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ANTIOXIDATIVE DEFENSE MECHANISMS IN THE AGING BRAIN

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Abstract - Aging is an extremely complex, multifactorial process that is characterized by a gradual and continuous loss of physiological functions and responses, particularly marked in the brain. A common hallmark in aging and age-related diseases is an increase in oxidative stress and the failure of antioxidant defense systems. Current knowledge indicates that the level of glutathione progressively declines during aging. Because nerve cells are the longest-living cells that exhibit a high consumption rate of oxygen throughout an individual's lifetime, the brain may be especially vulnerable to oxidative damage and this vulnerability increases during aging. In addition, the brain contains high concentrations of polyunsaturated fatty acids and transition metals and low antioxidative defense mechanisms. Although aging is an inevitable event, a growing volume of data confirms that antioxidant supplementation in combination with symptomatic drug treatments reduces oxidative stress and improves cognitive function in aging and age-related diseases. The present review discusses the neuroprotective effects of antioxidants in the aging brain.

Key words: Aging, brain, oxidative stress, glutathione, polyphenols

OXIDATIVE STRESS AND AGING

The aging process is associated with a progressive decline in multiple aspects of cognitive performance, including reductions in mental speed, attention, memory, hearing, vision and stamina, as well as decreases in brain structure size and white matter integrity (Park and Reuter-Lorenz, 2009). Current evidence supports the view that aging is a multifactorial process that leads to loss of function and the inability to respond adequately to stress. Numerous aging theories have been proposed; probably the most important are those that incorporate genomic and free radical theories. The "free radical theory of aging" has been one of the most studied and accepted hypotheses for the molecular basis of aging (Harman, 1956). More than 50 years ago, Denham Harman defined aging as the progressive "... accumulation of diverse deleterious changes in cells and tissues with advancing age that increase the risk of disease and death". Later, Harman added a slight modification to this theory to bring special attention to the role of the mitochondria in the aging process because these organelles are a major site of reactive oxygen species (ROS) generation (Harman, 1972). Harman's original hypothesis has been refined in such a way to address the role of many different forms of ROS in regulating the aging process and is now generally termed the oxidative stress theory of aging (Harman, 1998). The basis of this theory is that the imbalance between pro-oxidants and antioxidants leads to the accumulation of oxidative damage of cellular macromolecules that increases during aging. Age-related accumulation of oxidative damage in the brain contributes to a progressive loss in the function of cellular processes and cognitive deterioration. The oxidative stress theory and its correlate, the mitochondrial theory of

aging, are among the most studied and widely accepted of all hypotheses of the mechanism of the aging process.

A large body of experimental research has indicated that compared to other organs, the brain is particularly vulnerable to oxidative damage, due to its high metabolic rate, characterized by a highly active mitochondria metabolism. In addition, the brain is very susceptible to oxidative damage because of its high concentrations of polyunsaturated fatty acids and transition metals that are involved in the generation of the hydroxyl radical; moreover, the brain contains low activities of antioxidative defense mechanisms (Droge, 2003; Jovanovic and Jovanovic, 2011). Finally, neurons are the longest-living cells and oxidative damage of nerve cells tends to be cumulative over time. Consequently, ROS generation in the mammalian brain is intense. Oxidative stress is caused by an imbalance between the production of ROS and the cellular mechanisms responsible for the scavenging of ROS. Oxidative damage that occurs because of increased levels of ROS can target cellular components, consequently leading to altered physiological function of the cells (Sultana and Butterfield, 2011). Hydrogen peroxide (H_2O_2), superoxide anions ($O_2^{\bullet -}$), hydroxyl radicals ($HO^{\bullet}$) and other reactive compounds (singlet oxygen, alcoxyl and peroxyl radicals, etc.) derived from oxygen are collectively called "reactive oxygen species" (ROS). Once produced, ROS react with lipids, proteins and nucleic acids, causing oxidative damage to these macromolecules in the cells during the organism's lifespan, leading to a progressive decline of cellular functions (Evans et al., 2004; Chakravarti and Chakravarti, 2007). ROS-induced damage in biomolecules increases with aging, especially in the last quarter of the lifespan. There is a growing body of evidence supporting the strong role of age-related increases in protein oxidation as a primary mediator of the cellular dysfunction observed during normal aging and in age-related diseases (Squier, 2001; Sohal et al., 2002). Since proteins are the major components of biological systems and regulate multiple cellular pathways, oxidative damage of key proteins is considered to be the principal molecular mechanism leading to loss of cellular function in the aging process.

According to Salmon et al. (2010), if oxidative stress plays a role in aging, it is much more limited than previously thought. The results from mice with genetic manipulations in the antioxidant defense system suggest that oxidative stress plays a very limited role in aging, but a major role in lifespan. Salmon et al. (2010) speculates that the role of oxidative stress in aging depends on the environment. In an environment with minimal stress, oxidative damage plays little if any role; however, when an organism is exposed to chronic stress over its lifespan, oxidative damage plays a major role in the aging process. According to Sasaki (2010), production of ROS increases with age and this reactive oxygen may be a kind of signal for aging, and its levels in tissue may determine the aging process and lifespan. Decelerating the age-related increases of ROS production is expected to be a potent strategy for the development of anti-aging interventions.

It has long been known that aging is a major risk factor for many neurodegenerative diseases, including one of the most common forms of age-associated neural decline, Alzheimer's disease. Oxidative stress that is normally associated with aging is a prominent and early feature of Alzheimer's disease and plays a role in its pathogenesis and progression (Jovanovic, 2012; Sultana and Butterfield, 2013).

ANTIOXIDANTS IN THE AGING BRAIN

To maintain cell viability and homeostasis, aerobic organisms possess a defense mechanism to cope with the increased oxidative stress, or to prevent the onset of oxidative stress, through enzymatic scavengers of ROS (e.g. superoxide dismutases, catalase and glutathione reductase) or nonenzymatic sources (e.g. glutathione, melatonin, vitamins A, C and E and flavonoids). Additional redox-dependent protein repair pathways prevent the accumulation of misfolded or damaged proteins and protect the cell against potentially toxic proteins.

Enzymatic antioxidants are considered to be the first line of cellular defense against oxidative damage. The second line of defense against ROS is provided by non-enzymatic antioxidants.

When ROS production exceeds antioxidant protection, the resulting oxidative stress leads to macromolecular damage. The brain is poor in catalytic activity and has low levels of protective antioxidant enzymes, catalase and glutathione peroxidase. The glutathione system consists of reduced (GSH) and oxidized (GSSG) forms of glutathione. A large body of experimental evidence demonstrates that GSH can protect neurons against ROS, chiefly acting as an antioxidant and a redox regulator (Dringen and Hirrlinger, 2003; Shish et al., 2003). GSH is a tripeptide consisting of the amino acids glutamate, cysteine and glycine. GSH is essential for the detoxification of ROS in nerve cells. However, the concentration of glutathione is relatively lower in the brain compared to other organs of the body (Skaper et al., 1999). The results of Jovanovic and Jovanovic (2013a, b) suggest that the neurotoxic effect of cumene hydroperoxide in leech Retzius nerve cells was reduced in the presence of GSH applied in a concentration of 0.2 mM. The protective effects of GSH against cumene hydroperoxide-induced neurotoxicity may be due, at least in part, to its ability to scavenge ROS and to protect sulfhydryl groups on the ion transport proteins.

Several independent studies have suggested that the concentration of GSH progressively declines during aging and in some age-related diseases, such as neurodegenerative diseases (Dringen and Hirrlinger, 2003; Maher et al., 2005). Glutathione depletion in the brain has been connected with the oxidative stress occurring in aging. Sastre et al. (2005) found that cellular glutathione is slightly (by approximately 30%) decreased with aging.

ANTIOXIDANT DEFENSE OF THE BRAIN: A ROLE FOR ASTROCYTES

A growing body of data demonstrates that glial cells have a stronger antioxidant potential in comparison to neurons and that they can provide protection to neurons from oxidative damage. Microglial cells contain high amounts of GSH and show substantial activities of catalase, superoxide dismutase and glutathione peroxidase. The direct measurement of intracellular concentrations of brain glutathione has shown that GSH is somewhat more concentrated in the glia than in neurons. Rice and Russo-Menna (1998) demonstrated that glutathione is distinctly compartmentalized between neurons and glia, with an average intracellular concentration of 2.5 mM in neurons and 4 mM in glial cells. Astrocytes protect neurons from oxidative stress in several ways. According to Gupta et al. (2012), astrocytes can protect neurons against ROS, either through glutathione-dependent or glutathione-independent pathways. One route is via activation of the nuclear factor (erythroid-derived 2)-like 2 (Nrf2)-antioxidant responsive element (ARE) pathway. Nrf2 is referred to as the "master regulator" of the antioxidant response, modulating the expression of antioxidant enzymes; an increased level of GSH may be a major component of the neuroprotection mediated by Nrf2. Activation of this pathway protects cells from oxidative damage and cell death (Hur and Gray, 2011). Neurons are more susceptible than glia to oxidative damage. Neuronal viability is enhanced significantly by an increased supply of GSH precursors from Nrf2-overexpressing glia. Accumulating data suggests that astrocytes protect neurons from oxidative damage, in part, via maintaining sufficient neuronal glutathione levels. According to Stepkowski and Kruszewski (2011), the Nrf2/ARE signaling pathway is the main pathway responsible for cell defense against oxidative stress and in cellular redox regulation.

Additionally, cells have different protein repair pathways to rescue and repair oxidized and nonfunctional proteins and restore their functions. Recent studies have found that the ubiquitin-proteasome pathway plays a pivotal role in the recognition and degradation of oxidized proteins and thus limits oxidative damage in aging. The major function of the ubiquitin-proteasome pathway is to prevent the accumulation of misfolded or damaged proteins and

to protect the cell against potentially toxic proteins (Lecker et al., 2006).

ANTIOXIDANT SUPPLEMENTATION IN THE AGING BRAIN

Oxidative damage plays a role in limiting the lifespan of invertebrates. In the mammalian model, the effect of oxidative stress on lifespan is less clear. The expression of antioxidant enzymes has affected lifespan in a wide range of experimental animal models. However, the direct effect of antioxidant enzyme treatment on lifespan is less clearly defined in mammalian systems. Investigators have tried to determine whether dietary antioxidants can ameliorate ROS-mediated damage or slow the rate of aging. Current knowledge indicates that chronic intake dietary antioxidants, by preventing oxidative stress, may produce beneficial effects against multiple age-related deficits of the brain. A variety of antioxidants has been examined for a reduction of oxidative damage. These range from natural products with antioxidant properties such as melatonin, resveratrol, vitamin C, vitamin E, lipoic acid, coenzyme Q, green tea, *Ginkgo biloba* extract, L-carnosine and "thiol-delivering" glutathione-mimics (such as tricyclodecan-9-yl-xanthogenate).

Current evidence supports a contribution of polyphenols to the prevention of oxidative damage, but their mechanisms of action are not fully understood. One important cellular pathway affected by polyphenols is the activation of the transcription factor Nrf2 via the ARE, which mediates generation of phase 2 detoxifying enzymes (Erlank et al., 2011). According to Steele and Robinson (2012), drugs that stimulate Nrf2-mediated gene expression can increase the GSH level of nerve cells *in vitro* and *in vivo*. Such drugs include tert-Butylhydroquinone, sulforaphane, resveratrol and lipoic acid (Suh et al., 2004; Farr et al., 2012). Moskaug et al. (2005) found that dietary polyphenols such as flavonoids, increase expression of γ-glutamylcysteine synthetase, the enzyme which is rate limiting in the synthesis of the glutathione. Joseph et al. (2005) speculate that the combinations of antioxidant and anti-inflammatory fruit polyphenolic compounds may show efficacy in aging. According to Lebel et al. (2012), vegetal polyphenolic compounds might decrease the enhanced vulnerability to oxidative damage that occurs in the aging brain.

Resveratrol is a polyphenolic compound found in red wine, grapes, peanuts and some berries, which is reported to extend the lifespan in some (Valenzano and Cellerino, 2006; Wood et al., 2004), but not all studies in animals (Bass et al., 2007; Kaeberlein et al., 2005). Mokni et al. (2007) found that in the brains of healthy rats, resveratrol increases the activity of antioxidants such as superoxide dismutase and catalase, and decreases the level of oxidative stress. Liu et al. (2012) investigated whether resveratrol can reduce oxidative damage and reverse the cognitive deficit in senescence-accelerated mice after an 8-week treatment with resveratrol. It was found that resveratrol significantly improved learning and memory ability and increased the activities of antioxidant enzymes with a reduction in the content of malondialdehyde. According to Long et al. (2009), resveratrol is one of the active ingredients in grape extract that extends lifespan, protects mitochondria from oxidative damage and improves motor function in a drosophila model of Parkinson's disease. Accumulating data suggests that oral supplementation with GSH can mediate age-related changes in synaptic plasticity (Robillard et al., 2011; Mizuno et al., 2011).

Isothiocyanates are a group of naturally occurring compounds present in plants and cruciferous vegetables such as broccoli, Brussels sprouts, cabbage, kale, cauliflower, horseradish, radish and turnip. Based on recent animal and human studies, consumption of cruciferous vegetables may inhibit the development of tumors and slow the aging process (Conaway et al., 2002; Zanichelli et al., 2012). Grunwald et al. (2013) demonstrated that lyophilized broccoli, added to flour as a dietary source, significantly increases the longevity of the red flour beetle (*Tribolium castaneum*), and that its effect is mediated through signaling pathways involving Nrf-2, Jnk-1 and Foxo-1. According to Cheng et al. (2013), curcumin restores

age-related loss of synapse and produces an elevated level of glutathione in the hippocampus. As oxidative stress is implicated in the etiology of many neurodegenerative disorders, isothiocyanates may be a potential tool for the prevention and treatment of such diseases. Sulforaphane and 6-(methylsulfinyl)hexyl isothiocyanate (6-HITC) are naturally occurring isothiocyanates. Mizuno et al. (2013) demonstrated that pretreatment with sulforaphane and 6-HITC provided protection against the cytotoxicity induced by oxidative stress in rat striatal cultures and increased the intracellular glutathione content through the Nrf2-ARE pathway.

Vitamin C (ascorbic acid), a water-soluble vitamin, is a naturally occurring antioxidant and free radical scavenger. The literature implicating vitamin C in the reduction of oxidative damage and the promotion of cognitive function remains controversial. Many reports have documented the protective actions of vitamin C in various models of oxidative stress due to its high efficacy as a free radical scavenger and indirect antioxidant. According to Meister (1994), supplementation of ascorbic acid in glutathione-deficient mice and rats increases tissue and mitochondrial levels of glutathione. Treatments of mice with ascorbic acid significantly reduce the age-related increase in protein carbonyl level in the cerebral hemispheres in comparison with age-matched control mice, indicating that ascorbic acid ameliorates the age-related increase in protein carbonyl content (Dkhar and Sharma, 2011). In contrast, Tveden-Nyborg et al. (2012) found that a long-term poor vitamin C status does not accelerate oxidative stress in aging brains of guinea pigs. They compared the markers of oxidative stress (lipid oxidation, decreased glutathione, increased p53 mRNA expression and somewhat elevated DNA oxidation) of aging to that of vitamin C deficiency during a 6-month dietary intervention, by assessing vitamin C transport and redox homeostasis in the brain.

Asha Devi et al. (2012) examined the protective role of vitamins E and C in combating oxidative stress caused by intermittent cold exposure in aging rats' frontoparietal cortex. Supplementation with vitamins E (a daily dose of 50 I.U./kg body weight) and C (400 mg/kg body weight) together can protect against oxidative damage, particularly in middle-aged (18 months) and old (24 months) male Wistar rats. Dietary supplementation with high doses of vitamin E (5.0 g alpha-tocopherol acetate/kg of food from 28 weeks) extended the median lifespan by 40%, improved neurological functions by 25-28% and improved brain mitochondrial function in aging mice (Navarro and Boveris, 2010). Vitamin E crosses the blood-brain barrier, and chronic supplementation with vitamin E increases α-tocopherol levels 2.5-times in the mouse brain.

Melatonin, the pineal secretory product, is a potent free radical scavenger and an indirect antioxidant. Although many theories relating melatonin to aging have been proposed, the role of this pineal hormone in the aging process is still unclear. Melatonin is a multifunctioning molecule that may be neuroprotective. According to Reiter et al. (1996), melatonin is more effective than glutathione in neutralizing the highly toxic hydroxyl radical and is also superior to vitamin E as a peroxyl radical ($LOO^{\bullet}$) scavenger. In contrast to classical antioxidants, melatonin, because of its high lipophilicity, can cross the blood-brain barrier and has a widespread intracellular distribution. Melatonin stimulates several antioxidative enzymes (Fischer et al., 2013) and improves mitochondrial function and cellular bioenergetics. The results of Limon-Pacheco and Gonsebatt (2010) suggest that melatonin increases the expression and activities of the GSH-related enzymes and increases the levels of GSH. In addition, melatonin plays an important role in protecting neuronal cells from amyloid β-mediated oxidative damage and increases cell survival. Several animal and clinical studies have indicated that melatonin levels are decreased in aging and neurodegenerative diseases (Hardeland, 2012; Petrosillo et al., 2013; Pandi-Perumal et al., 2013). Additionally, melatonin exhibits immunomodulatory properties and a remodeling of the age-associated decline in immune function, known as immunosenescence (Espino et al., 2012). Future research should focus on molecular pathways that

contribute to senescence (especially among longer-lived species) and on potential targets for treatments of age-associated diseases.

CONCLUSION

Although the fundamental mechanisms in the pathogenesis of aging are still poorly understood, a growing body of evidence points to oxidative stress as to one of the primary determinants of the aging process. Glutathione depletion in the brain has been connected with the oxidative stress occurring in aging and age-related diseases. There is evidence that antioxidant treatment protects against age-related dysfunction, including cognitive decline. Dietary supplementation with fruit or vegetable extracts high in antioxidants might decrease the enhanced vulnerability to oxidative stress that occurs in aging and could have significant anti-aging effects. Future research will provide more insight into potential therapeutic targets and approaches for modulating aging, age-related diseases, and longevity.

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