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Central & Peripheral Nervous Systems

Natural antioxidants in Alzheimer's disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterised by severe cognitive impairment that ultimately leads to death. Current drugs used in AD are acetylcholinesterase inhibitors and antagonists to the NMDA receptors. These drugs may only slightly improve cognitive functions but have only very limited impact on the clinical course of the disease. In the past several years, based on *in vitro* and *in vivo* studies in laboratory animals, natural antioxidants, such as resveratrol, curcumin and acetyl-L-carnitine have been proposed as alternative therapeutic agents for AD. An increasing number of studies demonstrated the efficacy of primary antioxidants, such as polyphenols, or secondary antioxidants, such as acetylcarnitine, to reduce or to block neuronal death occurring in the pathophysiology of this disorder. These studies revealed that other mechanisms than the antioxidant activities could be involved in the neuroprotective effect of these compounds. This paper discusses the evidence for the role of acetylcarnitine in modulating redox-dependent mechanisms leading to the upregulation of vitagenes. Furthermore, future development of novel antioxidant drugs targeted to the mitochondria should result in effectively slowing disease progression. The association with new drug delivery systems may be desirable and useful for the therapeutic use of antioxidants in human neurodegenerative diseases.

Keywords: acetyl-L-carnitine, Alzheimer's disease, curcumin, haem oxygenase, heat-shock proteins, resveratrol, vitagenes

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1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterised by cognitive and memory decline, speech loss and personality changes, and it is one of the major cause of admissions to nursing homes [1]. Several lines of evidence have demonstrated clearly the importance of neuroinflammation and oxidative stress in the pathogenesis of AD. Among the major players involved in neuroinflammation are: i) β-amyloid, which is responsible for the generation of superoxide anion and α-carbon-centred radicals; ii) COX, which during its catalytic cycle produces both free radicals and prostaglandins; and iii) inducible nitric oxide synthase (iNOS), which is responsible for the formation of nitric oxide (NO) and reactive nitrogen species (RNS) [2-4]. All the above mentioned pro-oxidant species contribute to the massive destruction of some brain areas, in particular the entorhinal cortex and hippocampus in the early stage of AD. After several years the damage spreads to the temporal, frontal and parietal cortex [5]. Furthermore, choline acetyltransferase has been found to be decreased by up to 90% in several brain areas of patients with AD and this led to the hypothesis that

the deficit in the cholinergic system is a main consequence in AD [6,7]. Current therapy for AD is based on the administration of acetylcholinesterase inhibitors donepezil, rivastigmine or galantamine and the NMDA open-channel blocker memantine, but these drugs can only delay the onset and development of dementia [8]. Bearing in mind the claimed pathogenic role of inflammation in AD, the potential use of NSAIDs has, in the past, been postulated [9]. Unfortunately, although the results from *in vitro* studies were promising, clinical trials showed that NSAIDs had no effect on AD [10]. Recently, a new therapeutic approach for the therapy of AD was proposed. Based on the evidence that the heat-shock family of proteins (Hsps) exerts neuroprotective effects against oxidative stress-related injury and that nutritional antioxidants are able to upregulate Hsps in many cell lines including neurons, investigators proposed the administration of nutritional antioxidants such as resveratrol, curcumin and carnitine/L-acetyl-carnitine as 'pathogenetic' drugs in order to counteract the oxidative stress-induced brain damage in AD [11,12]. However, the bioavailability and pharmacokinetics of these substances should be considered when the use of these antioxidants is proposed.

2. Alzheimer's disease: why consider antioxidants?

AD, which rarely occurs before the age of 50, usually becomes clinically apparent as a subtly impaired cognitive function or a disturbance of affect. With time there is progressive memory loss and disorientation, which eventually progresses into dementia. Although most cases are sporadic, 5 – 10% or more are familial [13,14]. Gross examination of the brain in AD shows a variable degree of cortical atrophy with narrowed gyri and widened sulci most apparent in the frontal, parietal and temporal lobes. Microscopically, the features include neurofibrillary tangles, neurite (senile) plaques, the central core of which is amyloid-β peptide, derived from the transmembrane amyloid precursor protein, amyloid angiopathy, granulovacuolar degeneration and Hirano bodies [15]. The brain of an AD patient has been reported to be under oxidative stress, and this may play an important role in the pathogenesis and progression of AD [16-18]. Amyloid-β peptide (1-42) has been proposed to play a central role in the pathogenesis of AD [16,19]. β-Amyloid-associated free radicals can initiate lipid peroxidation, protein oxidation, reactive oxygen species (ROS) formation, intracellular and mitochondrial Ca^{2+} accumulation, and eventually lead to death of neurons [20]. A prediction of this model was that the antioxidant vitamin E should prevent or modulate these β-amyloid-induced effects to neurons [20-22]. Several other potential sources of oxidative stress were considered in the pathogenesis of AD. First, the concentration of iron, a potent catalyst of ROS generation, is increased in neurofibrillary tangle-bearing neurons [23,24]. Second, increased concentrations of iron would result in increased protein modifications, which are catalysed by metal ions and reducing sugars [25]. Third, microglial cells are activated and increased in AD, and represent a major source of free radicals [26,27]. Fourth, a decrease of complex IV activity has been reported in the cerebral cortex of individuals who died of AD [28]. Whilst the exact mechanism for this loss of activity is not clear, it is known that this enzyme complex is particularly susceptible to oxidative damage [29,30]. Recently, it has been shown that brains from patients with mild cognitive impairment (MCI) have increased protein oxidation and lipid peroxidation compared with aged-matched controls [31,32]. As MCI is considered as the transition zone between normal cognition and dementia in early AD, this finding suggests that oxidative stress is fundamental to the progression of AD and not simply a consequence of the disease. Therefore, it is mandatory to develop biomarkers of oxidative stress in easily accessible tissue in living individuals to learn more about AD, to monitor drug efficacy, and to follow disease progression. Recent evidence also suggests that NO and RNS may directly or indirectly be involved in neuronal death in AD and MCI [4,33]. There is also strong evidence to suggest that both p21ras and p21ras-dependent MAP-kinase pathways are strongly induced in AD, and an aberrant expression of p21 is highly colocalised with an aberrant expression of NOS in this condition [34,35].

3. Resveratrol and Alzheimer's disease

Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) (**Figure 1**) is a phytoalexin found in grapes, cranberries and peanuts [36,37]. Several *in vitro* studies have shown that resveratrol is a powerful molecule endowed with antioxidative, anticancer, anti-inflammatory and estrogenic activities [36,38]. Studies in rodents and humans have shown that after oral ingestion resveratrol is readily absorbed (at least 50% in the rat), reaching peak plasma concentration after 10 – 60 min of up to 2 μM total resveratrol (i.e., genuine resveratrol plus resveratrol derived from the hydrolysis of its conjugated products), whereas the amount of unchanged resveratrol is in the low nanomolar range [36-40]. In hepatic cells, resveratrol has been absorbed by passive diffusion and carrier-mediated processes [41]. Interestingly, the amount of resveratrol adsorbed did not change in the presence of ethanol [36]. Resveratrol binds to plasma proteins such as albumin and lipoproteins [41]. The plasma half-life was estimated to be 12 – 15 min in the rat and 9 – 12 h in humans [37,39]. Resveratrol undergoes massive metabolism both in gastrointestinal cells and liver. The main metabolites detected in humans are resveratrol monosulfate, resveratrol monoglicuronide (two isomeric forms), dihydroresveratrol monosulfate and monoglicuronide [37,38]. The serum half-life of these metabolites is ~ 9.2 h (i.e., significantly higher than that of unmodified resveratrol) [38,39] in both

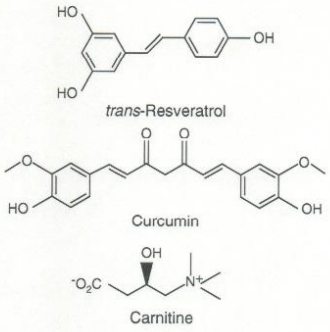

Figure 1. Chemical structures of *trans*-resveratrol, curcumin and carnitine.

rodents and humans; enteric recirculation of resveratrol metabolites has been proposed and may account for the increased half-life of these metabolites with respect to the unchanged form. Resveratrol, has been shown to inhibit CYP3A4 irreversibly and to be a reversible inhibitor of CYP2E1 [38,42]. Furthermore, in rat cardiomyocytes, resveratrol increased the activity of glutathione transferases, well known Phase II enzymes involved in the detoxification of drugs [43]. Resveratrol is mainly excreted by the kidney and only a small portion is recovered in faeces [36]. It is noteworthy to mention that in rodents and rabbits the tissue concentrations of resveratrol are always below 1 nmol/g fresh tissue [36,44], whereas in humans the organs in which resveratrol shows greater accumulation are intestinal mucosa, stomach, liver and kidney, but the exact concentrations have not been estimated [38].

The rationale to use resveratrol in the treatment of AD is based on the well-known antioxidant activity of this compound. Resveratrol has been shown to protect rat glioma cells from β-amyloid toxicity by reducing the expression of iNOS and COX-2, thus preventing the uncontrolled release of NO and prostaglandin F_2 [45]. These effects, could be ascribed to the ability of resveratrol to prevent the β-amyloid-induced nuclear translocation of NF-κB [45]. Furthermore, in PC12 cells, resveratrol counteracted the β-amyloid$_{25\text{-}35}$-induced toxicity by the downregulation of pro-apoptotic factors such as Bax and c-Jun N-terminal kinase proteins [46,47]. In rat hippocampal neuronal cell cultures, resveratrol protected cells from β-amyloid toxic effects by inducing protein kinase C [46,48]. The neuroprotective effect of resveratrol against β-amyloid toxic effects could also be mediated by promoting the intracellular degradation of β-amyloid through the ubiquitin proteasome system [49]. Interestingly, resveratrol reduced sodium nitroprusside-released NO toxicity in an experimental system of rat hippocampal mixed neuronal/glial cultures [50]. In the rat, the chronic administration of resveratrol (21 days) resulted in a marked inhibition of the cognitive impairment secondary to the intracerebroventricular administration of streptozotocin, and this effect has been related to the increase of brain glutathione levels [51].

Still under investigation is the neuroprotective effect of resveratrol through the overexpression of a family of proteins called sirtuins [46]. The Sir2 (a NAD-dependent class III histone deacetylase) homologs named Sirt1 – 7, are involved in the cellular protection against oxidative stress and belong to the sirtuin family. In particular, Sirt1 – 3, play a key role in protecting neurons in AD patients by at least three independent mechanisms: i) the repression of the pro-apoptotic protein p53 and forkhead transcription factor 3 expression; ii) the reduction of the hyperphosphorylation of τ protein; and iii) the reduction of ROS formation through the interaction with the uncoupling protein 4 [52].

Of note is the interaction of resveratrol with the vitagenes. The term vitagenes refers to a group of genes that are strictly involved in preserving cellular homeostasis during stress conditions. The vitagene family is composed of the Hsps haem oxygenase-1 (HO-1), Hsp70 and by the thioredoxin (Trx) system [53-55]. HO-1, also referred to as Hsp32, degrades haem, which is toxic if produced in excess, into ferrous iron, carbon monoxide and biliverdin (BV). BV is the precursor of bilirubin, a linear tetrapyrrole that has been shown to effectively counteract oxidative and nitrosative stress, due to its ability to interact with ROS, NO and RNS [3,56-59]. Hsp70 is a functional chaperone and acts by inhibiting key effectors of the apoptotic machinery [53,56]. Finally, Trx is responsible for the reduction of protein disulfide bonds whereas Trx reductase serves to maintain Trx in a reduced form [53]. Recently, resveratrol has been demonstrated to increase the expression of HO-1 in PC12 cells and primary neuronal cultures, presumably through the activation of NF-E2-related factor 2 (Nrf2) in PC12 cells [60,61]. Although not directly related to AD, these data are in good agreement with previous papers, which demonstrate how the overexpression of HO-1, and the related increase in antioxidant capacity, is neuroprotective in several models of AD. Indeed, Takahashi *et al.* found that cortical neurons cultured from mice expressing the Swedish mutation of AD had defects in bilirubin production with subsequent increases of hydrogen peroxide toxicity [62]. Furthermore, in transfected neuroblastoma cells overexpressing HO-1, the activity of this enzyme was increased, and conversely, the level of τ protein was significantly decreased when compared with antisense HO-1 or vector transfected cells [63]. The suppression of τ protein expression was almost completely counteracted by zinc deuteroporphyrin, a specific inhibitor of HO activity [63].

4. Curcumin and Alzheimer's disease

Curcumin (1,7-*bis*[4-hydroxy 3-methoxy phenyl]-1,6-heptadiene-3,5-dione) (**Figure 1**) is a phenolic compound

extracted from the rhizome of *Curcuma longa* Linn. (family *Zingiberaceae*) and it is commonly used in the Asian continent, in particular India, as a spice to colour and flavour food. Furthermore, traditional Indian medicine considered curcumin to be an effective drug on several disorders including anorexia, coryza, cough, hepatic diseases and sinusitis [64,65]. Recently, several studies reported on anti-inflammatory, anticarcinogenic and anti-infectious activities of this compound. The potential prophylactic or therapeutic use of curcumin in human diseases has, therefore, been proposed [66-70]. Following ingestion, almost 40 – 80% of curcumin is unaltered in the gastrointestinal tract [64]. However, curcumin undergoes marked first-pass metabolism, which limits its systemic bioavailability (~ 60%) as demonstrated in humans and rodents [71-73]. Interestingly, in order to increase its bioavailability, the co-administration of curcumin with piperine or its complexation with phospholipids to form a curcumin–phospholipids complex have been proposed [71,74,75]. Preclinical studies have shown that the administration of curcumin 1 g/kg to the rat allows the polyphenol to reach plasma concentrations of around 0.5 μg/ml; on the other hand, patients affected by malignant or premalignant conditions of the bladder, skin, cervix, stomach or oral mucosa, treated with high-dose curcumin (0.5 – 8 g/day for 3 months) had a plasma concentration of 1.75 ± 0.8 μM [71,76]. In the rat, the volume of distribution, which reflects the ability of a drug to bind to tissues or plasma proteins (i.e., a larger volume means the accumulation of the drug in tissues and vice versa) of curcumin is ~ 190 l, thus suggesting that this polyphenol may accumulate in many organs including colorectal tissue, liver and brain [71,74,77]. Studies in rodents and humans demonstrated that, after oral dosing, curcumin is conjugated to curcumin glicuronide and curcumin sulfate as well as reduced into dihydrocurcumin, tetrahydrocurcumin, hexahydrocurcumin, octahydrocurcumin and hexahydrocurcuminol [64,78,79]; curcumin, dihydrocurcumin and tetrahydrocurcumin can be further converted in monoglicuronide conjugates [78,80]. These metabolic changes seem to occur not only in the liver, the main organ deputed to biotransformation but also in the intestinal tract [64,79]. Interestingly, the metabolism of curcumin generates products such as tetrahydrocurcumin, which retains anti-inflammatory activity comparable to that of the parental compound [64,79]. In rodents and humans, curcumin inhibited CYP isoforms as well as other detoxifying enzymes such as glutathione transferases and gastrointestinal uridine diphosphate (UDP)-glucuronosyltransferases, therefore, the ingestion of this spice may alter the metabolism of several drugs thus increasing their plasma concentrations and originating potential toxic effects [81-84]. In the rat, curcumin is mainly excreted into the bile and then eliminated in the faeces, only a little amount is eliminated in the urine [72,73] and the half-life of elimination is ~ 1.5 h [74]. The urinary elimination of curcumin and its metabolites seems to be increased if curcumin is administered at large doses (e.g., 3.6 g/day for up to 4 months) [71,85]. With regard to the toxicity profile of curcumin, studies in rodents and primates have shown that the spice at doses of up to 3.5 g/kg body weight administered for up to 3 months were well tolerated by the animals [71]. In humans, curcumin at doses ranging from 2.1 to 8 g/day for up to 3 months did not initiate any toxic effect [76,86]. However, patients affected by advanced colorectal cancer treated with curcumin at 3.6 g/day developed diarrhoea whereas the dose of 0.9 g/day was associated with nausea, which resolved spontaneously. In the same patients, blood test abnormalities, such as a rise in serum alkaline phosphatase and lactate dehydrogenase, correlated with curcumin administration, but the possibility that they derived from the progression of cancer rather than curcumin toxicity cannot be excluded [71,85].

The first evidence of a protective role of curcumin in the onset of AD was derived from epidemiological studies. Ganguli and colleagues reported that the Indian population, who have a curcumin-enriched diet, has a reduced prevalence of AD compared to the US population [87]. Following this initial observation, many basic studies were conducted and the neuroprotective role of curcumin was corroborated. *In vitro* studies have shown that curcumin protects neuron-like PC12 cells from β-amyloid toxicity and, interestingly, the polyphenol displayed a neuroprotective effect greater than a well-known antioxidant such as α-tocopherol [88]. By using an Alzheimer transgenic APPSw mouse model (Tg2576), Lim and colleagues have shown that dietary curcumin suppressed inflammation and oxidative damage in the brain of these mice [89]. More recently, Garcia-Alloza *et al.* demonstrated in transgenic APPswe/PS1dE9 mice that curcumin, given intravenously for 7 days, crosses the blood–brain barrier, binds to β-amyloid deposits in the brain and accelerates their rate of clearance [77]. These latter results are in agreement with previous findings, which showed that curcumin disaggregates and inhibits β-amyloid aggregation [90,91].

Similarly to resveratrol, curcumin is a pleiotropic agent with multiple molecular targets and biological activities [92,93]. Curcumin has been described as an exceedingly potent direct antioxidant [94]. In addition, the upregulation of Nrf2-dependent gene expression is among the consequences of exposure to curcumin [95]. Because Nrf2-dependent genes encode for cytoprotective proteins (e.g., NQO1, HO-1, glutathione transferases, UDP-glucuronosyltransferases, thioredoxin reducatase) that detoxify oxidants and provide protection against oxidative stress, it is also possible to refer to curcumin also as a secondary antioxidant. Compounds such as curcumin (which possess both direct primary as well as indirect secondary antioxidant activity) have been designated as bifunctional antioxidants and could provide two levels of protection: i) instantaneous via direct scavenging of oxidants; and ii) long lasting via induction of cytoprotective proteins [96]. Importantly, the

indirect antioxidant activity through the induction of Nrf2-dependent genes is catalytic, long lasting (several days) and unlikely to have pro-oxidant effects [97].

5. Carnitines and Alzheimer's disease

L-carnitine (LC) (**Figure 1**) is a natural compound and its biological role is to facilitate the transport of fatty acids to the mitochondria. Dietary LC derives from the intake of red meats but the endogenous synthesis of LC from the amino acid precursors lysine and methionine has been also documented [98]. The dietary intake of LC in humans ranges from 1 to 15 µmol/kg body weight/day, whereas the rate of biosynthesis is ~ 1 – 2 µmol/kg body weight/day [99]. Recently, exogenous LC, given by oral (p.o.) or intravenous (i.v.) routes, has been used for the treatment of cognitive disorders such as AD and dementia [98]. After oral ingestion, dietary LC is well absorbed by simple or carrier-mediated diffusion and its bioavailability is 54 – 86%; conversely, the bioavailability of exogenous LC is much lower, in the range of 5 – 18% [98,99]. This paradoxical effect can be explained considering that the absorption of LC decreases as the intake of LC increases, thus keeping the concentration of LC constant [98,99]. The normal plasma concentration of LC in healthy adults with a mixed diet is 40 – 50 µM [98,100]. When administered at doses of 30 – 100 mg/kg p.o. in humans, LC peak plasma concentrations were 27 – 91 µM after 3 h, and returned to the baseline within 24 h [99,101]. LC undergoes acetylation in rodents and the human intestine thus forming esterified compounds such as ALC, which is endowed with biological activity *per se*. Interestingly, ALC diffuses across membranes much better than LC and its efflux in the systemic circulation has been calculated to be four-times greater than that of LC [99,102]. Data from AD patients have shown that after supplementation with pharmacological doses of ALC (2 g/day) for 55 days, its plasma concentrations increased from 7.2 to 10.3 µM [99]. In the plasma, neither LC nor ALC are bound to proteins [98]. The volume of distribution of LC differs considering the dietary or exogenous source being ~ 3000 l and 20 – 50 l, respectively [98]. This great difference in the volume of distribution between dietary and supplemental LC depends on the different degree of absorption, slow accumulation in tissues such as the muscle and rate of kidney elimination (see below) and, therefore, these numbers should be considered purely indicative [98]. It is interesting to note that ALC is able to cross blood–brain barrier; as shown by Parnetti *et al.* AD patients treated with ALC i.v. or p.o. for 10 – 60 days have an increased concentration of ALC in the cerebrospinal fluid of up to 3.55 nmol/ml [103]. In human subjects treated with LC i.v. its elimination half-life ranged from 3 to 12 h [98]. However, due to the long-lasting release of LC by the muscle, the total time of turnover from the body has been estimated to be 66 days [99]. LC is metabolised by the intestine to γ-butyrobetaine and trimethylamine, the former excreted in the faeces and the latter in urine [98,99]. Accordingly, the renal clearance of LC, which is ~ 1 – 3 ml/min, suggesting an extensive rate of tubular reabsorption, significantly increases at values close to the creatinine clearance with the increase in LC plasma concentrations indicating that tubular reabsorption approaches full saturation [98]. This last finding is very important and contributes to the explaination of how exogenous LC is almost completely excreted during the first 12 h after administration, whereas dietary LC is reabsorbed [98]. Due to its elimination mainly through the kidney, LC should be administered very carefully to patients affected by renal impairment [104].

ALC has been proposed to have beneficial effects in preventing the loss of brain function, which typically occurs during ageing and neurodegenerative disorders. The main mechanism of action of ALC is the improvement of mitochondrial respiration, which allows the neurons to produce the necessary ATP to maintain the normal membrane potential [105]. However, ALC has been shown to be neuroprotective through a variety of other effects, such as the increase in protein kinase C activity [105]. Interestingly ALC counteracted the loss of NMDA receptors in the neuronal membrane and increased the production of neurotrophins, two effects strictly related to synaptic plasticity [105]. Recent studies have shown that ALC reduces β-amyloid toxicity in primary cortical neuronal cultures by increasing both HO-1 and Hsp70 expression [106]. Studies in rats have shown that chronic ALC treatment increases lifespan, improves cognitive behaviour in aged animals and guarantees long-term memory performance [105]. Furthermore, chronic ALC treatment has been shown to prevent age-related changes in mitochondrial respiration and decreases oxidative stress biomarkers through the upregulation of HO-1, Hsp70 and superoxide dismutase-2 in senescent rat [107]. Taken together, these preclinical studies suggested that ALC treatment could be beneficial for the treatment of age-related diseases and the potential use in humans has been encouraged. Patients affected by AD and treated with ALC at doses ranging from 1 to 2 g/day for 6 – 12 months have shown an improved performance on several cognitive tests such as word recognition, name learning and world list recall with respect to placebo-treated patients, but none of these effects were significant [105,108]. In two clinical studies, ALC 3 g/day for 1 year significantly reduced cognitive decline in early-onset AD patients [109-111]. Consistently, the authors have demonstrated that acetylcarnitine induces HO-1 in a dose- and time-dependent manner and that this effect was associated with the upregulation of other Hsps as well as high expression of the redox-sensitive transcription factor Nrf2. The results from this study show for the first time that acetylcarnitine induces HO-1 and Hsp60 heat-shock proteins, and that this effect may involve the transcription factor Nrf2, implying the conceivable possibility that acetylcarnitine, by promoting the acetylation

of DNA-binding proteins, can induce post-translational modifications of critical target proteins endowed with DNA competence and transactivating activity [112]. Importantly, this new envisioned role of ALC as a molecule endowed with the capability of potentating the cellular stress response pathways seems to provide an alternative therapeutic approach for those pathophysiological conditions where the stimulation of the HO pathway is warranted, such as in AD [112].

6. Expert opinion

Unfortunately, AD currently remains an incurable neurodegenerative disorder and the drugs now available for treatment do not counteract or significantly delay the progression of the disease. Current treatment for AD is based on the use of acetylcholinesterase inhibitors (donepezil, rivastigmine, galantamine) and NMDA receptor antagonists (memantine) alone or in association [8]. By reducing the degradation of acetylcholine by acetylcholinesterase, acetylcholinesterase inhibitors increase the concentration of this neurotransmitter in the synaptic cleft and, therefore, contributes to the improvement or delay of the cognitive impairment in patients with mild-to-moderate AD [8]. Memantine slowed the cognitive and functional decline in patients affected by moderate-to-severe AD and its co-administration with donepezil was much more effective than donepezil alone [8,113].

The potential use of natural antioxidants in the therapy of AD has been proposed. In fact, these substances protect cells from free-radical induced damage by several mechanisms including a direct scavenging effect, the upregulation of protective genes/proteins and/or the downregulation of potentially damaging ones. However, despite the unquestionable protective activity in several *in vitro* models, clinical studies demonstrated only minimal effect in humans and this was related to the pharmacokinetics of these substances. In fact, the bioavailability of exogenous resveratrol and curcumin ranges from 20 to 50% and the plasma concentrations are around 2 µM [37,71]. For instance, it has been calculated that in order to take a therapeutic dose of 25 mg of resveratrol, it would be necessary to drink 25 glasses of red wine/day [64]. How would the 'potential' therapeutic effects of resveratrol reconcile the 'well known' toxic effects of ethanol? Furthermore, it is difficult to estimate the concentrations in tissues, which has been shown to be in the nanomolar range and much lower than those used *in vitro*. On the contrary, the pharmacokinetic parameters for LC/ALC are not as limiting and the plasma/tissue concentrations are higher than those of polyphenols. However, it is important to consider that exogenous treatment with LC/ALC results in plasma lower than expected carnitine concentrations, due to saturable reabsorption by the kidney [98]. Another important point to be stressed are the effects on Phase I and II detoxification enzymes. The inhibition of CYP isoforms by resveratrol and curcumin may have deleterious effects on the metabolism of other drugs taken concomitantly and this should be considered especially because older people have an age-related decline in liver function and might be taking many more drugs than younger people for chronic diseases [38,42,81-84]. In fact, through the inhibition of CYP3A4, resveratrol may alter the metabolism of terfenadine, cisapride and astemizole, commonly used drugs, thus increasing the risk of developing life-threatening ventricular arrythmias [38,114]. Taken together, the potential beneficial effects of natural antioxidants cannot justify the actual risk of severe side effects as well as the milder possibility of a 'no effect'. A possibility that could be addressed in the future is to modify natural antioxidants and increase their absorption and bioavailability. Maiti *et al.* have shown that, in the rat, the complexation of curcumin with phospholipids improved the pharmacokinetic parameters of this substance [74]. However, even in this case, the procedure necessary to perform all the preclinical and clinical studies could be quite long. Critically, in order to reach this goal, an active collaboration among chemists, pharmacologists, neurologists and general physicians is mandatory.

Stimulation of various maintenance and repair pathways through exogenous interventions (mild stress or compounds targeting the heat shock signal pathway), such as ALC, may have biological significance as a novel approach to delay the onset of various age-associated disorders [54], opening intriguing perspectives with a possible impact on cell survival during times of oxidative stress, hence contributing to the activation of cell life programmes and to the extent of cellular stress tolerance and resistance to AD pathogenic noxa. Notably, by maintaining or recovering the activity of vitagenes [54] it is possible to delay the ageing process and decrease the occurrence of age-related diseases with resulting prolongation of a healthy lifespan. Furthermore, future development of novel antioxidant drugs targeted to the mitochondria should result in effectively slowing disease progression. The association with new drug delivery systems may be desirable and useful for the therapeutic use of antioxidants in human neurodegenerative diseases.

Declaration of interest

The authors state no conflict of interest and have received no payment in the preparation of this manuscript.

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