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Neurobehavioral aspects of antioxidants in aging☆

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Abstract

Both aging and age-associated neurodegenerative diseases are associated with various degrees of behavioral impairments, and among the prime candidates responsible for producing the neuronal changes mediating these behavioral deficits appear to be free radicals and the oxidative stress they generate. Therefore, there have been a number of studies which have examined the putative positive benefits of antioxidants in altering, reversing, or forestalling these neuronal/behavioral decrements, with varying degrees of success. Additional experiments have examined the effects of diets rich in fruits and vegetables or herbal extracts in reducing certain types of cancer and cardiovascular diseases, and evidence emerging from such experiments suggests that these kinds of dietary modifications may be beneficial in altering neuronal/behavioral deficits in aging, as well. These kinds of diets are particularly rich in antioxidants such as vitamins A, C, E, and bioflavonoids (such as flavones, tannins, and anthocyanins), and thus, there may be synergistic effects among them. The present paper will review studies concerning the influence of dietary and synthetic antioxidants on normal, pathological age-related, and reactive oxygen species-induced behavioral changes in human and animal subjects. The antioxidants reviewed are vitamin E, α-lipoic acid, and the phytochemicals contained in herbals, fruits and vegetables. Published by Elsevier Science Ltd.

1. Introduction

The industrialized world's population is growing increasingly older, with increases in both life expectancy and in age-related neurodegenerative disorders such as Alzheimer's and Parkinson's Diseases (AD, PD). Approximately 15% of the population over age 65 years are afflicted with AD [160], and 1% by PD [50]. By the year 2000, more than 9 million people will be affected by one of these two diseases [160], and will be in need of extensive care. Importantly, these statistics do not include other types of dementia such as those that result from ischemic injury. As is well known, both aging and age-associated neurodegenerative diseases are associated with various degrees of behavioral impairments that significantly decrease the quality of life and severely tax the current health care system.

Among the prime candidates responsible for producing the neuronal changes mediating these behavioral deficits, however, appear to be free radicals and the oxidative stress they generate. The free radical hypothesis of aging has been utilized to suggest that age-related changes occur as a result of an inability to cope with oxidative stress that occurs throughout the lifespan. It has been utilized to explain the increased incidence of cancer and heart disease, but the brain may be even more vulnerable to oxidative stress, since it exhibits reduced free radical scavenging ability and utilizes high amounts of oxygen [3,7,18,33,44,49,160].

Normal and pathological aging [49], AD [7,133], and PD [115] have been associated with an increased sensitivity to reactive oxygen species, probably the result of pro-oxidant mediators (iron) and a decrease in antioxidants [5,24,135]. Precisely how oxidative stress causes its deleterious effects is not known, but some of this damage may include lipid and protein

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peroxidation [29,151], increases in DNA oxidation products [3,131], and deficits in calcium regulatory mechanisms that may eventually lead to cell death [58,97].

Since a large amount of evidence has accumulated over the years that has implicated oxidative stress as being intimately involved in the deficits seen in aging and age-related neurodegenerative diseases, there have been a great number of studies which have examined the putative positive benefits of antioxidants in altering, reversing, or forestalling these neuronal/behavioral decrements, with varying degrees of success (e.g., see [14,71,77,113,121]).

Additional experiments have examined the effects of diets rich in fruits and vegetables in reducing certain types of cancer and cardiovascular diseases [35,40,132], and evidence emerging from such experiments suggests that these kinds of dietary modifications may be beneficial in altering neuronal/behavioral deficits in aging, as well. These kinds of diets are particularly rich in antioxidants such as vitamins A, C, E, and bioflavonoids (such as flavones, tannins, anthocyanins, and quercetin) [26,27,35,77], and thus, there may be synergistic effects among them. Therefore, it might be important to examine the impact of the antioxidants contained in different foods on various neuronal and behavioral parameters known to change with age (e.g., signal transduction, cognitive behavior and motor behavior) and determine whether their course can be altered.

The present paper will review studies concerning the influence of dietary and synthetic antioxidants on normal, pathological age-related, and reactive oxygen species-induced behavioral changes in human and animal subjects. The antioxidants reviewed are vitamin E, α-lipoic acid, and the phytochemicals contained in herbals, fruits and vegetables.

2. Vitamin E

The lipid soluble vitamin E (vit. E) is an antioxidant compound that includes different tocopherols and tocotrienols. Tocopherols and tocotrienols exist in different isomers that vary in the number and the position of the methyl groups on the chroman ring and a saturated phytyl chain (α-, β-, γ-, δ-tocopherol), or an unsaturated phytyl chain (α-, β-, γ-, δ-tocotrienol). α-tocopherol is the most abundant form of vit. E [34]. Vit. E is recognized as a major lipid soluble chain-breaking antioxidant, preventing lipid peroxidation, and protecting membrane integrity; α-tocopherol is the most efficient isoform of vit. E in terms of its antioxidant capacity [30]. The major sources of vit. E are vegetable and seed oils, and the recommended dietary allowances vary with age, gender, and state of the person (e.g., infants require from 3 to 4 mg of α-tocopherol equivalents (α-TE), males 10 mg α-TE regardless of their age, females 8 mg α-TE, unless pregnant or lactating when the need increases up to 12 mg α-TE) [34].

The relationship between vit. E intake and neuronal function early in development has been established for a number of years. Naturally occurring chronic vit. E deficiency (which is a very rare occurrence, mostly due to severe and longstanding fat malabsorption) causes a syndrome of spinocerebellar degeneration with weakness, ataxia of limbs and gait, loss of deep tendon reflexes, and retinal pigmentation and myopathy [127]. The neurological symptoms generally occur by the second decade of life [47]. It has been suggested that the mechanism underlying these abnormalities is associated with inadequate antioxidant protection [138,139]. Treatment with large doses of vit. E (100 mg/kg/day orally) early in childhood prevents or reverses the insurgence of these neurological manifestations [48,136,137].

With respect to aging, there have been numerous experiments showing beneficial effects of vit. E on immune function [15,42,84,85,123,152], cancer [40,54,68,96,148], and coronary artery disease [76,103,155] in the elderly. Moreover, vit. E in general appears to play a beneficial role in maintaining human good health [100].

More relevant to this review, vit. E may be an important factor in maintaining neuronal integrity [77,90,95,138], and preventing cell loss [33,78,81,145]. Given these findings, it is important to determine if the neuronal protection provided by vit. E supplementation translates into improvements in behavioral function such as age-related decrements in cognitive performance.

2.1. Human studies

2.1.1. Normal aging

In survey studies of normally aging subjects [60,154], a certain percentage showed that vit. E intake and age-related cognitive decline did not correlate with past vit. E intake. However, Šrám and colleagues [140] showed that daily oral supplementation of 300 mg of vit. E (given concomitantly with 1000 mg/day vit. C) for 12 consecutive months improved short-term memory (digit span), psychomotor performance, and overall mood of both males and females, and verbal memory only in females.

One variable that may be important is the length of time that vit. E supplementation is utilized. La Rue and coworkers [65] have shown that healthy aging people who chronically (6 years) supplemented their diets with commercially available vit. E (median = 29.3 mg/day) had higher scores than controls on four of the cognitive measures analyzed in the

New Mexico Aging Process Study. This vit. E group scored better in measures of visuospatial skills, non verbal learning, non verbal memory, and abstract reasoning than controls.

2.1.2. Alzheimer's disease

Several years ago, an in vitro study showed that vit. E protects cell cultures from the toxic effects of amyloid β protein [6]. Since that time there have been a number of experiments that have attempted vit. E. therapy in order to prevent or forestall the progression of AD. Sano and colleagues [124,125] conducted a study on the potential therapeutic use of both selegiline (also known as deprenyl, an MAO inhibitor with additional antioxidant activity) and/or α-tocopherol in patients with AD of moderate severity. The patients were assessed for impairment in individual activities, behavioral disturbance, and clinical dementia over a 2-year period. α-Tocopherol (1000 IU, bid) and/or selegiline (5 mg, bid) treatment increased the level of independence of the patients and delayed the deterioration of daily living performance, but did not improve cognitive test scores. The authors suggest that treatment in the earlier stages of the disease with either selegiline or vit. E may delay the necessity of institutionalization in patients with moderate dementia. However, shortly after the publication of this study concerns were raised regarding the scientific rigor of the statistical analyses utilized [63,111]. These investigators stated that the assumptions of the statistical tests were violated and that other factors might be responsible for the effects (socioeconomic factors and educational levels).

In a subsequent discussion, van Reekum and colleagues [149] concluded that the earlier the treatment with vit. E can begin, the more effective might be the treatment. Therefore, in addition to the factors mentioned above, which affect the efficacy of vit. E, the time of initiation of the treatment may be important. Unfortunately, to date, these points have not been addressed in rigorous examinations.

2.1.3. Parkinson's disease

Perhaps the longest history that associates oxidative stress and age-related neurodegnerative disease can be found with PD [17]. This association began to be examined in the wake of data on 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP), a compound which could produce Parkinsonian-like effects in both animals (e.g., see [83] for review) and humans (e.g., see [69,70] for reviews) and which is postulated to produce its selective damage through oxidative stress mechanisms. In fact, because of the putative oxidative stress effects in PD, as early as the mid-1980s Cadet [17] discussed the potential use of antioxidants (vit. E and selenium) in treating this disease.

To investigate the possible role of vit. E in PD, treatment studies of two types were undertaken: (a) epidemiological, which investigated the correlation between intake of dietary antioxidants and incidence of PD; and (b) controlled administration of vit. E, which examined the clinical efficacy of this vitamin in PD. In the first type, participants are generally screened for PD and administered a food frequency questionnaire to characterize macro- and micronutrient intake frequencies. The results from these relatively uncontrolled studies, as might be expected, are mixed. Surveys of early-life intake of various foods showed that subjects that ate foods particularly rich in vit. E were less likely to develop PD later on in life [38,39]. The Rotterdam study [23] showed that higher dietary vit. E intake is associated with a decreased incidence of PD in a dose dependent manner. Other studies failed to find any type of association between amount of vit. E intake and incidence of PD [51,75,128].

In the clinical studies, high doses of both vit. C (3000 mg/day) and vit. E (3200 IU/day), when administered to PD patients for a period of time ranging between 1 and 8 years before the patients needed levodopa (L-DOPA) to control their symptoms [31,32], delayed the need for levodopa by 2–3 years. Additional studies, however, which have examined the efficacy of vit. E in PD, have produced mixed effects. For example, a combination antioxidant therapy with tocopherol (2000 IU/day) and/or selegiline (10 mg/day) for Parkinsonism (DATATOP) was studied in a multicenter, double blind, placebo controlled study [104–108]. The goal of the study was to delay the need for L-DOPA treatment by 2 years as the result of one of the treatments, and an endpoint was reached when a subject achieved a level of functional disability sufficient to warrant the initiation of L-DOPA treatment. While the findings suggested that there might be selective effects with respect to the monoamine oxidase B inhibitor, selegiline [104,105], in delaying the need for L-DOPA treatment, neither of these agents alone or in combination were successful in ameliorating the motor symptoms [106,107]. Moreover, these treatments were not effective in altering either cognitive performance [62] or mortality [108]. These latter findings differed from previous uncontrolled studies [9,10] that suggested extended life from selegiline treatment.

2.2. Animal studies

2.2.1. Vit. E deficiency

In some of the earliest investigations of the putative role of vit. E in behavioral aging, the effects of vit. E deficiency were examined. Lal and colleagues [66] used chronic vit. E deficiency to 'accelerate' aging and lipofuscin (an aging pigment) accumulation in 45 day old rats who were fed either standard Purina rodent chow or a vit. E deficient diet

for 14 months. For behavioral and histological determinations, three groups of animals were tested (16 month old rats on normal diet, 16 month old rats on low E diet, and 45–60-day old rats on control diet). The results indicated that the vit. E deficient rats showed decreases in weight of several organs including: brain, spleen, heart, kidney, lung and liver as compared to young and age-matched normal vit. E animals (no differences between young and 16 month old animals fed a normal diet were detected). Behaviorally, locomotor activity, conditional avoidance response (learning to escape an electric shock at the sound of a buzzer), one trial retention test (animals were taught to press a lever for food; on the fourth day of training, the last lever press delivered an electrical shock instead of a food pellet), and delayed alternate response were assessed in these animals. Although chronic deficiency of vit. E did not affect locomotor activity, for the 16 month old animals, the vit. E deficient group needed more trials to acquire the conditional avoidance response and showed deficits in the retention of the task compared to the control group. These animals also showed reductions in the retention of one trial learning and in the number of correct responses on the delayed alternate response test, under all delay intervals. Histological analysis of brain lipofuscin showed that young animals had very little lipofuscin, whereas all old animals showed considerable lipofuscin accumulation. Vit. E deficient animals had a higher content of lipofuscin in the hippocampus than the young and their age-matched controls. Thus, the effect of the vit. E deficient diet was greater than the effect of aging alone on both behavioral and histological parameters, and appeared to be independent of locomotor effects [66].

Similar findings were seen by Sarter and van der Linde [126] who found that vit. E deficient animals exhibited a higher content of lipofuscin in the hippocampus and showed no deficits in spontaneous locomotor activity or in reversal learning, but did show reductions in exploratory activity (in a tunnel maze). These findings suggest that the cognitive behavioral effects of vit. E deficiency may be task-specific in that it does not induce behavioral stereotypy or inflexibility (e.g., reversal learning) [126] but it may affect conditional [66] or passive avoidance [53].

Task-specificity effects of vit. E deficiency in locomotor behavior were also seen by Summerfield and Tappel [146] who showed that vit. E deficits are important in mediating the course of motor behavioral deficits in aging. They demonstrated that vit. E-deficient animals show age-related declines of motor activity, in comparison to a group fed a diet with vit. E. One group of animals that had initially been given vit. E treatment and then withdrawn showed an age-related decline in motor activity that was less than groups that did not receive vit. E.

2.2.2. Vit. E supplementation

Ichitani and colleagues [53] supplemented Sprague–Dawley rats from 1 to 25 months of age with vit. E (58.5 mg DL-α-tocopherol/100 g diet). At 25 months of age the rats were tested in a step-through passive avoidance (PAR), and the animals fed the vit. E supplemented diet showed a trend toward higher latencies with respect to control animals.

More positive effects of vit. E supplementation on behavioral and neuronal parameters sensitive to aging were analyzed in a recent study by Joseph and colleagues [57] in which Fischer 344 rats were fed a control diet or one supplemented with vit. E (500 IU) for 8 months. The animals fed the diet supplemented with vit. E had shorter distances to reach the platform in the Morris water maze compared to animals fed a control diet. Moreover, the vit. E supplemented animals did not show the decrements in muscarinic and noradrenergic receptor sensitivity that were seen in the control group (assessed via oxotremorine-enhanced, K^+-evoked dopamine release from striatal slices and Purkinje cells responsive to isoproterenol, respectively). Additionally, striatal synaptosomal calcium buffering ability following oxidative stress (H_2O_2) was greater in the supplemented group than in controls.

Freund [37] analyzed the effect of vit. E on a less conventional model of animal aging: alcohol consumption. The author argues that alcohol may accelerate aging and enhance free radical formation and propagation in the membrane, and that the behavioral deficits seen in aging are comparable to the ones seen in alcohol abuse. Freund's experimental design examined mice treated for five months with chow diets or diets containing ethanol or isocaloric glucose, with or without supplementation of vit. E (the vit. E rich diet supplied ~250 mg/kg body weight/day). It was found that vit. E decreased brain content of lipofuscin in all groups, but failed to prevent ethanol-induced learning deficits in active avoidance.

Other studies have attempted to examine the effects of vit. E supplementation on deficits in locomotor behavior that are induced by various toxic agents known to produce reactive oxygen species or to decrease antioxidant defenses. As an example, cadmium (Cd) reduces the level of endogenous antioxidants and decreases locomotor activity in young rats. Ali and colleagues [1] found that Wister rats, after 4 weeks of Cd treatment (0.4 mg/kg), showed decreased ambulatory time, number of stereotypic movements, and number of vertical movements, and increased resting time in an open field. Animals treated with both

Cd and vit. E (5 mg/kg) did not show any behavioral signs of the Cd treatment, and animals treated with vit. E only presented values comparable to control.

Another study showed that vit. E could antagonize the toxic effects of intrastriatal injections of 6-hydroxydopamine (6-OHDA) [19]. 6-OHDA is a selective catecholaminergic toxin, whose action involves the production of reactive oxygen species [18,21]. Cadet and colleagues [19] were able to show that unilaterally 6-OHDA-lesioned animals that received vit. E supplementation (50 IU/kg) for one month showed reduced apomorphine-induced rotations and antagonized the 6-OHDA effects on dopamine and its metabolites.

2.3. Conclusions

From the literature reviewed above, it appears that vit. E has some protective effects against reactive oxygen species injuries and age-related degenerative processes, although the aforementioned studies at times present interpretative and design problems.

Adult animals and humans, even under conditions of severe dietary vit. E deficiency, did not develop symptoms of deficiency [100]. Successful studies that employ vit. E deficiency as a model of aging have to use weanling animals, and the outcome analyzed may represent a developmental effect of the deficiency more than a reproduction of the aging process. Animal studies exploring vit. E supplementation treatment for age-related behavioral deficits show encouraging data, but it is necessary to systematically analyze the potential benefits of a long-term treatment, to prevent some of the symptoms of aging.

Vit. E supplementation was successful in retarding the need for institutionalization in patients with mild AD [124,125], but was not as successful with PD patients [104–108]. The fact that vit. E was positively correlated with a decrease in PD incidence in the Rotterdam study [23] and in other survey studies [38,39], rather than in the controlled trials of the DATATOP study, may reflect the fact that the survey studies are a retrospective analysis of life-long eating habits that may have started early enough in life to prevent the disease. The failure of the Rotterdam study to confirm findings of other retrospective studies suggests the need for a controlled long-term longitudinal study to assess the existence of this correlation, or lack thereof.

However, the DATATOP study is one which attempts to reverse the symptoms of PD. These symptoms appear when 80% of the nigra has already degenerated [50]. Once the symptoms of PD are evident, vit. E alone may not be enough to slow down the progression of the disease, whereas a life-long healthy diet may not prevent it, but could at least retard its appearance and slow down its progression. In light of the AD and the PD data, and of some of the animal studies reviewed, it would be interesting to study whether vit. E would be more effective in preventing age-related cognitive deficits than severe age-related motor deficits.

One other factor that may be important in this regard is whether or not combinations of antioxidants are utilized. For example, a pilot study of a high-dose antioxidant treatment in early PD showed that treatment with two antioxidants (vit. E and vit. C) in patients with early symptoms of the disease were effective in delaying the need for L-DOPA treatment [31,32], suggesting that vit. E may be more effective if given with other antioxidants. Vit. E can react with lipid peroxyl radicals, inhibiting the chain propagation of peroxidation to form lipid peroxides and tochopheroxyl radicals [13]. Although these radicals may, under certain conditions, mediate further lipid peroxidation [141], cooperative interaction between vit. E and other antioxidants like vitamin C can reduce the tochopheroxyl radicals to regenerate tocopherol [141,147].

3. Alpha-lipoic acid

Alpha-lipoic acid is an eight-carbon dithiol existing in two enantiomeric forms: the R-enantiomer (naturally occurring form) and the S-enantiomer; synthetic α-lipoic acid is a racemic mixture of the S- and R-enantiomers. It is available in a standard diet (especially from tissues with high metabolic activity, e.g., heart), and can be obtained by de novo biosynthesis from fatty acids and cysteine [8].

Alpha-lipoic acid is a coenzyme in the oxidative decarboxylation of α-keto acids [120]. Rosenberg and Culik [122] were the first to observe that α-lipoic acid has an antioxidant action; it has been shown to be a good scavenger of hydroxyl, peroxy radicals, singlet oxygen, and nitric oxide. α-Lipoic acid also appears to be a good chelator of transition metals (for a review see [101]) and has been shown to prevent the symptoms of both neuropathy in rats fed vit. E deficient diets (see [82] for discussion of neuropathy and vit. E) and scurvy in vit. C-deficient rats [114]. Rats fed α-lipoic acid diets were protected against ischemia-reperfusion injury in isolated perfused Langendorff heart system [46,129], in neuronal cultures [91], and in vivo models of ischemia in gerbils [20]. In the Cao and Phillis [20] study they showed that α-lipoic acid (20 mg/kg) significantly reduced the percent increase in spontaneous activity after ischemia when compared to both vehicle and saline controls. α-Lipoic acid also reduced hippocampal histological damage induced by the ischemic episode.

3.1. α-Lipoic acid and aging

In spite of a tremendous potential therapeutic use, unfortunately, just as can be seen with respect to vit. E, until recently, very little research has been carried out on the effects of α-lipoic acid on behavioral correlates of reactive oxygen species-mediated phenomena.

α-Lipoic acid (100 mg/kg body weight) has been shown to improve memory in aged mice [142] treated orally once a day for 2 weeks prior to behavioral testing. Both young (3 months) and aged (20/23 months) mice were tested in an open field, and the level of habituation over three exposures was analyzed. Young animals habituated to the apparatus and explored significantly less as the trials progressed, whereas aged mice, after an initial decline in exploration in the second trial (15 min after the first exposure), showed significantly increased horizontal movements on the third trial (24 h later). The authors found an age-dependent effect of α-lipoic acid, where treatment with α-lipoic acid improved performance in old animals, but did not further decrease young animals' performance. These investigators also showed that while α-lipoic acid did not increase the age-related reductions in the concentration of NMDA in whole brain and the frontal cortex of the mice, this treatment significantly increased the B_{max} of these receptors in the aged animals. However, since many of the NMDA assessments were carried out in whole brain, the correlational relevance to the behavioral evaluations is virtually useless.

In a later study these researchers [143] replicated their previous findings with respect to open field habituation and also examined the efficacy of α-lipoic acid on altering the age-related deficits in Morris water maze performance. As seen with respect to open field, α-lipoic acid was effective in improving performance of the old animals in the Morris water maze without having much effect in the young. The authors conclude that α-lipoic acid improves cognitive function in rodents, and may be useful as a treatment for age-related memory decline. However, there may be some problem with the generalizability of the findings in Morris water maze performance, since the α-lipoic acid was administered intraperitoneally (100 mg/kg body weight for 2 weeks).

3.2. Conclusions

In vitro studies of the protective effects of α-lipoic acid suggest a tremendous therapeutic potential in age-related pathologies, as well as ischemia-reperfusion injuries [102,161]. Moreover, α-lipoic acid appears to: (1) protect cultured neurons against hypoxic, glutamate or iron-induced injury [91]; (2) prevent mitochondrial damage induced by *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment in mice [41]; and (3) regenerate other antioxidants [99]. These features, and the experiments reviewed above, suggest that α-lipoic acid might be effective in slowing down the progression of aging and perhaps age-related neurodegenerative diseases such as PD. However, as can be seen from the citations above with respect to aging, there is a great deal of work that still needs to be accomplished before definitive statements concerning the possible therapeutic role of this compound can be made. To date, even the doses and routes of administration to achieve maximal effect on a particular behavior have not been characterized.

4. Phytochemicals

In the previous sections, prior to discussing the effects of particular dietary supplements that may alter behavior in aging, we focused on the behavioral effects of single putative antioxidants such as vit. E or alpha-lipoic acid. However, as pointed out in the Introduction, recent evidence that will be reviewed below suggests that there may be increased beneficial effects with combinations of antioxidant phytochemicals [e.g., allium compounds (diallyl sulfide, Allyl methyl trisulfide); carotenoids (α-carotene, β-carotene, lutein, lycopene); and of course, flavonoids (quercetin, kaempherol, myricetin, ellagic acid, genistein, diadzein, etc.)] that are contained in fruits, vegetables and herbals. Unfortunately, as mentioned above with respect to vit. E and α-lipoic acid, there is still a paucity of studies that are directed toward determining the positive behavioral effects of fruits, vegetables and herbals. In fact, with respect to herbals, the most intense study has been of EGb 761 (the extract of the dried leaves are standardized to contain 24% flavonoids and 6% terpenes) [118], which is derived from the *Ginkgo biloba* leaf. *Ginkgo biloba* is a traditional remedy that has been present in Chinese medicine for several thousand years [118].

4.1. EGb 761

EGb 761 contains flavonglycosides, terpenoids derivates, protoanthocyanidines and some organic acids [28]. Standard pharmacological tests have found that while EGb 761 does not possess sedative, antipsychotic, anxiolitic, anticonvulsant, or analgesic activity after acute administration [117], it is a potent antioxidant [11].

4.1.1. Reversal of memory impairments in AD and aging

Because of the association of AD with reactive oxygen species injuries and the action of EGb 761 as an antioxidant, *Ginkgo*'s extracts have been tried as a potential treatment for dementia of the Alzheimer's type

(DAT). In one study [79], assessments of the effects of *Ginkgo* extract EGb 761 were examined on DAT, using a placebo group and a EGb 761-treated (240 mg/ three times a day for 3 months) group. The control and EGb 761-treated group were compared on a battery of psychometric tests. The subjects in the two groups were unequally distributed so that the EGb 761 group had lower initial cognitive scores. The patients were subjected to the following tests: SKT-test (battery of tests including naming objects, immediate recall, naming numerals, arranging blocks, replacing blocks, counting symbols, reversal naming, delayed recall, and recognition memory; this assesses the patient's attention and memory), multiple vocabulary test (carried out to establish the premorbid general intelligence level of the patients), trail making test (ZVT), and the Alzheimer Disease's assessing scale (ADAS). The analysis of the SKT scores showed a significant improvement after three months of treatment with EGb 761. ZVT and ADAS scores showed a trend toward improvement without reaching statistical significance, probably due to the fact that 6 patients were given the highest possible scores because they could not complete the tests in the allotted time. Ninety percent of the subjects receiving the treatment requested continuation of the medication after the termination of the study. Similar results were obtained in comparable studies conducted on DAT and patients with mild signs of multi-infarct dementia (MID) treated with a lower daily dose of EGb 761 (240 mg/day; 120 mg/day respectively) [61,72].

These results supported those of a previous study in which elderly subjects with mild memory impairments showed improvement in speed of information processing after treatment with EGb 761 (320 or 600 mg/ day/3 weeks EGb 761) or placebo [2]. The subjects were tested for dual-coding of verbal material and images. The placebo group recalled significantly more images than words at the longest presentation time, whereas the EGb 761 treated groups significantly shifted the difference toward a shorter presentation time.

In animal aging studies, the effects of EGb 761 have been examined in both rats and mice. The aging process is associated with, among other things, modifications in membrane fluidity, and changes in the phospholipids to cholesterol ratio [144]. EGb 761 (100 mg/kg body weight/day/3 weeks) was tested in a group of young (3 month), middle age (12 month) and old (22–24 month) mice, to investigate its effects on passive avoidance learning and membrane fluidity. EGb 761 had some positive effect on membrane fluidity, without bringing the levels of anysotropy of the old animals back to the levels of the young ones. However, there was a profound effect of EGb 761 on passive avoidance learning, whereby EGb 761 treatment prevented the deleterious effects of aging on this parameter in all three age groups tested.

Aging rats were also tested for learning and memory in a delayed nonmatching to position task (DNMPT), and in a more common version of the RAM, following treatment with EGb 761 [159]. The animals started training in the RAM at the age of 6 weeks, and were trained throughout their life. DNMPT consisted of training the animals not to reenter arms that were baited, and to enter arms that were previously blocked and not baited. At the age of 21 months, EGb 761 (50 mg/kg diluted in condensed milk) had no significant effect on the acquisition of the radial arm maze task, but it did positively affect the DNMPT by decreasing the number of errors. There was also some indication that EGb 761 increased the life span of the animals.

4.1.2. Experimentally-induced neuronal and behavioral deficits

In animal models, extracts of *Ginkgo* leaves were found to have protective effects on free radical insults such as cerebral ischemia [45,73,162] and hypoxia [94], where both the signs of neuronal damage and the amount of lipid peroxidation were reduced. EGb 761 was also shown to be effective in neuronal cultures, reducing the damage from oxidative stress induced by hydrogen peroxide [98] or MPTP. Interestingly, however, it had no effect on 6-OHDA neurotoxicity [118].

Young rats (3 months) with hemiplegia induced by excision of the right somatomotor cortex showed improvement of their motor performance slightly earlier than their old counterparts (24 months) after treatment with *Ginkgo* extracts [12]. A higher priming (p) dose was given for 7 days, followed by 21 days using a lower maintenance (m) dose. Motor impairments due to the lesion were measured using a continuous deficit score (0 = no deficit; 6 = total incapacity). The animals were trained to balance themselves and walk on a narrow (2.5 cm) beam. On the first day after surgery, most animals could not complete the task, with the old animals performing worse than the young ones. From the second day on, all the animals progressively improved their performance with the young animals showing a faster trend to recovery than the old ones. EGb 761 treatment (p50–m10) starting on day 2 after surgery improved young rats performance; the positive effect was present several days following cessation of the treatment. In comparison to the young animals, the beneficial effects of EGb 761 treatment was slightly delayed in the aged animals. Their motor performance did not improve before the 7th day of the maintenance period. However, two higher doses (p100–m50 and p200–m100) of EGb 761 improved the old rats' motor performance as early as day 2 after surgery and the

effect was still present 2 days after the cessation of the treatment.

Mechanistically, one reason for the beneficial effects of EGb 761 may be due to the relatively high concentration of quercetin that is found in this compound. In studies recently completed [153], we have found that quercetin is a very potent antioxidant that is very effective in reversing the deleterious effect of hydrogen peroxide on Ca^{2+} in PC-12 cells. Decrements in cell calcium buffering can lead to a loss of cell viability, and the loss of Ca^{2+} homeostasis under OS has been found in both the hippocampus (e.g., see [67]) and striatum [22].

4.2. Ginseng

Similar positive benefits on behavior in aging have been reported with *Panax ginseng* in combination with other traditional Chinese prescriptions. *Ginkgo biloba* and *Panax ginseng*, alone or in combination, were tested in young (3 month) and old (26 month) rats, on a battery of negatively reinforced learning tests (two-way active avoidance; passive avoidance/step-down; passive avoidance/step-through), and on the MWM [110]. Ginseng (17, 50, 150 mg/kg body weight, orally), *Ginkgo* (10, 30, 90 mg/kg body weight, orally), and the combination of the two compounds increased the number of avoidance responses in the two-way passive avoidance at all doses tested. This test was performed in young animals only, and simply confirmed the facilitatory action of the treatments on learning. Again the two compounds, and their combination, at medium doses prolonged step-down latency and increased performance in the retention test of the step-down version of the passive avoidance in young animals. Old animals required higher doses to increase their performance. Step-through and MWM tests were administered only to young animals and only a minimal effect of the treatments was found.

A similar facilitatory effect of herbal combinations was seen in the senescence accelerated mice (SAM) model, which was used to evaluate the behavioral effects of DX-9386 [93] and S-113 m [92]. DX-9386 consists of *Panax ginseng, Polygala tenuifolia, Acorus gramineus*, and *Poria cocos* in the ratio of 1:1:25:50. This herbal combination has been used in Chinese medicine for the treatment of brain hypoxia and senile amnesia [93]. DX-9386 was supplemented in the diet in the ratio of 1%, for 8 months. DX-9386 improved memory retention in the passive avoidance/step-down test and spatial memory in the MWM in SAM P8 (senile prone) mice, but did not affect performance of the learning behaviors in SAM R1 (senile resistant) mice. Additionally, DX-9386 reduced motor activity (measured in a round tilting-type cage) in both strains. SAM P8 mice had higher levels of lipid peroxides in liver and serum than their reference strain (SAM R1). DX-9386 decreased the levels of lipid peroxides in SAM P8 in both liver and serum to levels comparable to the ones seen in the SAM R1 strain; the treatment did not affect the levels of lipid peroxides of the SAM R1 strain.

S-113 m consists of *Biota orientalis, Panax ginseng*, and *Schizandra chinensis* in the ratio of 1:1:3. Again this herbal combination is used in Chinese traditional medicine to improve memory, and to prevent amnesia and a variety of other conditions not related to the central nervous system [92]. Chronic administration (1% in the diet for 4–9 months) of this herbal combination to SAM P8 and SAM R1 mice showed no effect on motor activity (tilting type ambulometer) or two-way active avoidance, but decreased the number of errors in both strains in two tests of passive avoidance (step-down and step-through). No effect of S-113 m on lipid peroxides was found.

4.3. Fruits and vegetables

For many years now, studies have shown that an increased intake of fresh fruits and vegetables may lower the incidence of ischemic heart disease mortality [4,150] (See also [52,80]). Consumption of fruits and vegetables is also correlated with reducing the incidence and mortality rates of cancer in humans (e.g. [25,157,158]) and animals (e.g. [156]). Even extracts of single foods such as garlic (e.g. [112]) and tomato (lycopene [130]) can have some antitumor properties.

However, although there are numerous experiments that have been carried out with respect to the beneficial effects of fruits and vegetables in cardiovascular disease and ischemia, until recently their putative positive effects on CNS aging and behavior have not been examined.

In a similar series of experiments to the ones mentioned above, the SAM model was used to test aged garlic extract (*Allium sativum* extracted for more than 10 months in ethanol, this extract contains S-allycisteine, S-allymercaptocysteine, allicin and diallosulfides) [88,89]. Two different strains of SAM P (P8 and P10) responded similarly to aged garlic extract; motor activity was not affected by garlic extract treatment, however both groups showed increased survival and better performance in both passive and active avoidance. The authors attribute this effect to the antioxidant properties of the aged garlic extract.

In a recent study, long-term effects of dietary strawberry and spinach extracts were investigated in 15 month old Fischer 344 rats to examine if fruits and vegetables with high antioxidant activity were beneficial in preventing functional age-related central nervous system and cognitive behavioral deficits [57] (see Section 2). The chemical composition of these extracts

is the subject of ongoing investigations. The extracts were supplemented to a control diet so that each diet would supply equivalent antioxidant activity. All the animals were fed the diets for a period of 8 months (starting at age 6 months) and then tested in the MWM using a working memory paradigm. Parameters of receptor sensitivity loss (dopamine release in isolated striatal slices, cerebellar Purkinje cell activity), calcium buffering capacity (calcium efflux in striatal synaptosomes), and GTPase activity in striatal membranes were also recorded. Spinach, but not strawberry extracts, decreased the time and distance necessary to reach the platform in the reference memory trial of the MWM. The spinach extract group had a greater increase than the strawberry extract group in oxotremorine elicited, K^+-enhanced dopamine release from striatal slices, and the number of synaptosomes able to buffer calcium after exposure to H_2O_2; both spinach and strawberry extracts increased the number of Purkinje cells responsive to isoproterenol. Strawberry, but not spinach extracts, prevented the age-induced decrements in carbachol-stimulated GTPase activity. In a follow-up study [56], similar supplementation with diets high in antioxidant activity (strawberry, spinach, plus the addition of a blueberry diet) were investigated for their ability to reverse age-related declines. Old rats (19 months) were fed these diets for 2 months and the above parameters were assessed, with the inclusion of motor behavior. Overall, the blueberry-supplemented animals showed the greatest reduction of age effects on all parameters, while the strawberry and spinach also had some positive effects over the control-fed animals. The authors conclude that nutritional intervention with fruits and vegetables may play an important role in preventing and reversing the deleterious effects of aging on neuronal function and correlative cognitive and motor behaviors [55–57].

Mechanistically, it is still too early to identify the particular properties of the phytonutrients contained in these fruits and vegetables that may be involved in these effects. There may also be properties of the phytochemicals contained in spinach, strawberries and blueberries that may produce effects other than antioxidant protection. There is evidence that flavonoid compounds can increase membrane fluidity [44,119,144], and a previous experiment has shown [59] that experimental decreases in membrane rigidity (via s-adenosyl-l-methionine) can ameliorate deficits in striatal signal transduction in old animals. Phytonutrients (e.g., anthocyanins and other flavonoids) contained in the fruits and vegetable have also been shown to antagonize arachidonic acid transport [64] and suppress the 5-lipoxygenase pathway [86] and thus reduce inflammatory responses [11,36].

4.4. Conclusions

Compared to the variety of nutrients present in the diet, relatively little research has been done on the effects of phytochemicals on behavior and associated age-related neuronal deficits. Most of the research that has been done is on herbal extracts already known in traditional Chinese medicine for their action on the central nervous system. Although the extracts reviewed here have potent antioxidant capacities, their action is not confined solely to this aspect of their pharmacology. *Ginkgo biloba, Panax ginseng*, and even aged garlic contain multiple compounds that most likely act synergistically on a variety of processes. *Panax ginseng* can potentiate pentobarbitone induced-sleep and amphetamine induced motor activity; it also has anti-nociceptive properties, can modulate 5-HT and L-DOPA activity, attenuate stereotyped behavior induced by apomorphine and amphetamine, and antagonize foot shock induced aggression in mice [87]. In humans it has anti-stress and anti-fatigue actions accompanied by anxiolytic activity [87]. This wide variety of actions cannot be solely attributed to any specific antioxidant activity, but may be more of a general central nervous system modulatory activity.

EGb 761 extract has anti-inflammatory as well as antioxidant properties, and can also modulate neurotransmitter function [72]. It also lowers blood viscosity, improves blood flow [110], and alleviates a number of cognitive disorders. Aged garlic itself has also been demonstrated to have an anti-stress action [88].

All the experiments examined here considered the herbal extracts for their antioxidant capacity only, with very promising results. All the compounds possess potent antioxidant capacities, but little is known about which specific chemicals in the extracts possess this capacity and if the effects seen in these experiments are attributable to the antioxidant capacity alone or to a combination of effects. This may be especially true in the case of the flavonoids contained in fruit and vegetable extracts (see above).

5. General conclusions

Epidemiological data indicates that diets rich in antioxidants may play a pivotal role in maintaining good human health [35]. The literature reviewed here shows that dietary antioxidants play some role in preventing or slowing the progression of age-related neurological and behavioral impairments, possibly due to reactive oxygen species insults. The different results obtained in the various studies may be due to the diversity of the doses of the various antioxidants used, to the different modality of administration, to the diversity of methods used, to the timing of the treatments, and most impor-

tantly to the fact that in most studies only one antioxidant was analyzed at a time.

A design for the 'perfect diet' or the perfect dietary supplementation should take into consideration a number of factors, such as synergistic action of antioxidants and good balance with other components of the diet (that may have both nutritional and psychotropic activity). Another important factor to keep in mind is that the diets may have to be initiated early in life to be effective.

Antioxidants react with reactive oxygen species to form intermediate species bearing highly reactive radicals that need further inactivation to block the chain reaction. Endogenous and exogenous antioxidants work synergistically to block the propagation of oxidative insult [16]. If it is true that endogenous antioxidants decline with age and in some neurodegenerative diseases [5,43,134], supplementation with only one may not be effective enough to prevent the damage. It has long been known that substituting one missing antioxidant for a different one may not be an effective strategy [74]. Moreover, although most antioxidants can scavenge a wide array of reactive oxygen species, some antioxidants may be more effective on a particular reactive oxygen species than others. In fact, *Ginkgo biloba* was effective in antagonizing the effects of MPTP but not 6-OHDA [118], whereas vit. E was effective in antagonizing the effects of 6-OHDA [116].

Age-related behavioral problems are the end-point manifestation of a life-long process. Once the behavioral dysfunction is manifest, many systems have already degenerated. It could be difficult to rescue systems whose degeneration is too advanced, whereas it could be easier to prevent or to slow down the degeneration. Acute interventions with antioxidants in age-related neurodegenerative diseases like PD may not be as effective as a healthy diet carried on for an entire life span. In fact, as pointed out above, the Rotterdam study [23] surveyed eating habits of subjects with and without PD and found a correlation between increased vit. E supplementation and decreased incidence of PD, whereas the DATATOP results are not so encouraging [106,107]. These data are in accordance with another survey study that found an inverse correlation, albeit not as strong, between antioxidant intake and PD [51]. Perrig and colleagues [109] replicated the Rotterdam study findings in a survey study that also inversely correlated memory performance and antioxidant intake in the old and very old. Other surveys failed to find correlations between antioxidant supplementation and PD incidence [128] and cognitive function in elderly patients [60,154]. One study failed to find a correlation between PD and antioxidants, but did find increased PD in patients with diets that increased their overall pro-oxidant conditions [75]. The discrepancies between the studies could be due to a number of factors: (a) the surveys were not accurate; (b) the concomitant pro-oxidant conditions were so overwhelming that they covered up any antioxidant effect; (c) not all aspects of cognitive function respond to antioxidant treatment; and (d) antioxidants alone do not produce the effect and need to be associated with other compounds to be effective. As already mentioned some of these conditions are true in some of the studies: increased pro-oxidant conditions [75], and differential effects of antioxidants on different aspects of memory [65,140].

In this regard, all of the studies that did not find a correlation between antioxidants and cognitive performance use 30-point mini mental evaluations to determine cognitive performance in the subjects [60,128,154]; whereas the studies that did find an effect employ a more in depth analysis of cognitive performance. These studies find differential effects of the antioxidants on cognitive performance; working memory is the least affected, whereas semantic memory is the most sensitive to the treatment [23,51,79,109].

Similar results were obtained in animal studies: Ichitani and colleagues [53] found a vit. E effect in the passive avoidance task, but not in the spatial memory task; working memory was not affected by vit. E deprivation in an animal model of aging [126]; and *Ginkgo* extracts were effective in antagonizing aging effects in a delayed non matching to position task, but not in the radial arm maze [144,159]. According to this literature, the radial arm maze is not the best test to analyze the interaction between aging, memory and antioxidants.

Bioflavonoids were markedly more effective than vit. E in antagonizing the effects of aging or reactive oxygen species challenges. It is still unclear whether the effect is due solely to their antioxidant activity, or to a combination of effects. In the studies cited above, Joseph and colleagues [56,57] showed that although the antioxidant capacities of the fruits and vegetables used were held constant (based on assessments of their total antioxidant capacity) the neuronal and behavioral effects differed. This suggests that these extracts may contain more than one active principle with different activities.

In spite of dietary antioxidants overall positive influence on human well being, it should not be forgotten that bioflavonoids are characterized by a number of antithetic effects [36]. For instance, quercetin was found to have both carcinogenic and carcinostatic activity [36]. These contrasting effects of quercetin are thought to be the results of concomitant factors such as the relative oxidative status of the organism, different challenges from other potentially harmful compounds or maybe due to the kind of plant from which quercetin itself is isolated [36]. It is important to keep in mind the opposite effects that certain dietary antiox-

idants can exert in order to determine the optimal dosage of these antioxidants in the diet. The studies reviewed here take into consideration a wide array of antioxidants, doses, modalities of administration of the antioxidants, and sources of the antioxidants. None of these studies examine systematically whether there is a difference of effect or efficacy between the different modalities of administration of the various antioxidants, nor do they examine whether there is a difference of effects between dietary supplements and antioxidants directly provided by the diet.

Although the role of diet in human life has been studied for a long time, the impact of diet and its micronutrients is still in its infancy. All the literature reviewed above provides some insight into the interaction between oxidative damage, behavior and possible interventions, but there are still many questions unanswered and many methodological problems that need to be solved. Dietary antioxidants may provide some protection from reactive oxygen species sensitivity; increased intake of certain nutrients may provide that balance between the different antioxidants needed and other substances that may provide some other kind of protection. It is crucial to know whether a better diet may positively influence human behavior so as to decrease the deleterious effects of aging.

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