Biochimica et Biophysica Acta 1822 (2012) 616–624

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Review

Natural substances and Alzheimer's disease: From preclinical studies to evidence based medicine ☆

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ARTICLE INFO

Article history:
Received 25 July 2011
Received in revised form 2 September 2011
Accepted 5 September 2011
Available online 14 September 2011

Keywords:
Acetyl-L-carnitine
Alzheimer's disease
Curcumin
Ginkgo biloba
Pharmacodynamics
Pharmacokinetics

ABSTRACT

Over the last 10 years, the potential therapeutic effects of nutraceuticals to prevent or delay Alzheimer's disease were proposed. Among dietary antioxidants curcumin, *Ginkgo biloba* and carnitines were extensively studied for their neuroprotective effects. The rationale for this alternative therapeutic approach was based on several preclinical studies which suggested the neuroprotective effects for curcumin, *Ginkgo biloba* and acetyl-L-carnitine due to either a free radical scavenging activity or the inhibition of pro-inflammatory pathways or the potentiation of the cell stress response. However, although these are interesting premises, clinical studies were not able to demonstrate significant beneficial effects of curcumin, *Ginkgo biloba* and acetyl-L-carnitine in improving cognitive functions in Alzheimer's disease patients. The aim of this review is to summarize the main pharmacologic features of curcumin, *Ginkgo biloba* and carnitines as well as to underlie the main outcomes reached by clinical studies designed to demonstrate the efficacy of these natural substances in Alzheimer's disease patients. This article is part of a Special Issue entitled: Antioxidants and Antioxidant Treatment in Disease.

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1. Introduction

According to the Delphi study, about 24 million people suffered from dementia in 2001 worldwide and this figure was estimated to double in 2020 and quadruple in 2040 [1]. Alzheimer's disease (AD) is the most common form of dementia and is characterized by cognitive and memory decline, speech loss, personality changes and is one of the major cause of admission to nursing homes [2,3]. From an epidemiologic point of view, the incidence of AD was shown to increase with age and doubled every 5 years after 65 years of age with 1275 new cases/100,000 persons/year [3]. The prevalence of AD was calculated as about 1% in subjects aged 60–64 but increases up to 33% in people aged 85 or older, in the Western hemisphere [4]. However, the annual incidence worldwide ranges from 1% to 7% at the ages of 70 and 85, respectively [5]. Sporadic AD is the more common form of the disease, accounting for 90% of all cases, whereas only 1% accounts for the familial form [2]. Although it was not definitely proven, most of the sporadic AD is associated with the ε4 allele of the apolipoprotein E (APOE), a plasma protein implicated in the transport of cholesterol which binds amyloid-β-peptide (Aβ), whereas familial AD is an autosomal dominant disorder, whose early onset was associated with mutations in specific genes such as *amyloid-β precursor protein* (APP), *presenilin 1* and *presenilin 2* [2,6]. Despite the huge amount of data derived from preclinical and clinical studies about the pathogenesis of AD, a limited number of drugs were developed over the last years [7]. A growing interest was focused on dietary antioxidants contained in many foods due to their natural origin and the quite good safety profile [8–10]. However, although promising early evidence of therapeutic benefit, mainly provided by preclinical experimental findings, clinical studies demonstrated the relative inability of dietary antioxidants to delay the onset and progression of AD as well as to improve cognitive function and the activities of daily living.

The aim of this paper is to provide an overview of the main pharmacologic features of the more common dietary antioxidants proposed to have therapeutic activity in AD, such as curcumin (which belongs to the polyphenol family), *Ginkgo biloba* extract (which contains flavonoids and terpenes) and carnitines. In addition, the main outcomes reached by clinical studies designed to demonstrate the effects of dietary antioxidants in AD patients will be analyzed. Finally, the pros and cons of the use of curcumin, *Ginkgo biloba* and acetyl-L-carnitine (ALC) in AD patients will be outlined.

2. The rationale for the use of antioxidants in AD

A main role in AD pathogenesis is played by amyloid-β-peptide (Aβ) and hyperphosphorylated *tau* protein [3,7,11]. Amyloid-β-peptide is formed by the secretase-mediated cleavage of APP. β-secretase cleaves APP at the N-terminus, generating an extracellular soluble fragment (sAPPβ) and leaving an intramembrane fragment called C99 [3,12]. This latter is cleaved at the C-terminus by the γ-secretase and Aβ is released [3,12]. Once formed, Aβ aggregates as oligomers and fibrils, which form the central core of senile plaques (SP) [3,7,13]. Many

☆ This article is part of a Special Issue entitled: Antioxidants and Antioxidant Treatment in Disease.

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doi:10.1016/j.bbadis.2011.09.004

research studies demonstrated that Aβ oligomers are more toxic than aggregates [3,14,15]. β-amyloid peptide oligomers isolated from the brain of AD subjects and administered to mice significantly impaired synaptic function and disrupted memory of a learned behavior [14]. On the other hand, insoluble Aβ plaques from AD patients became toxic for mice if the aggregates were solubilized to release low molecular weight oligomers [14]. The evidence that Aβ oligomers are more toxic than plaques provides a plausible explanation of the reason why drugs targeted to facilitate the disgregation of Aβ fibrils into oligomers, *e.g.* bapineuzumab and solanezumab, failed to improve cognitive function in AD patients [12,16]. Amyloid-β-peptide exerts its neurotoxic effects through several mechanisms: (a) generation of superoxide anion and nitric oxide through the activation of NADPH oxidase [17] and inducible nitric oxide synthase (iNOS) [3,15,18], respectively, which react with each other and form peroxynitrite ($ONOO^-$); (b) mitochondrial impairment secondary to the inhibition of key enzymes involved in the respiratory chain and Krebs-cycle [3]; (c) stimulation of the ionotropic glutamate receptor NMDA and increase of Ca^{2+} overload thus leading to excitotoxic cell death [3]. Both ROS and $ONOO^-$ oxidize and/or nitrate proteins and lipids thus contributing to cell death [13,19]. The free radical-induced damage and opening of mitochondrial membrane transition pores, causes cytochrome *c* release, caspase-3 activation and apoptotic cell death [3,20]. On the other hand, Aβ promotes the hyperphosphorylation of tau, a microtubule-associated structural protein, by activating multiple kinases, such as GSK-3β and DYRK1A [21]. Hyperphosphorylated tau aggregates and forms neurofibrillary tangles (NFTs) [3]. Hyperphosphorylated tau inhibits mitochondrial complex I and synergizes with Aβ to damage mitochondria [3]. As a consequence of increased oxidative/nitrosative stress and the opening of mitochondrial membrane transition pores leading to cytochrome *c* release and caspase-3 activation, apoptotic cell death takes place [3,20].

In this frame, the use of polyphenols and carnitine/ALC may be useful since they enhance both the cell stress response and improve mitochondrial respiration thus allowing the neuron to counteract free radical-induced damage and produce the ATP necessary to maintain the normal membrane potential [8–10].

2.1. Curcumin

Curcumin (1,7-bis[4-hydroxy 3-methoxy phenyl]-1,6-heptadiene-3,5-dione, Fig. 1) is a polyphenol 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 make food colored and flavored. Furthermore, curcumin is considered by the traditional Indian medicine as a therapeutic effective on several disorders including anorexia, coryza, cough, hepatic diseases and sinusitis [22,23].

Curcumin is quite stable at acidic pH and almost 40–80% of this compound is found unaltered in the gastrointestinal tract upon oral administration [22]. However, curcumin undergoes a marked first-pass effect which limits its systemic bioavailability (~ 60%) as demonstrated in humans and rodents [24–26]. In order to increase its bioavailability, the co-administration of curcumin with piperine [27] or its complexation with phospholipids or other vehicles to form lipophilic adducts [28–32] was proposed. The rationale for the co-administration with piperine is that this compound inhibits both hepatic and intestinal glucuronidating enzymes as well as the efflux pump P-glycoprotein thus increasing the amount of free-curcumin [27,33]. In humans treated with 2 g curcumin plus 20 mg piperine the increase in curcumin bioavailability was about 200% [27]. The complexation with phospholipids or solid lipid nanoparticles (SLN) increased curcumin bioavailability of 5- and 39-fold factors, respectively [28–30]. The formation of curcumin adducts with lipophilic vehicles seems to be a better strategy to increase its bioavailability than the co-administration with piperine. Shaikh et al. found that the complexation with polylactide-co-glycolide (PLGA) improved curcumin oral bioavailability of a 9-fold factor compared to that reached with the co-administration of the polyphenol with piperine [32]. Preclinical studies showed as the administration of 100 mg/kg of curcumin *per os* to the rat allows the polyphenol to reach peak plasma concentration (C_{max}) values around 90 ng/ml (~0.24 μM) with a T_{max} (time to reach C_{max}) of 0.5 h which increase at 121.2 ng/ml (~0.33 μM) and Tmax 0.75 h in the presence of 10 mg/kg piperine [32]. The C_{max} increases also when curcumin is complexed with liposoluble vehicles. Rats treated with 50 mg/kg curcumin-SLN complex have a T_{max} of 0.5 h and C_{max} of 14.29 μg/ml (~38 μM), this latter being about 50-times higher than that reached in rats exposed to control curcumin formulation solubilized with Tween80 [30]. Similar results were obtained in rats treated with 100 mg/kg curcumin-PLGA *per os* in which the C_{max} approaches 260 ng/ml (~0.71 μM), about 3-times higher than that found in rats assuming standard curcumin suspension [32]. However, the T_{max} value for curcumin-PLGA was 2 h [32]. Lower C_{max} values of curcumin were found in humans. The oral administration of 450–3600 mg of curcumin/day for 1 week to patients affected by colorectal cancer produced a plasma concentration of ~3 nM [34]. Plasma levels up to 160 nM reached after 2–5 h from the administration, were obtained in healthy volunteers exposed to oral curcumin at supra-therapeutic doses (10–12 g/day) [35]. These low plasma concentrations of curcumin did not depend on the acute regimen of treatment. Indeed, chronic administration of curcumin (1–4 g/day for 6 months *per os*) initiated plasma concentrations in the range 60–270 nM [36]. In the rat, the volume of distribution of curcumin is around 190 l thus suggesting that this polyphenol may accumulate in many organs including colorectal tissue and liver and it decreases when the polyphenol is complexed with phospholipids or SLN [24,28,30]. Studies in rodents and humans demonstrated that, after oral administration, curcumin undergoes phase I reactions and is reduced into dihydrocurcumin (DHC), tetrahydrocurcumin (THC), hexahydrocurcumin, octahydrocurcumin and hexahydrocurcuminol as well as phase II reactions originating curcumin glucuronide and curcumin sulfate [22,37,38]. These metabolic changes were shown to occur not only in the liver, the main organ deputed to biotransformation, but also in the intestinal tract [22,38]. Interestingly, the metabolism of curcumin generates products such as THC which retains antiinflammatory activity comparable to that of the parental compound [22,38]. It is important to recall the interaction of curcumin with drug-metabolizing enzymes and the possible repercussions on health. Curcumin and its derivatives inhibit the activity of several isoforms of drug metabolizing enzymes such as cytochrome P450 (CYP), including CYP3A4, glutathione-S-transferase and UDP-glucuronosyltransferase [39–42] and this effect may be responsible for an undesired increase in plasma concentrations of drugs metabolized through these enzymes such as digoxin, acetaminophen and morphine [43]. Furthermore, even piperine, which has been used in combination with curcumin to increase its bioavailability, has been shown to be a non-competitive inhibitor of CYP3A4 [40]. The inhibition by curcumin, alone or in combination with piperine, of CYP3A4 is potentially harmful, in particular in the case of prolonged exposure to these substances. In fact, it was shown that CYP3A4 inhibitors may alter the metabolism of several drugs commonly used in old people, *e.g.* amiodarone and quinidine, thus increasing the risk to develop life-threatening ventricular arrhythmias such as *torsades de pointes* [43,44]. Donepezil and galantamine, commonly used in AD

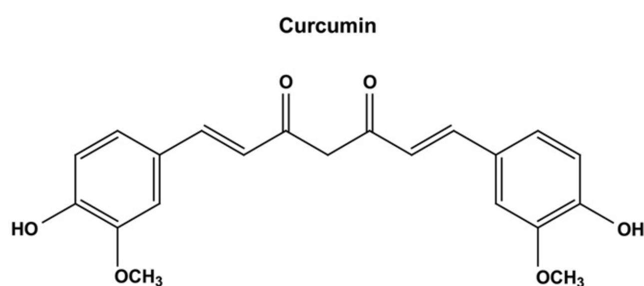

Fig. 1. The chemical structure of curcumin.

patients to improve cognitive function, are metabolized by CYP3A4. For this reason, the concomitant administration of donepezil and galantamine with curcumin alone or plus piperine could increase the half-life of these drugs and exacerbate their side effects such as fatigue, nausea, emesis and muscle cramps, dizziness and sinusitis [45,46]. In the rat, curcumin is mainly excreted into the bile and then eliminated by the feces, only a little amount is eliminated by the urine [25,26] and the elimination half-life is ~1.5 h [28]. The urinary elimination of curcumin and its metabolites seems to be increased if curcumin is administered at large doses (for example 3.6 g/day for up to 4 months) [24,47]. With regard to the safety profile, curcumin was shown to be carcinogenic and to cause DNA damage and chromosomal alteration in mammalian cell lines at concentrations close to those reported to exert beneficial effects [48]. In addition, curcumin behaved as an iron chelator and induced iron-deficient anemia in mice fed with diets poor in iron [48]. Studies in rodents and primates have shown that the spice at doses up to 3.5 g/kg body weight administered for up to 3 months were well tolerated by the animals [24]. However, patients affected by advanced colorectal cancer treated with curcumin 3.6 g/day developed diarrhea whereas the dose of 0.9 g/day was associated with nausea, which resolved spontaneously. In the same patients, blood test abnormalities correlated with curcumin administration were a rise in serum alkaline phosphatase and lactate dehydrogenase [24,47].

The early evidence of a protective role of curcumin in AD derived from epidemiological studies. Ganguli and colleagues demonstrated that Indian population, who has a curcumin-enriched diet, has a reduced prevalence of AD compared to United States [49]. Following this initial observation, several preclinical studies showed the cytoprotective effects of curcumin (Table 1). *In vitro* studies showed that curcumin and derivatives (0.2–27 μM) protected neuron-like PC12 rat cells and normal human umbilical vein endothelial (HUVEC) from β-amyloid toxicity and, interestingly, the polyphenol displayed a neuroprotective effect greater than a well known antioxidant such as α-tocopherol [50]. In addition, curcuminoids were shown to restore synaptic plasticity, evaluated as long term potentiation, significantly damaged by Aβ treatment in rat hippocampal slices [51]. Curcumin exerts indirect effects by either inhibiting pro-inflammatory transcription factors (NFkB and activator protein-1) and enzymes (cyclooxygenase, lipoxygenase, inducible nitric oxide synthase *etc.*) [52] or stimulating detoxifying enzymes such as glutathione-S-transferase [53] (Fig. 2 and Table 1). Furthermore, curcumin potentiated the cell stress response through the activation of the heme oxygenase-1/biliverdin reductase (HO-1/BVR) system as well as other members of the heat shock protein family in several rodent and human cell lines, including rat neurons and astrocytes [54] (Fig. 2). In particular, the ability of curcumin and other natural compounds, such as *Ginkgo biloba* (see below), to induce the cell stress response is quite interesting and put these natural compounds in the “hormetin arena”. In other words, a mild stressful condition elicited by the hormetins activates antioxidant systems, such as the HO-1/BVR pathway which, in turn, exerts it cytoprotective effect by degrading heme, which is toxic if produced in excess, and generating the powerful free radical scavenger bilirubin [54–56].

Table 1
Some of the main intracellular targets involved in the pharmacological effects of curcumin.

Curcumin			
Target(s)	Experimental model(s)	Pharmacological effect	Reference(s)
NFkB, AP-1	hML-1a cells Rat liver	Inhibition of inflammatory response	[118,119]
iNOS	hKBM-5 cells Rat liver	Inhibition of inflammatory response	[119,120]
Bcl-2, Bcl-xL	hKBM-5 cells	Activation of apoptotic cascade	[120]
COX-2	hKBM-5 cells Rat liver	Inhibition of inflammatory response	[119,120]
GST	Rat liver	Inhibition of carcinogenesis	[53]
HO-1/BVR	Rat neurons Rat astrocytes pLLC-PK1 cell line rNRK-52E cell line Bovine endothelial cells	Enhancement of the cell stress response	[121–123]
IL-1β	Tg2576 mice	Inhibition of inflammation	[57]
APP	Tg2576 mice APPswe/PS1dE9 mouse	Reduction of senile plaques	[57,58]

AP-1, activator protein-1; APP, amyloid precursor protein; Bcl-2, B-cell lymphoma-2; Bcl-xL, B-cell lymphoma-extra large; COX-2, inducible cyclooxygenase; GST, glutathione-S-transferase; h, human; IL-1β, interleukin-1β; iNOS, inducible nitric oxide synthase; HO-1/BVR, heme oxygenase-1/biliverdin reductase system; NF-kB, nuclear factor kB; p, porcine; r, rat.

By using an Alzheimer transgenic APPSw mouse model (Tg2576), Lim and colleagues showed that dietary curcumin (160–5000 ppm) suppressed inflammation and oxidative damage in the brain of these mice [57]. Garcia-Alloza et al. in transgenic APPswe/PS1dE9 mice demonstrated that curcumin 7.5 mg/kg/day intravenously for 7 days, crosses the blood–brain-barrier, binds to β-amyloid deposits in the brain and accelerates their rate of clearance [58]. Although these are promising preclinical findings, the beneficial effect of curcumin in AD patients was not proved so far. Curcumin 1–4 g/day for 6 months did not reduce peripheral biomarkers of inflammation, such as serum Aβ and isoprostanes, in AD patients; more importantly, curcumin did not ameliorate cognitive performance in these patients [36]. Since curcumin was hypothesized to have a protective role in cardiovascular diseases due to its ability to reduce cholesterolemia, the same AD patients were monitored also for the plasma lipid profile. The results clearly demonstrated that curcumin did not have any significant beneficial effect on plasma triglycerides or total, LDL and HDL cholesterol over 1 or 6 months, rather a slight increase in cholesterol plasma levels was detected in AD patients when the absorbed dose of curcumin was taken into consideration [59].

2.2. *Ginkgo biloba*

Ginkgo biloba, a plant whose places of origin are the remote mountainous valleys of Eastern China, is considered a “living fossil”. Although *Ginkgo biloba*'s extracts contain several active compounds, including flavonoids, terpenes, organic acids and polyphenols, the most important ones are flavonol-glycosides (quercetin, kaempferol and isorhamnetin derivatives) and terpene-lactones (Fig. 3). These latter are further divided into diterpenes (ginkgolides) and sesquiterpenes (bilobalide) [60,61]. Flavonol-glycosides and terpene-lactones are endowed with many pharmacological activities such as the antagonism on platelet activating factor and neuroprotection [60–62].

Ginkgo biloba extract is well absorbed by oral route: the bioavailability for flavonol-glycosides is >60%, for ginkgolides >98% and for bilobalide ~70% [63]. Both flavonol-glycosides and terpene-lactones are mainly adsorbed in the small intestine, even if the possibility of a gastric absorption of ginkgolide B could not be excluded [63–65]. Preclinical studies showed that in rats treated with *Ginkgo biloba* extract containing 20.3, 14.7 and 3.2 mg/kg of quercetin, kaempferol and isorhamnetin, respectively, the C_{max} values of each compound were 179.21 ng/ml (quercetin), 180.23 ng/ml (kaempferol) and 195.96 ng/ml (isorhamnetin) and the T_{max} were 1.21 h, 6.32 h and 7.21 h, respectively [62]. Similar results were obtained in rats treated orally with Egb 761® (a *Ginkgo biloba* extract containing 24% flavonol-glycosides and 6% terpenes) at the dose of 600 mg/kg [66]. Interestingly, repeated administration of 600 mg/kg Egb 761® for 8 days led to 4.5-fold, 11.5-fold and 10-fold increases in plasma concentration of quercetin, kaempferol and isorhamnetin, respectively [66]. With regard to terpene lactones, data in humans are available. After the administration of 160 mg of Ginkgoselect® (a product with the same composition of Egb 761®) to healthy male volunteers, C_{max} values of ~42 ng/ml, 5.6 ng/ml and 37.6 ng/ml for ginkgolide A, B and bilobalide, respectively, were reached after 2 h [67]. In the same subjects, the half-

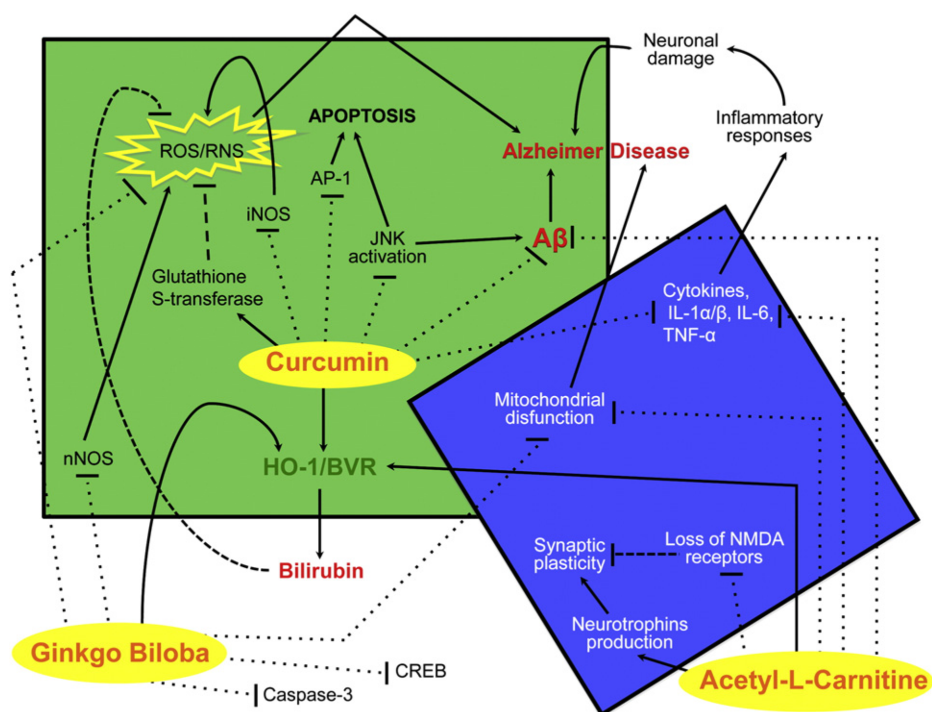

Fig. 2. The main intracellular pathways involved in the neuroprotective effects of curcumin, *Ginkgo biloba* and acetyl-L-carnitine. For further information see text and Tables 1–3. Arrows, stimulation; dotted lines, inhibition.

life of terpenes was calculated within the range 138–158 min [67]; these values are different from those found by Kleijnen and Knipschild who calculated elimination half-lives of 4–6 h and 3 h for ginkgolides and bilobalide, respectively [63]. Terpene-lactones were shown to cross the rat blood–brain barrier, and the concentrations in brain tissue were found to be 55 ng/g, 40 ng/g and 98 ng/g for ginkgolide A, ginkgolide B and bilobalide, respectively [68]. Similarly to curcumin, a phospholipidic complex of flavonol-glycosides and terpenes (Ginkgoselect® Phytosome®) was prepared to improve bioavailability. In rats treated orally with a phospholipidic formulation of *Ginkgo biloba* extract, equivalent to doses of 20.3, 14.7 and 3.2 mg/kg of quercetin, kaempferol and isorhamnetin, the C_{max} of each compound increased 4.04, 1.79 and 3.44 times, respectively, compared to *Ginkgo biloba* extract [62]. In human subjects treated with 160 mg of Ginkgoselect® Phytosome®, C_{max} values of ~108 ng/ml, 13.4 ng/ml and 60.3 ng/ml for ginkgolide A, B and bilobalide, respectively, where reached after 3–4 h from the administration [67]. Ginkgolides A and B are mainly excreted unchanged in the urine (70% and 50%, respectively), whereas for bilobalide this figure is about 30% [63]. The administration of *Ginkgo biloba* extracts is quite safe. Only mild adverse effects were recorded including mild gastrointestinal complaints, headache and allergic symptoms [63]. However, important interactions between *Ginkgo biloba* and common drugs should be underlined. Due to the ability

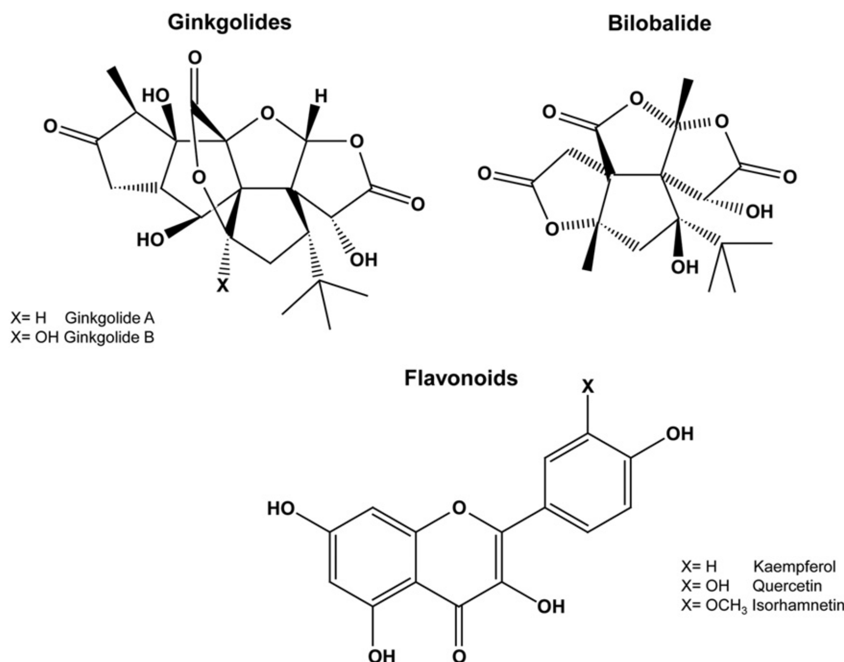

Fig. 3. The chemical structures of the main *Ginkgo biloba*'s active components.

to increase the activity of CYP2C19, *Ginkgo biloba* chronic treatment reduced omeprazole plasma concentration and this effect was marked in individuals sharing the status of poor metabolizers [69]. The induction of CYP2C19 by *Ginkgo biloba* was shown to increase metabolism and reduce plasma concentrations of antiepileptic drugs such as valproic acid and phenytoin, thus increasing the risk of fatal seizures [70]. Finally, ginkgo's flavonol-glycosides caused coma in a 80-years individual with AD treated with trazodone [71]. This severe side effect was hypothesized to be due to a marked stimulation of CYP3A4 activity which increased the transformation of trazodone into the active metabolite 1-(mchlorophenyl) piperazine which, in turn, increased GABAergic activity in the brain and precipitated coma [71].

Several preclinical studies in rodents put forth the neuroprotective role of *Ginkgo biloba*, mainly due to its ability to prevent free radical-induced damage, increase the intracellular levels of antioxidant enzymes and restore calcium homeostasis [72–75] (Fig. 2 and Table 2). Relevant to this paper are the several findings related to the neuroprotective effect of *Ginkgo biloba* in both *in vivo* and *in vitro* models of AD. EGb 761 failed to modify α- and β-secretase expressions and activities in rat hippocampus and cortex as well as in the Neuro-2A cell line and in the Tg2576 transgenic mouse [76,77], but inhibited the formation of Aβ fibrils in a neuroblastoma cell line overexpressing an AD-associated double mutation [78]. Furthermore, EGb 761 (10–100 μg/ml) dose dependently counteracted Aβ-induced toxicity in rat hippocampal primary cultured cells [79]. This neuroprotective effect was demonstrated when rat hippocampal cells were treated with EGb 761 before and concomitantly with Aβ [79]. The antioxidant effect of EGb 761 seemed to be attributable to the flavonoid fraction rather than the terpene-lactones fraction [79]. This last finding was also confirmed by the evidence that single flavonol-glycosides as quercetin are neuroprotective against Aβ-induced neuronal damage. As shown by Ansari et al., quercetin counteracted Aβ-induced protein and lipid oxidation and attenuated apoptosis in rat cortical neuronal cultures [80]. Since ROS and RNS are mainly involved in Aβ-induced neurotoxicity, the efficacy of *Ginkgo biloba* was also specifically tested against these free radicals. EGb 761 (10–100 μg/ml) improved survival and inhibited ROS and RNS accumulation in rat primary mixed hippocampal cell cultures exposed to sodium nitroprusside [81] or in neuroblastoma cell line overexpressing an AD-associated double mutation treated with hydrogen peroxide [82]. These Authors confirmed the selective neuroprotective effect of the flavonol fraction and the relative inefficacy of ginkgolides and bilobalides [81]. Among the mechanisms through which EGb 761 exerts its neuroprotective effects, the inhibition of the apoptotic cascade through the block of caspase-3 activation [78] and the phosphorylation of the pro-survival transcription factor CREB is worthy of mention [83] (Fig. 2). Similarly to curcumin, even *Ginkgo biloba* exerted neuroprotection through the activation of the HO-1/BVR axis in a mouse model of ischemia/reperfusion brain injury [84,85] (Fig. 2).

Table 2
Some of the main intracellular targets involved in the pharmacological effects of *Ginkgo biloba*.

Ginkgo biloba			
Target(s)	Experimental model(s)	Pharmacological effect	Reference(s)
Catalase, SOD	Rat brain	Neuroprotection	[72]
Voltage-gated calcium channels	rCGN	Restoration of calcium homeostasis	[75]
Caspase-3	rCGN N2a/N2aAPP695 cell lines	Inibition of the apoptosis cascade	[75,78]
Aβ	N2a/N2aAPP695 cell lines TgAPP/PS1 mouse	Inhibition of Aβ aggregation	[78,83]
CREB	TgAPP/PS1 mouse	Stimulation of neurogenesis	[83]
HO-1/BVR	Mouse I/R injury	Enhancement of the cell stress response	[84,85]

Aβ, amyloid-β-peptide; CGN, cerebellar granule neurons; CREB, cAMP response element-binding; HO-1/BVR, heme oxygenase-1/biliverdin reductase system; I/R, ischemia/reperfusion; r, rat; SOD, superoxide dismutase.

Although these are promising preclinical evidence, the efficacy of *Ginkgo biloba* was not confirmed in AD patients. As a matter of fact, earlier clinical trials demonstrated a slight improvement in cognitive functions in individuals suffering from mild-to-moderate or moderate-to-severe AD and treated with EGb 761 120–240 mg/day for short (12–24 weeks) or long time (up to 52 weeks) [86–90]. On the other hand, other clinical trials were not able to confirm the efficacy of EGb 761 (160–240 mg/day for 24 weeks) to ameliorate cognitive function in mild-to-moderate AD patients, and the effect of *Ginkgo biloba* extract was found comparable to placebo [91–93]. The inefficacy of *Ginkgo biloba* as a therapeutics in AD patients was confirmed by two larger clinical trial which evaluated the ability of this herbal product to prevent AD in elderly subjects with normal cognitive function and in individuals with mild cognitive impairment (MCI) [94,95]. The follow-up of this trial was 6.1 years. The results of this study showed that *Ginkgo biloba* extract, 120 mg twice a day, did not reduce the incidence of AD in both healthy individuals and those suffering from MCI [94,95]. A systematic review and meta-analysis confirmed that the neuroprotective effects of *Ginkgo biloba* are moderate, but their clinical relevance is difficult to evaluate [96].

2.3. L-Carnitine and derivatives

L-carnitine (LC, Fig. 4) is a natural compound and plays an important function in mitochondrion biologic function since it facilitates the transport of fatty acids to this organelle. Dietary LC derives from the intake of red meats, but the endogenous synthesis of LC from the amino acids lysine and methionine has been also documented [97]. The dietary intake of LC in humans is in the range 1–15 μmol/kg body weight/day, whereas the rate of biosynthesis is about 1–2 μmol/kg body weight/day [98]. Dietary LC is well absorbed by the gastrointestinal tract by simple or carrier-mediated diffusion and its bioavailability is

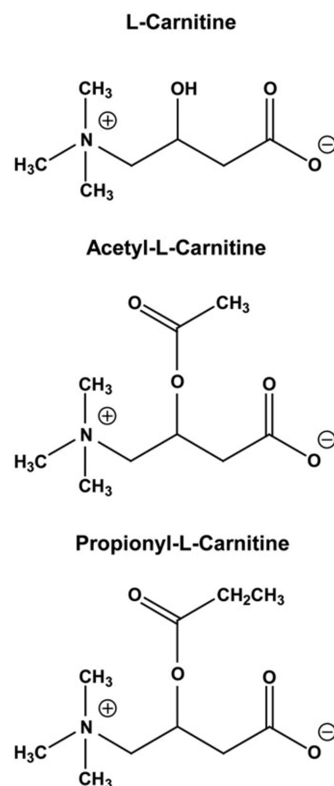

Fig. 4. The chemical structures of L-carnitine and its derivatives.

54–86%; conversely, the bioavailability of exogenous LC is much lower, in the range 5–18% [97,98]. This paradoxical effect can be explained considering that the absorption of LC decreases as the intake of LC increases, this is to maintain the concentration of LC constant [97,98]. When administered at doses 30–100 mg/kg by oral route to humans, the C_{max} of L-carnitine is 27–91 μM with T_{max} 3 h, and the plasma concentration returns to the baseline within 24 h [98,99]. L-carnitine undergoes extensive metabolism in rodents and human intestine thus forming esterified compounds such as ALC and propionyl-L-carnitine (PLC) (Fig. 4) which are 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 4-times greater than that of LC [98,100]. In healthy male volunteers treated with PLC 1–8 g as a single dose by intravenous infusion over 2 h, C_{max} values of 9.4–16.4 nmol/ml and 143–1049 nmol/ml were reached for ALC and PLC, respectively [101]. In the same study, the T_{max} values for both ALC and PLC were 2.4–3.1 h and 1.9–2.1 h, respectively [101]. Data from AD patients showed 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 [98]. Neither LC nor ALC nor PLC is bound to plasma proteins [97,101]. The volume of distribution (Vd) of LC differs considering the dietary or exogenous source being approximately 3000 l and 20–50 l, respectively [97]. 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 [97]. Similarly, PLC (1 g, single dose intravenously) has a Vd in the range 17.4–18.3 l [101]. The elimination half-life for LC, ALC and PLC ranged 3–12 h, 4 h and 1 h, respectively [97,101,102]. It is interesting to underlie that LC, ALC and PLC are able to cross blood–brain-barrier [103]; Parnetti et al. also showed as AD patients treated with ALC i.v. or p.o. for 10–60 days have an increased concentration of ALC in the cerebrospinal fluid up to 3.55 nmol/ml [104]. Of note and according to aforementioned data, the renal clearance of LC which is about 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 [97]. This last finding is very important and concurs to explain how exogenous LC is almost completely excreted during the first 12 h after administration whereas dietary LC is reabsorbed [97]. Due to its elimination mainly through the kidney, LC and should be administered very carefully to patients affected by renal impairment. The basal renal-excretory clearance of PLC, which is about 0.33 l/h, increases from 1.98 l/h to 5.55 l/h at doses of 1 g and 8 g, respectively, thus suggesting an extensive tubular reabsorption [101].

Acetyl-L-carnitine was shown to exert beneficial effects in preventing the loss of brain function which typically occurs during aging and neurodegenerative disorders (Table 3). Preclinical and clinical studies demonstrated that the main mechanism of action of ALC is the improvement of mitochondrial respiration which allows the neuron to produce ATP necessary to maintain the normal membrane potential [105–107] (Fig. 2). In the meantime, ALC was shown to be neuroprotective by regulating other intracellular pathways, such as PKC, in the rat [107,108]. Interestingly, ALC counteracted the loss of NMDA receptors in rat neuronal membrane and increased the production of neurotrophins, two effects strictly related to synaptic plasticity [107,109–111] (Fig. 2). With regard to the pathogenesis of AD, ALC reduces Aβ toxicity in rat primary cortical neuronal cultures by increasing the cell stress response through the activation of the heat shock proteins HO-1 and Hsp70 expression [112] (Fig. 2). Through the modulation of the heat shock protein family, ALC prevented age-related changes in mitochondrial respiration and decrease oxidative stress biomarkers in senescent rat brain [113]. Accordingly, ALC treatment increased life-span, improved cognitive behavior in aged rats and guaranteed long-term memory performance [107]. As a whole, these pre-clinical studies suggested that ALC treatment could be beneficial for the treatment of age-related diseases and the potential use in humans was explored. Patients affected by AD and treated with ALC at doses ranging from 1 to 2 g/day for 6–12 months, had 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 was significant [107,114]. In two clinical studies, ALC 3 g/day for 1 year significantly reduced cognitive decline only in early-onset AD patients [115,116], but this evidence was not confirmed in a later *ad hoc* designed study [117].

Table 3
Some of the main intracellular targets involved in the pharmacological effects of acetyl-L-carnitine.

Acetyl-L-carnitine			
Target(s)	Experimental model(s)	Pharmacological effect(s)	Reference(s)
Mitochondria	Rat brain	Improvement of synaptic plasticity	[105,106]
	Rat heart	Stimulation of energy metabolism	
PKC	Rat brain	Amelioration of cognitive function	[108]
NMDA receptors	Rat brain	Amelioration of cognitive function	[111]
NGF	Rat brain	Amelioration of cognitive function	[109,110]
HO-1/BVR Hsp70	Rat brain	Enhancement of the cell stress response	[112,113]

HO-1/BVR, heme oxygenase-1/biliverdin reductase system; NMDA, N-methyl-D-aspartate; NGF, nerve growth factor; PKC, protein kinase C.

3. Conclusions

On the basis of preclinical and clinical evidence, the role of curcumin, *Ginkgo biloba* and ALC in the therapy of AD is still under debate. Important pharmacokinetic limitations, such as the poor biovailability and harmful interactions with drug metabolizing enzymes, need to be carefully considered in case of curcumin and *Ginkgo biloba* administration. Conversely, despite favorable pharmacokinetics (*e.g.* the ability to cross blood–brain barrier) the lack of therapeutic effect of ALC in AD patients seems to be related to its pharmacodynamics. In this frame, the development of new drug-delivery systems which improve systemic bioavailability and brain penetrance and allow the administration of natural substances at low doses has to be considered a promising strategy. This approach could also minimize the risk for adverse effects related to unwanted fluctuations in nutraceutical plasma concentrations. It is an ambitious goal, however, and one that will require close, active collaboration by pharmacologists, chemists, and clinicians.

Acknowledgements

This work was supported by Fondi Ateneo to C.M.

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