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EFFICACY OF ADEMETIONINE IN ACUTE KIDNEY INJURY OF DIFFERENT GENESIS

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Abstract

Acute kidney injury is a complex clinical syndrome with different etiologies, multifactorial pathophysiological mechanisms and rapid progressive development, usually resulting from trauma, sepsis, kidney transplantation, or influence of toxic substances and is associated with an increased risk of mortality, cardiovascular events, and progression to chronic kidney disease. AKI occurs in approximately 15-50% of patients admitted to hospital, which increases mortality of 3% to 30%. The study was conducted to assess the nephroprotective effect of ademetionine on different animal models of acute kidney injury. It was established that treatment with ademetionine in ischemia-reperfusion and gentamicin-induced acute kidney injury ameliorated excretory kidney function of rats, which was confirmed by an increase in GFR and diuresis with a simultaneous decrease in azotemia and proteinuria. That was accompanied by a restoration of ion-regulatory kidney function, confirmed by the reduction of fractional excretion of sodium. Antioxidant effect of ademetionine resulted in inhibition of the lipid peroxidation against the background activation of the antioxidant system. The established nephroprotective potential creates a background for further research into mechanisms and pharmacological effects of ademetionine in conditions of kidney injury of different etiology.

Keywords: *nephroprotective activity, ademetionine, ischemia-reperfusion kidney injury, gentamicin-induced nephropathy*

Introduction

Acute kidney injury (AKI) has high rates of morbidity and mortality and is often accompanied with the development of multi-organ failure, which means that existing methods of its early diagnosis, prevention and management are not sufficiently effective. AKI is a complex clinical syndrome related to numerous etiologies and pathophysiological mechanisms and characterized by a rapid decline in glomerular filtration rate and accumulation of metabolic waste products [1]. AKI is associated with an increased risk of mortality, cardiovascular events, and progression to chronic kidney disease [2]. AKI occurs in approximately 10–15% of patients admitted to hospital, while its incidence in intensive care units has been reported in more than 50% of patients [3]. This leads to an increase of 3% to 30% in mortality and additional costs. The mortality among patients who become dependent on dialysis during their hospital stay is drastically increased and lies between 14% and 41% [4].

Ischemia-reperfusion injury (I/R injury) is one of the most common causes of AKI and is associated with adverse clinical outcomes resulting from the reduction of renal blood flow, severe inflammatory and oxidative response to hypoxia followed by reperfusion [5, 6]. Hypoxia causes dysfunction and failure of the Na+-K+-ATPase pump with an increase in intracellular calcium ions, thus inducing apoptosis, oxidative stress, and epithelial cells injury or necrosis. Furthermore, reperfusion itself induces production of reactive oxygen species, progression of oxidative stress, metabolic imbalance and inflammation [7].

No less common form (approximately 14-26% in prospective cohort studies of AKI) is drug-induced nephrotoxicity [8]. The incidence of nephrotoxicity is 5-10% in adults [9] and between 20 and 33% in children treated with aminoglycosides [10]. Aminoglycosides are freely filtered through the glomerulus and then partially taken up by, concentrated in, and produce damage to proximal tubular cells. The renal injury induced by these drugs is related to their preferential accumulation in the renal cortex. After administration, up to 5 to 10% of the parenteral dose is retained in the renal cortex, reaching concentrations greatly exceeding the concurrent serum concentration. Once inside the cell, aminoglycosides accumulate within lysosomes, the Golgi apparatus, and endoplasmic reticulum, inhibiting protein synthesis and associated functions. Binding to phospholipids and inhibition of phospholipase activity results in lysosomal phospholipidosis. Lysosomal cathepsins released into the cytoplasm may cause cell necrosis. Cytoplasmic aminoglycoside affects the mitochondria, activating the intrinsic pathway of apoptosis, disruption of electron transport and ATP production, and formation of reactive oxygen species [10].

In the considered types of toxic and ischemic AKI, the pathogenetic trigger is damage to the antioxidant defence system with impaired stability of the prooxidant-antioxidant balance, energy metabolism, and activation of inflammatory reactions, further leading to apoptosis and necrosis of the nephrocytes [11]. To date, the main mechanisms for the generation of reactive oxygen species in this pathology are identified. It is also known, that partial kidney ischemia and hypoxia are often unavoidable in clinical situations like kidney transplantation, severe trauma and blood loss, rhabdomyolysis, heart failure, sepsis, and use of some drugs. Thus, amelioration of oxidative stress is an important therapeutic approach to prevent and manage acute kidney injury of different origin. The protection of renal tissue from damage requires the use of drugs with nephroprotective properties. To date, however, they are not allocated into a separate group of drugs, and information on the nephroprotective properties of some drugs is fragmentary and not systematized. Promising is the search for pleiotropic drugs acting on different pathogenetic mechanisms of kidney injury progression including the processes of free radical oxidation.

Ademetionine (S-adenosylmethionine, SAMe) is a natural antioxidant synthesized in the liver and present in all cells of the body, while highest levels are found in the liver and brain. It is an active intermediate metabolite of methionine involved as a cosubstrate in anabolic reactions of transmethylation, transsulfuration and aminopropylation. Ademetionine is a precursor of choline, cysteine, taurine, glutathione, coenzyme A,

etc. [12]. The activity of ademetionine is provided by the presence of an active sulfur atom and methyl group in its structure. The transmethylation reaction promotes the synthesis of phosphatidylcholine and maintains the phospholipid bilayer, fluidity and polarization of the cell membrane. In addition to the structural function, phospholipids participate in the processes of molecular transport, cell division and differentiation, and stimulate the activity of various enzyme systems [13]. Since various pathogenic factors cause damage primarily to cell membranes, thus leading to disturbances in the cellular metabolism, ademetionine may be considered a cytoprotective [14]. It has been established that use of ademetionine normalizes the permeability of cell membranes and increase the energy potential of the cell. Due to its membrane-protective properties, ademetionine can prevent the development of mitochondrial dysfunction and bioenergetic stress [15-17]. Glutathione, sulphates, and taurine are integral components of the body's detoxification system; they are synthesized in the transsulfuration reaction. A transfer of the aminopropyl groups from ademetionine to polyamines essential for protein synthesis and ribosomes structure (putrescine, spermidine and spermine) leads to stimulation of cell proliferation and promotes cell regeneration [18, 19]. SAMe is currently used as a hepatoprotector for the treatment of liver diseases, as an antidepressant drug, and in a complex therapy of osteoarthritis and fibromyalgia due to its anti-inflammatory activity.

The research aimed to study the nephroprotective potential of ademetionine on the animal models of gentamicin-induced nephrotoxicity and ischemia-reperfusion acute kidney injury in rats.

Methods

Two series of experiments were conducted. The experiments were performed on 42 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).

In the first series of experiment, animals were randomly distributed into three groups (n=7): I group – control (pseudo-operated animals), II group – modelling of ischemia-reperfusion (I/R) AKI, III group – administration of ademetionine (Heptral, Abbott SpA, Italy) at a dose of 20 mg/kg during 3 days before I/R modelling. Dose of ademetionine was determined in accordance with the literature and the results of own experiments [16]. I/R AKI was reproduced by 60-minute bilateral renal ischemia followed by 24-hour reperfusion [20].

In the second series of the experiment, the animals were randomized into groups (n=7): I group – control, II group – gentamicin-induced AKI, group III – administration of ademetionine at a dose of 20 mg/kg, 40 min after each injection of gentamicin. Gentamicin-induced AKI was caused by intraperitoneal injection of 4% gentamicin sulphate solution (Galychpharm JSC, Ukraine) at a dose of 80 mg/kg, once a day during 6 days [20]. Animals were withdrawn from the experiment 24 h after the last injection, while blood, urine and kidneys were sampled for biochemical and histopathological assessments. 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).

Kidney function was assessed by diuresis, plasma creatinine level, GFR, urine protein excretion, fractional excretion and reabsorption of sodium. Plasma and urine creatinine levels were determined using the Jaffe reaction; urine protein content – using the sulfosalicylic acid precipitation test; sodium and potassium levels – using an electronic flame photometry method. In kidney tissue levels of malondialdehyde (MDA) and protein oxidative modification products (OMP), catalase and glutathione peroxidase (GPx) activity [21], and activity of succinate dehydrogenase (SDH) was determined [22].

Statistical processing of the obtained data was performed using the SPSS Statistics 17.0 software. All data are represented as a mean ± standard error of the mean (M±m). Estimation of the differences between the samples was conducted using a parametric Student's t-test and a nonparametric Mann-Whitney U test. The minimum significance level was $p<0.05$.

Results

In the first series of experiments, ischemic-reperfusion AKI was experimentally reproduced. Its development combines damage to the vascular and tubular apparatus of the kidney due to limitations in blood supply and organ hypoxia with its subsequent restoration and re-oxygenation. The results of the experiment showed the absolute functional disorganization of the vascular-tubular apparatus of the kidney in animals from the II group (see Table 1). The disorder of the tubular-glomerular balance resulted in a decrease in GFR and development of oliguria. It was accompanied by an increase in the plasma creatinine level and retention azotemia, and damage to the tubular epithelial cells resulted in significant proteinuria. Besides, inhibition of tubular reabsorption led to the loss of sodium ions, as evidenced by an increase in the fractional excretion of sodium.

Activation of free radical oxidation after the post-ischemic reperfusion induced the development of oxidative stress. Damage to the membranes of nephrocytes and impairment of metabolic and reparation processes manifested in an increase in the MDA and OMP levels in the kidney tissue of the operated animals. The exhaustion of the antioxidant defence system in animals from the II group was indicated by the decrease in catalase and glutathione peroxidase activity compared with the group of pseudo-operated animals (see Table 2).

In the second series of the experiment, the toxic effect of gentamycin on kidney resulted in an impairment of excretory and reabsorption function of nephrons. The nephrotoxicity manifested by a decrease in GFR and, consequently, diuresis, significant azotemia and proteinuria compared with indices of animals from the control group (see Table 3). The loss of functional capacity of the proximal tubular epitheliocytes led to a disruption of electrolyte transport, evidenced by a significant increase in the fractional excretion of sodium with a corresponding reduction of the reabsorption index compared with control data.

Considering that the key mechanism in the pathogenesis of gentamicin tubular damage is the hyperproduction of reactive oxygen species leading to the intensification of lipid and protein peroxidation, the investigation of the prooxidant-antioxidant balance in kidney tissue was a next step of the study. It was established that the accumulation of gentamicin in the proximal tubular cells promoted the generation of ROS and intensification of lipid and protein peroxidation in the kidney tissue, along with a decrease in the activity of antioxidant enzymes GPx and catalase (see Table 4).

Another important factor in the pathogenesis of AKI is the mitochondrial dysfunction and disruption of the energy balance in nephrons. In both series of experiments, a significant decrease in the SDH activity in animals with toxic or ischemic AKI compared with indices in the control group was observed, indicating development of cellular energy deficiency in the kidney (see Table 2, 4).

Discussion

Ischemia-reperfusion is accompanied by predominant damage to tubular epithelial and vascular endothelial cells with the initiation of oxidative stress, which subsequently leads to necrosis and apoptosis of nephrocytes. Since the activation of free radical oxidation occurs immediately after post-ischemic reperfusion, the maximum efficacy of drugs with antioxidant properties is observed in their prophylactic use; therefore, ademetionine was administered before I/R AKI induction.

Treatment with ademetionine prevented severe damage to cells and dysfunction of nephrons. Preservation of excretory kidney function is indicated by a significant increase in urine output, GFR, and decrease in creatininemia (see Table 1). Apart from its cytoprotective properties, this effect may be due to the ability of ademetionine to stimulate the synthesis of neurotransmitters in transmethylation reactions. An increase in dopamine levels contributes to dilation of the afferent arteriole, an increase in the filtration pressure and rate of glomerular filtration. In our research, maintenance of the filtration process in nephrons promoted the reduction of proteinuria compared with the I/R group. The protective effect of ademetionine contributed to the prevention of the significant ions loss evidenced by an increase in the sodium reabsorption and a corresponding

decrease in the fractional excretion of sodium (See Table 1).

In the second series of the experiment, the accumulation of gentamicin in the proximal tubular epitheliocytes caused their destabilization, development of mitochondrial dysfunction leading to energy imbalance, activation of apoptosis, generation of ROS and induction of oxidative stress, as well as the release of lysosomal cathepsins and massive proteolysis. Co-treatment with ademetionine partially counteracted the nephrotoxic effect of gentamicin, as evidenced by the restoration of diuresis due to maintenance of GFR, prevention retention azotemia due to a decrease in plasma creatinine, and a pronounced decrease in proteinuria compared with untreated animals (See Table 3).

Ademetionine maintained the reabsorption capacity of tubular cells, and it resulted in an increase in the sodium reabsorption and normalization of the fractional excretion of sodium (See Table 3). The nephrotoxic effect of gentamicin is produced through blockage of phospholipids in cell membranes of the proximal tubules, inhibition of phospholipase activity, and subsequent cell apoptosis. Established nephroprotective effect of ademetionine in this model of AKI is mediated by involvement in the biosynthesis of phosphatidylcholine of cell membranes and membrane-protective action [23].

Ademetionine showed a significant antioxidant effect on both models of AKI. According to the results of the study, the antioxidant effect of ademetionine inhibited the intensity of lipid (decrease in MDA levels) and protein (reduction of OMP levels) peroxidation in the kidney tissue, along with an increase in the activity of antioxidant enzymes - catalase and GPx in the kidney. The antioxidant effect promotes restoration of the function of renal tubular cell membranes and increases the resistance of nephrocytes to damage by reactive oxygen species. Ademetionine participates in the synthesis and restores the pool of endogenous glutathione - antioxidant and cofactor of antioxidant enzymes.

Maintenance of the cellular energy balance is an important mechanism of the nephroprotective effect [24, 25]. Co-treatment with ademetionine contributed to an increase in the activity of SDH by 60% compared with I/R AKI and fully restored the enzyme activity in gentamicin-induced AKI. Under the conditions of renal damage, ademetionine promotes the compensatory activation of the aerobic glycolysis and activates the energy-synthesizing function of nephrocytes.

Conclusion. The results of the experimental studies show the nephroprotective activity of ademetionine in conditions of AKI of different etiology. Ademetionine produces pleiotropic effects due to the influence on the main pathogenetic mechanisms of AKI. The nephroprotective effect manifests by the maintenance of the kidney function and restoration of the prooxidant-antioxidant and energy balance in kidneys of animals with gentamicin-induced and ischemia-reperfusion acute kidney injury. The obtained results substantiate the relevance of further research to broaden the spectrum of ademetionine use and optimize the pharmacotherapy of renal pathology.

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Table 1. Effect of ademetionine (20 mg/kg) on kidney function of rats with ischemia-reperfusion kidney injury

Index	Control	I/R AKI	I/R AKI + Ademetionine
Diuresis, ml/2 h	4.38±0.19	2.38±0.11#	3.66±0.13*
Plasma creatinine, μmol/L	63.21±6.05	165.37±9.23#	117.83±4.93*
Glomerular filtration rate, μl/min	532.7±47.3	173.1±9.9#	296.7±8.3*
Urine protein excretion, mg/100 μl	0.014±0.001	0.068±0.004#	0.040±0.002*
Sodium reabsorption, μmol/min	63.78±5.17	25.35±1.88#	40.38±1.60*
Fractional sodium excretion, %	0.38±0.05	2.46±0.13#	1.19±0.10*

#p<0.05 versus control; *p<0.05 versus ischemia-reperfusion AKI

Table 2. Effect of ademetionine (20 mg/kg) on prooxidant-antioxidant and energy balance in kidneys of rats with ischemia-reperfusion kidney injury

Index	Control	I/R AKI	I/R AKI + Ademetionine
Malondialdehyde, μmol/g	40.36±2.83	70.18±2.16#	58.83±0.96*
Oxidative protein modification products, units/g	8.43±0.43	12.56±0.76#	10.40±0.72*
Catalase, μmol/min×mg	7.13±0.35	5.29±0.23#	5.83±0.77
Glutathione peroxidase, nmol/min×mg	217.65±9.75	114.41±9.53#	144.64±11.90*
Succinate dehydrogenase, nmol/min×mg	14.13±0.12	4.45±0.91#	7.13±0.53*

#p<0.05 versus control; *p<0.05 versus ischemia-reperfusion AKI

Table 3. Effect of ademetionine (20 mg/kg) on kidney function of rats with gentamicin-induced AKI

Index	Control	Gentamicin-induced AKI	Gentamicin + Ademetionine
Diuresis, ml/2 h	4.63±0.19	2.47±0.10#	4.34±0.11*
Plasma creatinine, μmol/L	43.49±1.01	121.88±3.40#	64.06±3.95*
Glomerular filtration rate, μl/min	645.4±27.5	201.1±6.7#	524.0±51.4*
Urine protein excretion, mg/100 μl	0.079±0.01	0.188±0.02#	0.123±0.01*
Sodium reabsorption, μmol/min	114.46±6.71	39.05±2.12#	95.94±10.38*
Fractional sodium excretion, %	0.14±0.01	3.80±0.26#	0.45±0.03*

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

Table 4. Effect of ademetionine (20 mg/kg) on prooxidant-antioxidant balance in kidney tissue of rats with gentamicin-induced AKI

Index	Control	Gentamicin-induced AKI	Gentamicin + Ademetionine
Malondialdehyde, µmol/g	36.34±5.84	66.70±3.19#	45.09±3.37*
Oxidative protein modification products, units/g	11.01±0.25	17.04±0.52#	13.07±0.66*
Catalase, µmol/min×mg	11.97±0.11	8.92±0.42#	10.27±0.37*
Glutathione peroxidase, nmol/min×mg	161.31±8.15	101.59±7.27#	148.58±8.82*
Succinate dehydrogenase, nmol/min×mg	12.30±0.18	2.71±0.07#	9.62±0.11*

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