Relationship of catalase activity distribution in serum and tissues of small experimental animals

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Abstract. In the process of physiological activity of the body, a wide range of toxic metabolites is formed, in particular, hydrogen peroxide, superoxide and hydroxyl radical, which have a destructive effect on cells and cell membranes. One of the natural body protection mechanisms is the enzyme catalase, which accelerates the decomposition of constantly formed hydrogen peroxide to the final products, while oxidizing low molecular alcohols and nitrites, involved in the process of cellular respiration and does not require energy for activation. Catalase activity in blood and tissues is a marker of metabolic disorders. Catalase is localized mainly in the peroxisomes and cytoplasm of the cell, saturates the erythrocytes, liver and kidneys. Catalase activity allows assessing the level of endotoxicosis in the body. In order to study the relationship of the distribution of catalase concentration in serum and rat tissues, the following tasks were solved: the concentration of catalase in serum and tissues of the liver, brain, heart, as well as in skeletal muscle tissues of rats was determined; the correlation of catalase concentration distribution in blood serum and tissues of rat was revealed. The paper presents the results of nonparametric correlation analysis to assess the relationship of catalase activity distribution in serum and tissues of small experimental animals.

1. Introduction
The antioxidant defense system of the body is one of the multifactorial mechanisms of homeostasis maintenance. Its main component is a network of enzymes that controls the processes of free radical oxidation initiated by active oxygen species.

The set of various factors accompanying the processes of life, from negative environmental conditions to depressive emotional states, leads to the formation of pathological changes in the body, which are accompanied by increased formation of free radicals [1].

Free radicals and compounds that can lead to their formation in the course of chain reactions are considered within a single group, which is designated as reactive oxygen species (ROS), thereby emphasizing its ability to lead to various oxidative processes in the cell. ROS can be provisionally divided into two main groups: with low activity and high activity. Low-activity compounds are formed in small amounts during normal aerobic metabolism, and the damage that can be caused by it is easily corrected by the cell itself without additional intervention. However, in some cases, even low-level
oxygen species, such as superoxide radical, by oxidative reactions can be transformed into more aggressive compounds with the participation of some metals as coenzyme [1,2].

Free radical oxidation is a necessary condition for the renewal of cytoplasmic membranes and its normal functioning, so its low level is characteristic of any living organism. The level of lipid peroxidation can change, since it is a consequence of the interaction of two opposite mechanisms – the activity of free radicals and the activity of antioxidant systems that reduce its activity. Normally, in a healthy body, the formation of reactive oxygen species (ROS) occurs continuously. ROS and other prooxidants are involved in mechanisms of bactericidal activity, in synthesis of biologically active substances, in collagen metabolism, regulation of membrane permeability, in mechanisms of apoptosis, modulation of immune response, in inflammatory processes and gene expression.

Almost all functions and elements of the structure of a living cell are associated with the process of peroxidation of lipids (POL) – the structure and renewal of cell membranes, processes of phagocytosis and pinocytosis, synthesis of biologically active substances (prostaglandins, progesterones, steroid hormones, cholesterol, thromboxanes), regulation of oxidative phosphorylation, cell division processes. The speed of the process depends on the structural organization of lipids in the body, all factors that destroy the structural shell of lipids accelerate POL. Regulators of the POL system are the enzymes affecting reactive oxygen species in the body (oxidase, superoxide dismutase) and convert peroxides without isolation of the free radicals (catalase, peroxidase).

Under the conditions of pathological processes, the peroxide balance is disturbed, there is an accumulation of metabolites that change primarily the functional activity of the membranes-permeability, electrical stability, there is also a change in the structure of serum lipoproteins, hypercholesterolemia appears, and, consequently, homeostatic equilibrium changes at all metabolic levels. POL products (peroxides, aldehydes, ketones, acids, carbonyl compounds) are toxic and cause destruction of nucleic acids and coenzymes, modification of biomolecules, leading to severe diseases, sometimes by united term “free radical pathology”. Accordingly, for therapeutic or prophylactic purposes, antioxidant substances are used, which capture free radicals and activate endogenous mechanisms of protection of the body from oxidative processes [1,2,3].

Antioxidants are located in different tissue structures and cellular components, and have different substrate specificity, but they all have an affinity for active forms of oxygen and provide comprehensive protection of biopolymers. Although the process of peroxidation develops in the form of chain reactions in the lipid phase of membranes and lipoproteins, the initial (and possibly intermediate) stages of this complex system of reactions occur in the aqueous phase. Part of the protective systems of the cell is also localized in the lipid phase, and part – in the aqueous phase. Depending on this, we can talk about water-soluble and hydrophobic antioxidants.

Antioxidant protection can be carried out, firstly, by the direct removal of reactive oxygen species (physiological component of antioxidant protection), and secondly, by neutralizing active oxygen/nitrogen by interacting with their unpaired electrons (biochemical component of antioxidant protection). The third option may be inhibition of ROS production. Another protection option may be the effect on intermediate links, namely the binding of those metals that are necessary for the catalysis of ROS and regulation of endogenous oxidant protection.

Antioxidants can be divided into endogenous, which protect against those free radicals that are produced in a small amount (5 %) in the normal life of the cell, as well as exogenous.

The choice of antioxidant depends on the type of ROS that is formed in a particular process, the place of formation of free radicals (i.e. it is necessary to take into account the possibility of penetration of the oxidant to the action, for example, through the blood-brain barrier) and the severity of the injury.

There are enzymatic and nonenzymatic components of the antioxidant system. The enzymatic link is represented by glutathione peroxidase, superoxide dismutase, catalase, aldehyde dehydrogenase and sulfiredoxin. There is a certain specialization in relation to specific types of radicals and peroxides [2,3].
One of the indicators of the efficiency of the functioning of a warm-blooded organism is the activity of catalase. Catalase is a chromoprotein, a tetramer of four polypeptide chains comprising more than 500 amino acids, and also includes four groups of porphyrin heme capable of oxidizing by active oxygen species. Oxidized heme is a prosthetic group of catalases [4,5]. This is the first link of intracellular protection against reactive oxygen species [6]. The main function of catalase is the neutralization of the anion-radical $O_2^-$, hydroxyl radical, radicals of unsaturated fatty acids (of lipophilicity), the splitting of hydrogen peroxide generated during cellular respiration, to molecular oxygen and water. Catalase is an enzyme belonging to the class of oxidoreductases that catalyzes the heterolytic cleavage of the O-O bond in hydrogen peroxide. Although catalase is the main enzyme that eliminates excess amounts of hydrogen peroxide, however, due to its low affinity to the substrate, it is effective only at high concentrations of $H_2O_2$.

At low concentrations of hydrogen peroxide, catalase is able to catalyze its reduction only in the presence of additional hydrogen donors, such as ethanol, formic or ascorbic acids. Unlike other enzymes that contribute to the elimination of hydrogen peroxide, catalase does not require additional substrate and energy for activation and despite this is a highly active enzyme. Catalase is always present in systems where transport of electrons involving cytochromes is carried out, i.e. where hydrogen peroxide, toxic to the cell, is formed. It is localized mainly in cell peroxisomes and cytoplasm [5,6].

Thus, catalase is one of the main elements of antioxidant protection of the body, providing a dynamic balance between the formation of reactive oxygen species and its elimination. Catalase is formed in large quantities in organelles (peroxisomes) and cytoplasm of cells. High content of the representative of the class of oxidoreductases was noted in the liver, kidney, erythrocytes in homeothermic organisms [7,8,9,10].

In the development of pathology are leading membrane-destructive processes, including processes of free radical oxidation, determining the degree of endotoxicosis, and accordingly the level of oxidative stress. Currently, oxidative stress is recognized as one of the reasons for the formation of a number of pathological conditions – malignant neoplasms, atherosclerosis, diabetes mellitus, neurodegenerative brain lesions, pathologies associated with adverse environmental conditions [11].

The number and dynamics of catalase activity can indirectly judge the state of the body, the degree of oxidative stress and the level of endogenous intoxication, which are the result of pathological internal or external processes. For example, an indicator of some oncological diseases and neuropathologies (Parkinson’s disease) is a decrease in catalase activity in the kidneys and liver [12,13,14,15,16]. The activity of antioxidant enzymes protection, including catalase, is associated with the adaptation of heart tissue to hypoxia, which means a decrease in the likelihood of heart attack and ischemia [17,18,19].

Reduction of catalase activity occurs in excess of methionine, cystine, copper, zinc. In particular, the activity of catalase affects the age of the body, in young tissues the enzyme acts more effectively. Catalase activity is expressed by catalani number and is determined by the gauge or polarographic method with fixing the resulting biochemical reactions of oxygen. Spectrophotometric and titrimetric methods based on the measurement of the current concentration of hydrogen peroxide are also used. Thus, the analysis of catalase activity allows using this parameter to assess the degree of disturbance of homeostasis of the body. [20].

All of the abovementioned suggests that the assessment of this indicator is necessary to determine the causes and mechanisms of the development of one or another pathological process and offers ways to treat diseases. Similar studies to identify the correlation of catalase activity distribution in serum and tissues outside pathological processes in small experimental animals have not been conducted.

Thus, the aim of our research was to study the relationship of catalase activity distribution in serum and tissues of white mongrel rats.
To achieve this goal, it was necessary to solve the following tasks: to determine the activity of catalase in serum and tissues of the liver, brain, heart, as well as in skeletal muscle tissues of rats; to identify the relationship of the distribution of catalase activity in serum and tissues of rats.

2. Materials and methods

The study was carried out using white mongrel sexual mature healthy male rats of one month of birth, weighing 190-210 g in the amount of 150 pieces, which were contained in the vivarium under standard conditions.

Determination of catalase activity was carried out by the standard method of Koroluk M. A. [21]. The reaction was started by adding 0.1 ml of homogenate (blood serum) to 2 ml of 0.03% hydrogen peroxide solution. Distilled water was added to the blank sample instead of the test liquid. The reaction was stopped after 10 minutes by adding 1 ml of 4% ammonium molybdenum. Color intensity was measured on photoelectrocolorimeter at a wavelength of 410 nm on the background of a control sample (2 ml of water, 0.1 ml of blood plasma, 1 ml of ammonium molybdenum).

Catalase activity is calculated by formula 1:

\[ E = \frac{A_{\text{blank}} - A_{\text{test}}}{V + t \cdot K} \]  

Calculation: (mkat/l), where

E - catalase activity, mkat/l;
A - optical density of blank and test samples;
V - volume of introduced samples;
t - incubation time;
K - coefficient of millimolar extinction of hydrogen peroxide, equal to 1.6·10^5.

Catalase concentrations were studied in the liver, heart, brain and skeletal muscle tissue of rats, as well as in serum. To do this, the rats were killed in accordance with ethical norms under ether anesthesia by decapitation, then the necessary tissues were extracted, which (except for blood serum) were washed by saline solution and immediately frozen. Homogenates were prepared by mechanical grinding of tissues weighing 1 g with 9 ml of tris-buffer (pH 7.4), at a speed of 5000 rpm in a vessel with double walls, constantly cooled by running water [21].

The digital material was statistically processed by nonparametric Spearman correlation analysis, as well as using gamma correlation coefficients and Kendall Tau.

3. Results of the study

As a result of the experiments, an array of numerical data of catalase activity in blood serum and tissues of rats was obtained. The results were subjected to statistical processing (table 1). At the first stage of statistical analysis, the normal distribution of catalase activity in blood serum and tissues of rats was checked. The Kolmogorov – Smirnov single-sample criterion was used for this purpose. As a result, it was found that the distribution of catalase activity in serum and tissues does not correspond to normal. In this regard, in the further statistical processing we have used nonparametric methods of analysis.

| Table 1. Distribution of catalase activity values in serum and tissues of small experimental animal. |
| --- |
| **Descriptive statistics of combined groups** | N | M | Me | Min | Max | 25 Perc | 75 Perc | 10 Perc | 90 Perc |
| Blood serum | 150 | 19.72 | 19.60 | 17.40 | 22.10 | 18.90 | 20.40 | 18.35 | 21.40 |
| Liver | 150 | 58.04 | 58.20 | 55.40 | 60.40 | 57.30 | 58.90 | 56.20 | 59.70 |
| Brain | 150 | 11.08 | 11.20 | 9.20 | 12.70 | 10.50 | 11.70 | 9.75 | 12.20 |
| Heart | 150 | 29.25 | 29.40 | 27.10 | 30.90 | 28.50 | 30.10 | 28.10 | 30.60 |
| Skeletal muscle | 150 | 35.20 | 35.20 | 33.10 | 37.40 | 34.50 | 35.80 | 33.70 | 36.45 |
To assess the relationship between the distribution of catalase activity in serum and tissues of small experimental animals, a study of correlations within the observation group was carried out using Spearman’s nonparametric correlation coefficient (table 2), and using gamma correlation coefficients (table 3) and Kendall Tau (table 4).

### Table 2. Spearman correlation coefficient on catalase activity distribution in blood serum and tissues of rats and p value.

| Correlation by Spearman all the joint measurements | Valid N | Spearman R | p-level |
|----------------------------------------------------|---------|------------|---------|
| Blood serum & liver                                | 150     | 0.053101   | 0.518689|
| Blood serum & brain                                | 150     | -0.054795  | 0.505419|
| Blood serum & heart                                | 150     | -0.072130  | 0.380397|
| Blood serum & muscles                              | 150     | -0.205246  | 0.011749|

According to the data presented in table 2 it is clear the presence of weak forces is a significant inverse correlation between the catalase activity in serum and skeletal muscle tissue (of 0.21 at p ≤ 0.011749).

Since no other relationships between catalase activity in blood serum and tissues of rats were detected using Spearman correlation coefficient, it was decided to conduct an analysis using gamma correlation criteria (table 3) and Kendall Tau (table 4).

### Table 3. Gamma correlation coefficient for distribution of catalase activity in blood serum and tissues of rats.

| MD pairwise deleted Marked correlations are significant at p <.05000 | Valid N | Gamma | Z     | p-level |
|---------------------------------------------------------------------|---------|-------|-------|---------|
| Blood serum & liver                                                | 150     | 0.040228 | 0.70940 | 0.478076 |
| Blood serum & brain                                                 | 150     | -0.040822 | -0.71553 | 0.474282 |
| Blood serum & heart                                                 | 150     | -0.050561 | -0.88672 | 0.375229 |
| Blood serum & muscles                                               | 150     | -0.144658 | -2.54344 | 0.010977 |

### Table 4. Kendall Tau coefficient correlation of catalase activity distribution in serum and tissues of rats.

| MD pairwise deleted Marked correlations are significant at p <.05000 | Valid N | Kendall Tau | Z     | p-level |
|---------------------------------------------------------------------|---------|-------------|-------|---------|
| Blood serum & lever                                                | 150     | 0.039066    | 0.70940 | 0.478076 |
| Blood serum & brain                                                 | 150     | -0.039403   | -0.71553 | 0.474282 |
| Blood serum & heart                                                 | 150     | -0.048831   | -0.88672 | 0.375229 |
| Blood serum & muscles                                               | 150     | -0.140064   | -2.54344 | 0.010977 |

According to the data presented in tables 3 and 4, it can be seen that there is only one significant weak inverse correlation between the activity of catalase in serum and skeletal muscle tissues.

### 4. Conclusions

Thus, all three applied methods of nonparametric correlation analysis for assessing the relationship between the distribution of catalase activity in the blood serum and tissues of small experimental animals revealed that only weak inverse correlation between the activity of catalase in blood serum is determined reliably with catalase activity in rats within the physiological norm and skeletal muscle tissue.

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