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Age-Dependent Changes of Redox Ratio in Rat Brain Parts, Internal Organs and Blood Plasma

Natalia Andreevna Krivova[1*], Olga Borisovna Zaeva[1], Irina Vladimirovna Sukhodolo[2], Ivan Vasilevich Milto[2] and Valeria Aleksandrovna Grigorieva[1]

[1]Laboratory of Experimental Physiology, Institute of Biology and Biophysics, Tomsk State University, 36 Lenina Ave, Tomsk, Russian Federation; nakri@res.tsu.ru

[2]Department of Morphology and General Pathology, Siberian State Medical University, Moskovsky Trakt 2, Tomsk, Russian Federation

Abstract

Aging of the body is accompanied by a gradual development of the oxidative stress. However, this process has different rates in various systems of the body; so, it seems interesting to compare the levels of oxidative stress in different systems during aging. The luminol-dependent chemiluminescence method with the Fenton reaction was used to investigate the antioxidant capacity. The light sums of chemiluminescence for antioxidant capacity and free radicals of the oxidized molecules were determined to find the ratio of these sums (redox ratio) in the indicated tissues. Changes in the light sums of chemiluminescence for antioxidant capacity and free radicals in the internal organs generally agree with the commonly accepted ideas on the role of oxidative stress in aging. All the studied parts of the brain showed a substantial increase in the light sums of chemiluminescence for antioxidant capacity and free radicals. A comparison of the level of free radicals and the antioxidant capacity has revealed differences in their ratio in the internal organs, blood plasma and brain parts during aging and different levels of oxidative stress. Results of the comparative study gave grounds to suggest that the brain has some other components with antioxidant capacity, which are absent in the heart, lungs, liver and skeletal muscles. Such components in the brain may be glycan, which possess the antioxidant capacity and are widely represented in the brain parts.

Keywords: Aging, Antioxidant Capacity, Brain, Internal Organs

1. Introduction

Aging is an inevitable stage of ontogenesis; however, the rate of age-related changes differs for different systems of the body. The idea of non-uniform aging was supported by studying the biological processes at different levels of vital activity of the body. If all processes occurred at equal rates upon aging, this would lead to a rapid extinction of the vital activity. This is exactly the non-uniformity of changes that maintains activity of the body during aging. Weakening of some control units and mobilization of others is the leading factor of the body adaptation upon aging1. Among the mechanisms underlying the aging process is the oxidative damage caused by free radicals[2].

Manifestation of the oxidative stress in various organs is determined by their structural, functional and metabolic features, which may produce different oxidative damages. Thus, it was found that in aged rats the amount of diene conjugates in heart tissue was four times smaller as compared to brain tissue; however, the level of malonic dialdehyde in brain was lower than in liver and heart, and Superoxide Dismutase (SOD) and Glutathione Transferase (GST) activities in brain were substantially lower than in liver and virtually did not differ from activities of these enzymes in young rats[3]. Opposite results were obtained by4: young rats showed a higher MDA content in brain as compared to heart and lungs; in aged rats this difference was even more pronounced. These

* *Author for correspondence*

data are not useful for determination and comparison of redox homeostasis in different organs because features of the interaction between pro- and antioxidant mechanisms in different systems should also be taken into account. For example, antioxidant enzymes decrease the amount of ROS and thus control the formation of the oxidized forms of macromolecules; and/or the oxidized macromolecules interact with each other, which leads to their mutual inactivation, therewith, they go out of metabolic processes and decrease functional activity of the organ; other, yet unknown types of the interaction are also possible. As was demonstrated by Yoshida et al.[5]: "antioxidant capacity in vivo is determined by several factors such as bioavailability, metabolism, localization, distribution, fate of antioxidant-derived radicals, and interaction with other antioxidants as well as reactivity toward free radicals. It is important to elucidate each factor by employing basic studies for the sound interpretation of experimental results. However, it is quite difficult to estimate the antioxidant capacity in vivo based on these individual factors". It became clear that there is a wide variety of redox mechanisms, which have systemic and tissue features; so, they should be supplemented with the proteomic approach and new information technologies.

Ortuno-Sahagun et al.,[6] noted that: "it is unquestionable that the step-by-step approach of studying one gene or one protein at a time, even if their partners could eventually be unveiled, is a parsimonious endeavor. Therefore, a more integral and holistic approach is required. Within this context, genomic and proteomic studies, mainly by microarray for messenger RNA (mRNA) and Two-Dimensional (2D) electrophoresis for protein expression profiles, would eventually elicit the comprehension of the entire process of cell function, and also that at tissue and organ levels, which in turn will provide us with a wider panorama and lead us to a more comprehensive understanding of the aging mechanisms and the intrinsic role of Reactive Oxygen Species (ROS) at a molecular level" . This may open new prospects for understanding the molecular mechanisms of aging. However, a strictly molecular approach has not yet provided significant advantages for the development of therapeutic and/or preventive agents for aged patients. Howes[7] noted that: "Overly exuberant and exaggerated past expectations and claims of the free radical theory have been quieted by extensive randomized, double-blind, controlled human studies. A half century of data demonstrates its lack of predictability and it has not been validated by the scientific method. Widespread use of antioxidants has failed to quell the current pandemic of cancer, diabetes, and cardiovascular disease or to stop or reverse the aging process. Electronically modified oxygen derivatives contribute to the modulation of cellular redox status, which is of primary importance in disease prevention and homeostasis." Not only the cellular redox status, but also the tissue and organ redox status are of functional importance for physiological state of organs and systems. These characteristics may be essential for studying the non-uniformity of aging processes and the development of compensatory mechanisms that provide "healthy aging". To this end, it seems reasonable to investigate the redox status in brain, internal organs and blood system of aged animals.

The level of oxidative stress can be estimated also by ChemiLuminescence (CL) analysis, which is based on the primary physiological effect of free radicals. The reviews on the application of luminal-dependent chemiluminescence for investigation of reactive oxygen species and antioxidant capacity in biological and model systems revealed many limitations and advantages of this method[8–12]. An important advantage of CL analysis is its high sensitivity; so, applications of this method are being constantly developed in new fields. The main limitation of luminal-dependent chemiluminescence analysis is non-specificity of the signal. Thus, CL analysis is often developed for specific tissue specimens using various analytical procedures, enzymes and other methods to restrict the reacting components[13–16].

However, to compare redox status in different systems, the study is likely to be performed under similar conditions. Thus, in the present study, results of analysis of the redox ratio in different brain parts, internal organs and blood plasma of adult and aged rats are reported. The antioxidant and pro-oxidant activities were investigated under similar conditions of luminal-dependent chemiluminescence.

2. Materials and Methods

2.1 Animals

All animal procedures were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the Animal Research Ethics Committee at the Institute of Biology and Biophysics, Tomsk State University.

Male Wistar rats, 2 and 10 months of age, were purchased from a nursery at the Institute of Pharmacology, Siberian Division of the Russian Academy of Medical Sciences. The rats were kept in an isolated and ventilated room in the vivarium at the Institute of Biology and Biophysics, Tomsk State University. A temperature of 20 ± 2.0°C and air humidity of 60% were maintained in the room with a 12 h light: 12 h dark diurnal cycle. The animals were housed in 1,575 cm2 cages during 4 weeks prior to sacrifice. Young adult rats (2 months of age) were housed in groups of 5 rats per cage, while aged rats (10 months of age) in groups of 2–3 rats per cage. All the animals were given ad labium access to food and water (a standard rat diet). Prior to sacrifice, all the animals used in this experiment showed no signs of diseases according to the state of their fur, eyes, respiratory rate, and behavior.

2.2 Experimental Design

Rats were monitored during a four week period. Two groups of rats were used, with 10 animals in each group. Animals from the first group ("young adult rats") were sacrificed at the age of 3 months (body weight of 310 g). Animals from the second group ("aged rats") were sacrificed at the age of 11 months (body weight of 570 g). Prior to sacrifice, rats were weighed, temperature of the body nucleus was measured, and exercise tolerance (time of hanging on a metal grid with the mesh size 1.5×1.5 mm, an average of three attempts) was evaluated. Prior to sacrifice, the rats received light inhalation anesthesia. Blood was collected from femoral vein to prepare blood plasma. Rats were sacrificed instantly with a guillotine.

2.3 Tissue Homogenates

Immediately after sacrifice, the brain was harvested to isolate the olfactory bulb, cerebellum, pons, frontal lobe cortex, mesencephalon and thalamic area. Samples (up to 150 mg in weight) of the isolated brain parts were weighed, placed in 1 mol of cold normal saline solution, and stored at a temperature of –20°C prior to assay. After the isolation of femoral muscle, lungs, heart, testicles and liver, samples (up to 150 mg in weight) were weighed, placed in 1 mol of cold normal saline solution (0.15 M NaCl) and stored at –20° C prior to assay.[17] Blood plasma samples were also stored at a temperature of –20°C. At the day of assay, the tissue samples were defrosted, brought to a concentration of 50 mg tissue/1 mL with normal saline, and homogenized. The homogenates were centrifuged for 25 minutes at 8000 g. The resulting supernatants were used to determine antioxidants and oxidants in each studied tissue. The assay was performed by a pairwise method with the same samples from young adult and aged rats.

2.4 Chemiluminescence Analysis of Oxidants and Antioxidants

The study was carried out on a Lumat LB 9507 (Berthold Technologies) two-flask high-resolution chemiluminometer with spectral sensitivity in the wavelength range of 390 to 620 nm. The intensity of Chemiluminescence (CL) was recorded in Relative Light Units (RLU). The intensity of luminol-dependent CL was measured for 5 minutes.

2.4.1 Antioxidants

Antioxidant capacity of the tissue samples was estimated using the modified method[15], where a source of radicals — the conjugate of Horseradish peroxidase with goat anti-mouse immunoglobulin — was replaced by the radicals produced by the Fenton reaction at the alkaline pH.[12] In the radical-producing system, 1 mL H_2O contains 30 µL of a 0.01 M luminal solution in a phosphate buffer pH 8.5, 20 µL of a 0.05 M $FeSO_4$ solution, and 10 µL of 0.1 M H_2O_2. 1 mL of the radical-producing system was introduced into each of two Lumat LB 9507 flasks; the first flask was then supplemented with 10 µL H_2O, and the second flask with 10 µL of supernatant of the test sample. The CL intensity was measured in the first and second flasks during 5 minutes. The difference between CL intensities of the radical-producing system (1st flask) and the radical-producing system supplemented with supernatant of the test sample (2nd flask) was used to plot a CL intensity curve characterizing the Antioxidant Capacity (AC). The light Sum (Sm) of CL AC was found as the area under the curve using the Microsoft Excel program. The dimensions of Sm CL AC were presented as units/g tissue, where units = RLU·10^9.

2.4.2 Oxidants

Free radicals (reactive oxygen species – ROS) were determined from the activated luminal chemiluminescence under the same CL conditions. 1 mL

H_2O of the system for ROS measurements contains 30 µL of a 0.01 M luminal solution in a phosphate buffer pH 8.5 mL of the system was placed into a Lumat LB 9507 flask and supplemented with 10 µL of supernatant of the test sample; CL intensity was measured for 5 minutes. After plotting the CL intensity curve for ROS characterization, Sm CL ROS was found as the area under the curve using the Microsoft Excel program. The dimensions of Sm CL ROS were presented as units/g tissue, where units = RLU·10^9.

2.4.3 Redox Ratio

The redox ratio was found as the quotient of Sm CL AC and Sm CL ROS in the same sample.

2.5 Statistical Analysis

Statistical analysis was carried out using the Statistical 6.0 software package for nonparametric samples. The data were expressed as the mean value ± SD. The groups were compared using the nonparametric Mann–Whitney test; a comparison of the internal organs or brain parts within a group was performed with the Wilcoxon test. The p values of < 0.05 were considered to be significant. The Microsoft Excel program was used for processing the CL intensity curves and preparing the documents.

3. Results

3.1 Characteristics of Young Adult and Aged Animals

The body weight of young adult rats prior to sacrifice was 311±10.7 g, and in aged rats 571±22.6 g ($p < 0.05$). The body temperature was equal to 37.1±0.1°C in both groups. Exercise tolerance (time of hanging on a metal grid) in young adult rats was 9.5±2.5 s, and in aged rats 1.2±0.1 s ($p < 0.05$). These values correspond to normal physiological state of the young adult and aged rats.

3.2 Redox Ratio in Brain Parts of Young Adult and Aged Rats

According to the results obtained, Sm CL AC was higher than Sm CL ROS in all the studied brain parts of young adult and aged rats, and this determined a high level of the redox ratio (Figures 1 and 2).

The highest level of redox ratio within each age group was noted in the frontal lobe cortex. Therewith, in the group of young adult rats, a significantly decreased redox ratio was observed in the olfactory bulb and pons as compared to the frontal lobe cortex. In aged rats, the redox ratio decreased significantly in the pons and cerebellum with respect to the frontal lobe cortex. These differences can be attributed to different arrangement of cyto- and myeloarchitectonics of the studied parts. In aged rats, all the studied characteristics (Sm CL AC, Sm CL ROS, and redox ratio) in all brain parts significantly exceeded those of young adult rats (except the redox ratio in mesencephalon).

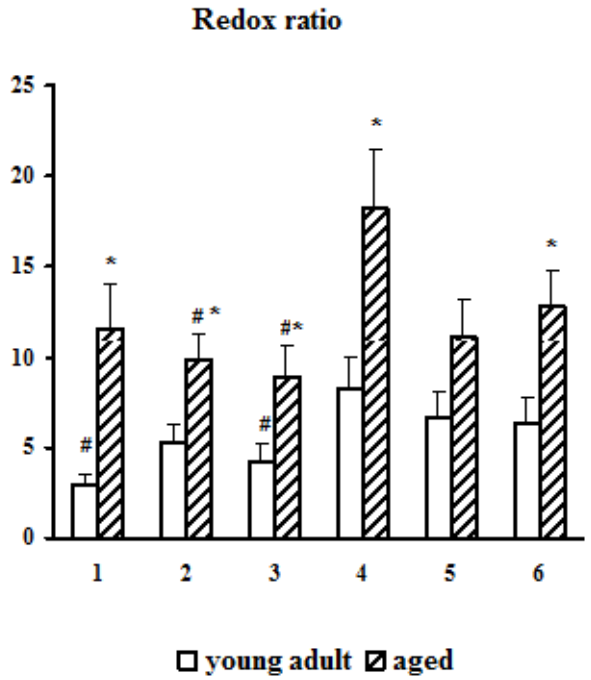

Figure 1. Redox ratio in the brain parts in young adult and aged rats. Number of young adult rats (n = 10) and aged rats (n = 10). Brain parts: 1 – olfactory bulb, 2 – cerebellum, 3 –pons, 4 – frontal lobe cortex, 5 – mesencephalon, 6 – thalamencephalon. Data are presented as the mean value ± SD. * $p < 0.05$ (the differences between groups of young adult and aged rats). # $p < 0.05$ (the differences between brain parts within each age group with respect to the frontal lobe cortex

3.3 Redox Ratio in Skeletal Muscles and Internal Organs of Young Adult and Aged Rats

Opposite changes were found when studying the internal organs of aged rats (Figures 3 and 4).

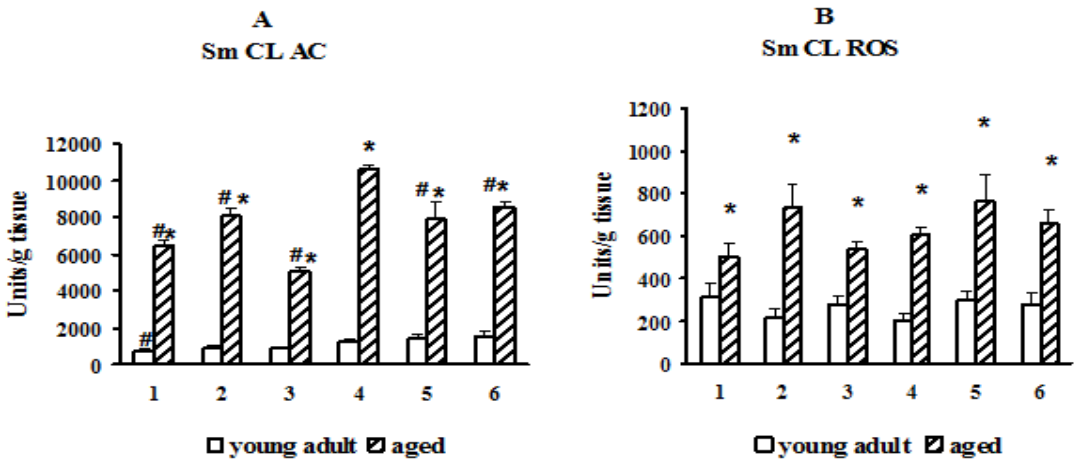

Figure 2. The light sums of chemiluminescence for antioxidants (A, Sm CL AC) and oxidants (B, Sm CL ROS) in the brain parts in rats. Number of young adult rats (n = 10) and aged rats (n = 10). Brain parts: 1 – olfactory bulb, 2 – cerebellum, 3 –pons, 4 – frontal lobe cortex, 5 – mesencephalon, 6 – thalamencephalon. Along the Y axis, units = RLU·109 (RLU is the relative light units). Data are presented as the mean value ± SD. * $p < 0.05$ (the differences between groups of young adult and aged rats). # $p < 0.05$ (the differences between brain parts within each age group with respect to the frontal lobe cortex).

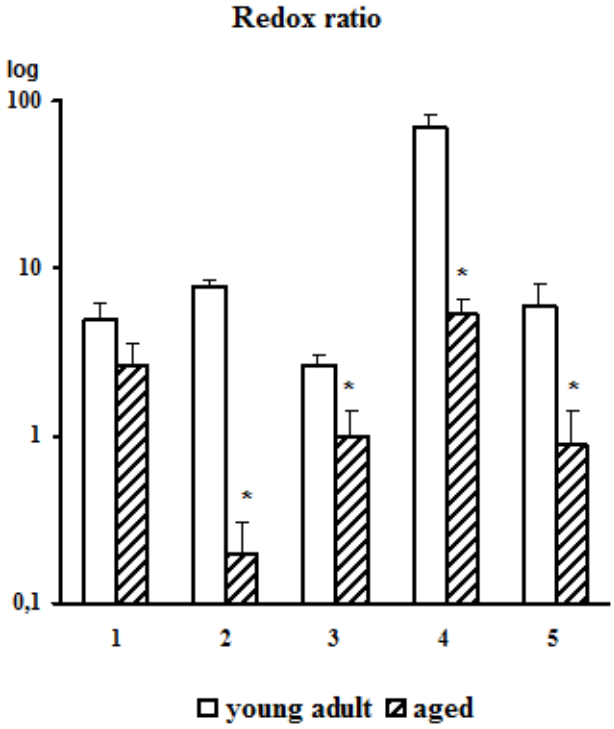

Figure 3. Redox ratio in the internal organs in young adult and aged rats. Number of young adult rats (n = 10) and aged rats (n=10). Internal organs: 1 – femoral muscle, 2 – lungs, 3 – heart, 4 – testicles, 5 – liver. The logarithmic Y axis. Data are presented as the mean value ± SD. * $p < 0.05$ (the differences between groups of young adult and aged rats).

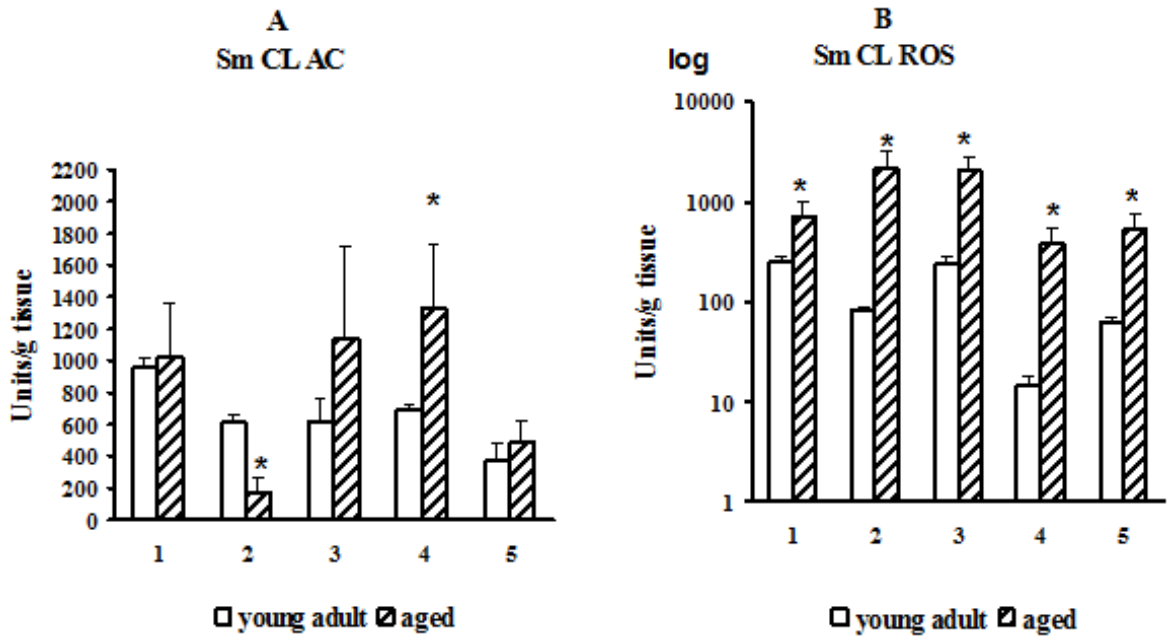

Figure 4. The light sums of chemiluminescence for antioxidants (A, Sm CL AC) and oxidants (B, Sm CL ROS) in the internal organs in rats. Number of young adult rats (n = 10) and aged rats (n = 10). Internal organs: 1 – femoral muscle, 2 – lungs, 3 – heart, 4 – testicles, 5 – liver. The logarithmic Y axis for plot B. Units = RLU·109 (RLU is the relative light units). Data are presented as the mean value ± SD. * $p < 0.05$ (the differences between groups of young adult and aged rats).

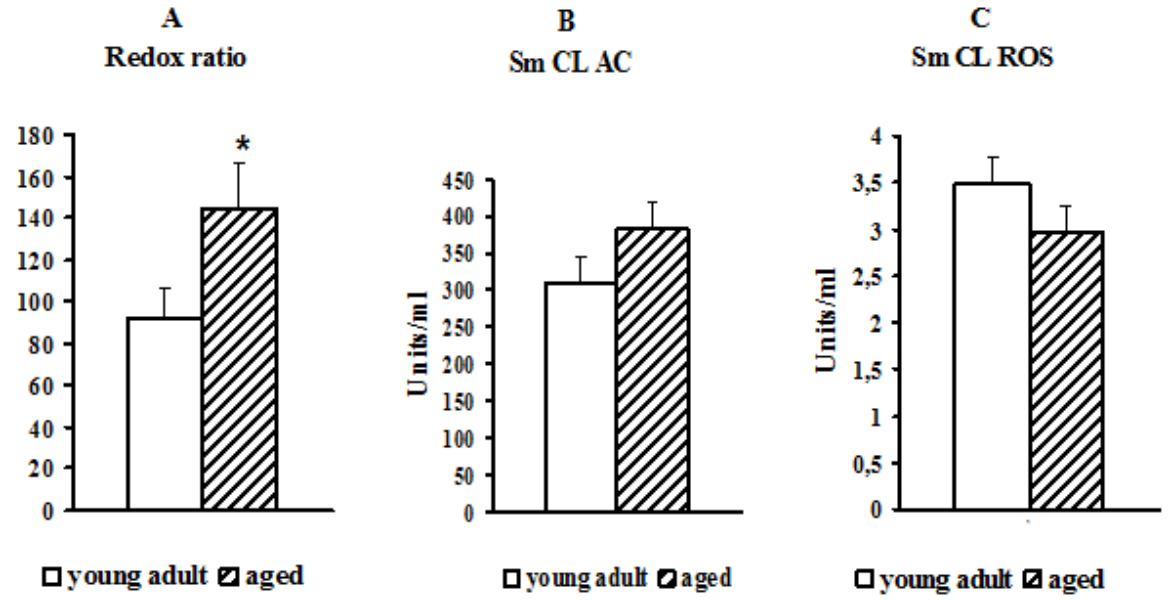

Figure 5. Redox ratio (A), light sum of chemiluminescence for antioxidants (Sm CL AC) (B), and light sum of chemiluminescence for oxidants (Sm CL ROS) (C) in blood plasma of young adult (n = 10) and aged (n = 10) rats. Along the Y axis, units = RLU·109 (RLU is the relative light units). Data are presented as the mean value ± SD. * $p < 0.05$ (the differences between groups of young adult and aged rats).

The redox ratio decreased in aged rats with respect to young adult animals for all the studied organs except skeletal muscle. In all the studied internal organs of aged rats, Sm CL ROS increased in comparison with young adult animals. Their Sm CL AC significantly decreased in the lungs, did not change in homogenates of the skeletal muscle, heart and liver, and increased only in testicles with respect to young adult animals (Figures 3 and 4). Nevertheless, the increase of Sm CL AC in the testicles of aged rats was less pronounced than the increase of Sm CL ROS; so, redox ratio in the testicles of aged rats also decreased in comparison with young adult rats, but remained at the highest level with respect to all other organs examined in the study.

3.4 Redox Ratio in Blood Plasma

Aged rats showed an increase of redox ratio in blood plasma in comparison with young adult rats (Figure 5).

In aged rats, the redox ratio in blood plasma was 1.5 times higher as compared to young adult animals. This was caused by a minor decrease of Sm CL ROS and an increase of Sm CL AC in aged rats with respect to young adult rats. The direction of age-related changes of redox ratio in rat blood plasma coincided with the changes that were found in the brain. It should be noted that age-related changes of redox ratio in blood plasma did not correlate with changes in the internal organs of rats.

4. Discussion

Determination of oxidized products in biological systems is the prevalent method for measuring the oxidative stress level[18–21]. In this work, AC of supernatants of tissue homogenates was estimated by the luminal-dependent CL method using products of the Fenton reaction, which is initiated in the alkaline medium, mostly by superoxide radicals[22]. AC of the products of protein oxidative damage[23], and AC of glycoproteins associated with hexosamines and N-Acetylneuraminic Acid (NANA) were reported earlier. This property of monosaccharide units of glycan was revealed for the first time; in our opinion, it can help to elucidate their physiological role. Our previous studies were carried out with the samples of purified mucus glycoproteins, for which the level of CL AC and the total biochemical composition of monosaccharides were determined[24]. Sm CL ROS was measured under the same conditions of CL reaction to determine the extent of organic molecules damage and to make a comparative analysis of the Sm CL AC and Sm CL ROS ratio (to determine the redox ratio) in different tissues.

Changes in Sm CL AC and Sm CL ROS in the internal organs generally agree with the existing concepts on the role of oxidative stress in aging. In aged rats, the redox ratio substantially decreased in the heart, lungs, liver and testicles as compared to young adult rats; this decrease was caused by an increase in Sm CL ROS. Under in vitro conditions of the experiment, the main contribution to Sm CL ROS was made by the long-living radicals generated during the oxidative modification of macromolecules. This was demonstrated for the cardiac muscle[25,26], femoral muscle[27,28], lungs[29] and liver[30,31]. Sm CL AC in the tissues of femoral muscle, heart and liver of aged rats remained at the level of young adult animals, in the lung tissue it showed a significant decrease, and in the testicles increased as compared to young adult rats. Sm CL AC can be related both to AC of the oxidation products of proteins and glycan and to the low-molecular antioxidants, which have different representation in each tissue. Testicles of young adult rats showed the highest redox ratio, i.e., the AC value strongly exceeded the amount of oxidative damaged molecules. It is known that spermatogenesis activity is accompanied by extremely high consumption of oxygen[32]; so, the medium of this tissue is quite aggressive. Glycolipids are the main component of spermatic plasma. This suggests fast damage of glycan, release of terminal residues of sialic acid, binding of the radicals, and thus the restriction of oxidative stress. In young adult animals, this process is quite rapid, which is determined by the high rate of metabolic processes. In aged animals, the process decelerates, which leads to an increased amount of damaged molecules along with an increased AC. Therewith, the absence of DNA damage in the testicles of aged rats was noted[30]. A constantly high level of AC in male rats younger than 24 months was demonstrated in[33]. Redox ratio in the testicles of aged rats decreased, but remained at a higher level than in other internal organs examined in the work. In blood plasma, changes in Sm CL AC and Sm CL ROS were insignificant but had opposite directions; so, the redox ratio showed a significant increase. Obviously, Sm CL AC of the plasma is related to low-molecular antioxidants, which are able to manifest AC in the alkaline medium. Kim et al. demonstrated that the decrease in antioxidant ability of

blood plasma upon aging is affected also by an increase in the level of peroxide and nitrated proteins and by a decrease in thiol content[34].

In our experiments, the following results were obtained for the brain parts of aged rats. The level of redox ratio was different in different parts of the brain: in olfactory bulb, pons and cerebellum this value significantly differed from the redox ratio in the frontal lobe cortex within each age group. This can be attributed to different arrangement of cyto- and myeloarchitectonics of the studied parts. In all the studied brain parts except mesencephalon (olfactory bulb, cerebellum, pons, frontal lobe cortex, and thalamic area), a substantial increase in redox ratio caused by the increase in Sm CL ROS and Sm CL AC was found for aged rats. The indicated changes are unusual; they disagree with some literature data and with changes in the internal organs.

According to the review by Gemma et al.,[35] the AC mechanisms diminish with physiological aging of the brain, and accumulation of oxidative damaged macromolecules deteriorates its functions. The conclusion about AC deterioration is based mainly on investigation of enzymatic antioxidants. Besides, there is disagreement in the studies elucidating the role of lipid peroxidation, mDNA and oxidized proteins in the aged brain[30,36–38]. The absence of pronounced age-related oxidative damage of polyunsaturated fatty acids in hippocampus and cerebellar tonsil and the presence of high level of α-tocopherol in these parts of the brain upon aging were established[39]. A comparison of the intensity of lipid peroxidation and AC in the brain tissue revealed even an invert correlation of these processes in young adult animals; AC was estimated using the FRAP analysis[29]. It was shown that the normal brain tissue contains a great amount of oxidized proteins[38]. The protein carbonyl formation was shown to increase in the aged brain[6]. However, this process occurs in all the organs[19]. Thus, a comparison of the data obtained allowed us to suggest that there are some other components in the brain: they possess AC and differ from components of the heart, lungs, liver, and skeletal muscles. This role can be played by glycan, which are present in different parts of the brain[40]. Glycan can contribute to Sm CL AC because they possess AC associated with hexosamines and N-Acetylneuraminic Acid (NANA). There are difficulties in determination of glycan and their conjugates with proteins and lipids; so, for a long time they were not considered in relation to the aging of the brain.

In 2010, Sato and Endo[41] published a paper reporting for the first time the literature and comprehensive original data on the alteration of brain glycoproteins during aging. However, the authors did not examine the AC products of glycan degradation and did not mention their involvement in the oxidative stress upon aging. Our experiments demonstrated high levels of Sm CL ROS and Sm CL AC for components of the aged brain. Sm CL ROS is related to the oxidative damage of macromolecules, probably including glycoconjugates. Sm CL AC is determined by the presence of antioxidants, in particular, the degradation products of glycoconjugates and glycan, which can also possess AC, i.e. they can be involved in the restriction of oxidative stress during the aging of the brain.

5. Conclusion

A comparison of the level of ROS and the antioxidant capacity has revealed differences in their ratio in the internal organs, blood plasma and brain parts during aging and different levels of oxidative stress. Results of the comparative study gave grounds to suggest that the brain has some other components with antioxidant capacity, which are absent in the heart, lungs, liver and skeletal muscles. Such components in the brain may be glycan, which possess the antioxidant capacity and are widely represented in the brain parts.

6. Acknowledgement

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7. Conflict of Interest

There is no conflict of interest in this paper.

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