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AMELIORATIVE EFFECT OF EXOGENOUS GLUTATHIONE IN RENAL ISCHEMIA/REPERFUSION INJURY

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Abstract

Acute kidney injury (AKI) is associated with high morbidity and mortality rate worldwide. Ischemia/reperfusion (I/R) is the cause of AKI in 20-30% of cases and is commonly accompanied by acute tubular necrosis with the need for hemodialysis. Renal I/R is associated with progressive glomerular and tubular damage, induction of inflammation, oxidative stress, apoptosis and a rapid decline in kidney function. According to the pharmacological classification, reduced glutathione belongs to the class of hepatoprotectors with antioxidant and detoxifying properties. The objective of the current study was to evaluate the renal effects of exogenous glutathione on the animal model of I/R AKI. Pre-treatment with glutathione reduced the severity of damage and prevented kidney dysfunction associated with I/R. The obtained results demonstrate the nephroprotective effect of glutathione due to the restoration of the pro-oxidant/antioxidant balance and energy supply in the kidney and show its therapeutic potential for the prevention of renal I/R injury.

Keywords: *nephroprotective activity, glutathione, ischemia-reperfusion kidney injury, antioxidant*

Introduction

Renal ischemia-reperfusion (I/R) injury is a common complication in organ transplantation, trauma, sepsis, cardiac arrest, shock and has complex pathogenesis and a rapidly progressive course [1]. Acute kidney injury (AKI) due to renal I/R is a major clinical problem with high morbidity and mortality rate and no specific treatment available [2]. Despite the advancement in renal replacement therapy, the mortality rate in patients with AKI complicated by multiorgan dysfunction remains high worldwide and reaches 50% [3]. Pathology is accompanied by dysfunction of microcirculation, hypoxia, induction of inflammatory cascade and oxidative stress, further leading to necrosis and apoptosis of nephrocytes with a predominant injury of tubular epithelial cells. Generation of an excessive amount of reactive oxygen species (ROS) at the reperfusion phase is a major pathophysiological mechanism initiating a cascade of cellular responses leading to inflammation, oxidative stress and cell death [4, 5]. Cellular damage occurs mainly via peroxidation of membrane lipids, mitochondrial dysfunction with an inhibition of oxidative phosphorylation and ATP depletion as well as oxidative damage of proteins, fragmentation of DNA, and induction of apoptosis, resulting in vacuolation of the cytoplasm and nucleus, gradual destruction of the cell membrane and cell death [6, 7].

Currently, the therapeutic management of patients with I/R AKI includes the elimination of the etiological and pathogenetic factors, avoiding potentially nephrotoxic drugs, maintenance of renal blood flow, and use of nephroprotective agents to prevent the occurrence of irreversible morphological and functional changes via antioxidant and anti-inflammatory effects.

Glutathione is a key intracellular antioxidant playing an essential role in the vital activity of cells and the organism as a whole [8]. Exogenous glutathione is currently an object of extensive study as a promising tool for normalizing the redox balance in various pathologies. According to the pharmacological classification, reduced glutathione (TAD 600, Biomedika Foskama, Italy) belongs to the class of hepatoprotectors with antioxidant and detoxifying properties. Clinical uses of glutathione include prevention of nephrotoxicity and hepatotoxicity of cisplatin and similar compounds, detoxification therapy in the treatment of poisonings, and complex therapy of liver diseases [9].

The current research aimed to study the nephroprotective potential of glutathione (TAD 600) on the animal model of I/R AKI.

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 (pseudo-operated animals), group II – modelling of I/R AKI (60-minute bilateral renal ischemia followed by 24-hour reperfusion) [10], group III – administration of glutathione (TAD 600, Biomedica Foscama, Italy) at a dose of 30 mg/kg during 3 days prior to I/R AKI modelling. Dose of glutathione 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).

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, and activity of succinate dehydrogenase (SDH) was determined [12]. The activity of gamma-glutamyltranspeptidase (GGTP) in urine was determined using a test kit of reagents PJSC «Reagent» (Ukraine).

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 non-parametric Mann-Whitney U test. Spearman's (r) test was used to measure the correlations. The minimum significance level was $p<0.05$.

Results

In animals from group II, the I/R injury and renal hypofiltration resulted in a decrease in urine output and GFR, and progression to the oliguric stage of AKI (Table 1). A significant decrease in glomerular filtration led to an increase in plasma creatinine level compared with control and the development of retention azotemia. In addition, the glomerular and tubular injury resulted in significant proteinuria, as evidenced by a significant increase in protein excretion compared with control. I/R injury of the tubular epithelial cells along the entire length of the nephron resulted in the decrease in the tubular reabsorption in both proximal and distal parts and manifested by an increase in fractional excretion of sodium, and an increase in potassium loss and development of hypokalemia in animals with I/R AKI.

Correlation analysis (Table 2) revealed a failure of the kidney autoregulation mechanisms in animals with I/R AKI in form of a breakdown of the tubulotubular and glomerulotubular balance manifested by a decrease in the strength of the correlation between GFR and proximal transport of sodium (TpNa), distal transport of sodium (TdNa) and TpNa.

The protective effect of glutathione resulted in the restoration of the excretory kidney function with an increase in GFR, urinary output and, consequently, a reduction of plasma creatinine levels and proteinuria in animals from group III compared with untreated animals with I/R AKI. Glutathione prevented the significant damage to tubular cells as evidenced by prevention of sodium and potassium loss with an increase in tubular sodium reabsorption in both proximal and distal parts of the nephron and normalization of plasma potassium level in animals from group III. The preservation of the active reabsorption capacity of tubular epithelial cells resulted in the restoration of glomerulotubular balance confirmed by the direct relationship between GFR and TpNa, and TdNa, and tubulotubular balance with a compensatory activation of the reabsorption in the distal nephron confirmed by the inverse relationship between TpNa and TdNa.

In animals from the I/R AKI group, a significant increase in gamma-glutamyl transpeptidase (GGT) activity in urine – one of the early markers of proximal epithelial cells cytolysis was found (Figure 1). Cytoprotective effect of glutathione is confirmed by a decrease in GGT activity in urine compared with untreated animals.

Discussion

I/R injury is usually initiated by an increased generation of ROS by endothelial cells and their further interaction with lipids, proteins and carbohydrates resulting in disturbances in the processes of methylation and oxidative deamination and leading to the formation of various toxic substances: peroxides, hydroperoxides, ketones, aldehydes, etc., and a significant decrease in antioxidant protection of cells. Under the conditions of hypoxia, an overproduction of nitric oxide is an extra source of free radicals, leading to endothelium dysfunction and impaired microcirculation, and, as a consequence, to endothelial edema. A decrease in the intensity of microcirculation and an increase in arteriovenous output is the basic compensatory mechanism triggered in response to reperfusion injury. In turn, the passage of fluid into the interstitial space provokes blood thickening, slowing down blood flow and the formation of blood clots [2, 6, 13]. A significant role in the reperfusion injury belongs to leukocytes, which accumulate in the ischemic zone and release free radicals, proteolytic enzymes, followed by further damage to the endothelium with the progression of endothelial dysfunction [14].

It is known that one of the main causes of the induction of oxidative stress in I/R is the process of ATP degradation, resulting in the formation of hypoxanthine, which accumulation contributes to the enhanced generation of ROS. Subsequently, hypoxanthine is metabolized into xanthine with the formation of hydrogen peroxide characterized by a high reactivity and interacting with iron ions providing the formation of superoxide ion, followed

by the formation of peroxynitrite, which contributes to cellular damage through the nitrosylation of cellular proteins and peroxidation of lipids. On the other hand, the development of ischemia stimulates the formation of nitric oxide in epithelial cells of the renal tubules, which interacts with hydrogen peroxide resulting in the formation of cytotoxic hydroxyl radical causing damage to cellular DNA and induction of apoptosis [15].

Table 3 shows the state of the pro-oxidant/antioxidant balance in kidneys of rats with I/R AKI. Activation of lipid and protein oxidation processes caused an increase in malondialdehyde (MDA) and oxidative protein modification products (OMP) levels in kidney tissue of animals from group II compared with control. It was accompanied by a significant decrease in catalase and glutathione peroxidase (GPx) activity indicating the development of oxidative stress.

Since the activation of free radical oxidation occurs immediately after postischemia reperfusion, the maximum efficiency of the drugs with antioxidant properties is seen in their prophylactic and early use; therefore, the glutathione was administered in a prophylactic therapeutic regimen. In our research, glutathione partially alleviated the manifestations of the I/R injury due to its ability to neutralize the cytotoxic products of peroxidation and their active metabolites. Pre-treatment with glutathione normalized pro-oxidant/antioxidant balance in kidney tissue of rats with I/R AKI as evidenced by inhibition of lipid peroxidation and decrease in MDA level, as well as a reduction of the OMP level in kidney tissue of animals from group III compared with group II. Glutathione maintained the activity of the antioxidant enzyme catalase on the level of control and prevented the decrease in GPx activity (see Table 3). Apparently, administration of the exogenous glutathione leads to replenishment of the pool of endogenous glutathione. The running of the glutathione system maintains several important physiological processes and functions: neutralization of toxic substances provided by the ability of glutathione to block and remove free radicals and activate biotransformation enzymes – glutathione peroxidase and glutathione transferase; regulation of immune function via potentiation of the cytokines IL-1, IL-2, IL-6, and interferons synthesis; detoxification of xenobiotics or their metabolites due to the ability to form conjugates with these substances and their removal; regulation of the redox status of thiol proteins involved in the process of apoptosis; and serving as a reserve of cysteine in the cell. The pronounced nephroprotective effect in conditions of I/R is apparently associated with its antioxidant and pharmacokinetic properties. The main site of the breakdown of glutathione is the kidney, where the oxidized form of glutathione is metabolised after completing its biological functions. In the proximal tubular cells, enzymes of the outer surface of the brush border membrane cysteinyl glycine peptidase and GGT carry out glutathione hydrolysis [9, 16-17].

Energy deficiency due to a mitochondrial dysfunction and decrease in ATP synthesis is an equally important triggering mechanism for the development of pathobiochemical reactions arising from damage to nephrocytes. It causes dysfunction of the ion transport channels and the progression of mitochondrial dysfunction itself. Energy deficiency leads to the accumulation of calcium ions with the following activation of proteinases, endonuclease and phospholipase A2, destabilization of cell membranes and disintegration of the cytoskeleton [4, 18]. Succinate dehydrogenase (SDH) provides a compensatory function of energy supply for the restoration of normal vital activity of nephrocytes; therefore, the study of its activity is an important criterion for assessing the nephroprotective action. In the group of animals with I/R AKI, a significant decrease in the activity of the SDH enzyme was found (Figure 2). The use of glutathione led to an increase in the activity of SDH, which indicates the restoration of the energy-synthesizing function of nephrocytes as one of the important mechanisms of nephroprotection in I/R injury.

Conclusion. In conditions of the renal I/R injury glutathione shows a nephroprotective effect, contributing to the restoration of the renal tubular function and an increase in the resistance of nephrocytes to damage. The ameliorative effect of glutathione manifests by the normalization of the excretory kidney function and is mediated by the cytoprotective and antioxidant effects with the restoration of the energy-synthesizing function of the nephrocytes. Results of research complement

existing data on the nephroprotective activity of glutathione and substantiate its therapeutic potential in renal pathology.

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Table 1. Effect of glutathione (30 mg/kg) on kidney function of rats with I/R AKI

Index	Control	I/R AKI	I/R AKI + Glutathione
Diuresis, ml/2 h	4.38 ± 0.19	2.38 ± 0.11$^{\#}$	4.31 ± 0.14*
Plasma creatinine, μmol/L	63.21 ± 6.05	165.37 ± 9.23$^{\#}$	78.39 ± 2.37*
Glomerular filtration rate, μl/min	532.71 ± 47.29	173.08 ± 9.90$^{\#}$	463.69 ± 24.19*
Urine protein excretion, mg/100 μl	0.014 ± 0.001	0.068 ± 0.004$^{\#}$	0.021 ± 0.002*
Fractional sodium excretion, %	0.38 ± 0.05	2.46 ± 0.13$^{\#}$	0.57 ± 0.05*
Sodium reabsorption, μmol/min	63.78 ± 5.17	25.35 ± 1.88$^{\#}$	55.73 ± 3.80*
Proximal transport of Na, mmol/2 h	7.13 ± 0.61	2.70 ± 0.20$^{\#}$	6.17 ± 0.43*
Distal transport of Na, μmol/2 h	527.56 ± 34.45	346.14 ± 29.29$^{\#}$	514.66 ± 25.58*
Plasma potassium, mmol/L	5,18 ± 0,49	4,46 ± 0,10$^{\#}$	5,25 ± 0,13*

$^{\#}$p<0.05 versus control; *p<0.05 versus I/R AKI

Table 2. Effect of glutathione (30 mg/kg) on kidney autoregulation mechanisms in I/R AKI

Group	GFR ↔ T_p Na^+	GFR ↔ T_d Na^+	T_p Na^+ ↔ T_d Na^+
Control	r = 0.94, p<0.05	r = 0.89, p<0.05	r = -0.89, p<0.05
I/R AKI	r = 0.89, p<0.05	r = 0.35, p>0.05	r = -0.51, p>0.05
I/R AKI + Glutathione	r = 0.85, p<0.05	r = 0.75, p<0.05	r = -0.89, p<0.05

T_pNa^+ – proximal transport of sodium, T_dNa^+ – distal transport of sodium, r – Spearman's correlation coefficient

Table 3. Pro-oxidant/antioxidant balance in kidney tissue of rats with I/R AKI

Index	Control	I/R AKI	I/R AKI + Glutathione
Malondialdehyde, μmol/g	40.36 ± 2.83	70.18 ± 2.16$^{\#}$	46.48 ± 2.44*
Oxidative protein modification products, units/g	8.43 ± 0.43	12.56 ± 0.76$^{\#}$	8.94 ± 0.42*
Catalase, μmol/min×mg protein	7.13 ± 0.35	5.29 ± 0.23$^{\#}$	7.42 ± 0.20*
Glutathione peroxidase, nmol/min×mg protein	217.65 ± 9.75	114.41 ± 9.53$^{\#}$	196.32 ± 4.37*

$^{\#}$p<0.05 versus control; *p<0.05 versus I/R AKI

Figure 1. Gamma-glutamyltranspeptidase activity in the urine of rats with I/R AKI

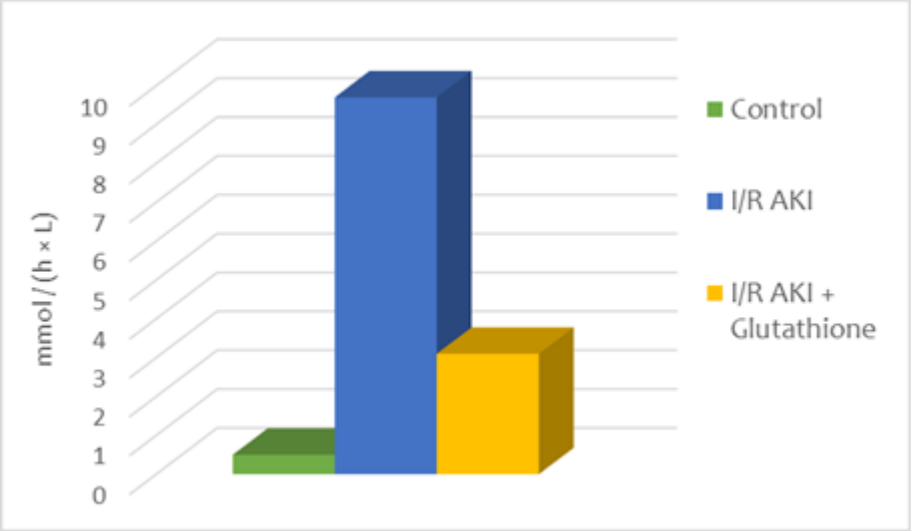

Figure 2. Succinate dehydrogenase activity in the kidney tissue of rats with I/R AKI