Correlation of glutathione reductase activity distribution in the blood serum and tissues of white unborn rats

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Abstract. It is known that the majority of pathological processes take place against the background of formation of active oxygen species and intensification of free radical oxidation of bio-substrates. In response to this, the antioxidant system of the cell is activated, and the glutathione system is an important link in this system. The latter can take part in the maintenance of the optimal state of biomembranes, in the processes of detoxification, antioxidant protection, etc. The biological role of glutathione reductase is to maintain high intracellular concentration of reduced glutathione. The aim of our study was to study the relationship between the distribution of glutathione reductase activity in blood serum and rat tissues. In order to achieve the goal of the study the following tasks were solved: the activity of glutathione reductase in blood serum and tissues of liver, brain, heart, as well as in skeletal muscle tissues of rats was determined; the interrelation of the activity distribution of glutathione reductase in blood serum and tissues of rats was revealed. The article presents the results of nonparametric correlation analysis to assess the relationship between the distribution of glutathione reductase activity in blood serum and tissues of small experimental animals.

1 Introduction

Habitats constantly and continuously affect living organisms through abiotic and biotic factors. The process of survival of living organisms in constantly changing conditions is called adaptation.

Increasing the mobilization capacity of the body, such as the high-speed deployment of functional and metabolic reactions, is the main way to adapt and is expressed in a more efficient functioning of physiological systems, increasing the limits of the body and intensifying recovery processes. The ability to maintain high levels of functioning of physiological systems in conditions of maximum intensity of loading is designated as functional stability and it allows maintaining maximum efficiency. Functional stability is a multi-component property of an organism, including a complex of factors, which determine the stability of physiological systems of an organism, and stability of mental and psychomotor functions [1].

As a result of the action of any irritant in accordance with the studies of F.Z. Meerson (1981, 1988) there is a process of urgent adaptation, in which the body functions with the maximum mobilization of internal reserves. In this mode, the reserves are quickly exhausted; the body's reaction becomes short-term and turns into a stress-response. Sustainable (long-term) adaptation is formed gradually as a result of long and repeated exposure to the environment, when quantitative changes are transformed into qualitative, economical reactions that allow a body functioning within the limits of physiological norms. Therefore, by activating the «stress-limiting systems of the body» it is possible to provide adaptation to changing environmental conditions [2].

Thus, the adaptation is associated with a constant tension of physiological, neurochemical mechanisms and with the strengthening of constant disturbing stressful effects there is a depletion of physiological reserves and the emergence of a state of disadaptation.

Disadaptation is a peculiar state of mismatch between the body and the environment, leading to the disruption of physiological processes, behavioral reactions and development of pathologies [3].

Any pathological process represents a new unfavorable condition of existence in which the body has to function. For this purpose, endogenous mechanisms of adaptation aimed at maintaining homeostasis are included, despite the functional (and often anatomical) defect [4].

The leading role of exhaustion of regulatory systems under various stressors in the development of a number of pathological conditions is justified by the works of G. Selliers and is called the adaptation syndrome [5].

It has been established that a complex system of molecular changes is launched during the process of deadaptation, in which free radical processes leading to cell degradation and DNA damage occupy one of the key places.

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Lipid peroxidation and free-radical oxidation play an important physiological role: they participate in the process of renewing the composition and maintaining the functional properties of biomembranes; in the energy processes of the cell; in the process of cell division and intracellular signaling, as well as play a leading role in adaptation and deadaptation [6].

Free radicals can come from a variety of sources. Endogenous sources of free radicals include those in which they are generated intracellularly and show their action inside the cell, as well as those in which free radicals, forming intracellularly, are then released into the surrounding space and there they show their action. Endogenous free radicals are generated during oxidation and auto-oxidation of various molecules, in the chain of electron transport (terminal oxidation chain), in the functioning of various enzymes – oxidase, cyclooxygenase, lipoxygenase, dehydrogenase, etc. Free radicals can be generated in almost all cellular components: mitochondria, lysosomes, peroxisomes, endoplasmic reticulum, plasma membranes, as well as in some cytosol compartments.

The failure of biochemical regulation of the body in the process of disadaptation leads to the disturbance of homeostasis in the system of lipid peroxidation – antioxidants and free-radical reactions are out of control. In turn, this leads to progressive accumulation of damage in the body. Various stress factors trigger complex interrelated molecular processes that are involved in the survival and death of the cell. Free radical processes, which trigger cellular degeneration and death, play a key role in this survival system. Active oxygen forms (AOF) are formed in the body as products of biochemical reactions and cause multiple damaging effects and, above all, degradation and death of proteins, lipids, nucleic acids. Under the influence of AOF, membranes, collagen, DNA, chromatin, structural proteins are damaged; AOF participate in the epigenetic regulation of the expression of nuclear and mitochondrial genes affect the intracellular level of calcium, trigger a cascade leading to apoptosis, etc [7].

The pro-oxidant system is opposed to the antioxidant system. Antioxidants are substances that have the ability to interact with various reactive oxidants – active oxygen species and other free radicals – and cause their partial or complete inactivation [8].

Disadaptation leads to imbalance of pro-oxidant and antioxidant systems, leading to oxidative stress. In case of deadaptation, the endogenous system of antioxidant protection works inefficiently and the products of interaction between free radicals and macromolecules are constantly found in organs and tissues of the body, and cells are subjected to oxidative stress [9].

Oxidative stress caused by deadaptation, endogenous and exogenous factors and antioxidant system dysfunction causes 4 main damaging processes: oxidation of DNA, proteins, lipids and glucose oxidation. More than 100 conditions and diseases are considered, in which free radicals are an important pathogenetic factor, and among them the first place is occupied by pathological conditions and brain diseases, including those related to the state of deadaptation. The brain is the most vulnerable organ for the development of oxidative stress, as it has a high rate of metabolic processes, low rate of cell division, high content of lipids (more than 50 % of brain dry matter), in some areas a high content of iron and copper. The brain, despite its low weight, consumes about 30 % of oxygen and is hypersensitive to hypoxia, microcirculation disorders, changes in energy balance, etc. [10, 11].

In recent years, especially abroad, attention to substances with antioxidant effects has increased dramatically. The number of endogenous and exogenous substances classified as antioxidants is constantly growing.

In response to the intensification of free radical oxidation (FO) of biosubstrates, the antioxidant system (AOS) of the cell is activated. Glutathione reductase/glutathione peroxidase (GR/GP) system is the most important component of the body's antioxidant protection [12], which supports the intensity of free radical processes at the stationary level [13, 14]. Due to the functioning of GR/GP systems in mammalian cells, hydroperoxides and peroxides, which are the main source of hydroxyl radicals, are detoxified [15, 16].

Glutathione reductase is considered to be an enzymatic link in the body's endogenous antioxidant protection system. The substrate of glutathione reductase is oxidized glutathione, which is converted into reduced glutathione with the participation of НАДФ·Н It has also been established that glutathione itself has an independent antioxidant effect. Glutathione reductase is a flavin enzyme and is a dimer with a molecular weight from 50,000 to 55,000 D. It is known that dissociation to stable monomers leads to increased activity of GR. GR subunits have cross-linkages through disulfide groups. In addition to disulfide or thiols, there are arginine and histidine in the active site. Arginine parts of the molecule of glutathione reductase are responsible for the restoration of glutathione. Histidine in the active GR center functions as a proton donor in catalysis. Catalysis follows a two-stage mechanism through flavoprotein.

Enzyme activity increases with increased concentration of reduced forms of peridinucleotides and oxidized glutathione. Thus, glutathione reductase – glutathione peroxidase – forms a closed antiperoxidase complex in which peroxidase neutralizes peroxides to hydrogen and water, while glutathione oxidizes and glutathione reductase restores the oxidized glutathione, turning it into a substrate for glutathione peroxidase activity [17].

Taking into account the fact that most of the known pathological conditions are accompanied by the increase of CO [18] of biosubstrates and changes in the activity of AOS, the assessment of the condition of these systems for the purpose of early diagnosis and choice of tactics of treatment of diseases is a very urgent problem.

Thus, **the aim of our study was** to study the interrelationships between the distribution of glutathione reductase activity in blood serum and tissues of white non-pedigree rats.

In order to achieve this goal it is necessary to determine the activity of glutathione reductase in blood serum and tissues of liver, brain, heart, as well as in rat

skeletal muscle tissues; to reveal the interrelationships between the distribution of GR activity in blood serum and rat tissues.

2 Materials and methods

The study was carried out on white, non-patented, healthy male rats of one month of birth, weighing 190–210 g and contained in a vivarium under standard conditions.

Glutathione reductase activity was determined spectrophotometrically at a wavelength of 340 nm. The decrease in optical density during the reaction is the result of oxidation of PADFN in NADF due to the glutathione reduction reaction under the influence of the enzyme. The GR activity was measured in 50 mM potassium-phosphate buffer, pH 7.4 (Vecton, Russia), containing 1 mM EDTA (Vecton, Russia), 0.16 mM NADPH (AppliChem GmbH, Germany) and 0.8 mM GSSG (Carl Roth GmbH + Co. KG, Germany).

Glutathione reductase activity was studied in the liver, heart, brain and skeletal muscle tissue of rats, as well as in blood serum. For this purpose, rats were ethically murdered under etheric anesthesia by decapitation, and then the necessary tissues were extracted, which (except for blood serum) were washed with saline solution and immediately frozen. Homogenates were prepared by mechanical grinding of tissues weighing 1 g with 10 ml of tris-buffer (pH 7.4), at a rate of 3000 rpm in a vessel with double walls, constantly cooled by running water [19].

Digital material was statistically processed by nonparametric Spearman correlation analysis, as well as by using gamma correlation coefficients and Kendella Tau.

3 Results

As a result of the experiments, an array of numerical data on the activity of glutathione reductase in blood serum and rat tissues was obtained (Fig. 1). The obtained results were statistically processed (Table 1).

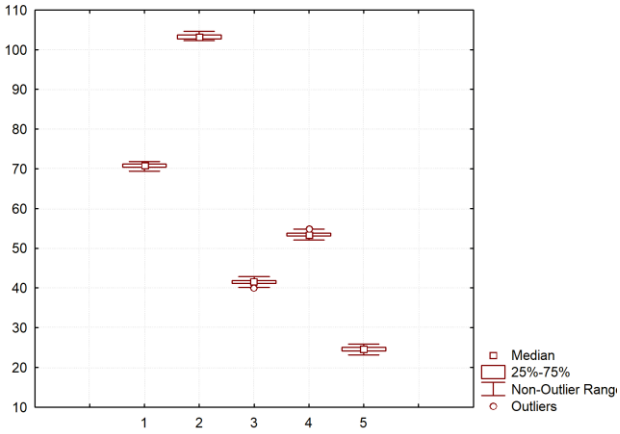

Fig. 1. Distribution of glutathione reductase activity in blood serum and rat tissues

At the first stage of the statistical analysis we tested for compliance with the normal distribution of GR activity in blood serum and rat tissues. For this purpose Kolmogorov-Smirnov single-sample criterion was used. As a result, it was found that the distribution of GR activity in blood serum and tissues does not correspond to normal. In connection with that at the further statistical processing we applied nonparametric methods of analysis.

In order to assess the relationship between the distribution of GR activity in blood serum and tissues of small experimental animals, we studied the correlations within the observation group using the nonparametric Spearman correlation coefficient (Table 2), as well as using the gamma correlation coefficients (Table 3) and Kendell Tau (Table 4).

According to the data presented in Table 2, there is no correlation between glutathione reductase activity in blood serum and tissues of white non-pedigree rats.

Since no correlation between the distribution of glutathione reductase activity in blood serum and rat tissues was revealed using the Spearman correlation coefficient, it was decided to carry out the analysis using the gamma correlation criteria (Table 3) and Kendella Tau (Table 4).

Table 1. Distribution of glutathione reductase activity values in the blood serum and tissues of white, unborn rats

Descriptive statistics of merged groups	N	M	Me	Min	Max	25 Perc	75 Perc	10 Perc	90 Perc
Blood serum	150	70.75	70.80	69.40	71.80	70.40	71.20	69.80	71.60
Liver	150	103.26	103.20	102.30	104.60	102.70	103.70	102.50	103.90
Brain	150	41.62	41.70	40.10	42.90	41.20	41.90	40.80	42.40
Heart	150	53.44	53.40	52.10	54.90	53.10	53.80	52.60	54.30
Skeletal muscles	150	24.65	24.60	23.10	25.90	24.20	25.10	23.80	25.70

Table 2. Spearman's correlation coefficient for GR activity distribution in blood serum and rat tissues and p

Spearman correlation in all combined dimensions	Valid N	Spearman R	p-level
Blood serum & liver	150	0.083693	0.308567
Blood serum & brain	150	–0.052943	0.519936
Blood serum & heart	150	0.059254	0.471360
Blood serum & muscle	150	–0.024625	0.764856

Table 3. Gamma-ray correlation coefficient on the distribution of GR activity in blood serum and rat tissues

MD pairwise deleted Marked correlations are significant at p <0.05000				
	Valid N	Gamma	Z	p-level
Blood serum & liver	150	0.064887	1.100367	0.271172
Blood serum & brain	150	–0.041535	–0.706306	0.479998
Blood serum & heart	150	0.045872	0.780006	0.435388
Blood serum & muscle	150	–0.022371	–0.380995	0.703207

Table 4. Kendella Tau's coefficient of correlation on the distribution of GR activity in serum and rat tissues

MD pairwise deleted Marked correlations are significant at p <0.05000				
	Valid N	Kendall Tau	Z	p-level
Blood serum & liver	150	0.060596	1.100367	0.271172
Blood serum & brain	150	–0.038895	–0.706306	0.479998
Blood serum & heart	150	0.042954	0.780006	0.435388
Blood serum & muscle	150	–0.020981	–0.380995	0.703207

According to the data presented in Tables 3 and 4, it can be seen that the correlation between the activity of glutathione reductase in blood serum and white non-pedigree rat tissues was not revealed when studying the distribution of GR activity in blood serum and rat tissues using gamma correlation coefficients and Kendella Tau.

4 Conclusion

Thus, all three methods of nonparametric correlation analysis used to assess the relationship between the distribution of glutathione reductase activity in blood serum and rat tissues did not reveal any correlation between GR activity within the physiological norm in blood serum and tissues of small experimental animals.

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