EFFECT OF ADEMETHIONINE ON THE PROTEOLITIC AND FIBRINOLYTIC ACTIVITY IN KIDNEYS OF RATS WITH RABDOMYOLITIC ACUTE KIDNEY INJURY

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Abstract. In an experimental study on a model of rhabdomyolysis-induced acute kidney injury, the dynamics of the main indicators of the proteolytic and fibrinolytic system were found. In the animals of the group of model pathology, inhibition of the fibrinolysis system was found, which was realized by a decrease in enzymatic fibrinolytic activity, and as a result, total fibrinolytic activity. In terms of the proteolytic activity of the kidney tissue, inhibition of the lysis of collagen, low- and high-molecular proteins was found. The use of ademethionine led to the restoration of proteolytic and fibrinolytic activity in the kidneys under the conditions of the development of rhabdomyolytic acute kidney injury.

Key words: proteolysis, fibrinolysis, rhabdomyolysis-induced acute kidney injury, ademethionine.

Introduction

In recent years, the concept of AKI has undergone significant changes and finally fixed the main mechanisms of development and symptom complexes of this pathology depending on the etiology, although it has been proven that the pathogenesis of AKI is complex and only to a certain extent depends on the cause of its occurrence. Taking into account the significant prevalence of AKI, which is on average 5% of all hospitalized patients, about 20% of patients in intensive care units [1], with the majority of surgical and obstetric hospital patients, mortality from AKI can reach up to 80% in certain groups of patients (children and elderly age, multiple organ failure), especially where the loss of renal function is combined with the development of multiorgan failure [2]. Therefore, for the pharmaceutical and medical community, the issue of finding new and improving existing methods of pharmacotherapy, including etiological, pathogenetic and symptomatic ways of correcting AKI, does not lose its relevance.

Rhabdomyolysis is a frequent cause of AKI, and accounts for about 10-30%, while the mortality rate of patients can reach 8%. The occurrence of rhabdomyolysis can be preceded by factors of both traumatic and non-traumatic genesis, caused by the toxic effect of alcohol, drugs, metabolic or electrolyte disorders, infections, etc. [3]. The main pathogenetic mechanism of the development of rhabdomyolysis is the formation of extensive muscle myolysis, the release of a large amount of heme-containing myoglobin, which leads to myoglobinuria, renal vasoconstriction and vascular dysfunction with the development of obstruction of renal tubules by myoglobin cylinders and acute tubular necrosis. In addition, during muscle damage, a large amount of thromboplastin is released, which leads to the activation of intravascular blood coagulation, and, as a result, the formation of microthrombi in the kidney parenchyma with the development of ischemia, as a result of the activation of the coagulation system with the simultaneous inhibition of the fibrinolytic system, which is often accompanied by the development of fibrosis [5, 6]. Therefore, the system of regulation of the aggregate state is, on the one hand, an important factor in the development of AKI, and on the other hand, a component that can be controlled and influenced to improve the course of AKI.

Taking into account the pathogenesis of rhabdomyolysis-induced AKI, the development of which is accompanied by microcirculation dysfunction, changes in the structure and functions of tubular epithelial cells, the development of hypoxia, oxidative stress, due to the generation of a large number of free oxygen radicals, the initiation of a cascade of inflammatory reactions, cytokines, chemokines, activation of leukocytes, adhesion molecules, with a predominant damage to tubular epithelial and vascular endothelial cells, which subsequently leads to necrosis and apoptosis of nephrocytes, an important stage of research in rhabdomyolysis-induced AKI was the evaluation of the proteolysis and fibrinolysis system, since its dysfunction is one of the key factors in the activation of inflammatory reactions and fibrinogenesis, due to the breakdown of regulatory proteins [6, 7].

Ademethionine is an active sulfur-containing metabolite of methionine, a natural antioxidant, the activity of which is ensured by the presence of an active sulfur atom and CH3- group in its chemical structure. Participates in three main reactions: transmethylation, transsulfuration and aminopropylation, is a precursor of glutathione and taurine. According to the pharmacological classification, it belongs to the group of hepatoprotectors [8, 9]

The purpose of the experimental work was to study the changes in proteolysis/fibrinolysis of kidney tissues after administration of ademetionine under conditions of development of rhabdomyolysis-induced AKI.

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 rhabdomyolysis-induced AKI, which were intramuscularly injected with a 50% solution of glycerol at a dose of 8 mg/kg and decapitated for 24 hours of the experiment under light ether anesthesia [10], group III – administration of

Ademethionine (Heptral, "Abbott spa", Italy, Italy) at a dose of 20 mg/kg. The drug was administered within 6 days after simulation of rhabdomyolysis-induced AKI. Dose of Ademethionine was determined in accordance with the literature and the results of own experiments [11]. 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) [12]

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) [13].

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) [14].

Results and discussion.

With rhabdomyolysis-induced AKI, a significant decrease in enzymatic fibrinolytic activity by 5.7 times was recorded in kidney tissue, which led to a decrease in total fibrinolytic activity by 1.3 times compared to the indicators of intact controls. The same difference was observed when assessing non-enzymatic fibrinolytic activity (Table 1). Probably, inhibition of the fibrinolysis system was a consequence of damage to the proximal tubules of the nephron by myoglobin, which led to the development of urothrombosis and a decrease in the filtration capacity of the kidneys. Changes in the non-enzymatic link were also observed: in animals with model pathology.

Table 1 - State of fibrinolysis in kidney tissue of rats during administration Ademethionine 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 + Ademethionine	20,51±0,52[**]	11,95±0,77[**]	10,97±0,84[**]

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

When using ademetionine against the background of Rhabdomyolysis-induced AKI, an increase in the activity of fibrinolysis was noted, where the drug contributed to the recovery of the activity of the total fibrinolytic activity: by 58.8%, probably due to the non-enzymatic fibrinolytic activity by 41.7%, with a significant increase in the enzymatic component.

From the side of proteolytic activity of the kidney tissue, collagen lysis was inhibited by 1.6 times, which is a factor in the chronicity of the pathological process in the kidneys. A significant suppression of the lysis of low-molecular-weight proteins by 2.2 times was also found (see Table 2).

The use of ademethionine normalized the state of proteolysis in kidney tissue. Administration of the drug was characterized by a 2.3-fold increase in the lysis of low-molecular-weight proteins and 2.3-fold increase in collagen lysis, compared to the group of animals with model pathology. Also, in the group of treated animals, there was a tendency to increase the proteolytic activity of the kidney tissue in relation to high molecular weight proteins by 35.2%, which became more reliable under the influence of the studied agent (see Table 2).

Table 2 - The state of proteolysis in the tissue of the kidneys of rats with the introduction of ademethionine 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 + Ademethionine	47,94±0,35**	19,95±0,91**	2,23±0,08**

$^{\#}p<0.05$ *versus control;* $^{*}p<0.05$ *versus rhabdomyolysis-induced AKI*

These effects of the drug are probably due to its physiological functions, the ability to promote the restoration of epitheliocytes by stimulating the synthesis of phosphatidylcholine of cell membranes throughout the nephron, due to the anti-inflammatory properties of the drug. In addition, as a result of the transsulfuration reaction, ademethionine acts as a precursor of taurine and glutathione and provides a redox mechanism of cellular detoxification, increases the energy potential of cells, reduces the content of methionine in the blood plasma, and normalizes the metabolic reactions of cells.

Conclusions. The obtained results indicate the ability of ademetionine to restore proteolytic and fibrinolytic activity in the kidneys under the conditions of the development of rhabdomyolytic acute renal failure, which reduces the risk of chronicity of the pathological process. probably due to the antioxidant and cytoprotective effects of ademethionine.

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