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Changes in oxidative stress in Wistar albino rats during senescence

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Objectives: The purpose of this study was to determine the changes in oxidative stress in Wistar albino rats during senescence.

Methods: The concentration of malondialdehyde and glutathione, which are indicators of oxidative stress, were measured in the whole brains of 3,6,12 and 24 month old Wistar albino rats.

Results: The glutathione levels of 24 months group were significantly decreased compared with those of 6 months old rats. In contrast, the malondialdehyde levels of the 24-month old group were significantly elevated compared with those of the all young groups.

Conclusion: In conclusion, our findings have supported the hypothesis that senescence might cause increased oxidative stress.

Key words: Glutathione (GSH), malondialdehyde (MDA), aging, brain, rat

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Introduction

Aging is a multifactorial process that leads to the gradual loss of capability of an individual to maintain homeostasis, which is characterized by progressive decline in function at tissue and cell levels. It causes a decrease in the individual's ability to respond to a wide range of challenges or stress and increases the susceptibility of the subject to age-associated diseases and death.[1]

Many theories have been advanced to account for the biochemical and molecular basis of aging. Among them, the free radical theory of aging, which was first proposed decades ago by Harman,[2] has received much attention from scientists in biomedical fields. He proposed that the accumulation of damage to biomolecules, caused by the attack of free radicals during normal aerobic metabolism, plays a major role in aging. He implemented this

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theory with the suggestion that mitochondria are the major target of free radical attack that leads to aging.[3] Free radical theory has been widely tested and has gained great support from work done in many laboratories involved in the molecular and cellular biological research of aging. Miquel and coworkers[4] showed that mitochondrial DNA damage and lipofuscin pigment formation in animal tissues were concurrently increased during aging.

Under normal physiological conditions, about 1-5% of the oxygen consumed by mitochondria is converted to superoxide anions, hydrogen peroxide and other reactive oxygen species (ROS).[5] ROS are highly reactive and capable of damaging many biological macromolecules such as DNA, RNA, proteins and lipids.[6] A particular consequence of this ROS-mediated attack is the accumulation of oxidatively damaged macromolecules, which may lead to genetic mutation and cellular senescence, if not timely removed in vivo.[7] The superoxide and hydroxyl radicals stimulate lipid peroxidation and disrupt membrane integrity.[8]

Tissues are normally protected from the toxic effects of ROS by enzymatic and nonenzymatic systems. These systems include nonenzymatic compounds glutathione, vitamin E and beta-carotene as well as a series of enzymes specializing in reducing free radicals to more stable species. The most important antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GR).[9]

Earlier studies suggest that with advancing age there may be a decrease in the ability of the brain tissue to sustain lipid peroxidation.[10,11]

The purpose of this study was to determine the changes in oxidative stress in Wistar albino rats during senescence.

Materials and Methods

Male Wistar albino rats (3, 6, 12, and 24 months old, n=12) were included in the study. They were fed on normal laboratory diet (as pellets) and water ad libitum. The rats were anesthetized with ether. A cannula was inserted into the left ventricle of the heart and the animal was perfused with ice cold 0.9% NaCl containing heparin (5 u/ml). The whole brain was immediately removed and homogenized in ice cold 0.15 M KCl (10% w/v). The concentrations of malondialdehyde (MDA) and glutathione (GSH), which are indicators of oxidative stress, were measured in the whole brains of 3, 6, 12 and 24 month old Wistar albino rats. MDA level was measured using the method of Ushiyama and Mihara.[12] The results were expressed as nmol MDA/g whole brain. GSH level was assayed using the methods of Ellman.[13] The results were estimated as mmol/g whole brain.

Statistical analysis

Data were expressed as means ± SD. The student's t test was used to compare the results of groups. $p<0.05$ being considered statistically significant.

Results

Figures 1 and 2 show the concentrations of GSH and MDA, respectively. When the results of GSH in all groups were compared to each other, the GSH levels of whole brain homogenate were slightly increased from 3 month to 6-month-old rats but began to decrease from 6 month to 24-month-old rats. We found that there was only a significant difference in GSH levels between 6 and 24 month old groups ($p<0.01$) (Figure 1). MDA levels of whole brain were slightly began to elevate in rats from 3 to 6 months old, but decreased from 6 to 12 months rats. MDA levels in the 24 month old group were significantly increased ($p<0.01$) by 29.6%, 28% and 40% as compared with the 3, 6, and 12 months old groups, respectively (Figure 2).

Discussion

The brain contains large amounts of polyunsaturated lipids that are rich in polyunsaturated fatty acids. The free radical theory of aging proposes that ROS, mainly singlet oxygen and hydroxyl radicals,

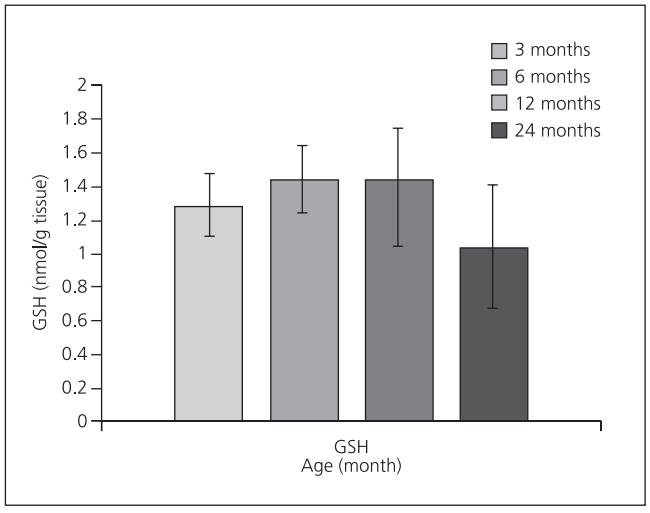

Figure 1
The levels o GSH with age.

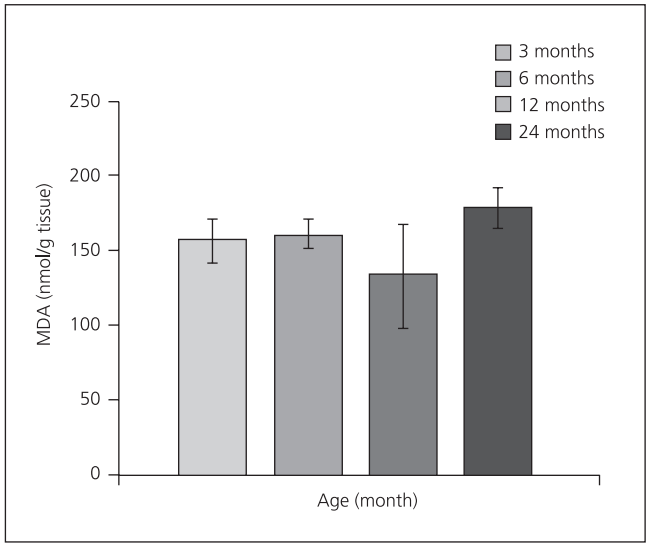

Figure 2
The levels of MDA with age.

cause peroxidation of the phospholipid bound polyunsaturated fatty acid. The lipid peroxidation of polyunsaturated fatty acids, which leads to the formation of MDA, is indicated in a variety of pathological states[14] and has been proposed as a mechanism of the aging processes.[15]

GSH is an important antioxidant that functions directly in elimination of toxic peroxides and aldehydes and indirectly in maintaining Vitamin C and E in their reduced and functional forms.[16,17] In the present study, the GSH levels in whole brain homogenate were significantly decreased in the 24-month old rats compared to the levels in the 6-month animals. Our results are in agreement with the previous studies concerning GSH. GSH concentrations in the brain homogenate were reported to decline with aging in six different mammalian species including the rat and mouse.[18] GSH levels were also demonstrated to decrease in different regions of Wistar albino, Spraque Dawley rats, and gerbils during aging.[19] Lower GSH concentration in the aging brain than the concentration in the young ones is confirmed by a study on senescence accelerated mice (SAM) compared to age-paired mice, a strain that ages normally.[20] Results from a number of studies have suggested that GSH-deficiency state is a general phenomenon in aging organisms and tissues.[21]

A severe GSH depletion decreases protein and DNA synthesis because GSH provides the reducing equivalents for the glutaredoxin system supplying electrons to ribonucleotide reductase. The GSH depletion affects many GSH-dependent enzymes (glutathione syntetase, glutathione peroxidase, glutathione transferase, leukotriene C4 synthetase, glutaredoxin system, glyoxylase I and II) making the cells more susceptible to any further change.[22]

GSH is a vital antioxidant defense because, besides serving as a substrate in the GSH-peroxidase reaction, it also acts as a free radical scavenger and helps regulate the thiol disulphide concentration of a number of glycolytic enzymes and Ca-ATPase.Thus indirectly preserving intracellular Ca^{2+} homeostasis.[23] Moreover, GSH regenerates other scavengers and antioxidants like α-tocopherol.[24]

In our study, MDA levels in the whole brain homogenates of 24 month- old rats were significantly higher than in 3, 6 and 12 month-old ones. Lipid peroxidation in the 36 month-old brains of Sprague Dawley rats was demonstrated to increase as compared to 2.5 month-old rats.[25] A significant increase in the generation of superoxide, hydrogen

peroxide and hydroxyl radicals was reported in the brain mitochondria of aged rats as compared to young ones.[25-27] There are many contradictory results concerning the MDA levels of tissue. Driver et al[28] showed that basal levels of ROS production were similar in 7-14- and 21-day old rats, increased in adults, (3-6 month old), highest in aged rats (24 month-old), and did not differ between brain regions. Ansari et al[29] found that in vitro MDA formation declines with age in the cerebrum, basal ganglia, cerebellum, medulla and cervical cord. Some authors found no significant changes in the tissue contents of MDA with age.[4,30]

Increased generation and release of ROS give damage to mitochondrial function, genome and other cellular constituents.[31] Aging has in fact been associated with altered membrane composition and function, enhanced protein oxidation, increase in both mitochondrial DNA oxidative damage and in the frequency of mutations.[26,32]

The free radicals react in the membrane with scavenger agents and are detoxified and eliminated. During aging, the decrease in GSH availability and function may result in increasing amounts of hydroxyl radicals, which can cause iron to be decompartmentalized from the normal deposit sites, thereby enhancing the production of ROS.[33] The most important of the results obtained with the GSH lowering compounds, is that peroxidative stress has a greater effect in older than in adult or mature rats.

In the present study, we can speculate that senescence might cause the increased levels of MDA but decreased GSH because of the elevated generation of free radicals in the course of senescence. In conclusion, our findings have supported the hypothesis that senescence might cause increased oxidative stress.

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