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CHANGES IN FIBRINO- AND PROTEOLYTIC ACTIVITY IN RATS KIDNEYS UNDER THE INFLUENCE OF EXOGENOUS GLUTATHIONE ON THE BACKGROUND OF RHABDOMYOLYSIS-INDUCED ACUTE KIDNEY INJURY

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Abstract. *The research aimed to study the effect of exogenous glutathione on the dynamic of changes in proteolytic and fibrinolytic activity in the kidneys of rats with rhabdomyolytic acute kidney injury. Disorders of the functioning of the activators and inhibitors of the proteolytic system are considered the nonspecific pathogenetic mechanisms of kidney damage. In animals with AKI, a decrease in enzymatic and total fibrinolytic activity was found, indicating the inhibition of the fibrinolytic system. At the same time, suppression of the lysis of low-molecular and high-molecular proteins and collagen demonstrates a decrease in tissue proteolytic activity. The administration of glutathione resulted in the normalization of proteolytic and fibrinolytic activity in the kidneys of rats with rhabdomyolytic AKI.*

Key words: *glutathione, proteolysis, fibrinolysis, rhabdomyolytic acute kidney injury.*

Introduction

To date, the results of research confirm the idea of AKI as a large-scale, worldwide, medico-social problem, the frequency of which in the general population of the pathology reaches 0.25%, which competes with the incidence rate of acute myocardial infarction. The causes and, accordingly, the forms of AKI are divided into 3 main groups - pre-renal, renal and post-renal. The renal form of AKI is usually accompanied by acute tubular and cortical necrosis during hemolysis, myolysis, exposure to nephrotoxic products, and obstruction of nephron tubules. The frequency of its development is 2-5% among all hospitalized patients and 10-15% among patients in the intensive care unit [1, 2]. It has been proven that the risk of mortality depends on the form of AKI, and in pre-renal and post-renal forms, mortality reaches only 7.5%, whereas in the case of renal - 30-40% [3, 4].

One of the most frequent causes of the renal form of AKI is myorenal syndrome or pigmented myoglobinuric nephrosis caused by massive rhabdomyolysis [5-6]. It is known that the main toxic compound in rhabdomyolysis is myoglobin, which normally binds to plasma globulin. However, due to the intensity of the process, the plasma is unable to bind all the hemoglobin, as a result of which it is filtered through the glomerular filter and enters the kidney tubules, where it causes their obstruction and impaired kidney function [7, 8]. At the same time, the non-protein component of myoglobin, heme, is able to increase free radical processes both in the vascular bed and in tissues, which leads to active ROS stimulation of the immune response, which in turn causes activation of the endothelium, promotes the accumulation of leukocytes and the formation of microvascular clots; and, accordingly, together with this – a change in the activity of the nitric oxide system, which leads to a violation of microcirculation with the development of mitochondrial dysfunction [9, 10].

As a result, myoglobinuric damage is accompanied by the development of oxidative stress with depletion of endogenous reduced glutathione reserves [11]. That is why, in this pathology, the potential pharmacotherapeutic points of treatment are the reduction of ROS formation and the degree of oxidative stress, and the drug of choice in this experimental study was exogenous glutathione - TAD 600 (Biomedica Foscama, Italy) [12, 13]

The system of endogenous glutathione, which is key in protecting the cell from oxidative stress [14] includes glutathione itself, glutathione peroxidase, glutathione reductase, and glutathione transferase, which form the glutathione antioxidant system. The antioxidant function of glutathione is largely carried out by HP, which reduces the formation of hydrogen peroxide and lipid peroxides, when reduced glutathione turns into an oxidized form, with the formation of glutathione conjugates. [15]. Accumulated clinical and experimental material allows us to single out the glutathione system as an important component of antioxidant protection and a factor influencing the formation of protective adaptive reactions of the body, which are activated during ischemia [16].

Summarizing the above information, we conclude that due to pleiotropic effects, exogenous glutathione can be considered as something that can replenish the pool of endogenous glutathione, and acts as a universal hepatoprotector with antioxidant and detoxifying properties.

Meanwhile, it is well known that in the pathogenesis of r radbomyolisis-induced AKI, an important role is played by hypercoagulable shifts and suppression of the tissue fibrinolytic system of the kidney system, a decrease in urokinase activity, which can naturally lead to the deposition of fibrin in tubules and vessels [17]. On the other hand, with AKI, inhibition of the proteolytic activity of renal tissues leads to an imbalance of proteolysis and collagenogenesis, due to a decrease in the activity of proteolytic enzymes, which is accompanied by a decrease in the breakdown of protein molecules and causes an increase in collagen synthesis and the formation of diffuse fibrosis in the kidneys. Along with this, due to an increase in the concentration of TxA2 in the glomeruli, natural renal vasoconstriction occurs, which also causes the constriction of the afferent glomerular arteriole and a decrease in glomerular filtration, and leads to intraglomerular thrombosis and subsequently leads to acute bilateral cortical necrosis [18].

Therefore, **the aim of the work** was to study the influence of glutathione on the system of proteolysis and fibrinolysis in kidney tissue under the conditions of the development of rhabdomyolytic acute kidney damage.

Materials and methods.

The experiments were conducted on 21 nonlinear mature white rats weighing 130-180 g, kept in the vivarium conditions at constant temperature and humidity, free access to water and food (full value fodder for the laboratory animals). Animals were randomly distributed into three groups (n=7): group I – control, group II – animals with rhabdomyolytic AKI, which were intramuscularly injected with a 50% glycerol solution at a dose of 8 mg/kg and decapitated for 24 hours of the experiment under light ether anesthesia [19], group III – administration of Glutatione (TAD 600, Biomedica Foscama, Italy) at a dose of 30 mg/kg. The drug was administered within 6 days after simulation of AKI. Dose of Glutatione was determined in accordance with the literature and the results of own experiments [20]. All studies were carried out following the criteria outlined in the European Union Directive 2010/63/EU "On the protection of animals used for scientific purposes" (2010).

The study materials were kidney homogenates. The state of proteolytic activity was determined based on the reaction with azo compounds (azoalbumin, azocasein, and azocollagen ("Biomark", Lviv). The principle of the method is based on the lysis of albumin, collagen, and casein associated with an azo dye, which gives a bright red color in an alkaline environment. Determination of optical densities were performed at a wavelength of 440 nm, proteolytic activity was expressed in E440/(ml/h) [21].

The principle of the method of tissue fibrinolytic activity is that when azofibrin is incubated with a standard amount of plasminogen in the presence of fibrinolysis activators, which are contained in urine, blood plasma or in tissues, plasmin is formed. The activity of the latter is estimated by the degree of coloration of the solution in an alkaline environment due to the lysis of azofibrin in the presence of ε aminocaproic acid (non-enzymatic fibrinolysis) or without it (total enzymatic activity). Enzymatic fibrinolysis is determined by the difference between total and non-enzymatic tissue activity. Indicators of fibrinolytic activity were expressed in E440/(ml/h) [22].

Results and discussion.

When studying the state of fibrinolysis in rat kidney tissue against the background of the development of radbomyolisis-induced AKI, inhibition of fibrinolytic activity was revealed in animals with model pathology. Namely, a decrease in enzymatic fibrinolytic activity by 5.7 times, which led to a decrease in total fibrinolytic activity by 33.8%, and indicators of non-enzymatic fibrinolytic activity decreased by 37% compared to the indicators of untreated animals (Tabl. 1), these changes probably caused intraglomerular thrombosis and decreased glomerular filtration.

The use of glutathione against the background of radbomyolisis-induced AKI led to an increase in the activity of fibrinolysis almost to the level of untreated animals. Under the action of the drug, the recovery of total fibrinolytic activity was recorded: by 40.1%, non-enzymatic fibrinolytic activity by 21.6%, with an increase in the enzymatic component by 5.3 times.

The activity of the proteolytic system also underwent significant changes. In the group of animals with model pathology, the intensity of lysis of low-molecular-weight

proteins exceeded the indicators of animals of the control group by 2.2 times, with a pronounced inhibition of lysis of low-molecular-weight proteins (Tabl. 2). Collagenolytic activity decreased by 59.1%, probably as a result of damage to the proximal parts of the tubules of the nephron.

Table 1 - State of fibrinolysis in kidney tissue of rats during administration Glutathione under rhabdomyolysis-induced AKI (M±m, n=7)

A group of animals	Total fibrinolytic activity, E440/(h×mg)	Non-enzymatic fibrinolytic activity, E440/(h×mg)	Enzymatic fibrinolytic activity, E440/(h×mg)
Intact control	17,27±1,16	11,55±0,80	7,84±0,98
Rhabdomyolysis-induced AKI	12,91±0,59#	8,43±0,32#	1,36±0,99#
Rhabdomyolysis-induced AKI + Glutathione	18,09±0,94*	10,25±0,72*	7,20±0,93*

*#p<0.05 versus control; *p<0.05 versus rhabdomyolysis-induced AKI*

The use of glutathione led to the normalization of the state of proteolysis in the kidney tissue, probably due to its antioxidant, cytoprotective and detoxification potential, contributing to the restoration of the functions of the cell membranes of the renal tubules and increasing the resistance of nephrocytes to damage. Proteolytic destruction of low-molecular-weight proteins decreased by 83.1% with a slight increase in the lysis of high-molecular-weight proteins. Collagenolytic activity under the influence of the drug increased by 45.9%, compared to the group of animals with model pathology (Tabl. 2).

Table 2 - The state of proteolysis in the tissue of the kidneys of rats with the introduction of Glutathione under the conditions of the development of Rhabdomyolysis-induced AKI in rats (M±m, n=7)

A group of animals	Lysis low-molecular proteins E440/(h×mg)	Lysis of high molecular weight proteins E440/(h×mg)	Lysis collagen E440/(h×mg)
Intact control	45,52±0,59	16,44±0,66	1,56±0,99
Rhabdomyolysis-induced AKI	20,73±0,82#	14,76±0,17	0,98±0,06#
Rhabdomyolysis-induced AKI + Glutathione	37,97±1,58*	15,69±0,49	1,43±0,09*

*#p<0.05 versus control; *p<0.05 versus rhabdomyolysis-induced AKI*

These effects of the drug are probably related to the antioxidant and pharmacokinetic properties of the latter. The main site of glutathione breakdown is the kidneys, where oxidized glutathione, formed from reduced glutathione, breaks down after performing its biological functions. In the cell, oxidized glutathione, freely diffusing through the cell membrane, is transported by the blood system to the kidneys, where it undergoes enzymatic hydrolysis in the proximal part of the renal tubules with the help of enzymes on the outer surface of the brush border membranes: cysteinylglycine dipeptidases, gamma-glutamyltransferases, and cystine reductases, where the interorgan exchange is completed of glutathione [13]. The use of exogenous glutathione, in our opinion, contributed to the restoration of the pro-oxidant-antioxidant balance due to cytoprotective and membrane-stabilizing effects.

Conclusion.

The results of the experimental study testify to the ability of glutathione to improve the functional capacity of nephrocytes against the background of the development of radbomyolisis-induced AKI, as evidenced by the strengthening of the proteolytic activity of kidney tissue and the restoration of fibrinolytic activity indicators.

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