RESEARCH STUDIES

Influence of new Schiff bases and their combinations with 3d metals on the glutathione and thiol-disulfide metabolism in the liver in physiological conditions

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Abstract

Background: Glutathione and glutathione metabolism enzymes are essential for the normal functioning of cells and tissues and maintenance of body homeostasis. The aim of our study was to investigate the influence of the new Schiff bases, and their combinations with 3d metals, on the glutathione metabolism in the liver in physiological conditions.

Material and methods: An experimental study of laboratory animals (rats) was performed. New local biologically active compounds (BAC) – CMD-4, CMD-8 and CMJ-23, were injected to healthy rats for 14 days. The content of glutathione (total, reduced and oxidized) and the activity of glutathione reductase (GR), glutathione peroxidase (GPO), glutathione-S-transferase (GST) and glutaredoxin (GRX) were determined in the liver homogenates.

Results: All tested BAC statistically significant decreased the level of reduced and total glutathione (about 20-30%), and GMJ-23 also reduced the oxidized glutathione in the liver (-48%, $p<0.05$). Influence on the activity of enzymes of glutathione metabolism was more ambiguous. Administration of CMD-4, CMD-8 and CMJ-23 did not produce conclusive changes of the enzyme activity of the glutathione metabolism, and their induction had only tendency of GR, GST and GRX activity decrease, while CMT-28 and CMT-67 preferentially modulated the activity of enzymes.

Conclusions: Local BAC exerts an individual influence on the glutathione metabolism in physiological conditions, which may be used for a particular regulation of the processes according to the cell needs.

Key words: Schiff bases, 3d metals, glutathione, liver.

Introduction

Glutathione (GSH) and glutathione metabolism enzymes are essential for normal cellular, tissue and organism functioning. Glutathione is able to neutralize the free radicals and other reactive oxygen species, interact with various xenobiotics (acetaminophen, bromobenzene, etc.), eliminate some carcinogens (formaldehyde), interfere in the metabolism of eicosanoids, adjust the level of nitric oxide (NO) through the thioredoxin system. Maintaining the optimal level of glutathione and activity of enzymes of the glutathione metabolism is essential for body homeostasis [12, 11, 13].

The reactions of production and reduction of mixed disulfides, including glutathionylation and deglutathionylation of the proteins occur permanently in the cells. These processes are catalyzed and directed by several enzymes – a complex network of thiol/disulfidoxide reductase, that are located in the endoplasmic reticulum. Glutaredoxines (GRX) and thioltransferases, which catalyze the reduction of disulfides or of the mixed disulfides of GST, belong to this group of enzymes [9, 10]. GRX and thioredoxine systems play the protective action in patients with cardiovascular diseases and cataract by deglutathionylation of cardiac and lens proteins [1, 19].

Thus, the synthesis and testing of new chemical compounds, with the potential to strengthen reserves of intracellular glutathione and control the activity of glutathione enzymes, present interest for modern biomedical science and practice.

Biologically active compounds (BAC) are represented in a very wide area of scientific research, given extremely large variety of substances both natural and synthetic, which exerts potent action on different processes in living organisms. Synthesis of new BAC and study of their prophylactic and/or therapeutic potential is permanently on the agenda of researchers, including those in Moldova.

Several laboratories and scientific groups in Moldova (Gulea A. et al. [7, 8], Macaev F. et al. [14], Gudumac V. et al. [6]) investigated BAC with various chemical structures, physico-chemical and biological actions. Previous research of Moldovan scientists has determined that these BAC have a great pharmacological potential due to their antimicrobial, antifungal, antitumor, cytostatic, immunomodulatory, bone formation induction, and hepato-protective action [16, 17, 15, 18].

The aim of our study was to establish the influence of the local BAC offered by Professor Aurelian Gulea (CMD-4, CMD-8, CMJ-23) on glutathione metabolism in the liver in physiological conditions.

Material and methods

The research was approved by the Research Ethics Committee of the Nicolae Testemitsanu State University of Medicine and Pharmacy (June 20, 2011).

Experiments were done with the male white rats weighing 160-250 g. All animals were maintained in similar standard vivarium conditions. Considering the mechanisms of regulating the circadian biological rhythms (diurnal, seasonal, etc.), and their impact on the concentration, structure and

Table 1

Influence of local BAC on the content of glutathione and protein SH-groups in the liver in physiologic conditions

Experimental groups	Total glutathione, µmol/g	Reduced glutathione, µmol/g	Oxidized glutathione, µmol/g	Protein SH-groups mol/g
Control	12,70±0,40 100%	11,24±0,49 100%	1,45±0,25 100%	22,11±1,72 (100%)
CMD-4	10,22±0,49* 80,47%	9,06±0,47* 80,60%	1,54±0,39 106,21%	19,92±0,50 (90%)
CMD-8	9,27±0,99** 73,00%	7,94±1,01* 70,64%	1,33±0,23 91,72%	22,37±0,91 (101%)
CMJ-23	9,13±0,58** 71,89%	8,36±0,57* 74,38%	0,76±0,09* 52,41%	21,75±1,50 (98%)

Note: The statistical significance of the differences compared with controls: * - p <0,05, ** - p <0,01.

distribution of intracellular components [5], experiments were done during the same season, and investigation material was collected at the same time of the day.

All studied local BAC – CMD-4, CMD-8, CMJ-23 were administered intramuscular during14 days with the daily dose 1,0 mg/kg body weight.

Animals were sacrificed under light anesthesia with sulfuric ether, and liver was taken after 24 hours from the last administration of BAC. All operations were performed at temperature +4oC. For biochemical investigations livers were subjected to homogenization. The whole process of preparing the liver homogenates was determined under specific conditions for assessing enzyme activity.

The following indices of glutathione and thiol-disulfide metabolism: the level of total, reduced and oxidized glutathione, the content of protein SH-groups, the activity of glutathione reductase (GR), glutathione peroxidase (GPO), glutathione-S-transferase (GST) and glutaredoxine (GRX) were determined in the liver homogenates according to the procedures described by Gudumac V. and co-authors [6].

All methods for determination of enzyme activity and contents of chemical compounds have been used by techniques in our modification, adapted for application to the spectrophotometer Power Wave HT (BioTek Instruments, USA) and the microplate spectro-fluorimeter Synergy H1 (Hydride Reader, BioTek Instruments, USA).

The statistical analysis of the data has been performed by non-parametrical Mann-Whitney U-test. The differences have been considered significant for p<0.05. The data are presented as a M±SEM.

Results and discussion

The research of the local Schiff base BAC showed their significant influence on the level of glutathione and content of protein SH-groups in physiological conditions (tab. 1).

It was established that content of reduced glutathione was significantly higher than of the oxidized glutathione – 11,24 ± 0,49 µmol/g tissue vs 1,45 ± 0,25 µmol/g tissue (p<0,001) in the liver of the control animals. At the same time, the amount of protein thiol groups was almost 2 times higher than of the total glutathione (p <0,001).

BAC (CMD-4, CMD-8 and CMJ-23) administration at the dose of 1,0 mg/kg body weight, induced changes of different magnitude of the contents of glutathione and protein thiol groups in the liver of the healthy animals.

The contents of total and reduced glutathione were decreased by all BAC included in this study. The level of total glutathione was decreased by CMD-4 by 20% (p<0,05 in both cases), by CMD-8 by approximately 30% (p<0,01, and respectively, p<0,05) and CMJ-23 by 26-28% (p<0,01, and respectively, p<0,05). At the same time, the level of oxidized glutathione was not statistically conclusively influenced by the local BAC. Only CMJ-23 reduced by 48% (p<0,05) the content of the oxidized glutathione in the liver of control animals (Tab. 1). Values of the protein thiol groups, as well were not changed significantly by the tested BAC. Just CMD-4 induced a descending trend of the level of protein thiol groups (-10%, p>0,05).

Local Schiff base type coordination compounds and their complexes with metals did not have significant impact on the activity of enzymes of the glutathione metabolism (tab. 2).

Table 2

Influence of local BAC on glutathione metabolism enzymes in the liver in physiologic conditions

Experimental groups	GR (nmol/s·g prot.)	GPO (nmol/s·g prot.)	GST (nmol/s·g prot.)	Glutaredoxine (nmol/s·g prot.)
Control	30,12±2,91 (100%)	29,44±2,11 (100%)	31,22±1,92 (100%)	14,16±2,37 (100%)
CMD-4	27,56±1,02 (92%)	29,44±1,73 (100%)	30,07±1,12 (96%)	13,45±3,39 (95%)
CMD-8	24,59±1,71 (82%)	30,39±2,26 (103%)	27,51±1,02 (88%)	12,73±0,89 (90%)
CMJ-23	26,92±1,96 (89%)	31,17±3,43 (106%)	28,18±1,40 (90%)	9,68±0,82 (68%)

Note: GR – glutathione reductase; GPO – glutathione peroxidase; GST – glutathione-S-transferase.

None of the studied local BAC produced significant changes of the activity of GR, GST and glutaredoxine. However, all tested BAC induced the decreasing of the GR activity by about 8-18% (p>0,05), of GST - by about 4-12% (p>0,05), and glutaredoxine - by about 5-32% (p>0,05). Glutathione peroxidase activity was not influenced by any of the studied BAC, its activity was mantained within 100-106% limits of the reference values.

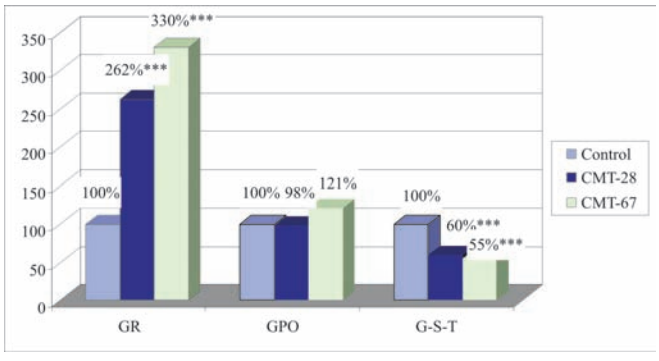

Fig. 1. Influence of CMT-28 and CMT-67 on the activity of glutathione reductase (GR), glutathione peroxidase (GPO) and glutathione-S-transferase (GST) in the liver of healthy rats (%).

Note: The statistical significance of the differences compared with controls: *** – p<0,001.

Thus, the influence of BAC – CMD-4, CMD-8 and CMJ-23 didn't change the activity of GPO, which is needed for the reduced glutathione using it for protective function against peroxides of lipids. The level of its activity was similar to the level of control animals. At that time the activity of GR, which is the enzyme for regeneration of the oxidized glutathione (GSSG) to its reduced form (GSH), decreased by action of BAC. This causes the depletion of reduced glutathione and the capacity of glutathione system to neutralize various chemical compounds.

The results of the influence of local Schiff base type with copper-containing coordination compounds - CMT-28 and CMT-67, on the activity of glutathione reductase and glutathione peroxidase in the liver in physiological conditions did not reveal statistically conclusive changes of the activity of these enzymes (fig. 1).

Both compounds increased statistically conclusive activity of GR - CMT-28 about 2.6-fold (p<0.001), and CMT-67 about 3.3-fold (p<0.001) compared to the values found in the animals of the control group. Activity of glutathione peroxidase was not influenced by CMT-28 (98%) and increased after administration of CMT-67 by 21% (p<0.01).

At the same time, CMT-67 and CMT-28 decreased statistically significant activity of GST by 40% (p<0.001) and 45% (p<0.001), compared to the values identified in the liver of the healthy animals (fig. 1).

Thus, the exertions of CMT-28 and CMT-67 provided different influence from those specific ones of CMD-4, CMD-8 and CMJ-23, which opens up possibilities for using the studied compounds in order to modulate the activity of enzymes of the glutathione metabolism. Considering that maintaining of high levels of GSH and decreased values of GSSG is cardinal for ongoing of the glutathione-dependent processes, because the glutathione realizes its majority of biological functions in reduced form, beneficial effect on the metabolism of glutathione in physiological conditions exert CMT-28 and CMT-67, which were increasing the GR activity. The enzyme converts oxidized glutathione to GSH, considerably reduces de novo synthesis of GSH and maintains the antioxidant activity of the enzymes responsible for the reduction of peroxides (GPO, GST) and disulfids (glutaredoxins) [3, 20, 21].

System GR-GSH has an important value in maintaining of the thiol-disulfide status in mammalian cells. This system plays a key role in protecting of cellular macromolecules against damage caused by free radicals that are generated in excess. Also systems GR-GSH and TrxR/TRX TrxR-TRX are involved in several cellular signaling pathways (control the activity of transcription factors and other factors that regulate apoptosis and cell division). Inhibition of mentioned pathways has dual consequences - can induce programmed cell death (apoptosis), or increase the sensitivity of cells, including tumor cell, to the action of drugs [2, 4, 10].

Conclusions

The exertions of local biologically active compounds provided the different influence on the indices of glutathione metabolism in the physiological conditions. New Schiff bases in combination with 3d-metals, CMD-4, CMD-8 and CMJ-23, caused changes in the content of total, reduced and oxidized glutathione, but did not act on the activity of enzymes of the glutathione metabolism , while CMT-28 and CMT-67 preferentially modulated the enzyme activity. Thus, for the local BAC takes place the specific individual influence on the glutathione metabolism, that may be used for a particular regulation of the processes according to the cell needs.

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Evaluation of antibiotics consumption in therapeutic intensive care department

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Abstract

Background: Monitoring of aggregate, ward-supply data and analysis of the anatomical therapeutic chemical/defined daily dose system, adjusted for bed-occupancy, provides a clear picture of antibiotics consumption frequency and time-trends within hospitals and especially intensive care departments.
Material and methods: For this study we used data of a five-year (2010-2014) period, in therapeutic intensive care department of the Emergency Medicine Institute, which show the consumption dynamics of anti-infectives for systemic use of drugs in grams and value indexes.
Results: The defined daily doses (DDD) per 1000 occupied-bed days (DDD/OBD) of antibiotics in therapeutic intensive care department decreased from 1524 in 2010 to 1206 DDD/1000 in 2014 or by 20.87%, however, it is by 11.77% higher than medium consumption of 1052.25 DDD/1000 in intensive care units with the same activity in international hospitals. The value of 54948 lei per DDD/1000 OBD in 2010 recorded a decline to 40754 lei or by 25.84% in 2014. The cost of one medium DDD from 36.05 lei in 2010 decreased to 33.77 lei or by 6.33% in 2014. The average antibiotics annual institution consumption constituting 464.1 DDD/1000 in 2014 was higher by 1.06% comparatively with medium consumption of 459.20 DDD/1000 registered in 1706 international hospitals, and by 35.31% in comparison with global consumption of 343 defined daily doses per 1000 patient-days.
Conclusions: The decrease of DDD/1000 OBD and their cost took place as a result of efforts for rational use of antibiotics during the evaluated period. Great opportunities were found for improving rational utilization of anti-infectives for systemic use.
Key words: antibiotics, defined daily dose, consumption, rational use, hospitals.

Introduction

The main function of the Therapeutic intensive care department of Emergency Medicine Institute consists in providing measures to recover patients after anesthesia of surgical, neurosurgical, traumatological and other investigations as well as from other possible critical conditions of hospitalized patients. Often these patients are exposed to multiple invasive procedures and have to administrate a multiple broad spectrum of antibiotics. At the same time, the prescriptions are often empiric and lead to overuse or misuse of antibiotics, unnecessary side effects, growth of pathogenic microbes resistance to antibiotics and increasing treatment costs. The above mentioned requires surveillance, stringent consumption control and suppose rational antibiotic prescription [1, 2, 3, 4]. Unfortunately, in the Republic of Moldova the information about antibiotics prescription, consumption patterns, and cost analysis in hospitals with only few scientific publications [5, 9, 10, 20] is rather limited.

National Scientific-Practical Centre of Emergency Medicine of the Republic of Moldova reorganized in 2014 into Emer-