Oxidative stress protection and vulnerability in aging: putative nutritional implications for intervention


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Abstract

Research indicates that vulnerability to oxidative stress (OSV) may increase in aging, suggesting that age-related neurodegenerative diseases such as Alzheimer’s disease (AD) or vascular dementia (VAD) may be superimposed upon a vulnerable neuronal environment. Determinations in cell models have suggested that the enhanced OSV may be the result of, (a) increases in membrane lipids, especially sphingomyelin and the sphingomyelin metabolite, sphingosine-1-phosphate, (b) decreases in glutathione, and (c) CNS distribution of OS-sensitive neuronal muscarinic receptor subtypes (e.g. M1, M2 and M4). These changes appear to enhance, (a) decrements in cellular calcium buffering following KCl-induced depolarization, and (b) cell death under OS conditions. Among the most effective agents that antagonized cellular OSV were the combination of polyphenolics found in fruits (e.g. blueberry extract) with high antioxidant activity. Subsequent experiments using dietary supplementation with fruit (strawberry) or vegetable (spinach) extracts have shown that such extracts are also effective in forestalling and reversing the deleterious effects of behavioral aging in F344 rats. Thus, it appears that the beneficial effects of the polyphenolics found in fruits and vegetables in neuronal aging and behavior may be similar to those seen with respect to carcinogenesis and cardiovascular disease. © 2000 Published by Elsevier Science Ireland Ltd.

Keywords: Membranes; Receptors; Dietary supplementation; Polyphenolics

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1. Introduction

There is a plethora of research indicating the occurrence of numerous neuronal and behavioral deficits during aging, even in the absence of neurodegenerative disease. These changes may include decrements in calcium homeostasis (see Landofield and Eldridge, 1994) and in the sensitivity of several receptor systems, most notably, (a) adrenergic (Gould and Bickford, 1997); (b) dopaminergic (e.g. Joseph et al., 1978; Levine and Cepeda, 1998); (c) muscarinic (Joseph et al., 1990; Yufu et al., 1994; Egashira et al., 1996); and (d) opioid (Kornhuber et al., 1996; Nagahara et al., 1996). These decrements can be expressed, ultimately, as alterations in both motor (Joseph et al., 1983; Kluger et al., 1997) and cognitive behaviors (Bartus, 1990). The alterations in motor function may include decreases in balance, muscle strength and coordination (Joseph et al., 1983; Kluger et al., 1997), while memory deficits appear to occur primarily in secondary memory systems and are reflected in the retrieval of newly acquired information. Indeed, these characterizations have been supported by a great deal of research both in animals (Bartus, 1990; Ingram, 1990; Joseph and Roth, 1993; Ingram et al., 1994; Shukitt-Hale et al., 1998) and humans (West, 1996; Muir, 1997).

The mechanisms involved in these changes remain to be determined, but data are accumulating which suggest that the most important among them may be enhanced vulnerability to oxidative stress (OSV) shown by the CNS (see Olanow, 1992), which may increase further in aging (see Joseph et al., 1996; Cantuti-Castelvetri and Joseph, 1999). As an example, Cantuti-Castelvetri et al. (in preparation) have shown that senescent rats exhibited significantly greater motor behavioral deficits and reduced tyrosine hydroxylase immunoreactivity (pars compacta) than young rats following intra-nigral applications of dopamine. Although the exact nature of these increases in OSV remain to be elucidated, previous research has indicated that they may be the result of increases in the ratio of oxidized to total glutathione (Olanow, 1992), significant lipofuscin accumulation with bcl-2 increases, increases in membrane lipid peroxidation (Migheli et al., 1994; Yu, 1994), reduced glutamine synthetase (Carney et al., 1994), alterations in membrane lipids (see discussion in Denisova et al., 1997) and regional variations in the distribution of OSV sensitive receptor subtypes (Joseph et al., 1997). Research from our laboratory has concentrated upon the latter two of these factors, and on possible nutritional interventions to reduce the age-related increases in OSV. The present review is directed toward the discussion and elaboration of the findings from this research.

2. OSV and membrane changes in aging

Aging is accompanied by significant alterations in the molecular structure of membranes. These changes include, (a) size and dynamic cholesterol (CHL) domains, and (b) populations of glycerophospholipid molecular species (e.g. chemical structure of the polar head, type of linkage to the glycerol moiety, and the structure of the aliphatic chains at sn-1 and sn-2 positions, etc.) (Wood et al., 1984; Tacconi...
et al., 1991; Maniongui et al., 1993; Sunshine and McNamee, 1994; Lopez et al., 1995; Delion et al., 1996; Zhang et al., 1996; Denisova et al., 1998). One of the net effects of these alterations is the induction of significant changes in the biophysical properties of the membrane such as asymmetry and fluidity (Choe et al., 1995; Choi and Yu, 1995; Schroeder et al., 1996; Wood et al., 1999). For example, in aging the exofacial leaflet becomes less fluid (Wood et al., 1999).

One lipid that plays a major role in membrane fluidity and is significantly affected by aging is cholesterol (CHL, Igbavboa et al., 1996; Dietschy, 1997). The synaptic plasma membrane CHL content is over 40 mol% of the total membrane lipids (Wood et al., 1996, 1999) and appears to be located in two pools, one of which is relatively labile, can be easily depleted and is associated with bulk membrane fluidity (Leibel et al., 1987). In aging, it has been shown that there is a translocation from the inner plasma membrane leaflet to the exofacial leaflet, thus reducing both overall membrane fluidity and membrane asymmetry between the two leaflets (see Wood et al., 1999). It has been suggested that transbilayer alterations in CHL could also affect surface proteins (e.g. apolipoproteins and receptors), as well as other lipids, such as sphingomyelin (SPH) (Igbavboa et al., 1996, 1997). SPH is an important lipid that also regulates the trafficking of CHL within the membrane leaflets (Leppimaki et al., 1998). Thus, it has been shown that as levels of SPH in the exofacial leaflet were hydrolyzed by sphingomyelinase, the rate of CHL movement to the inner leaflet of the membrane increased (Slotte and Bierman, 1988). Additional findings have indicated that CHL that is oxidized in the outer leaflet of synaposomes induced significant reductions in the activity of the Ca^{2+} ATPase 'pump' (Schachter et al., 1983). This pump is extremely important in the removal of calcium from the cell following depolarization. If it is compromised, it would seriously hamper a cell’s ability to extrude or sequester calcium and ultimately alter vulnerability to oxidative stress (see below).

It has also been shown that SPH and its metabolites might have potent interactions with oxidative stressors that could result in increased sensitivity to free radicals. Denisova et al. (1997) have shown that PC-12 cells, in which SPH and CHL were increased to levels seen in some brain areas in aging (Denisova et al., 1998), exposed to hydrogen peroxide showed greater decrements in the ability to buffer (sequester or extrude) calcium following 30 mM KCl-induced depolarization than control cells. Also, previous research has indicated that long-lasting increases in cytosolic calcium may contribute to cell death by several mechanisms, most notably, free radical induction through xanthine oxidase activation (Cheng et al., 1994), as well as the generation of nitric oxide synthase and phospholipases (Lynch and Dawson, 1994). As pointed out above, OS may increase calcium dysfunction (see Cheng et al., 1994) in cells that are already compromised, subsequently enhancing pro-oxidant generation, loss of functional capacity of the cell, and ultimately cell death.

In addition to altering the calcium buffering capacity of the cell following OS, increases in the levels of SPH and CHL have been found to induce significant reductions in the levels of a major antioxidant, glutathione (GSH), in these cells (Denisova et al., submitted for publication). In fact, in a recent experiment

examinations were made of the effects of increasing the levels of ceramide, sphingosine, or sphingosine-1-phosphate independently, or via sphingomyelinase, on the ability of PC-12 cells exposed to hydrogen peroxide to buffer calcium following depolarization with 30 mM KCl. The results indicated that both sphingomyelinase and the SPH metabolite, sphingosine-1-phosphate, significantly increased the sensitivity of the cells to OS. It appeared that sphingosine-1-phosphate was extremely effective in lowering the cellular levels of glutathione, and that this may have induced the increased OSV, since repletion of glutathione with N-acetylcysteine antagonized the deleterious effects of sphingosine-1-phosphate on the cells at the low (5 μM) but not high (300 μM) hydrogen peroxide concentration. An additional determination suggested that ceramide might also be involved, since cells treated with ceramide showed increased free radical activity (assessed by dichlorofluorescein fluorescence) following hydrogen peroxide exposure as compared with controls, although the ceramide-treated cells did not show reduced glutathione activity.

Thus, it appears that there might be several membrane changes in aging that could enhance the vulnerability to oxidative stress. These may include alterations in CHL transfer or levels, SPH regulation of CHL transfer, or SPH reductions in glutathione levels. Moreover, there are also findings which indicated that membrane fatty acid binding proteins (e.g. BFABP) may also decrease in aging, further inhibiting normal CHL trafficking (Pu et al., 1999). Their role in OSV remains to be determined.

One other consideration is that the changes in the lipid-induced alterations in aging that may increase OSV could also produce an environment conducive to the development of Alzheimer’s disease (AD). Although the data regarding the levels of CHL under AD conditions are controversial, Sparks (1997) reported a significant increase in the CHL levels in frontal cortex. However, other studies reported that significant membrane lipid modifications, such as a decreases in phospholipids, cholesterol, and gangliosides (marker for axodendritic arborization), had been observed in the brains of patients with AD (Svennerholm and Gottfries, 1994; Svennerholm et al., 1991), and pronounced loss of nerve endings in early onset AD had been suggested (Svennerholm and Gottfries, 1994; Svennerholm et al., 1991). In addition, it has been shown that the concentration of apolipoprotein in AD patients was significantly reduced (Leppimaki et al., 1998).

The specifications of CHL/SPH-apolipoprotein interactions have not been studied to any great extent. However, there are some indications that ApoE may be involved in transporting CHL to neurons from glial, but the role of this CHL remains to be determined (Ptas et al., 1987). A more recent study has shown that ApoE polymorphism is an important determintor of CHL transport with 30% less [3H]cholesterol released into plasmas of apoE2/2 and apoE4/4-individuals as compared with plasmas of apoE3/3-subjects. Additionally, the plasma obtained from apoE3/3 individuals accumulated 50 and 65% more cell-derived [3H]cholesterol in \( \alpha \)-LpA-I2 than plasmas of apoE4/4 and apoE2/2-subjets, respectively (Huang et al., 1995). Research had also shown that while the presence of CHL facilitated the removal of emulsion particles from plasma in rats, SPH inhibited such removal. A
subsequent determination indicated that this effect was the result of SPH-induced decreases in the binding of apoE which increased the circulation time of emulsion particles in plasma (Arimoto et al., 1998). These interactions with various apoE polymorphisms have not been examined, but it is clear that their specification in AD would be of extreme importance and may even influence the OSV in the course of the disease.

3. Receptor subtype and OS vulnerability

In addition to lipid modifications, a second factor that might be important in determining OSV in aging may involve qualitative/quantitative differences in receptor subtypes in various neuronal populations. While there is a great deal of work that has characterized the role of OS with respect to glutamate receptors (see Michaelis, 1998 for review) and transporters (see Trotti et al., 1998 for review) in neurotoxicity, the putative role of other receptors and receptor subtypes that may confer regional sensitivity to OS have not been examined. As an example, it has been known for many years that some areas of the brain ‘age’ at a faster rate than others, in particular, the striatum. If variations in OS sensitivity are involved in these selective regional variations in function, it may reflect the composition of the receptor populations. As a first step in making these determinations, we chose to examine differential sensitivity to OS among the five muscarinic receptor (MACHr) subtypes, since research has indicated that they may be critically important in a variety of neuronal functions and show a loss in sensitivity as a function of age and AD (see Roth et al., 1995; Fowler et al., 1997 for reviews). This apparent decrease in sensitivity appears to be the result of age- or disease-related declines in G-protein mediated signal transduction, an index that has been shown to be extremely vulnerable to OS (Joseph et al., 1992) and which decreases as a function of age (Joseph et al., 1996).

There is also strong evidence to suggest that muscarinic receptors (MACHr) are intimately involved in various aspects of both neuronal (APP processing, Rossner et al., 1998) and vascular functioning (Elhusseiny et al., 1999). Additionally, findings indicate that M1AChR may modulate excitatory hippocampal synaptic transmission (Marino et al., 1998). Moreover, there is a preponderance of vulnerable MACHRs in memory control areas (Levey, 1996) and the vasculature (Elhusseiny et al., 1999), pre-disposing them to increased OSV.

In order to determine selective OSV among the 5 MACHr subtypes, we exposed COS-7 cells transfected with one of five muscarinic receptor subtypes (M1–M5 AChR) to low concentrations of H2O2 (0, 300 or 500 μM for 30 min in growth medium) or dopamine (DA, 1 mM for 4 h) and examined intracellular Ca2+ levels prior to and following 500 μM oxotremorine (oxo, to induce depolarization), as well as cell death following OS exposure. COS-7 cells were utilized for these determinations, since they do not express MACHr and have a long history of usefulness in studying various structural-activity relationships among the MACHr subtypes in isolation (Jakubic and Wess, 1999).
Following H$_2$O$_2$ exposure, the number of cells showing oxo-induced depolarization and Ca$^{2+}$ recovery varied as a function of transfected MACHR subtype. With respect to calcium buffering following H$_2$O$_2$ or DA exposure and oxotremorine-induced depolarization, COS-7 cells transfected with M1, 2 or M4 AChR showed the greatest decreases (25–100%). Calcium buffering in M3 and M5 transfected DA- or H$_2$O$_2$-exposed cells was not significantly decreased. Using M1 and M3-transfected COS-7 cells as models for ‘vulnerable’ and ‘non-vulnerable’ receptors, respectively, we examined the degree of alterations in cell viability (Live/Dead Eukolight kit) 4 and 24-h post DA treatment. Results indicated that the degree of cell death by apoptosis following DA exposure (Apo-Tag Kit) was about 10% of the 40% cell death in M1-transfected cells. No cell death was observed in M3-transfected, DA-exposed cells. Thus, it may be that receptor differences in OSV may determine, in-part, regional susceptibility to cell death. For example, it has been shown in the striatum that, (a) M1 receptor protein is expressed in 78% of the neurons; (b) M2 receptors may be the predominant muscarinic receptor; (c) M4 receptors were localized to 44% of striatal cells (Hersch et al., 1994); and (d) there are high concentrations of DA (which form a variety of OS-based toxic products, Hastings and Zigmond, 1994; Ben-Shachar et al., 1995). Thus, the profound age-related changes in the striatum may be reflective of the interactions between DA and specific populations of OS vulnerable receptors.

As an example, we recently (Joseph et al., 1998a) examined differences in DA receptor subtype (D1 and D2) OSV induced by the exposure of striatal slices obtained from young (6 months) and old (24 months) rats following exposure to 1 mM DA. OS vulnerability was assessed by determining differences in D1- or D2-stimulated GTPase activity following SKF 38393 or quinelorane (D1 or D2 agonists, respectively) application to the slices. The results indicated that while DA significantly reduced GTPase activity in both age groups and in both D1 and D2 receptors, the effects of DA were significantly greater in the striatal slices obtained from old animals, with respect to the D1 receptors, even though striatal DA uptake was significantly lower as a function of age. These findings indicate that there are age-related increases in OSV that may be receptor subtype selective.

Finally, since as mentioned above, the mAChR are intimately involved in APP processing and in other aspects of vascular functioning, and AD and VAD can occur simultaneously in the aged, we examined the selectivity of Aβ 25–35 toxicity on M1- and M3-transfected COS-7 cells (assessed as described above). Interestingly, the results indicated that the Aβ sensitivity was similar to that seen with DA- or H$_2$O$_2$-induced OS, with Aβ-exposed M1 transfected cells showing greater disruptions in calcium buffering than the M3-transfected cells. A subsequent experiment showed that pretreatment of the M1-transfected cells with the nitrone trap, α-phenyl-n-tert-butylnitrone (PBN) or the calcium channel antagonists, nifedipine or conotoxin, prevented the Aβ-induced decrements in calcium buffering.

Since there has been some success in AD treatment with the antioxidant vitamin E (Sano et al., 1997) we also examined the efficacy of Trolox (a soluble form of vitamin E) in this model with mixed success. However, vitamin E is but a single antioxidant. Therefore, given the positive results seen in numerous experiments with
respect to cancer, cardiovascular and cerebrovascular diseases, which show that the combinations of polyphenolic compounds found in diets high in fruits and vegetables may reduce their incidence (see below), we believed that Aβ toxicity on M1-transfected cells could be reduced through application of fruit or vegetable extracts prior to Aβ application.

Research (Prior et al., 1998, see below) had indicated that blueberries (BB) are higher than most other fruits and vegetables in antioxidant activity, and contain numerous polyphenolic compounds. Thus, we examined the effects of pre-treatment of the M1-transfected cells with blueberry on preventing Aβ-induced decrements in calcium buffering. Results indicated that the BB extract was extremely efficacious in preventing deleterious effects of the Aβ, as well as dopamine. We are presently attempting to determine the most effective BB polyphenolic or combination that may be inducing this protection. However, it should be clear that in age-related neurodegenerative diseases (AD and PD), the additional insults are superimposed upon a nervous system that is already showing increased responsiveness to OS. Therefore, it may be possible to reduce this sensitivity through antioxidant supplementation with the combinations of antioxidants found in fruits and vegetables. To this extent, we have been examining these possibilities in rodent models, and these efforts are described below.

4. Nutritional modulation of neuronal and behavioral aging

As alluded to above, there is a long history of evidence showing that there is a significant association between fruit and vegetable intake and ischemic heart disease mortality (see Armstrong et al., 1975; Verlangieri et al., 1985; Hughes, 1995; Mayne, 1996). Consumption of fruits and vegetables appears to reduce the incidence and mortality rates of cancer in humans (Doll, 1990; Willett, 1994) and animals (Wattenberg and Coccia, 1991). It appears that even extracts of single foods such as tomato (lycopene, Sharoni et al., 1997) and garlic (Pinto et al., 1997) can have some antitumor properties.

While the studies examining the effects of fruit and vegetable consumption on cancer and cardiovascular disease are well documented and numerous, such documentation is far less with respect to the CNS. Indirect evidence has been provided that it is possible to increase the antioxidant protection in aged animals and humans by the consumption of the flavonoid glycosides of Ginkgo Biloba and improve cognitive performance (Rai et al., 1991), difficulties in concentration (Kleijnen and Knipschild, 1992a,b) and Ca^{2+}-induced increases in the oxidative metabolism of brain neurons (Oyama et al., 1993, 1994). More recent evidence suggests that administration of Ginkgo Biloba Egb 761 (Kanowski et al., 1996) slowed the progression of AD.

It has also been shown that decreasing serum levels of vitamin E were associated with poor memory performance in the elderly (Perkins et al., 1999), while Perrig et al. (1997) have shown that higher ascorbic acid and beta-carotene plasma levels are associated with increased memory performance in the elderly. Additionally, van
Reekum et al. (1999) have suggested that vitamin E may play a preventative role in the development of AD. Therefore, it appears that altering the dietary intake of antioxidants may have similar protective effects on CNS function to those seen with respect to cancer and cardiovascular disease. However, one important question here is whether this can be accomplished by increasing the dietary amounts of fruits and vegetables.

In this regard, our research has suggested that fruit and vegetable extracts that have high levels of anthocyanins and other flavonoids also show increased levels of total antioxidant activity as assessed via the oxygen radical absorbance capacity assay (ORAC). This assay employs β-phycoerythrin (β-PE) or R-PE as an indicator protein, 2,2′-Azobis(2-amidino-propane) dihydrochloride (AAPH) as a peroxyl radical generator, and Trolox as a standard (Cao et al., 1995). Results from the assay have shown that the foods with the highest ORAC activity include spinach and strawberries, (Cao et al., 1996; Wang et al., 1996) and blueberries (Prior et al., 1998).

In a previous study, we examined whether long-term (from 6 to 15 months of age; F344 rats) feeding with a control diet (AIN-93) or supplemented (strawberry extract or spinach extract or vitamin E) diet would prevent age-related decrements in cognitive or neuronal function. Results indicated that the supplemented diets could prevent the onset of age-related deficits in several indices (e.g. signal transduction, such as oxotremorine-enhanced striatal dopamine release (ox-K+ -ERDA)), as well as cognitive behavior (e.g. Morris water maze performance) (Joseph et al., 1998b), with spinach having the greatest effects. These results suggested that phytochemicals present in antioxidant rich foods might be effective in forestalling functional age-related deficits.

However, while prevention of these decrements is important, it is also critical to determine if these interventions would be effective in reversing these deficits once they occur. It is critical to note that at present, the world population comprised of people over 65 years of age represents over 50% of all those who have ever lived having attained this age. Therefore, determinations of whether these dietary supplementations might be effective in aged organisms is of paramount importance. Thus, in a subsequent experiment (Joseph et al., 1999), we examined whether dietary supplementations (for 8 weeks) with spinach, strawberry or blueberry extracts in an AIN-93 diet (at 14.8, 9.1 or 18.6 g dried aqueous extract per kg of diet, respectively) would be effective in reversing age-related deficits in neuronal and behavioral (motor and cognitive) function in aged (19 months) Fischer 344 rats. The results showed that overall these supplements, particularly blueberry, were effective in this regard and reversals were observed in age-related deficits in several neuronal and behavioral parameters including, oxotremorine-enhancement of K+ -evoked release of dopamine from striatal slices, carbachol-stimulated GTPase activity, striatal Ca45 buffering in striatal synaptosomes, motor behavioral performance on the rod walking and accelerod tasks, and Morris water maze performance. In fact, the changes seen with respect to motor behavior are particularly interesting since it is well known that age-related decrements in motor behavior (e.g. deficits in balance and coordination) have been very resistant to reversal. These findings suggest that,
in addition to their known beneficial effects on cancer and heart disease, phytochemicals present in antioxidant rich foods may be beneficial in reversing the course of neuronal and behavioral aging. The mechanisms involved in these changes are being investigated but it may be that the flavonoids and other polyphenolic compounds contained in these foods may have potent anti-inflammatory (Krischer et al., 1997) and membrane fluidizing (Halder and Bhaduri, 1998) properties, in addition to their antioxidant characteristics that may account for these beneficial effects. We are currently investigating these possibilities.

References


