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GLUTHATIONE CONTENT IN BLOOD AND KIDNEY IN GLYCEROL INDUCED ACUTE RENAL FAILURE

SUMMARY

Acute renal failure (ARF) induced by intramuscular injection of hypertonic glycerol solution was followed by free radical production. In these experimental conditions antioxidative profile is changed especially in kidney. Glutathione (GSH) as a very important nonprotein sulfhydryl component has a significant role in tissue protection from oxidative stress. In this study Wistar male rats treated with glycerol (8 ml/kg BW, 50% solution) are sacrificed after 48,72 and 96 hours. The control group was injected by saline solution. GSH content was measured in blood and kidney tissue by Ellman reagent. Concentration of urea and creatinine, parameters of renal failure, were significantly increased in ARF group sacrificed after 48 and 72 hours, compared to the control ($p<0.05$). Ninety six hours after glycerol induced ARF, renal function was improved and these parameters reached normal values. The content of GSH in blood was not significantly changed in comparison to the control group, while in kidney it showed a significant decrease 48 hours after glycerol induced ARF ($p<0.001$). The obtained results point out the important role of GSH in kidney and suggest the possibility of preventing renal failure using GSH esters.

Key words: **glutathione, acute renal failure, oxidative stress**

INTRODUCTION

Renal illnesses are common medical problem in which pathogenetic mechanisms of cell damage are not completely resolved.

Acute renal failure (ARF) is serious pathologic condition with high mortality rate. Etiological factors of ARF are numerous (nephrotoxic drugs, contrast means in radiology, passing ischemia, ' immunotoxins) and they all cause homeostasis disorder of renal cells.

The basic molecular mechanisms of cell damage are:

- Reduced concentration of purine nucleotides
- Acidosis and swelling of the kidney cell
- Increased production of free radicals (1).

Free radicals are molecular forms with unpaired electron that makes them very reactive. They can react mutually or with non radicals and this process spreads as the serious of chain reactions. In reaction with other bimolecular in their surroundings (proteins, lipids, nucleic acid), free radicals may cause changes in their structure and functions, damage and death of the cell (2).

In physiological conditions the presence of free radicals doesn't express their toxic effect because of antioxidative systems in cell. This system aims at preventing the formation of free radicals, enabling their removal or stopping the chain reactions.

Glutathion, (GSH-y-glutamyl-cisteinyl-glicin) - is the most important nonprotein sulfhydryl component in all-living beings! Its concentration in cells is,

generally, at least one order of magnitude greater than that of other known thiols. The limiting component of production of GSH is cysteine, while kidney and liver are the participating organs in the elimination of GSH (3).

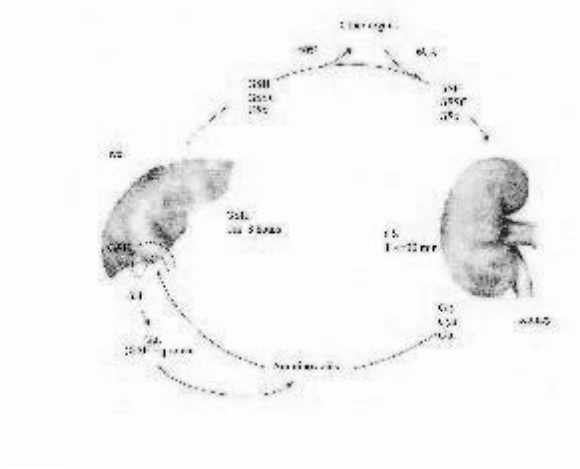

Figure 1. Inter-organ distribution of glutathione

The main role of GSH is cell protection from free radicals, in reaction with them or over glutathione dependent reduction of H_2O_2 and other hydro peroxidase, along with glutathione peroxidase or conjugation with xenobiotics with GSH S-transferase^ Glutathione participates in maintaining: the integrity of cell membrane, the synthesis of eicosanoids, nucleic acid, and the transportation of amino acid (4). Several features, including the rapid GSH turnover in the kidney, and the ability of the kidney to effectively clear the circulation of GSH, distinguish GSH metabolism in the kidney from other organs (5).

Kidney may use GSH from plasma demolishing it over reaction with y-glutamyl-transpeptidase or carrying GSH directly into the cell (6) (50-60% of total GSH from plasma extracts are in kidney). After the glomerular filtration GSH is demolished by y-glutamyl-transferases.

Non filtrate component can be demolished, oxidized or used again (7). Red blood cells also present important source of GSH, which participate in the process of detoxication of plasma and red blood cells by conjugation with other toxins. These conjugations are thrown out through gall, liver and kidney.

The aim of this study is to follow concentration of GSH in red blood cells and kidney tissue of rats in glycerol induced acute renal failure in different time intervals. Evaluation of kidney function is accompanied by measuring concentration of urea and creatinine, parameters of renal failure.

EXPERIMENTAL DESIGN AND METHODS

Male Wister rats (250-280 g), maintained under standard vivarium conditions were used for all experiments. Four groups of 6 animals each dehydrated 18 h before the induction of ARE was studied. Rats were injected with 50% (v/v in sterile water) glycerol (8ml/kg, i.m.) and sacrificed after 48, 72 and 96 hours (using sodium pentobarbital at 50mg/kg administered i.p.). The control group was injected by saline solution. Plasma was obtained for urea and creatinine assays, and red blood cells and kidney tissue were used for determining the content of GSH. Concentration of urea and creatinine in blood were measured on Synchron Cx-3 ana-lyzer-Beckman.

GSH content was measured by Ellman reagent (5,5'-dithiobis(2 nitro benzoic acid-DTNB) (8). Intracellular GSH content is given as |u.mol/gr hemoglobin. In 10% kidney homogenates the content of GSH was measured by the method of Sedlak and Lindsay (9) and expressed as jimol per mg protein. Proteins were measured by Lowry method (10).

All data are reported as mean±standard deviation. Statistical analyses employed Student's /-test.

RESULTS

Figure 2 presents concentration of urea and creatinine in blood of the examined groups.

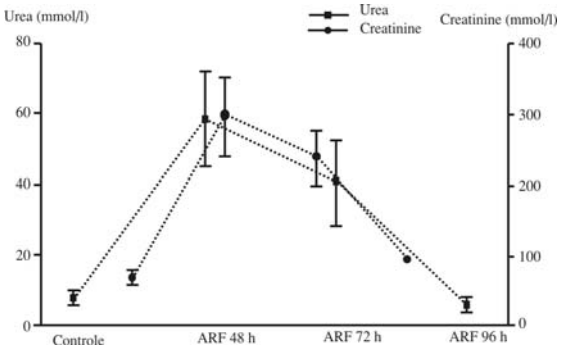

Figure 2. Values of urea and creatinine in experimental groups

The concentration of urea and creatinine were significantly increased in ARF group sacrificed 48 and 72 hours after compared to the control ($p<0.05$), and a group sacrificed 96 hours after ($p<0.05$). The increase of concentration of creatinine was statistically significant also in the group sacrificed 72

hours after, compared to control ($p<0.01$) and group were sacrificed after 96 hours ($p<0.05$).

Table 1 presents concentration of GSH in blood, which was not significantly changed in comparison to the control group.

Table 1. Concentration of glutathione in blood of experimental groups

Group	Glutathione (μamol/mg Hb)
Control	14.29 + 4.01
ARF - 48 hours	12.82 ±3.49
ARF - 72 hours	13.66 ± 1.27
ARF - 96 hours	13.16 ±0.89

Figure 3 represents concentration of GSH in kidney tissue. There was a significant decrease in kidney 48 hours after glycerol induced ARF in comparison to the control group ($p<0.001$). In other groups concentration of GSH was not significantly changed.

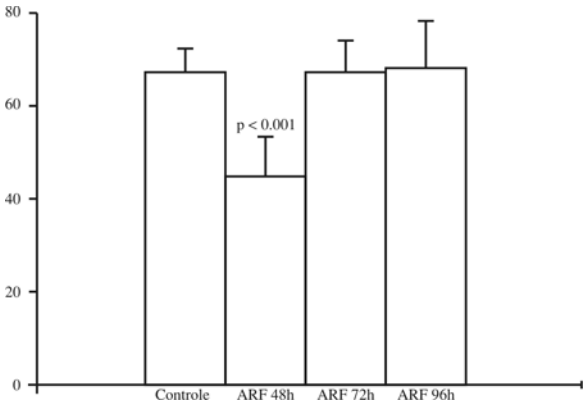

Figure 3. Concentration of glutathione in kidney tissue in experimental groups

DISCUSSION

Almost 10% of all ARF are the result of the rhabdomyolysis after crush syndrome or nontraumatic muscular necrosis. Hem proteinuria, which occurs during ARF, causes formation of deposits, early obstruction of tubules and expresses toxic effect of them. The pathogenesis of glycerol-induced myoglobinuric ARF involves, among other causes, ischemia, vascular congestion and reactive oxygen metabolites (11). In myoglobinuric acute renal failure, Fentone reaction and the increase of concentration of free iron, exept physiological source of free radicals, are very important mechanisms (12). Iron-induced oxidative stress has become a generally accepted mediator of tissue damage. Guidet and Shah (13) documented increased H_2O_2 generation in rat cortex following experimental myohemolobinuria, as assessed by the aminotriazole tehnique. During this experimental conditions concentration of malondialdehyde (MDA) was increased.

Concentration of urea and creatinine, parameters of renal failure, were significantly increased in ARF group sacrificed after 48 and 72 hours, in comparison to the control ($p<0.05$). Ninety six hours after glycerol induced ARF, renal function was improved and these parameters reached normal values.

Glutathione is an important intracellular agent, which protects the cell from the damage caused by free radicals. The intracellular concentration of GSH is regulated by a complex process comprising the transport of precursor amino acid across the cell membranes, intracellular synthesizing enzymes activities, feedback regulation by GSH itself, and intracellular utilization of GSH. Kidney has an important role in metabolism of GSH. Concentration of GSH in blood of rats with ARF wasn't significantly changed compared to the control group, which is in accordance with the data in literature (14,15). The intracellular ratio GSSG/GSH, as an indicator of oxidative stress increased significantly in liver and blood of uremic rats. In the model of glycerol induced ARF a decrease of serum total antioxidant status level within 24 h with spontaneous recuperation 72 h after was shown (16). In kidney tissue there was a statistically important decrease after 6 hours (5) and that fall was reflected 48 hours after the i.m. injection of glycerol. Our results show that 72 and 96 hours after glycerol induced ARF concentration of GSH returned to the control level.

Intravenous injection of GSH ester resulted in significant functional protection as measured by concentration of urea and creatinine levels (17). Much of the current evidence favors the concept that GSH is broken down to its constituent amino acids or dipeptides, which are then transported in cells where intracellular enzymes catalyze synthesis of GSH. Administration of GSH is associated with glutathionuria (18,19). Thus, the protective effect of GSH may be due to either increased intracellular GSH levels or the presence of GSH in tubular fluid, or both. Results of numerous studies confirm the importance of GSH esters in the preventions and in medical treatment of acute renal failure.

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SAŽETAK

Intramuskularnim davanjem hipertoničnog glicerola pacovima izazvana je akutna bubrežna insuficijencija (ABI) koja se karakteriše povećanom produkcijom slobodnih radikala. Pri ovakvim eksperimentalnim uslovima menja se i antioksidativni profil naročito u tkivu bubrega. Glutation (GSH) kao vrlo važna neproteinska sulfhidrilna komponenta ima značajan udeo u zaštiti tkiva od oksidativnog stresa. Pacovi Wistar soja, kojima je ABI izazvana glicerolom (8ml/kg TM, 50% rastvor), podeljeni su u tri ekspeirmentalne grupe i žrtvovani posle 48, 72 i 96 sati. Kontrolna grupa je primala fiziološki rastvor. Koncentracija GSH određivana je u krvi i tkivu bubrega Ellmanovim reagensom. Procena stanja bubrega vršena je određivanjem koncentracije ureje i kreatinina koji su statistički značajno bili povećani u grupi sa ABI žrtvovanoj posle 48 i 72 časa u odnosu na kontrolu ($p < 0.05$). Posle 96 sati bubrežna funkcija se popravlja vraćanjem vrednosti ovih parametara na kontrolni nivo. Koncentracija glutationa nije bila značajno promenjena u kriv u odnosu na kontrolnu grupu dok se u tkivu bubrega zapaža smanjenje 48 sati nakon izazivanja ABI ($p < 0.001$). Ovo ukazuje na značajnu ulogu GSH u zaštiti bubrega od toksičnog delovanja slobodnih radikala pri ABI.

Ključne reči: **glutation, akutna bubrežna insuficijencija, oksidativni stres**