

Lipid Metabolism and Oxidative Stress in Aging Processes in Experimental Rats

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Abstract

Lipid metabolism and oxidative processes were studied in young (4-5 months) weighing 150-200 g and old (25-28 months) 250-350 g of white out bred rats. The increased rate of lipid peroxidation (hydroperoxides, malondialdehyde), as well as the accumulation of products of the oxidative modification of proteins, was observed in the mitochondrial fraction of rat brain tissues. The study of lipid diversity in brain tissues of old rats demonstrated that aging is accompanied by changes in the qualitative and quantitative lipid composition. It was found that changes in the metabolism of glycolipids result in a decrease in the expression of cerebrosides and sulfatides. Also, an increase was observed in the sphingosine level (a product of hydrolysis of neutral glycolipids). It was shown that disorders in lipid metabolism play a key role in pathological changes during aging.

Keywords: Aging; Lipid peroxide; Mitochondria; Cerebrosides; Gangliosides; Sphingosine; Oxidative modification of proteins

Introduction

Currently the ageing problems attract attention in molecular and cell biology. It is well known that the aging process is accompanied by the development of age-related pathological processes, such as stroke, cerebrovascular accident, atherosclerotic encephalopathy, malignant growth, endocrine and immune disorders. Understanding the molecular mechanisms underlying the aging process, may provide the best strategy to address the problems associated with aging. Among the hypotheses and theories to explain the primary mechanisms underlying aging, an important place belongs to the free radical theory linking the age-related changes to the accumulation of molecular damage to membranes and the genetic apparatus of cells by free radicals and lipid peroxidation product, proteins [1,2] According to this theory, free radicals are formed as a result of various oxidation reactions in the body, have multiple damaging effects on macromolecules (lipids, nucleic acids and proteins), causing their degradation and aging. Under the influence of reactive oxygen species between the bases of two different strands of DNA, DNA and protein, or two amino acid residues of the protein can form covalent bonds, or "joining"? Formation of such links is extremely dangerous, as this violated the functional activity of proteins, genes, and mutations can occur [3] Genetic changes and oxidative stress are important for human cell aging [4,5]. Oxidative reactions lead to: cellular homeostasis disorder, which contributes to the development of diseases or premature aging. Lipids are not only structural components of cell membranes; they also play an important role in functional activity. The activity of membrane ferments and receptors, as well as cell phagocytosis and adhesion, depend on the phase properties of

the lipid membrane, such as viscosity, surface charge, and polarity. Impaired lipid metabolism in an aged organism can result in a number of pathological processes in humans and animals. Among the most important apoptosis regulators are sphingosine, ceramid, sphingosine-1-phosphate which plays the role of the secondary messengers of the apoptotic signal. Ceramid and sphingosine modulate the activity of a variety of enzymes and transcription factors; particularly, they activate various protein kinases involved in the transduction of pro-apoptotic signal [6,7].

Materials and Methods

The study was conducted on white outbred rats weighing 150-200 g (young) and 250-350 g (old). 7 series (gangliosides and neutral glycolipids), 10 series (protein oxidative modifications and lipid peroxidation) of experiments were performed on 20 rats in each experiment. All experiments were performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. Brain mitochondria were isolated in a medium containing 0.25 M sucrose and 0.01 M Tris HCl buffer with pH 7.4 by differential centrifugation at speed force of 13 000 g after the precipitation of nuclei at a speed force of 600 g. Gangliosides were determined by thin layer chromatography using plates from Merck (Germany) and the solvent system chloroform: methanol: 2.5 M ammonium (60:35:8). Ganglioside content was evaluated by the amount of N-acetylneuraminic acid [8,9]. The precipitation of cerebrosides and sulfatides depends on their ability to form a dense white layer at the interface of water and chloroform layers following the chloroform methanol lipid extract treatment with trichloroacetic acid and water. Fractionation of cerebrosides and

sulfatides was performed by TLC (TLC plates, Merck, Germany) using chloroform: methanol: concentrated ammonia (80:20:0.4) as a mobile phase. The amount of cerebroside was determined *via* the interaction of sugar residue with resorcin and amount of sulfatide was determined *via* the interaction of the sulfate group with azure [8,9].

To isolate sphingosine residues, the mixture of cerebroside and sulfatides was subjected to acidic methanolysis by an H₂SO₄:methanol mixture (1:20) at 78-80°C for 6 h, followed by the extraction of sphingosine residue with diethyl ether. The amount of sphingosine was determined by the color producing reaction with methyl orange measuring the absorbance at wavelength of 415 nm [8,9]. Lipid peroxidation (LPO) was measured by the level of malondialdehyde (MDA). The level of MDA was detected by the reaction with thiobarbituric acid [8,10]. Protein oxidative modification was detected by interaction of oxidized amino acid residues with 2, 4-dinitrophenylhydrazine (2, 4 DNPH), which led to formation of 2,4-dinitrophenylhydrazones [10]. The protein content was determined according to the Lowry procedure [11]. Statistical significance of results was evaluated using Student's test; differences were considered as statistically significant at $p \leq 0.05$.

Results and Discussion

Studying ganglioside content in the brain (Figure 1) we found 4 gangliosides differed by content of neuraminic acids, which are

characterized by high exchangeability. It was found there was a decrease in all main ganglioside fractions (mono-, di-, tri- and tetrasialogangliosides). These glycolipids are located in the outer monolayer of plasma membrane so that their carbohydrate chain carrying total negative charge is exposed to the outer environment. Regulation of plasma membrane glycosphingolipid composition may also influence neuron excitability (because gangliosides are saturated with sialic acids carrying negative charge). In spinal ganglia neurons with damaged axons ganglioside metabolism is changed and this is accompanied by increased content of sialic acids on the cell membrane. It is believed that the increase of negative charge on the plasma membrane (due to sialic acids) results in neuronal hyperexcitability [5,12]. Gangliosides are synthesized from N-acylsphingosine (ceramide) *via* cerebroside formation, and their main difference consists in the presence of N-acetylneuraminic acid in their structure. The study of neutral glycolipids in the brains of control animals led to the discovery of two factions of cerebroside and sulfatides, and two fractions of sulfatides that differ in the content of fatty acids (Figure 2). Results of this study show that a decrease in the content of both the total and fractional composition of neutral glycolipids is observed in the brain with aging. While in young rats the total content of neutral glycolipids is 18.03 mg/g, a decrease to 9.39 mg/g is observed in old rats. Cerebroside is localized primarily in the myelin, whereas sulfatides are found in nonmyelin white

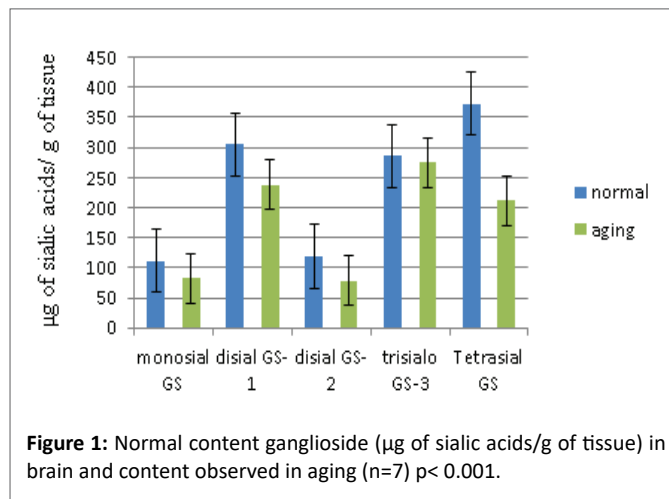


Figure 1: Normal content ganglioside (µg of sialic acids/g of tissue) in brain and content observed in aging (n=7) $p < 0.001$.

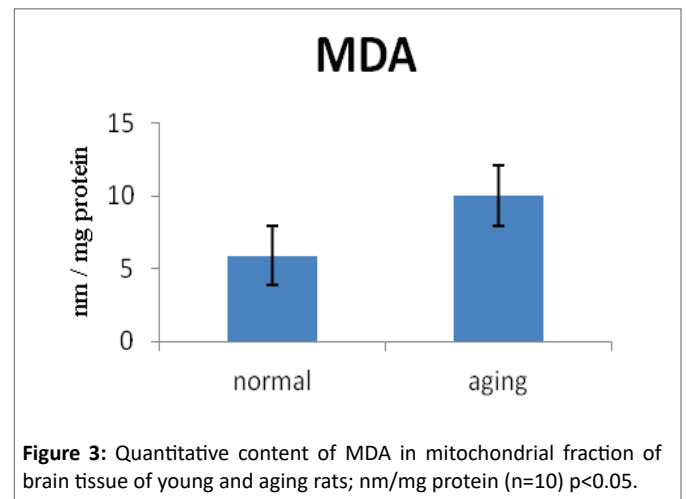


Figure 3: Quantitative content of MDA in mitochondrial fraction of brain tissue of young and aging rats; nm/mg protein (n=10) $p < 0.05$.

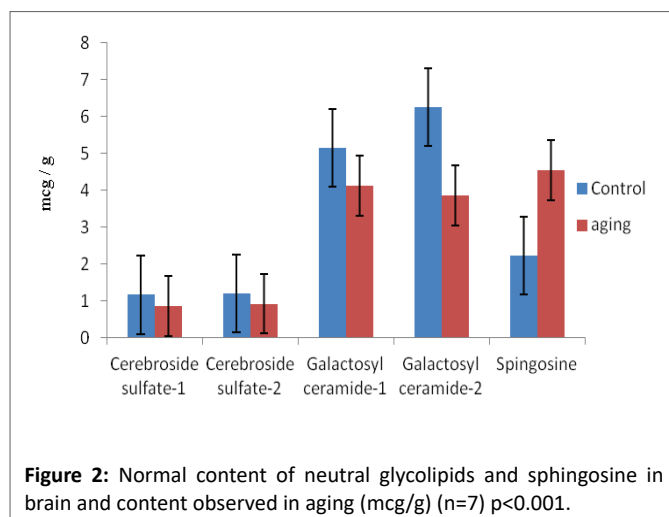


Figure 2: Normal content of neutral glycolipids and sphingosine in brain and content observed in aging (mcg/g) (n=7) $p < 0.001$.

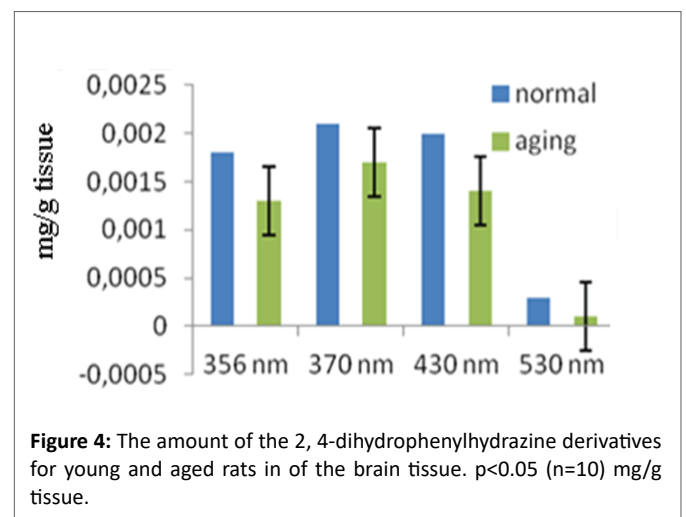


Figure 4: The amount of the 2, 4-dihydroxyphenylhydrazine derivatives for young and aged rats in of the brain tissue. $p < 0.05$ (n=10) mg/g tissue.

matter. The main function of myelin is the fast propagation of nerve impulses via axons surrounded by a myelin sheath. In addition to the transmission of nerve impulses, the myelin sheath serves as a source of nutrition for the nerve fiber and also provides structural support and protection for the nerve. We believe that the decrease in the studied fractions in old rats may be one of the underlying causes of impaired brain function observed during aging. The increase in the sphingosine content in the brains of aged rats is of particular interest. The results of the study of sphingosine, which is a product of the hydrolytic decomposition of neutral glycolipids, demonstrated the increase in its content in the brains of old animals. Free sphingosine is formed from sphingomyelins and cerebrosides via enzymatic cleavage by ceramidase and as a result sphingomyelinase forms sphingosine and fatty acid. Sphingomyelinase is found in almost all cells, but most of it is contained in the myelin of brain cells. Recently published data suggests that the sphingomyelinase activation depends on oxidative processes in the cell. When free radical processes are activated, the sphingomyelinase activity increases, which leads to the accumulation of ceramide and sphingosine. Sphingosine participates in the regulation of cell proliferation and cell death because it can inhibit the activity of protein kinase C [13]. Ceramide and sphingosine mediate apoptosis, and their accumulation in brain cells leads to the activation of apoptosis. Changes in the lipid content in the cell membrane, along with metabolic changes, are often caused by processes of free radical oxidation. The certain stable level of free radical reactions was detected in the brain tissues of young rats. An increase in MDA content was found during the study of oxidative processes in mitochondrial fraction of brain tissues of old rats. High content in the brain tissues of easily oxidized substrates such as polyunsaturated fatty acids and non heme iron (which is an activator of lipid peroxidation) contributes to increased levels of lipidperoxides in the mitochondrial fraction of the brain tissues. Under these conditions, the mitochondrial electron transfer chain becomes an important source of reactive oxygen species (ROS), which are an unstable and highly reactive byproduct of the metabolism. (Figure 3) ROS cause oxidative protein modification associated with an increase in membrane damage. It is believed that, under oxidative stress, ROS first attack the membrane proteins, rather than lipids [14]. Data analysis shows that (see picture) the level of carbonyl groups in the mitochondrial fraction of brain tissues of old rats is significantly higher than in tissues of young animals. This indicates an age associated increase in oxidative protein damage. (Figure 4)

356 nm (basic aldehyde derivatives)

370 nm (basic ketone derivatives)

430 nm (neutral aldehyde derivatives)

530 nm (neutral ketone derivatives)

In fact, all the amino acid residues of proteins are targets of oxidation, which leads to changes in their functions. Sulfo-oradacent amino- and hydroxyl groups of amino acids are oxidized which leads to the formation of cross links between proteins or between proteins and other molecules containing NH_2 group. Amino acids oxidation can cause the breakdown of the secondary and tertiary structure of protein, which can lead to inactivation of membrane associated enzymes [14].

Conclusion

The results show that the oxidative processes in the old rats are activated, the amount of malondialdehyde and the

2,4-dinitrophenylhydrazone count increases as a result of protein oxidative modifications and lipid peroxidation that contribute to the destruction of the structure of the macromolecular cells in the brain tissue. Is broken structural integrity of brain cell membranes in old rats, breaks the metabolism of neutral and acidic glycolipids, as a result, the amount of ceramide and sphingosine increases, which can lead to cellular apoptosis. Thus, the data we obtained on changes in oxidation and lipid metabolism can be useful for better understanding the mechanisms of aging.

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