April 30, 2020

PharmacologyOnLine

Archives • 2020 • vol.1 • 113-120

EFFECT OF GLUTATHIONE ON OXIDANT-ANTIOXIDANT SYSTEM AND THE CONTENT OF HYDROGEN SULPHIDE IN THE BLOOD BY EXPERIMENTAL NEPHROPATHY

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Abstract

The objective of the study was to examine the involvement of oxidative stress in the progression of nephropathy and to evaluate the influence of glutathione on oxidant-antioxidant state and content of H2S in the blood. The experiment was conducted on 131 male albino rats with the body weight of 0.16-0.18 kg. Experimental nephropathy was modeled by injection of a single intraperitoneal dose of folic acid (250 mg/kg). Glutathione was introduced daily (100 mg/kg) by the intragastric way for 3 and 7 days after the injection of folic acid.

The level of OMP370 by nephropathy was higher by 36% and OMP430 - by 14.6% on the 3d day of the experiment. Ceruloplasmin levels in animals with nephropathy incresed by 28 % and 43 % on 3 and 7 days. Glutathione decreases the content of protein by 35 % on 7 day. The level of SH-groups in the blood of rats with nephropathy were lower by 26% on the 3d day and by 9% on 7 days of the experiment. Catalase activity decreases by 25% on both days. We observed the increased activity of catalase following 3 days glutathione introduction by 15 % and decreased GPx activity in blood by 40% on 3 day and by 20% 7 day. The introduction of glutathione increases the activity of GPx to the control value during all experimental days. H2S levels were lower by 35.5% on the 3d day and by 25.7% on 7 days of the experiment. The glutathione increases the level of gasotransmitter by 14.3 % in blood plasma of rats on 3 day and by 11 % on 7 day of the experimental period.

We established the positive effects of glutathione on the oxidant-antioxidant state and content of H2S in the blood by nephropathy induced by high dose of folic acid. The close interrelation between the oxidant-antioxidant blood system and the development of nephropathy has been established.

Keywords: *experimental nephropathy, glutathione, antioxidant system.*

Introduction

Kidney diseases are a global public health problem [1], because the incidence of renal disease has increased steadily in recent years. The structural modifications in the kidney cause oxidative stress related to an imbalance between free radical's production and antioxidant capacity. Lipid and protein oxidation products are metabolized by non-enzymatic and enzymatic mechanisms to eliminate oxidative stress of the organism. Currently, the therapeutic options available for managing renal disease are not quite efficient and therefore, there lies a subsequent need for effective drug therapies that can stop progressive damage to the kidneys.

Recent papers show [2-4] that hydrogen sulfide (H_2S), a novel gaseous signaling molecule similar to nitric oxide and carbon monoxide, can exhibit renoprotective effects in the animal model of kidney injury via antihypertensive, antioxidative, antiapoptotic and anti-inflammatory mechanisms.

Glutathione (GSH) is the most abundant non-protein thiol and has many functions in vivo. The major role of GSH is the maintenance of cellular redox balance. The physiological role of GSH as an antioxidant has been described and substantiated in studies of numerous disorders reflecting the increased oxidation is a result of abnormal GSH metabolism. GSH is thought to be an important factor in cellular function and defense against oxidative stress, such as radiation and drug resistance. Recently, much attention focused on the role of oxidative stress in the various forms of kidney diseases. Many reports have demonstrated that GSH acts as an endogenous antioxidant [5-7]. However, there are no studies demonstrating an effect of GSH oxidant-antioxidant state and content of H_2S in the blood under the condition of nephropathy.

The aim of the study was to examine the involvement of oxidative stress in the progression of nephropathy and to evaluate the influence of glutathione on oxidant-antioxidant state and content of H_2S in the blood.

Methods

The experiment was conducted on 131 male albino rats with the body weight 0.16-0.18 kg. Experimental nephropathy was modeled by injection of a single intraperitoneal dose of folic acid (250 mg/kg, Sigma-Aldrich) [8]. The study was performed in the department of Bioorganic and Biological Chemistry and Clinical Biochemistry of Bukovinian State Medical University in 2018-2019 years. To confirm the pathology, the kidneys were examined by morphometric analysis. Glutathione (Sigma-Aldrich) was introduced daily (100 mg/kg) by the intragastric way for 3 and 7 days following after the injection of folic acid. Animals were narcotized and sacrificed on the next day after the last glutathione introduction. All manipulations with animals were carried out according to the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes and law of Ukraine "On protection of animals from cruelty". Rats were kept under the standard vivarium conditions at a constant temperature and basic allowance. All procedures were executed separately from other rats. Plasma concentration of H_2S was measured with spectrophotometry [9] using spectrophotometer Agilent Cary 60. In the blood plasma was determined content of ceruloplasmin [10], content of SH-group [11] and TBA-active products [12], oxidative modification of proteins (OMP) [13], in the hemolisate of erythrocytes was determined activities of superoxide dismutase (SOD) [14], glutathione peroxidase (GPx) [15, 16], and catalase [17]. The type of distribution was estimated using the Shapiro-Wilk test. Significant differences between group were evaluated by using the Wilcoxon test and Kolmogorov–Smirnov test with $P < 0.05$ considered [18]. All results in figures are represented as median minimum-maximum values (Me[min-max]).

Results

Using morphometric analysis we determined that the rapid appearance of folic acid crystals inside the renal tubules caused alteration of the epithelium of the proximal tubule of the kidney, inflammatory cell infiltration and caused necrosis and the cortical scarring. Damage of the epithelium of the proximal tubules with the subsequent proliferation of tubulointerstitial disintegration on the renal cortex, renal medulla, and renal papillae led to a decrease of filtering capacity of the kidneys. This changing on 3 experimental day was associated with a specific

volume of epithelial cells of proximal tubules of the kidney. In particular, the specific volume of epithelial cells of proximal tubules in the state of alteration is 84.8% and the oxidation modification of proteins increases (according to the R/B coefficient stained with bromophenol blue for Mikel Calvo) that suggest the development of nephropathy.

The degree of oxidative modification of proteins was evaluated in the blood by the level of aldehyde and ketone derivatives of neutral (OMP370) and basic (OMP430) composition. The level of OMP370 in rats with nephropathy was higher by 36% on the 3d day of the experiment compared to control rats.

The glutathione decreases the level of OMP370 by 24 % on 3 day of the experimental period compared with the group of animals without introduction of tripeptide. According to our study results, the indices of oxidative modification of proteins of the aldehyde and ketone derivatives of the neutral character were without significant changes on 7 day (see Table 1).

The level of OMP430 in the blood of rats with nephropathy was higher by 14.6% on the 3d day of the experiment than those in control rats. In animals with nephropathy on the seventh day activation of processes of oxidative modification of proteins confirmed by an increase (32.6%) of indices of the aldehyde and ketone derivatives of the basic character in the serum. The increase of oxidative modification of proteins is one of the pathogenetic links in the development of pathological conditions due to oxidative stress. The glutathione decreases the level of OMP430 to the control value on 3 day and by 15 % on 7 day of the experimental period compared with the group of animals without introduction of tripeptide.

Enhanced oxidative stress modifies lipids and lipoproteins causing essential alterations of their biological properties. In our experiment, TBA-active products in nephropathy-groups are increased by 21% on 3 day and 22,5% on 7 day, which indicates oxidative stress. The glutathione significantly decreases the level of TBA-active products to the control value on 7 day of the experimental period.

Ceruloplasmin, a copper-carrying metalloenzyme, acts as an antioxidant through its ferroxidase activity [19]. We conducted a study to evaluate whether the serum ceruloplasmin level is a predictive biomarker for the progression of folic acid-induced nephropathy. During the follow-up period, ceruloplasmin levels were higher in the rats with nephropathy than in the control group. Elevated serum ceruloplasmin levels in animals with nephropathy have been incresed by 28 % and 43 % on 3 and 7 experimental days accordingly. Thus, serum ceruloplasmin is a biomarker for first stage progression of nephropathy. The effect of glutathione on level of serum ceruloplasmin is shown as a significant decrease of the content of protein by 35 % in blood plasma of rats on 7 day of the experimental period compared with group of animals without introduction of glutathione.

Results our study demonstrated that SOD-activity was changed but significant changes were observed in the group of animals with nephropathy on the seventh day relative to the control group.

After the introduction of folic acid was observed the decrease of activities of catalase in the blood rats by 25% on both days compared to animals of the control group. However, we observed the increased activity of catalase following 3 days glutathione introduction by 15 % compared to the group with disease.

The level of SH-groups in the blood of rats with nephropathy were lower by 26% on the 3d day and by 9% on 7 days of the experiment than those in control rats. SH-groups have antioxidant properties, significant reactivity in redox reactions, and their amount is reduced by oxidative stress resulting from the violation of the balance between the production of free radicals and mechanisms of antioxidant control. The glutathione increases the level of SH-groups to the control value on 3 day and 7 day of the experimental period.

Results our study demonstrated that GPx was changed with various days of kidney disease: decreased GPx activity in blood by 40% on 3 day and by 20% 7 day of the experiment compared with control rats.

The introduction of glutathione increases the activity of GPx to the control value during all experimental days compared with the group of animals without introduction of tripeptide.

H2S levels in the blood of rats with nephropathy were lower by 35.5% on the 3d day and by 25.7% on 7 days of the experiment than those in control rats. Blood H2S level on 3 experimental day was associated with a specific volume of epithelial cells of proximal tubules of the kidney. The glutathione increases the level of gasotransmitter by 14.3 % in blood plasma of rats on 3 day and by 11 % on 7 day of the experimental period compared with the group of animals without introduction of tripeptide.

Discussion

Oxidative modification of proteins is associated with damage to both the polypeptide chain and individual amino acids with the formation of several types of radicals. The process of oxidative modification of proteins has a complex and specific nature, which is established by the amino acid composition of proteins [20, 21]. It is the oxidative modification of proteins that plays a leading role in the balance between synthesis and degradation of simple and complex proteins, which determine the ability of cells to generate and produce regulatory impulses, to exert receptor, mediator, and energy functions. Oxidative modification of proteins can contribute to changes in amino acid residues or changes in the valence and coordination of metals, which leads to disruption of protein structure and facilitate proteolysis processes. An increase in the concentration of reactive oxygen species is accompanied by the blocking and disintegration of the major enzymes of the antioxidant system. With the significant intensification of peroxide production and oxidative modification of macromolecules, it is followed by subsequent inactivation of antioxidant enzymes, which leads to depletion of antioxidant activity, reduction of the pool of antioxidant substances and cell death due to membrane depolymerization.

Thus, the intensity of oxidative modification of proteins can be a marker of the degree of peroxide processes and a factor that affects the state of the antioxidant system. Protein oxidation can occur through the oxidation of cysteine sulfhydryl (SH-groups), which results in the formation of disulfide bonds between its two molecules. SH-groups are chemically active groups of proteins that play an important role in the processes of cellular respiration, oxidative phosphorylation reactions, regulation of membrane permeability, are part of the active centers of many enzymes and coenzymes (determine their catalytic activity), and are also actively involved in maintaining the tertiary structure of proteins. The number of SH groups can be judged on the metabolic activity of enzymes. The sulfhydryl groups of proteins not only exert a catalytic function but also protect the proteins from the damaging effect of adverse environmental factors [22].

Since proteins are the primary target for reactive oxygen species, cysteine and reduced glutathione play an important role in protecting them against endogenous reactive oxygen species. Their sulfhydryl groups are easier to oxidize than SH-groups of proteins while protecting the proteins themselves from oxidative modification.

Catalase, which is responsible for the reduction of H2O2 to water, is expressed in most cells, organs, and tissues and at high concentrations in the liver and erythrocytes [23]. It may also be a key enzyme in antioxidant defense in the kidney during injury. Glutathione peroxidase is important selenium-containing enzymes that reduce hydrogen peroxides and protect against oxidative stress. GPx is primarily synthesized in the kidneys [24, 25] and is found in mammalian blood and tissues. Decreased GPx activity in blood is a common symptom in patients with kidney disease.

The decreased catalase activity in response to development of nephropathy might reduce the protection against free radicals and could lead to an increased lipid and protein peroxidation. The simultaneous reduction in the activity of both GPx and catalase makes the kidneys more vulnerable to folic acid-induced oxidative stress.

Our results suggest the potential usefulness of GSH introduction to reduce complications of kidney diseases. The main function of exogenous GSH is to suppress lipid peroxidation, which occurs in the plasma membrane and damages the membrane's structure and permeability.

One of its key functions is to combine with, and thereby inactivate (detoxify), reactive oxygen species, other oxidative molecules, and certain drugs, exogenous chemicals, and toxins. Because

GSH is depleted in these reactions, it must continually be replenished to maintain cell and organ viability and to support normal cellular functions.

GSH depletion impacts a wide variety of cellular processes, ranging from DNA synthesis and gene expression to sugar metabolism and lactate production. A wide variety of inflammatory and metabolic stimuli common during active disease increase the production of intracellular oxidants. In addition, neutrophils and other cells present at sites of inflammation release oxidants (reactive oxygen and nitrogen intermediates) that enter other cells and add to the internal oxidant burden. GSH provides the main defense against toxic oxidative intermediates by reducing and thereby inactivating them. However, in so doing, GSH is oxidized to GSH disulfide (GSSG). GSSG is then either rapidly reduced to GSH by GSSG reductase and NADPH or is excreted from the cell and only in part recovered from the circulation. Factors that may contribute to GSH deficiency include GSH losses that occur when GSH is enzymatically conjugated to exogenous chemicals (drugs, dietary components, and toxins) and excreted from the cell as GSH or acetylcysteine mercapturates (conjugates). In addition, disease processes may decrease the cellular uptake or synthesis of cysteine or cystine, increase GSH efflux [26], or increase the loss of cysteine/GSH sulfur due to accelerated oxidation to the final oxidized forms (sulfate and taurine). Because a balance between cysteine supply and GSH utilization must be maintained, if oxidant production or levels of substrate for GSH conjugation are high and cysteine supplies for GSH replenishment become limiting, severe GSH deficiency may occur. H_2S is known to play a role in redox homeostasis and antioxidant responses, regulation of inflammatory responses, ATP production26. It was previously demonstrated that H_2S protects cells against oxidative stress via increasing endogenous GSH production [27]. It can be assumed that the cysteine present in glutathione is used in the synthesis reactions of hydrogen sulfide, and our experiment confirms this hypothesis [28, 29].

We proposed that cysteine present in glutathione may act is through its enzymatic breakdown to produce hydrogen sulphide, which likely plays an indirect role in the antioxidant capacity of cells through its signalling functions.

The results indicate that we can judge the degree of kidney damage by blood findings. In the paper have shown that animals with kidney disease have impaired antioxidant system of the blood as indicated by decreased glutathione peroxidase, superoxide dismutase and catalase activities, reduced level of H_2S-concentration and SH-groups and increased the content of TBA-active products, the level of oxidative modification of proteins and ceruloplasmin.

The results of our studies confirm the existence of protective and antioxidant properties of glutathione. In our opinion, exogenous glutathione by increasing the level of SH-groups and the content of hydrogen sulfide promotes the correction of the activity of glutathione peroxidase and has an indirect positive effect on the activity of other antioxidant protection enzymes by decreasing the content of TBA-active products, the level of oxidative modification of proteins and ceruloplasmin.

It has been suggested that glutathione therapy might give helpful results in the first stage development of experimental nephropathy by the regulation of metabolism through GSH-dependent pathways. The cysteine present in glutathione is used in the synthesis reactions of hydrogen sulfide, and our experiment confirms this hypothesis. It will greatly improve our understanding of general metabolism changes in the body and also provide new insights into the control of nephropathy.

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Table 1. Indicators of the blood of rats under conditions of experimental nephropathy and introduction of glutathione (M±m)

Investigated indicators	**Control, n=36**	**Nephropathy, 3day, n=25**	**Nephropathy+glutathione, 3day, n=23**	**Nephropathy, 7day, n=24**	**Nephropathy+glutathione, 7day, n=23**
H_2S-concentration, µmol/l	75,05±1,05	53,9±1,14**	61,66±2,52#	58,22±1,97**	64,65±2,63#
Ceruloplasmin, mg/l serum	180,47±5,72	233,5±14,7**	186,1±18,5	314±23,02**	205,7±19,8##
TBA-active products µmol/l	4,34±0,24	5,37±0,31**	4,92±0,54	5,6±0,17**	4,31±0,23##
OMP_{370} µm/g of protein	0,641±0,014	1,005±0,03**	0,769±0,03**##	0,774±0,05	0,652±0,01
OMP_{430} µm/g of protein	0,358±0,01	0,410±0,01**	0,354±0,02#	0,52±0,04**	0,446±0,03#
SH-groups µmol/l	2,49±0,06	1,82±0,11**	2,2±0,11#	2,25±0,16**	2,48±0,102#
Catalase, µmol/min/l of protein	13,17±0,416	10,5±0,3**	12,46±0,24##	10,6±0,53**	11,25±0,6
Glutathione peroxidase nmol/min/ mg of Hb	6,93±0,36	4,34±0,8*	6,66±0,48#	5,74±0,13*	6,85±0,48#
Superoxide dismutase U/mg of Hb	1,913±0,054	1,742±0,09	1,968±0,08	1,749±0,02**	1,801±0,06

Notes: * - p<0.05, relative to control, ** - p<0.01, relative to control; # - p<0.05, relative to animals with nephropathy; ## - p<0.01, relative to animals with nephropathy. M±m; the data are presented as mean±SEM.