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Thiol-disulfide metabolism in kidney tissue at the administration of some copper coordination compounds

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Abstract

Background: Thiol-disulfide metabolism is essential for normal function of the organism. Thus the interest of the scientists in this area of research continues to grow.
Material and methods: Copper coordination compounds (CCC), derivatives of thiosemicarbaside (CMD-4, CMJ-33, CMT-67), action on thiol-disulfide metabolism in the healthy *Ratta albicans* kidneys were studied. The animals were divided in 6 groups of 7 rats each. The control group included healthy rats which were injected i/m physiological solution 3 times a week, for 30 days. The rats from groups 2-6 have got 3 times a week, for 30 days, i/m injections of CCC. The activity of following thiol-disulfide metabolism enzymes in the renal supernatant has been measured: glutathion-reductase (GR), glutathion-peroxidase (GPO), glutathion-S-transferase (G-S-T), γ-glutamyl transpeptidase (γ-GTP), glutaredoxin (Grx), as well the amount of the protein SH-groups and of the total glutathione, reduced glutathione (GSH) and oxidized glutathione (GSSG) in renal tissue.
Results: The compounds exhibit different actions: CMT-67 in the dose of 0.1 μM/kg influenced the activity of the glutathione metabolism enzymes – activated γ-glutamyl transpeptidase (γ-GTP) and glutaredoxine (Grx) and inhibited glutathione reductase (GR), while CMD-4 in doses of 0.1 μM/kg and 1.0 μM/kg and CMJ-33 in the dose of 1.0 μM/kg significantly diminished the reduced glutathione (GSH) level and increased the amount of the oxidised one (GSSG).
Conclusions: Selective action of the copper coordination copounds established by this study opens new possibilities of their usage in the therapy of kidney diseases.
Key words: thiol-disulfide metabolism, copper coordination compounds, kidney tissue.

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Introduction

Thiol-disulfide metabolism is a factor, which largely determines the functional activity of human body. Its intensity conditions the speed of the most diverse reactions of biosynthesis, growth and development of cells and organs, transport and repair processes and many other aspects of vital activity. This explains the constant interest of scientists in the exploring of various aspects of thiol-disulfide metabolism [1-3].

A very topical issue is the need to study the new coordination compounds of transition metals, especially the thiosemicarbazon derivatives, which can serve as a basis in the development of the medicinal preparations for the prophylaxis and the treatment of various diseases, including the renal ones and the stimulation of the regenerative processes in the tissues. Many medicinal remedies that contain metals exhibit modulatory effects on the thiol-disulfide system, which provides an opportunity to understand the molecular mechanisms of their action on the body and allow the development of new strategies for the treating of different diseases [4].

Due to their high biological activity, especially, to influence the thiol redox system, the thiosemicarbazon derivatives are increasingly used for the treatment of various diseases, including cancer [5]. The performed preliminary researches have revealed their therapeutic efficacy and the prospects for using of these compounds [6].

In the scientific studies with reference to the problem in question, the data that reveal the changes of thiol-disulfide metabolism in the renal tissue in the use of new thiosemicarbazon derivatives, complexed with metal ions are lacking.

The aim of the study was to elucidate the peculiarities of thiol-disulfide metabolism and its pathogenic importance in the renal tissue of laboratory healthy animals while administering of some new copper coordination compounds (CCC), derivatives of thiosemicarbazide.

Material and methods

The new local CMD-4, CMJ-33 and CMT-67 copper coordinative compounds, derivatives of the thiosemicarbazide, synthesized in the Laboratory of Advanced Materials in Biopharmacy and Technique of the State University of Moldova, have been studied [6-8].

Experiments have been performed on white laboratory rats (No 42) weighting 180-240 g. The research protocol was approved by the Research Ethic Board of *Nicolae Testemitanu* State University of Medicine and Pharmacy (No 43 of June 18, 2015) and the tests have been done according to the contemporary principles in biological standardization of experiences and Declaration of Helsinki with further amendments (Somerset West Amendment, 1996).

The animals have been randomly divided in 6 groups of 7 rats each, with chow and water available *ad libitum*. The control group included healthy rats which were injected i/m physiological solution 3 times a week, for 30 days. The rats from groups 2-6 have got 3 times a week, for 30 days, i/m injections of copper coordination compounds as following: group 2 – CMD-4 (0.1 μM/kg), group 3 – CMD-4 (1.0 μM/kg), group 4 – CMJ-33 (0.1 μM/kg), group 5 – CMJ-33 (1.0 μM/kg) and group 6 – CMT-67 (0.1 μM/kg). The experimental animals have been euthanized 24 hours after the last administration of CCC and the kideys have been removed.

The kidneys have been homogenized in 0.1 M (pH 7.4) phosphate buffer solution that contained 1 mM EDTA, final dilution 1:10. Membranes have been destroyed by Triton X-100 processing (final concentration 0.1%). All procedures have been performed in glacial media. The obtained homogenate has been subjected to centrifugation for 15 min at 5000 rotations per minute and the supernatant has been stored at (-) 40°C.

The activity of following thiol-disulfide metabolism enzymes in the renal supernatant has been measured: glutathion-reductase (GR), glutathion-peroxidase (GPO), glutathion-S-transferase (G-S-T), γ-glutamyl transpeptidase (γ-GTP), glutaredoxin (Grx), as well the amount of the protein SH-groups and of the total glutathione, reduced glutathione (GSH) and oxidized glutathione (GSSG) in renal tissue. Spectrophotometric micromethods adapted for Synergy H1 Hybrid Rider (BioTek Instruments, USA) have been applied [9, 10].

Data were subjected to statistical analysis in StatsDirect Statistical Software (version 1.9.5., 2001) using *U Mann-Whitney* non-parametric test to determine the differences between means at the significant level of $p<0.05$.

Results

Table 1 shows the activities of the thiol-disulfide metabolism enzymes (GR, GPO, G-S-T, γ-GTP and Grx) in the kidney homogenate after the administration of the CCC. The data reveals that the CCC exhibit different action upon the studied glutathione-dependent enzymes activities in the kidneys. The GR and G-S-T activities decreased by 14-35%, while GPO – non-significantly, under the influence of all studied compounds. γ-GTP was inhibited by CMJ-33 administrated in dose of 1.0 μM/kg, while the rest of the CCC have not changed the activity of the enzyme in comparison with the control group. Compound CMD-4 in both doses has not induced significant changes of Grx activity, which was considerably increased (up to 39-95%) by both doses of CMJ-33 and CMT-67 in the dose of 0.1 μM/kg.

The research results of the influence of studied compounds on the level of total, reduced and oxidized glutathione and protein SH groups in the kidney tissue are presented in table 2. Studied compounds have not induced statistically significant changes of the total glutathione level in the kidneys. Administration of both doses of CMD-4 and CMJ-33 in the dose of 1.0 μM/kg has produced a significant decrease (by 75-86%) of GSH along with a considerable increase of GSSG level – more than 2 times in comparison with the control group. Subsequently the GSH/GSSG ratio was notably reduced. The amount of protein SH groups manifested a decreasing tendency by the studied CCC, but the attested decrease has proved to be of no statistical relevance.

Discussion

The influence of CCC on the thiol-disulfide metabolism in the kidneys of healthy rats has been studied. GPO, G-S-T, GR, γ-GTP, Grx and reduced glutathion (GSH) are the elements of a complex antioxidant system that neutralizes the hydrogen peroxide and organic peroxides both in the cellular environments and biological membranes. Organic peroxides having a high damaging capacity can induce a series of chain reactions that will deteriorate the cellular structures [11].

We have identified a statistic significant decrease of the GR and G-S-T activities after the administration of the minimal dose of CCC (0.1 μM/kg), while the high dose (1 μM/kg) has not produced a significant effect on the enzymes activity ($p>0.05$).

The biological role of GR is to maintain a high level of GSH and low one of GSSG, which is particularly important, because glutathion performs its biological functions only in the reduced form. GR, having high activity in the kidneys, represents the enzyme that reduces GSSG and thus plays a cardinal role in the GSH recycling. We have attested the reduction of the GR activity in the kidneys after the administration of low doses of CCC, thus the enzyme can not restore the GSH level by reducing GSSG. The inability of GR to reduce GSSG and maintain the optimal level of GSH will disturb the proper functions of GPO and GST, that require GSH for peroxides reduction, and/or of Grx, that reduces the disulfides [11-13]. Thus, the CCC, diminishing the activity of GR, can influence the activities of GPO, G-S-T and Grx through the reduction of GSH amount in the kidneys.

Our study revealed a significant decrease of the G-S-T activity in the kidneys after CCC administration. In the kideys G-S-Ts are expressed in the tubules and acount for 2% of the total soluble cytoplasmic proteins. Recently was established that G-S-Ts are involved in the metabolism of xenobiotics and can neutralize the final products of lipid

peroxidation – a,b-highly reactive unsaturated aldehydes, such as 4-hydroxynonenal (4-HNE), and the latter compound, via a feedback mechanism, is involved in the regulation of the multiple cell signaling pathways.

The pronounced inhibition of GST activity in the renal tissue, detected in our research, may induce the increase of 4-HNE concentration, which in turn causes inhibition of pro-proliferative mechanisms, as well as DNA and protein synthesis, which may result in apoptotic or necrotic eventual death of cells [14]. Thus, inhibition of GSTs at the cellular level, along with other mechanisms, could underlie the antiproliferative, antitumor properties of these compounds and, it is not excluded, could produce effects, manifested also by a certain degree of nephrotoxicity. Participation of G-S-Ts as mediators of the signaling pathways involved in cell proliferation and death have been highlighted by numerous previous studies [15-17]. In other words, GSTs regulate cellular homeostasis by modulating intracellular levels of 4-HNE, and the latter functions as a sensor that at low concentrations directs cells to proliferation, survival, differentiation, and at increased concentrations causes inhibition of pro-proliferative processes, which induces apoptosis and necrosis [18, 19].

Therefore, G-S-T activity can be an important tissue marker of the effects on cell signaling, but also of the mechanisms underlying toxic/side effects of the chemical compounds tested as new pharmaceutical remedies.

GPO is a family of antioxidant enzymes, present in the tissues under several isoenzymes. The kidney isoform – GPO-3, is a tetrameric selenoprotein that is attached to the basal membrane of the proximal renal tubes. The GPO-3 is responsible for the reduction of hydrogen peroxide and of some organic peroxides using GSH as a donor of reducing equivalents. The GPO activity was not significantly decreased by the administration of CCC and the ability to reduce H_2O_2 to water and the potential for annihilation of organic peroxides was preservered, thereby the kindey cells were protected from oxidative damage.

The kidney is the main organ that regulates the plasma level of GSH, due to the high activity of γ-GTP in the renal convoluted tubules [20]. The enzyme is essental in the γ-glutamyl cycle, a pathway responsible for amino acids and peptides transfer through membranes into the cell, as well as in protein synthesis, synthesis and cleavage of glutathione, detoxification of medicines and xenobiotics [11, 13, 21, 22]. γ-GTP may also exhibit prooxidative properties in some circumstance and induce metal-dependent DNA damage [23-25].

Studied CCC maintained the γ-GTP activity at the level specific for control group animals that proves that the CCC do not influence the γ-GTP-dependent processes mentioned above. As an exception a significant decrease was revealed after the administration of CMJ-33 in maximal dose of 1.0 μM/kg.

This fact, probably, not only affects the translocation of amino acids and proteosynthetic processes in the renal cell, but can also be regarded as a process oriented to the reducing of the oxidative damage of cellular DNA dependent on metal ions, such as Fe, Cu, etc. In the literature it has been reported that γ-GTP may also exhibit prooxidative properties in some circumstance and in the presence of metal ions, such as Fe^{2+}, Cu^{+}, increases the level of DNA damage and nitrogenous bases [24, 25]. Subsequently CMJ-33 is not only having an impact on the amino acids transfer into the cell and protein synthesis in the kidneys, but also can prevent the oxidative damage of the DNA from metal ions like Fe^{2+}, Cu^{+}, etc.

Thus, the tissue level of γ-GTP activity can be an important marker of protein synthesis and DNA metal-ion dependent damage in preclinical and clinical studies of potential medicines, as long in the research of pathogenic mechanism of kidney diseases.

Grx was activated by CMJ-33 and CMT-67 revealing the capacity of these compounds to induce the reactions of reduction/deglutathionilation of proteins. Such reactions are catalysed by glutaredoxines/thioltransferases (Grx/TT) – members of the GSH-dependent thioldisulfide oxidoreductases, involved in reduction of disulfides or GSH mixed disulfides [26-28]. Grx and thioredoxin are catalyzing the reduction of protein disulfides, sulfoxides and sulfenic acid, as well as the reduction of ribose in the pathway of deoxyribonucleotide synthesis [29]. It is known that GSH modulates the DNA synthesis by maintaining glutaredoxin and/or thioredoxin in a reduced state, which is required for the activity of ribonucleotide reductase, enzyme that limits DNA synthesis in the cells [30].

Grx regulates through redox mechanisms the nuclear kB factor (NF-kB) expression and subsequently the chemokine production and inflammation in the kidneys [31]. Also these mechanisms involve Grx in the regulation of the eneregy state of the kidneys [32]. Therefore, Grx can be a valuable tissue marker, not only for assessing the detailed mechanisms of action of various chemical compounds tested as new therapeutic agents *in vivo*, but also for an in-depth study of the pathogenesis of kidney diseases.

The changes of GSH and GSSG amounts, as long of the GSH/GSSG ratio have been studied in the kidneys after administration of the CCC in different doses. The statistic significant decrease of GSH, increase of GSSG and decline of the GSH/GSSG ratio have been revealed in our study. Those changes can be a consequence of the reduction of the GSH recycling rate at enzyme level or/and enhancement of GSSG formation due to oxidative stress exacerbation.

The optimal GSH/GSSG ratio is important for cell vitality, and alteration of the intracellular GSH balance has been reported in many diseases, including tumors [33, 34]. Abnormal GSH/GSSG ratio can trigger significant changes of the redox-dependent cellular signaling mechanisms that are involving G-S-T and Grx.

Likewise, it is important to note that shifting the GSH/GSSG redox toward the oxidizing state activates several signaling pathways, like nuclear factor κB, c-Jun N-terminal kinase, apoptosis signal-regulated kinase 1, protein kinase

B, protein phosphatases, etc., thereby reducing cell proliferation and increasing apoptosis [35].

It is known that reduced glutathion plays an important role in antioxidant protection and is involved in regulation of major cellular processes like gene expression, protein synthesis, apoptosis, signal transduction, cytokine production and immune response and glutathionylation of proteins [2, 27, 33, 34, 36]. In reduced form, this tripeptide is involved in many reactions and processes, due to the high reactivity of the SH group, which by giving an electron pair can achieve a wide range of reversible or irreversible chemical combinations. GSH can directly or through some enzymatic reactions, acting as a coenzyme of GPO or GST, efficiently capture free radicals and other reactive oxygen species and neutralize endogenous or/and exogenous toxic compounds [20, 37, 38]. Thus, its decrease can have a significant impact on the mentioned processes.

The overall changes of the thiol-disulfide metabolism in the kidneys after the administration of the CCC offer new incites on this metabolic branch role in tissue homeostasis and possibilities of CCC usage in the treatment of the kidney diseases, as well as new directions of research of the therapeutical and preventive potential of the novel copper coordination compounds derivatives of thiosemicarbazide in kidney diseases.

Conclusions

1. The main indices of thiol-disulfide metabolism that can reveal the efficiency of the novel copper coordination compounds derivatives of thiosemicarbazide, in the kidneys of healthy animals have been estimated.

2. Evaluation of the activities of the thiol-disulfide metabolism enzymes in the kidney homogenate after the ad-

Table 1

The influence of copper coordination compounds derivatives of the thiosemicarbazide – CMD-4, CMJ-33 and CMT-67 on the indices of thiol-disulfide metabolism in the kidney tissue of rats

Groups of study	GR (nM/s.g.prot.)	G-S-T (nM/s.g.prot.)	GPO (nM/s.g.prot.)	γ-GTP (μM/s.g.prot.)	Grx (nM/s.g.prot.)
Control group	17.2±1.21 (100%)	16.4±0.66 (100%)	42.3±2.90 (100%)	8.2±0.35 (100%)	9.7±1.07 (100%)
CMD-4 0.1 μM/kg	**13.2±1.16* (77%)**	**14.1±0.38** (86%)**	34.8±2.53 (82%)	7.7±0.71 (93%)	8.7±0.63 (89%)
CMD-4 1.0 μM/kg	17.9±2.37 (104%)	14.58±0.85 (89%)	40.8±2.63 (96%)	7.54±0.55 (91%)	11.6±1.91 (118%)
CMJ-33 0.1 μM/kg	**13.0±0.81** (75%)**	**13.9±0.42** (85%)**	40.1±3.74 (95%)	8.6±0.19 (104%)	**13.6±1.52* (139%)**
CMJ-33 1.0 μM/kg	15.5±1.70 (88%)	15.1±0.71 (92%)	38.9±2.89 (92%)	**4.6±1.02*** (56%)**	**19.0±2.13*** (195%)**
CMT-67 0.1 μM/kg	**11.1±1.00*** (65%)**	**12.1±0.41*** (74%)**	40.8±5.81 (96%)	8.8±0.83 (106%)	**15.3±2.19* (157%)**

Note: * – *statistically significant difference with the control group, p <0.05; ** – p <0.01; *** – p <0.001.*

Table 2

The influence of copper coordination compounds derivatives of the thiosemicarbazide – CMD-4, CMJ-33 and CMT-67 on the indices of thiol-disulfide metabolism in the kidney tissue of rats

Groups of study	Total GSH (μM/g tissue)	GSH, (μM/g tissue)	GSSG, (μM/g tissue)	GSH/GSSG ratio	SH-group of prot. (mol/g.prot.)
Control group	3.72±0.07 (100%)	3.29±0.09 (100%)	0.44±0.09 (100%)	7.48±1.03 (100%)	17.18±1.48 (100%)
CMD-4 0.1 μM/kg	3.56±0.17 (96%)	**2.48±0.13** (75%)**	**1.07±0.10** (243%)**	**2.32±0.93*** (31%)**	15.08±0.76 (88%)
CMD-4 1.0 μM/kg	3.56±0.18 (96%)	**2.56±0.19* (78%)**	**1.00±0.10** (227%)**	**2.56±1.76* (34%)**	15.68±1.61 (91%)
CMJ-33 0.1 μM/kg	4.27±0.24 (114%)	3.21±0.06 (97%)	**1.06±0.22* (240%)**	**3.03±0.29** (41%)**	15.81±0.71 (92%)
CMJ-33 1.0 μM/kg	3.41±0.19 (86%)	**2.83±0.09* (86%)**	0.58±0.11 (132%)	**4.88±0.68* (65%)**	16.84±0.4 (98%)
CMT-67 0.1 μM/kg	3.89±0.85 (104%)	3.36±0.21 (102%)	0.53±0.15 (120%)	6.34±0.81 (85%)	15.21±0.58 (88%)

*Note: * – statistically significant difference with the control group, p <0.05; ** – p <0.01; *** – p <0.001.*

ministration of the CCC revealed that GR and GST activities were inhibited by CMD-4, CMJ-33 and CMT-67 (0.1 μM/kg), γ-GTP – by CMJ-33 (1.0 μM/kg), Grx activity was considerably increased by CMJ-33 (both doses) and CMT-67 (0.1 μM/kg), while GPO function did not change.

3. CMD-4 (both doses) and CMJ-33 (1.0 μM/kg) induced a significant increase of GSSG along with a considerable decrease of GSH and of the GSH/GSSG ratio.

4. The study of the copper coordination compounds derivatives of the thiosemicarbazide – CMD-4, CMJ-33 and CMT-67 influence on the thiol-disulfide metabolism, opens new possibilities of research with focus on the elucidation and analysis of the mechanism of CCC preventive and therapeutical action in health and disease.

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Authors' contribution
VS – designed the research, did statistics and interpreted the data; VP – conducted/performed the laboratory work; AG – interpreted the data and drafted the manuscript; OT – conceptualized the project and designed the research; LA – conducted/performed the laboratory work; IS – conducted/performed the laboratory work; VT – drafted the manuscript.
VG – conducted the laboratory work, revised the manuscript critically. All authors revised and approved the final version of the manuscript.

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Ethics approval and consent to participate
The research protocol was approved by the Research Ethic Board of Nicolae Testemitanu State University of Medicine and Pharmacy (No 43 of June 18, 2015) and the tests have been done according to the contemporary principles in biological standardization of experiences and Declaration of Helsinki with further amendments (Somerset West Amendment, 1996).

Conflict of Interests
No competing interests were disclosed.