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Antioxidant strategies for Alzheimer's disease

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Oxidative damage is present within the brains of patients with Alzheimer's disease (AD), and is observed within every class of biomolecule, including nucleic acids, proteins, lipids and carbohydrates. Oxidative injury may develop secondary to excessive oxidative stress resulting from β-amyloid-induced free radicals, mitochondrial abnormalities, inadequate energy supply, inflammation or altered antioxidant defences. Treatment with antioxidants is a promising approach for slowing disease progression to the extent that oxidative damage may be responsible for the cognitive and functional decline observed in AD. Although not a uniformly consistent observation, a number of epidemiological studies have found a link between antioxidant intake and a reduced incidence of dementia, AD and cognitive decline in elderly populations. In AD clinical trials molecules with antioxidant properties such as vitamin E and *Ginkgo biloba* extract have shown modest benefit. A clinical trial with vitamin E is currently ongoing to determine if it can delay progression to AD in individuals with mild cognitive impairment. Combinations of antioxidants might be of even greater potential benefit for AD, especially if the agents worked in different cellular compartments or had complementary activity (e.g. vitamins E, C and ubiquinone). Naturally-occurring compounds with antioxidant capacity are available and widely marketed (e.g. vitamin C, ubiquinone, lipoic acid, β-carotene, creatine, melatonin, curcumin) and synthetic compounds are under development by industry. Nevertheless, the clinical value of these agents for AD prevention and treatment is ambiguous, and will remain so until properly designed human trials have been performed.

Alzheimer's disease: Oxidative stress: Antioxidants: Vitamins: Dietary supplements

A large number of studies indicate that oxidative injury is present in the brains of patients with Alzheimer's disease (AD) and may play a role in the development of AD (Pratico & Delanty, 2000; Rottkamp *et al.* 2000; Smith *et al.* 2000). Oxidative damage has been found in all classes of organic molecules that are critical for maintaining neuronal structural and functional integrity. Excessive lipid peroxidation (e.g. malondialdehyde, 4-hydroxynonenal, isoprostanes), protein oxidation (e.g. protein carbonyls, nitrotyrosine, dityrosine etc.), DNA oxidation (DNA strand breaks, base modification) and glyco-oxidation (e.g. advanced glycation endproducts) have all been documented in the brain in AD.

Studies have demonstrated an increase in oxidized lipids using a variety of methods. Malondialdehyde and 4-hydroxynonenal are products of lipid peroxidation. Studies indicate both increased malondialdehyde concentrations in the brain in AD (Lovell *et al.* 1995; Marcus *et al.* 1998), as well as 4-hydroxynonenal protein adducts in neurofibrillary tangles (Markesbery & Lovell, 1998). F-2 isoprostanes (isomers of prostaglandins derived from free radical oxidation of polyunsaturated fatty acids) are elevated in plasma, urine and cerebrospinal fluid (CSF) of patients with AD (Pratico *et al.* 2000). F-4 isoprostanes, derived from free radical oxidation of docosahexaenoic acid (DHA) are also increased in AD (Nourooz-Zadeh *et al.* 1999).

Protein carbonyls, a measure of protein oxidation, are present in both tangle- and non-tangle-bearing neurons of brains in AD (Smith *et al.* 1996). Nitrotyrosine is similarly found in neurons of patients with AD, suggesting peroxynitrite-mediated protein damage (Good *et al.* 1996; Smith *et al.* 1997). Oxidative injury to DNA is suggested by elevated levels of DNA strand breaks and oxidized bases. Brain samples from patients with AD show approximately a twofold higher number of DNA strand breaks than those of controls (Mullaart *et al.* 1990).

Abbreviations: AD, Alzheimer's disease; CSF, cerebrospinal fluid; DHA, docosahexaenoic acid.
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Hydroxyl radical attack on deoxyguanosine is indicated by a threefold increase in 8-hydroxy-2-deoxyguanosine in mitochondrial DNA from brain samples from patients with AD (Mecocci *et al.* 1994). In AD hippocampal neurons demonstrate intense cytoplasmic staining with 8-hydroxy-2-deoxyguanosine antibodies (Nunomura *et al.* 1999). 8-Hydroxy-2-deoxyguanosine is increased in the ventricular CSF from patients with AD (Lovell *et al.* 1999*a*).

Oxidative processing of monosaccharides can result in the formation of abnormal glycosylated proteins (advanced glycation endproducts). Plaque fractions of brains from patients with AD contain a threefold increase in advanced glycation endproduct adducts compared with controls (Vitek *et al.* 1994). Advanced glycation endproducts are also increased in neurofibrillary tangles and neuritic plaques, suggesting a close link between abnormal oxidation of monosaccharides, glycosylation of proteins and fundamental AD pathology (Guevara *et al.* 1998; Yan *et al.* 1994).

Oxidative stress in AD may result from aging, energy deficiency, inflammation or excessive production of β-amyloid. β-Amyloid, present in amyloid plaques, can induce cell death through a mechanism involving H_2O_2 (Behl *et al.* 1994). Overproduction or impaired clearance of β-amyloid is believed by many researchers to be a critical step in the development of AD. Interestingly, oxidative stress also induces the expression and misprocessing of amyloid precursor protein leading to the generation of amyloidogenic fragments (Gabuzda *et al.* 1994; Misonou *et al.* 2000). This process can result in a potentially vicious cycle whereby oxidative stress leads to β-amyloid and β-amyloid, in turn, leads to more oxidative stress, neuronal dysfunction and ultimately neuronal death.

A variety of compounds and antioxidants have been suggested for reducing the oxidative stress associated with AD (Table 1). Many of these agents have been shown to protect cells from β-amyloid-induced neurotoxicity. Table 2 summarizes studies that have compared antioxidant concentrations in AD patients and controls. A number of studies suggest that antioxidant concentrations in the plasma, CSF and brains of AD patients may be altered. It is not clear, however, whether these changes contribute to or exacerbate

Table 1. Some agents with antioxidant properties proposed for prevention or treatment of Alzheimer's disease

Agent	References
Vitamin E*	Behl *et al.* (1992), Zhou *et al.* (1996), Subramaniam *et al.* (1998), Pereira *et al.* (1999), Yatin *et al.* (1999)
Vitamin C*	Behl *et al.* (1994), Yallampalli *et al.* (1998)
Vitamin A	Jama *et al.* (1996), Perrig *et al.* (1997)
Ubiquinone	Beal & Matthews (1997)
Idebenone*	Hirai *et al.* (1998), Pereira *et al.* (1999)
Creatine*	Brewer & Wallimann (2000)
Lipoic acid	Packer *et al.* (1997), Hager *et al.* (2001)
Cholesterol*	Zhou & Richardson (1996)
Docosahexaenoic acid	Hossain *et al.* (1999), Jeyarajah *et al.* (1999)
Zn*	Lovell *et al.* (1999*b*), Huang *et al.* (2000), Moreira *et al.* (2000)
Li*	Wei *et al.* (2000)
Se*	Jimenez-Jimenez *et al.* (1996)
Ginkgo biloba extract*	Bastianetto *et al.* (1999), Yao *et al.* (2001)
Ginseng	Kim *et al.* (1998)
Acetylcarnitine*	Behl *et al.* (1994)
Melatonin*	Pappolla *et al.* (1997), Daniels *et al.* (1998), Bachurin *et al.* (1999)
Indole-3-proprionic acid*	Chyan *et al.* (1999)
Curcumin*	Kim *et al.* (2001)
Resveratrol	Roth *et al.* (1999)
Quercetin	Roth *et al.* (1999)
Vinpocetine	Pereira *et al.* (2000)
Ferulic acid	Yan *et al.* (2001)
Pycnonegenol	Liu *et al.* (2000)
Garlic extract, aged	Borek (2001)
Deferoxamine	Rottkamp *et al.* (2001)
Tacrine*	Fagarasan & Efthimiopoulos (1996), Svensson & Nordberg (1998)
Donepezil*	Svensson & Nordberg (1998)
Huperzine A*	Xiao *et al.* (2000*a*)
Nicotine*	Kihara *et al.* (1997)
Flupirtine*	Muller *et al.* (1997)
Indomethacin*	Fagarasan & Aisen (1996)
Glutathiamine ethyl ester*	Pereira *et al.* (1999)
N-acetylcysteine*	Olivieri *et al.* (2001)
β-FGF*	Mark *et al.* (1997)
Oestrogen*	Behl *et al.* (1997)
Kaempferol*	Roth *et al.* (1999)

β-FGF, β-fibroblast growth factor.
*Agents shown to be protective against β-amyloid toxicity.

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Table 2. Case-control studies examining antioxidant concentrations in patients with Alzheimer's disease (AD) and controls

Antioxidant	Antioxidant levels relative to controls		References
Vitamin E	Plasma:	↓	Jeandel *et al.* (1989), Zaman *et al.* (1992), Jimenez-Jimenez *et al.* (1997), Sinclair *et al.* (1998), Foy *et al.* (1999), Bourdel-Marchasson *et al.* (2001)
		—	Riviere *et al.* (1998), Schippling *et al.* (2000)
	CSF:	↓	Jimenez-Jimenez *et al.* (1997)
		↓ trend	Schippling *et al.* (2000)
	Brain:	—	Metcalfe *et al.* (1989), Adams *et al.* (1991)
Vitamin C	Plasma:	↓	Jeandel *et al.* (1989), Riviere *et al.* (1998), Foy *et al.* (1999), Schippling *et al.* (2000)
		—	Sinclair *et al.* (1998)
	CSF:	↓	Schippling *et al.* (2000)
Vitamin A	Plasma:	↓	Jeandel *et al.* (1989), Zaman *et al.* (1992), Jimenez-Jimenez *et al.* (1997), Foy *et al.* (1999), Bourdel-Marchasson *et al.* (2001)
		↓ α-Carotene	Schippling *et al.* (2000)
		— β-Carotene	Schippling *et al.* (2000)
		—	Sinclair *et al.* (1998)
	CSF:	—	Schippling *et al.* (2000)
Ubiquinone	Plasma:	—	de Bustos *et al.* (2000), Schippling *et al.* (2000)
	Brain:	↑	Soderberg *et al.* (1992)
Se	Plasma:	↓	Jeandel *et al.* (1989)
		—	Meseguer *et al.* (1999)
	CSF:	—	Meseguer *et al.* (1999)
	Brain: –except ↑ in amygdala		Cornett *et al.* (1998)
Zn	Plasma:	—	Molina *et al.* (1998), Maes *et al.* (1999)
		↓	Jeandel *et al.* (1989)
	CSF:	↓	Molina *et al.* (1998)
	Brain:	↑	Samudralwar *et al.* (1995), Deibel *et al.* (1996), Cornett *et al.* (1998), Suh *et al.* (2000)

CSF, cerebrospinal fluid; (↓), reduced; (↑), increased; (—), no change.

Table 3. *Association of antioxidants with Alzheimer's disease (AD) or cognitive impairment (CI) in community studies*

Antioxidant	Association between higher intake or serum concentration of antioxidant and risk of AD or CI		References
Vitamin E	AD:	↓	Morris *et al.* (1998), Engelhart *et al.* (2000)
		—	Masaki *et al.* (2000)
	CI:	↓	La Rue *et al.* (1997), Schmidt *et al.* (1998), Perkins *et al.* (1999), Masaki *et al.* (2000), Morris *et al.* (2000)
		—	Jama *et al.* (1996), Perrig *et al.* (1997)
Vitamin C	AD:	↓	Morris *et al.* (1998), Engelhart *et al.* (2000)
		—	Masaki *et al.* (2000)
	CI	↓	La Rue *et al.* (1997), Perrig *et al.* (1997), Paleologos *et al.* (1998), Masaki *et al.* (2000)
		—	Jama *et al.* (1996), Schmidt *et al.* (1998), Perkins *et al.* (1999), Morris *et al.* (2000)
Vitamin A	AD:	—	Engelhart *et al.* (2000)
	CI	↓	Jama *et al.* (1996), La Rue *et al.* (1997), Perrig *et al.* (1997), Schmidt *et al.* (1998)
		—	Perkins *et al.* (1999)
Se	AD:	—	Engelhart *et al.* (2000)
	CI:	—	Perkins *et al.* (1999)
		↓	Berr *et al.* (2000)
Red wine	CI:	↓	Orgogozo *et al.* (1997)

(↓), Reduced risk; (–), no association.

AD, or are simply epiphenomenona with only minimal, if any, impact on the rate of cognitive and functional decline. Table 3 summarizes reports that have evaluated the relationship between antioxidants and the development of AD or cognitive impairment in community studies. There have been very few randomized double-blind clinical trials evaluating antioxidants for AD. Most of our knowledge to date relies on epidemiological studies. In these investigations antioxidant intake is non-random and determined by the individual subject based on their own dietary preferences, thereby making it difficult to generalize results to other subjects. The following discussion highlights those dietary antioxidants that have been most frequently studied.

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Antioxidant vitamins and minerals

Vitamin E

Vitamin E is a lipid-soluble (membrane) antioxidant. There are several postulated mechanisms for α-tocopherol to exert such an effect, including protection of neurons from β-amyloid protein toxicity (Behl *et al.* 1992), trapping of free radicals and inhibition of lipid peroxidation.

There is a reasonable amount of evidence that vitamin E metabolism is altered in patients with AD. Jeandel *et al.* (1989) found that serum vitamin E concentrations were decreased compared with normal controls. This finding is consistent with more recent findings by other investigators (Zaman *et al.* 1992; Jimenez-Jimenez *et al.* 1997; Sinclair *et al.* 1998; Foy *et al.* 1999; Bourdel-Marchasson *et al.* 2001). However, Riviere *et al.* (1998) did not detect a significant difference in plasma vitamin E concentrations between normal controls and patients with AD. Jimenez-Jimenez *et al.* (1997) found lower vitamin E concentrations in the CSF of patients with AD and Schippling *et al.* (2000) found a similar trend. Metcalfe *et al.* (1989) found no difference in cerebral tocopherol concentrations between patients with AD and controls. Adams *et al.* (1991) also observed no differences in vitamin E concentrations in most brain regions, but found an increased concentration of vitamin E in the midbrain of patients with AD.

In cross-sectional data from The Austrian Stroke Prevention Study (Schmidt *et al.* 1998) cognitive performance, as measured by the Mattis Dementia Rating Scale, was compared with plasma levels of serum antioxidants. Only α-tocopherol remained associated with cognitive function after linear regression analysis for possible confounders. Decreasing serum concentrations of vitamin E per unit cholesterol were also associated with lower memory performance in a large multi-ethnic elderly sample in the USA (Perkins *et al.* 1999).

Vitamin E supplementation has also been asserted to exert effects specific to AD. A recent report from the Rotterdam Study (Engelhart *et al.* 2000) found that high dietary intake of vitamin E decreased the risk of subsequent development of AD. Conversely, a study reported by Masaki *et al.* (2000) did not find a protective effect of vitamin E on the development of AD, although use of vitamin E or C was associated with less cognitive decline. In a study published by Morris *et al.* (1998) a group of 633 disease-free patients older than 65 years was followed prospectively, with vitamin users identified at entry. None of the subgroup using vitamin E (*n* 27) developed AD at the time of follow-up, whereas it was predicted that 3·9 patients in the subgroup would have developed AD.

A 1997 report from the Alzheimer's Disease Cooperative Study (Sano *et al.* 1997*b*) demonstrated approximately an 8-month delay to substantial worsening (death, institutionalization, loss of activities of daily living, decline in clinical dementia scale from 2 to 3) in patients with moderately-severe AD. This group had a lower rate of institutionalization over 2 years than did a placebo group (39 % *v.* 26 %). Unfortunately, the study did not demonstrate a difference in cognitive testing between the treated and untreated groups, possibly due to the relatively advanced stage of the patients in the study.

Presently underway is the Memory Impairment Study (Grundman, 2000), in which patients with memory impairment who don't yet have a diagnosis of AD, but are defined as having mild cognitive impairment, have been recruited at approximately seventy-five centres across North America. There are three arms in the trial, including vitamin E, donepezil and placebo. The goal of the trial is to determine whether vitamin E or donepezil can delay the onset of a clinical diagnosis of AD. The dose of vitamin E that is being administered is 2000 IU/d, which is the same dose as that used in the previous vitamin E clinical trial (Sano *et al.* 1997*b*).

Vitamin C

Vitamin C is a water-soluble (cytoplasmic) antioxidant. Jeandel *et al.* (1989) reported a decrease in vitamin C concentrations in the plasma of patients with AD compared with normal controls. Similarly, Riviere *et al.* (1998) and Foy *et al.* (1999) observed lower plasma vitamin C levels in subjects with AD. Schippling *et al.* (2000) found that ascorbate levels were lower in both the plasma and CSF of patients with AD *v.* non-demented controls. In contrast, Sinclair *et al.* (1998) did not find a significant difference in plasma vitamin C concentrations between patients with AD and controls.

A report from the Rotterdam Study (Engelhart *et al.* 2000) found that high dietary intake of vitamin C decreased the subsequent risk of AD. Masaki *et al.* (2000) reported no benefit of vitamin C on the development of AD. Morris *et al.* (1998) prospectively followed an AD-free population sample >65 years of age, in whom vitamin users were identified at intake. At follow-up (4·3 years), ninety-one of 633 subjects had developed AD. None of the twenty-three vitamin C users developed AD, whereas it was predicted that 3·3 subjects would develop it. Paleologos *et al.* (1998) reported on a cohort study of 117 subjects recruited from a retirement community. Vitamin C intake was assessed at baseline, and cognitive testing was performed 4 years later. Consumption of vitamin C supplements was associated with a lower prevalence of cognitive impairment. Perrig *et al.* (1997) found that among 442 subjects aged 65–94 years, a higher plasma concentration of vitamin C was associated with better memory performance.

Vitamin A

Vitamin A (retinol) is a lipid-soluble antioxidant derived from more complex carotenoids in the diet. Jeandel *et al.* (1989) found that serum vitamin A concentrations were decreased in patients with AD. Similarly, other researchers have reported lower serum vitamin A concentrations in patients with AD (Zaman *et al.* 1992; Jimenez-Jimenez *et al.* 1997; Foy *et al.* 1999; Bourdel-Marchasson *et al.* 2001). Schippling *et al.* (2000) reported lower plasma α-carotene levels but normal β-carotene concentrations. Sinclair *et al.* (1998) found no difference in plasma β-carotene concentrations between patients with AD and controls.

A well-executed prospective study of the effects of vitamin A supplementation for AD has yet to be performed. Perrig *et al.* (1997) found that among 442

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subjects aged 65–94 years a higher plasma concentration of β-carotene was associated with better memory performance. In a cross-sectional study from The Netherlands, Jama *et al.* (1996) found that a higher intake of β-carotene was associated with better cognitive performance. Similarly, Schmidt *et al.* (1998) found that individuals with higher plasma levels of β-carotene had better cognitive performance, although this association only showed a trend toward significance after adjusting for other variables. Other epidemiological studies have failed to find an association between cognitive performance and vitamin A (Schmidt *et al.* 1998; Perkins *et al.* 1999; Engelhart *et al.* 2000).

Selenium

Se has been suggested as a dietary supplement in AD, owing to its role in the reduction of oxidative stress, particularly the detoxification of peroxides. Clinical studies, however, have failed to reveal a clear relationship between Se and AD or cognitive impairment. A report from the Rotterdam study (Engelhart *et al.* 2000) found no correlation between Se intake and the subsequent development of AD. Perkins *et al.* (1999) compared serum antioxidant levels with cognitive performance in a multi-ethnic study of 4809 elderly Americans. They found no association between Se concentrations and memory performance. In contrast, Berr *et al.* (2000) reported that low serum Se levels were associated with an increased risk of cognitive decline in an elderly cohort after 4 years.

Other recent studies have further failed to discern a deficiency of Se as contributing to AD. Meseguer *et al.* (1999) studied serum and CSF levels in both AD subjects (*n* 27) and matched controls (*n* 34). No significant differences between the two groups were identified. Cornett *et al.* (1998) found that Se levels were comparable in brains from patients with AD and controls, except for a small elevation in the amygdala.

Zinc

Using histochemical methods to study the brains of subjects with AD, Suh *et al.* (2000) found vivid Zn staining in the amyloid deposits of dense-core (senile) plaques, in the amyloid angiopathy surrounding diseased blood vessels, and in the somata and dendrites of neurons showing characteristic neurofibrillary tangles. Since brains from age-matched non-demented controls revealed only scattered neuronal staining for Zn, the authors postulated abnormal Zn metabolism in AD. Corroborative findings were reported by Cornett *et al.* (1998). They detected statistically significant elevations in Zn in multiple areas of brains from patients with AD compared with controls. Deibel *et al.* (1996) reported elevated levels of Zn in the hippocampus and amygdala of patients with AD. Molina *et al.* (1998) found that CSF Zn concentrations were decreased in patients with AD, with no significant difference in serum Zn concentrations. The latter finding was confirmed by Maes *et al.* (1999). Additionally, Gonzalez *et al.* (1999) found an association between higher serum Zn concentrations in patients with AD and the apolipoprotein E4 allele.

Against this background Lovell *et al.* (1999*b*) performed a study assessing the effect of varying Zn concentrations on β-amyloid toxicity in cultured hippocampal neurons. The data obtained suggest protection against β-amyloid toxicity with low Zn concentrations, but enhanced toxicity at higher Zn concentrations. This finding is in agreement with earlier reports suggesting increased aggregation of β-amyloid at high concentrations of Zn (Bush *et al.* 1994). It appears that Zn could be either potentially harmful or beneficial for AD. Supplementation beyond the recommended dietary allowance is probably not advisable until its role is more thoroughly studied and understood.

Antioxidant dietary supplements and herbs

Ubiquinone

Ubiquinone is an essential cofactor of the electron transport chain in mitochondria and a lipid-soluble antioxidant (Beal & Matthews, 1997). Soderberg *et al.* (1992) reported increased concentrations of ubiquinone in brain tissue from subjects with AD. A recent study by de Bustos *et al.* (2000) found no significant difference in plasma ubiquinone concentrations between patients with AD and controls. Schippling *et al.* (2000) similarly found no alteration in ubiquinone concentration in plasma from patients with AD. No large clinical studies assessing the cognitive effect of oral supplementation of ubiquinone in AD have been performed.

α-Lipoic acid

α-Lipoic acid is a disulfide compound that serves as the coenzyme for mitochondrial α-keto acid dehydrogenases. It is a powerful antioxidant and can recycle other antioxidants such as vitamin C, vitamin E and glutathione (Packer *et al.* 1997). In a recent open clinical trial by Hager *et al.* (2001) 600 mg α-lipoic acid was given daily to nine patients with AD and related dementias for an average of 337 d. Cognitive measures remained stable over this time period. Although the study was small and not randomized, the findings suggest that further studies with α-lipoic acid might be worthwhile.

Acetyl-L-carnitine

Acetyl-L-carnitine is an esterified form of L-carnitine. Its function is to transfer long-chain fatty acids from the cytoplasm to the mitochondria, facilitating neuronal energy production. Clinical trials of acetylcarnitine have been disappointing. A 1996 study followed 431 AD subjects given 1 g acetylcarnitine three times daily for 12 months. Using standard cognitive measures for such trials the researchers found no significant differences between the treatment and placebo groups (Thal *et al.* 1996). A trend towards slower decline in the younger patients was noted, and Brooks *et al.* (1998) discussed this possibility further. Subsequently, however, Thal *et al.* (2000) published a trial of acetylcarnitine in early-onset AD. Again, there was no significant difference in rate of cognitive decline between the active and placebo treatment groups. Overall, there is no

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compelling evidence to recommend acetylcarnitine for treatment of AD at the present time.

Creatine

Creatine is a guanidino compound produced endogenously and found in meat products. Creatine and phosphocreatine provide a temporal energy buffer in times of high energy demand and a spatial energy buffer between the cytosol and mitochondria (Tarnopolsky & Beal, 2001). Creatine probably functions as an antioxidant by enhancing energy transduction. Brewer & Wallimann (2000) demonstrated that β-amyloid and glutamate toxicity to rat hippocampal neurons is ameliorated by creatine. The creatine buffer system may play a role in compensating for impaired energy metabolism in AD. Using magnetic resonance spectroscopy Pfefferbaum *et al.* (1999) found that among AD subjects higher grey-matter creatine plus phosphocreatine concentrations correlated with poorer performance on recognition memory tests. Oral loading can increase brain creatine. A study by Dechent *et al.* (1999) demonstrated that excess oral intake of creatine monohydrate increased brain levels of creatine over a period of several weeks. At the present time there are no reports of creatine treatment for AD.

Docosahexaenoic acid

DHA is a polyunsaturated fatty acid found in brain phospholipids. It is reported to have antioxidant properties, inhibiting NO production and enhancing cellular antioxidant enzyme activity (Hossain *et al.* 1999; Jeyarajah *et al.* 1999). An autopsy study published in 1991 (Soderberg *et al.* 1991) found that DHA concentrations were decreased in the brains of patients with AD compared with normal controls. A more recent study reported reduced concentrations of DHA in the hippocampus of patients with AD (Prasad *et al.* 1998). Schippling *et al.* (2000) reported lower concentrations of polyunsaturated fatty acids in the CSF of patients with AD.

There have been a number of interesting epidemiological studies relating to DHA. A Rotterdam study (Kalmijn *et al.* 1997) found that individuals in The Netherlands who consumed more fish (a marker for polyunsaturated fatty acids including DHA) had a reduced risk of developing AD. There is also data from the Framingham cohort (Kyle *et al.* 1999) suggesting that a lower DHA level is a predictor of all-cause dementia, including AD. Terano *et al.* (1999) reported that DHA supplementation resulted in improvement in patients with moderately severe dementia on the basis of thrombotic cerebrovascular disease.

Ginkgo

Herbal extracts from *Ginkgo biloba*, are capable of scavenging free radicals, a property that is thought to be due, in part, to their flavonoid components (Bastianetto *et al.* 2000*b*). A ginkgo extract has been shown to be neuroprotective against β-amyloid toxicity (Bastianetto *et al.* 2000*a*). While ginkgo suffers from a relative lack of good clinical trials to support its use, it is perhaps one of the better-studied supplements taken for cognition. A meta-analysis by Oken *et al.* (1998) found only four studies of fifty evaluating its use in patients with AD that met adequate criteria for inclusion. The conclusion from this review was that patients with AD receiving ginkgo had a slight improvement in cognition. There was inconclusive evidence to determine the effect of ginkgo on non-cognitive behavioural measures, functional measures, or a clinician's global rating. Recently, Le Bars *et al.* (2000) published the results of a double-blind placebo-controlled parallel-group 26-week, multicentre study comparing ginkgo (40 mg three times daily) with a placebo. The study included results for the subset of patients with mild to moderate AD, with outcomes assessed by cognitive and global measures. There was an improvement of 1·7 points at 26 weeks in the cognitive component of the Alzheimer Disease Assessment Scale. This level of improvement is somewhat less than that seen with donepezil and other US Food and Drug Administration-approved cholinesterase inhibitors for AD, which generally demonstrate approximately a 3-point improvement on the same cognitive scale (Grundman & Thal, 2000). Unlike the cholinesterase inhibitors that have been approved thus far, there was no significant effect seen in the clinical global impression of change, which means that the clinician evaluating the subjects could not detect a difference in the treated subjects. It appears that the doses of ginkgo used in that study were comparable with suboptimal therapeutic doses of currently-marketed cholinesterase inhibitors. On the other hand, ginkgo appears to be relatively free of side effects that can occur with some cholinesterase inhibitors. It is unknown if higher doses of ginkgo might be more effective. Additional clinical trials of ginkgo in patients with AD are underway.

Huperzine A

Huperzine A is a reversible and selective acetylcholinesterase inhibitor (Cheng & Tang, 1998; Wang & Tang, 1998; Ye *et al.* 1999) derived from the Chinese club moss *Huperzia serrata*. Huperzine A and other cholinesterase inhibitors (e.g. donepezil and tacrine) were recently found to offer neuroprotection against β-amyloid toxicity, possibly through nicotinic receptor activation or induction of antioxidant enzymes (Svensson & Nordberg, 1998; Xiao *et al.* 2000*b*). It is conceivable, therefore, that these neuroprotective properties may contribute to the clinical efficacy of cholinesterase inhibitors in the treatment of AD. Huperzine can be purchased as a dietary supplement in pharmacies and health food stores. Two trials have been reported in patients with AD, both in China. The first to be published (Xu *et al.* 1995) reported on an 8-week double-blind placebo-controlled multicentre trial of Huperzine A tablets (100 μg twice daily in 103 subjects with AD). The authors noted improvement in 58 % of the treated patients *v.* 36 % of the placebo group in areas including memory, cognition and behaviour. Comparison with studies typically conducted in Western countries, however, should be made with caution; for example, half the patients in this study had only an elementary school education or less. Also, the mean mini-mental status test score was only 14–16, whereas in most studies used for regulatory approval in the USA the mean mini-mental status test scores tend to be about 20. Approximately 10 % of the patients had gastrointestinal side

effects. The second, even shorter study (Zhang *et al.* 1991) claimed that Huperzine A was efficacious, even though the treatment period for senile and pre-senile memory disorders was only 2 weeks. Huperzine is an interesting compound that may well have some efficacy in AD, but it is difficult to draw firm conclusions based on the current data. Additional clinical trials with this agent are indicated.

Curcumin

Curcumin is an antioxidant derived from turmeric, the spice that provides curry with its yellow colour. Kim *et al.* (2001) found that curcumin was able to protect PC12 cells from β-amyloid toxicity. A recent report by Frautschy *et al.* (2000) found that curcumin could protect against behavioural deficits and lipid peroxidation induced by β-amyloid infusion in an animal model. Given these findings, further studies with curcumin in human subjects would be of interest.

Ginseng

Certain ginsenosides isolated from *Panax* spp. ginseng herb have been shown to reduce glutamate-induced neurotoxicity in neuronal cell cultures (Kim *et al.* 1998). In these experiments pretreatment with ginsenosides inhibited the overproduction of NO and malondialdehyde, and the influx of Ca. No studies of ginseng in AD have yet been reported. Recently, Wesnes *et al.* (2000) published a study examining a ginseng–ginkgo combination given to healthy middle-aged volunteers. A small improvement was seen in a memory index derived from a computerized battery in the active treatment group compared with controls. It is questionable, however, as to whether the subjects detected any benefit, as there was no improvement in a variety of other measures, including a number of subjective ratings of alertness, calmness, contentment, mood or well-being. Also, since the formulation studied was a combination of ginseng and ginkgo, it is difficult to draw any conclusions regarding the potential value of ginseng alone.

Vinpocetine

Vinpocetine is an alkaloid derived from *Vinca* spp. once favoured as a treatment for stroke (Bereczki & Fekete, 1999; Gulyas *et al.* 1999), owing to its effects on cerebral blood flow and glucose utilization following ischaemia (Rischke & Krieglstein, 1990). Vinpocetine has also been shown to be a free radical scavenger and to protect PC12 cells from β-amyloid toxicity. Findings of relatively short duration trials of this agent, both in healthy volunteers (Subhan & Hindmarch, 1985) and in patients with varying dementing illnesses (Balestreri *et al.* 1987; Hindmarch *et al.* 1991), suggested some benefit in cognitive performance measures. A 1-year open-label study of an escalating dose of vinpocetine in fifteen subjects with AD by Thal *et al.* (1989), however, revealed a decline in all cognitive measures at the same rate as those of a matched control group. They concluded that vinpocetine was likely to be ineffective in improving cognitive deficits or slowing the progression of AD.

Other dietary and lifestyle strategies for prevention and treatment of Alzheimer's disease

There is accumulating evidence that a diet high in fat and cholesterol may increase the risk of dementia and AD (Kalmijn *et al.* 1997; Notkola *et al.* 1998; Grant, 1999). In experimental animals a high-cholesterol diet is associated with increased deposition of brain β-amyloid. Rabbits fed high-fat high-cholesterol diets demonstrate increased β-amyloid in the brain (Sparks *et al.* 2000). Similarly, transgenic mice that produce β-amyloid produce even greater amounts of β-amyloid when fed a high-cholesterol diet (Refolo *et al.* 2000). Recently, the use of certain cholesterol-lowering agents (statins) has been associated with a reduced risk of dementia and AD in epidemiological studies (Wolozin *et al.* 1999; Jick *et al.* 2000). These agents have been shown to reduce intracellular and extracellular levels of β-amyloid in hippocampal neurons (Fassbender *et al.* 2001). Despite these promising findings it is likely that a diet low in fat and cholesterol may be most helpful during middle age. Several studies were unable to establish high cholesterol as a risk factor for AD in the years just before diagnosis (Notkola *et al.* 1998; Romas *et al.* 1999; Breteler, 2000). In fact, serum cholesterol may already be somewhat lower in the years just preceding and following diagnosis (Foy *et al.* 1999; Romas *et al.* 1999; Lerner *et al.* 2000). In contrast to a high-fat diet, a recent study (Engelhart *et al.* 2000) found that individuals who consumed more vegetables had a lower risk of dementia and AD.

In a prospective community study in the Bordeaux area of France, Orgogozo *et al.* (1997) found that moderate red wine drinking was associated with a lower incidence of AD at follow-up. This epidemiological study is supported by other experiments indicating that red wine constituents such as resveratrol can protect against NO toxicity in hippocampal neurons (Bastianetto *et al.* 2000*c*) and inhibit lipoprotein oxidation (Chopra *et al.* 2000).

Learning new things and maintaining a high level of intellectual activity throughout the lifespan, as well as exercising, may also reduce the risk of AD (Friedland *et al.* 2001), possibly by increasing synapses, initiating angiogenesis or by promoting neurogenesis (Black *et al.* 1990; Isaacs *et al.* 1992; Kempermann *et al.* 1997).

Conclusions

Recent clinical trials in AD have shown that we cannot rely on supportive basic science and epidemiological data to make clinical decisions regarding the use of putative agents in patients with AD. Oestrogen has long been thought likely to be effective in AD; however, recent clinical trials in patients with AD have shown no benefit in this population (although their use for prevention of AD is still an open question). It was hoped that cyclo-oxygenase-2 inhibitors might be neuroprotective in AD due to their anti-inflammatory effect. Thus far, however, they do not appear to be effective treatment in clinical trials. Acetylcarnitine and idebenone are yet additional examples of promising agents that have been disappointing in clinical trials.

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Combinations of vitamins, minerals and herbal antioxidants are likely to offer greater potential benefit for AD than any single antioxidant, especially if the agents work in different cellular compartments or have complementary mechanisms of action (e.g. vitamins E, C and ubiquinone). Nevertheless, it is not a simple matter to develop the ideal mixture of antioxidants for human use. While in theory this approach is appealing, by trying to deal with several sources of oxidative stress simultaneously, it is not so clear how to optimize the dose of each component or assure that when they are mixed they won't have an interacting toxicity or loss of efficacy. In a clinical trial of selegiline and vitamin E in AD the combination of selegiline and vitamin E was no better than each agent alone (Sano *et al.* 1997*a*). Preclinical safety studies in animals may be helpful for detecting likely toxicity, but extrapolating optimal dosing from current animal models and scaling that to man is challenging. Large-scale testing of many compounds in human subjects is also complicated, since reduction of oxidative damage is not a valid clinical outcome. Oxidative markers could be used, but it needs to be demonstrated that such surrogates correlate with clinical improvement. In the case of antioxidant trials attempting to prevent or delay AD, antioxidant mixtures need to be administered to normal elderly subjects or individuals with mild cognitive impairment before they develop clinical AD. Given that only a minority of such individuals develop AD over the course of a few years, such trials will be time consuming and expensive. Despite these concerns, it seems we have little choice but to conduct such trials if we are to get beyond our current impasse and develop optimal antioxidant therapy for prevention and treatment of AD.

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