the tubules of the kidneys, which can cause obstruction of the tubules and the consequent decrease in glomerular filtration rate (GFR) and

renal function violation [1, 5, 7]. Besides, MG is characterized by direct nephrotoxic effect [1]. Because of necrotic or degenerative changes in renal tubular epithelial cells, which develop due to the action of MG, obstruction of tubular cell with detritus and reverse movement of the tubular content with regurgitation of primary urine in the blood and lymph occur. Today cases of rhabdomyolytic kidney injury are 5–15% of total AKI cases, and the mortality rate is 8% [7]. In view of the relevance of AKI, pharmacological correction of this syndrome is still an important task of medicine and necessitates search for new approaches to its treatment or prevention.

At rhabdomyolysis glutathione (G) as an antioxidant plays an important role in protecting cellular structures from oxidative stress, acting as donor of electrolytes for peroxidase, which prevents the development of free-radical processes [9]. Another important function of G is the formation of mixed disulfides with proteins, which can be an additional element in the regulation of biological processes [8]. Glutathione can exist in oxidized (G-S—S-G) and restored (G-SH) forms. Restored form of G protects SH-groups of proteins from oxidation by various free radicals. The mechanism of protection is the oxidation of SH-groups of the G with the formation of its oxidized form and preservation of SH-groups of proteins in the active reduced form.

Therefore for confirmation of nephropro-

tective influence of glutathione at rhabdomyolytic AKI, we have studied the morphological changes in kidney tissues of laboratory rats in experimental model of rhabdomyolytic AKI [3]. Thus, the experiment proved that glutathione improves functional activity of kidneys at rhabdomyolytic AKI, which was manifested by an increase in urine output, a corresponding increase in GFR, a decrease in creatinine content in blood plasma and a decrease in proteinuria parameters.

Also, when using the drug positive changes in the prooxidant-antioxidant balance in the kidneys and blood plasma of animals were observed: the content of TBA-active products and oxide-modified proteins decreased, while indicators of activity of glutathione peroxidase and ceruloplasmin, and content of SH-groups increased significantly [3].

**Objective:** to study of morphological changes of kidney tissues at the correction with glutathione at the experimental model of rhabdomyolytic AKI.

**Materials and methods.** Experiments were performed on 28 white outbred rats (weighing 140-200 g). AKI was caused by intramuscular injection of 50% glycerin solution in amount of 10 mg/kg. Glutathione (TAD 600 "Biomedica Foscoma», Italy) was injected at a dose of 40 mg/kg once intraperitoneally in 40 min after injection of glycerol. In 24 hours after simulation of AKI the blood sampling and the kidney tissues sampling were performed after decapitation of animals under ether anesthesia in strict accordance with the requirements of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

Kidney tissues for histological study were fixed in 10% solution of neutral buffered formalin for 48 hours, dehydrated in alcohols of ascending concentrations, embedded in paraffin at 64°C with the following obtaining of histological sections of 5 microns thick, stained with hematoxylin and eosin. For objectification and improving reproducibility of the results of quantitative research computerized morphometry of objects in histological samples was carried out. For this digital copies of optical image of areas of microscopic samples were obtained with a digital camera Olympus (model

C740UZ) and microscope LYUMAM-P8 with the following creation of a bank of digital micrographs, which were further analyzed by the software "VideoTest - Size 5.0" ("VideoTest", Russia).

**Results and discussion.** Study of the morphological structure of kidney tissues of animals revealed that in intact animals the vessels are moderately blood-filled, hemorrhages are absent, the tubular epithelium is not changed, the tubular lumen is easily visible, the glomeruli are not changed (Figs. 1, 2).

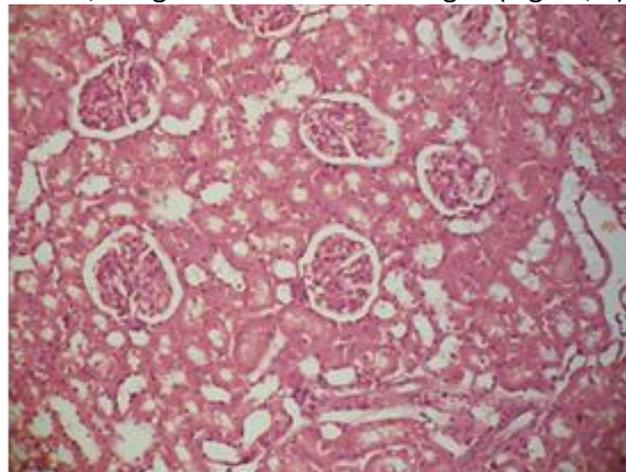


Fig. 1. The kidney sample (the cortical substance) of an intact rat. Stained with hematoxylin and eosin. Magnification: eyelens×40, objective ×10.

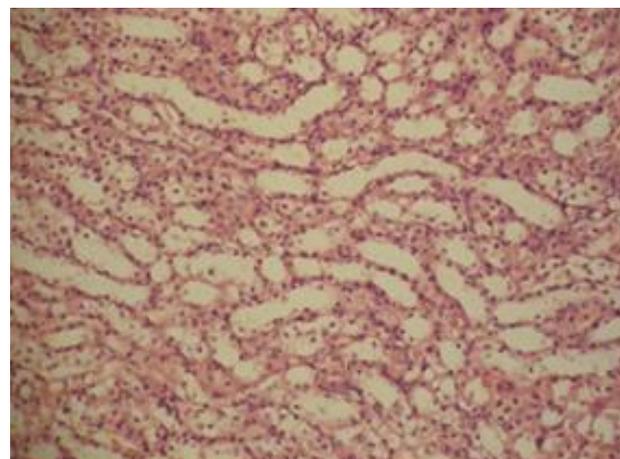


Fig. 2. The kidney sample (the medullary substance) of an intact rat. Stained with hematoxylin and eosin. Magnification: eyelens×40, objective ×10.

In contrast to the intact animals, in the animals with simulation of rhabdomyolytic AKI significant changes in the kidney histostructure are observed (Figs. 3, 4).

Thus there is an obstruction with myoglobin casts of lumens of  $28 \pm 0,8\%$  of convoluted tubules of cortical substance (Fig. 3) and

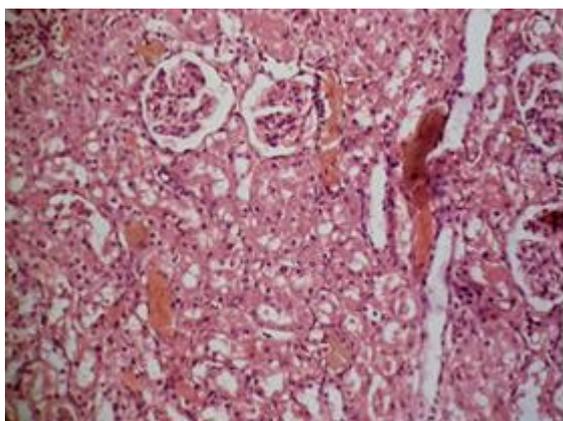


Fig. 3. The kidney sample (3 – the cortical substance) of a rat with rhabdomyolytic AKI. Stained with hematoxylin and eosin. Magnification: eyelens $\times 40$ , objective  $\times 10$ .

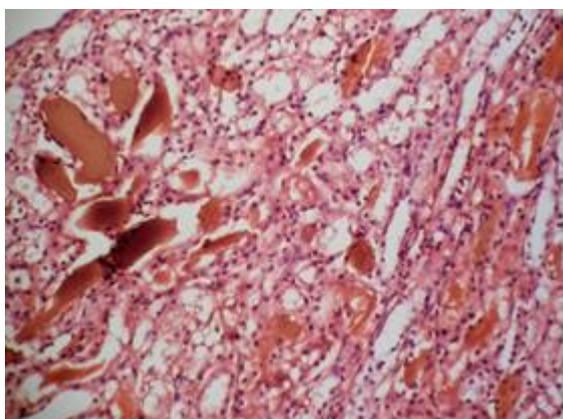


Fig. 4. The kidney sample (4 – the medullary substance) of a rat with rhabdomyolytic AKI. Stained with hematoxylin and eosin. Magnification: eyelens $\times 40$ , objective  $\times 10$ .

61 $\pm$ 1,2% of excretory tubules of medullary substance (Fig. 4). Myoglobin casts in most cases expand the lumens in the field of their localization strongly. The nuclei of the epithelium are not seen clearly, the lumen is almost absent. In the convoluted tubules 94  $\pm$  1,0% of epithelial cells have signs of granular and hydropic dystrophy, and 2  $\pm$  0,1% of epithelial cells are in a state of coagulation necrosis, which is manifested by the cytoplasm induration and karyopyknosis (Fig. 3). In the excretory tubules of the medullary substance 37  $\pm$  1,4% of epithelial cells are affected by hydropic dystrophy, which is the reverse process (Fig. 4).

At the correction of rhabdomyolytic AKI with glutathione on the 24<sup>th</sup> hour of the experiment the morphological picture of kidneys improves slightly (Figs. 5, 6), but there is still an obstruction of the lumens of the tubules of the

cortical substance with myoglobin casts, covering 6  $\pm$  0,4%, and 11  $\pm$  0,9% of the excretory tubules of the medullary substance. Myoglobin casts have varying degrees of staining, that is they are of varying density and slightly expand diameters of tubules in the places of their localization. In the convoluted tubules 82  $\pm$  1,3% of the epithelial cells have signs of granular and hydropic dystrophy. Dystrophic process in the form of granular dystrophy in the excretory tubules of the medullary substance covers 69  $\pm$  0,9%, and 2,0  $\pm$  0,3% of epithelial cells are in a state of coagulation necrosis with the cytoplasm induration and karyopyknosis.

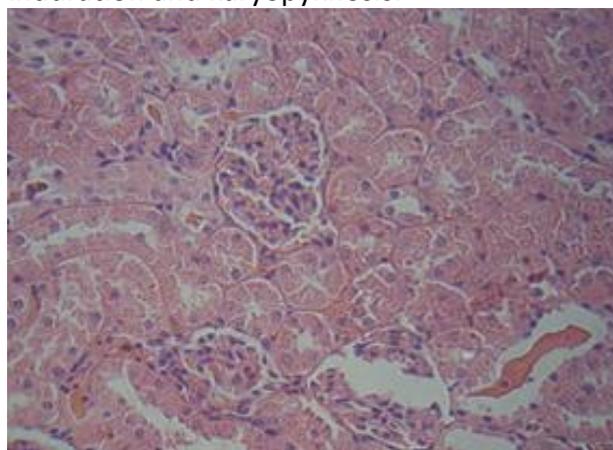


Fig. 5. The kidney sample (5 – the cortical substance, 6 – the medullary substance) of a rat with rhabdomyolytic AKI after glutathione injection. Stained with hematoxylin and eosin. Magnification: eyelens $\times 40$ , objective  $\times 10$ .

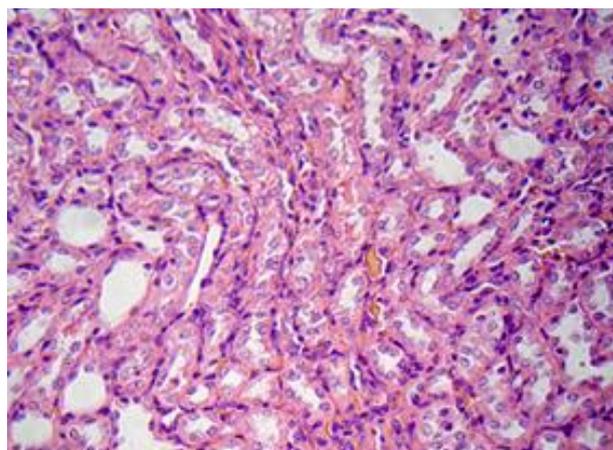


Fig. 6. The kidney sample (6 – the medullary substance) of a rat with rhabdomyolytic AKI after glutathione injection. Stained with hematoxylin and eosin. Magnification: eyelens $\times 40$ , objective  $\times 10$ .

**Conclusion.** 1. Rhabdomyolytic AKI leads to significant dystrophic and necrotic changes both

in the cortical and medullary substances of the kidneys.

2. The use of glutathione reduces incidence of dystrophic and necrotic processes in the kidneys, confirming the nephroprotective effectiveness of glutathione.

**Prospects for further research.** The use of glutathione at rhabdomyolytic kidney damage makes it possible to reduce the damaging effect of myoglobin on renal tubules due to its powerful antioxidant effect, which, in turn, is the prospect for the use of this tool for correction of AKI.

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