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Lipoic acid as an anti-inflammatory and neuroprotective treatment for Alzheimer's disease ☆

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that destroys patient memory and cognition, communication ability with the social environment and the ability to carry out daily activities. Despite extensive research into the pathogenesis of AD, a neuroprotective treatment – particularly for the early stages of disease – remains unavailable for clinical use. In this review, we advance the suggestion that lipoic acid (LA) may fulfil this therapeutic need. A naturally occurring cofactor for the mitochondrial enzymes pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, LA has been shown to have a variety of properties which can interfere with the pathogenesis or progression of AD. For example, LA increases acetylcholine (ACh) production by activation of choline acetyltransferase and increases glucose uptake, thus supplying more acetyl-CoA for the production of ACh. LA chelates redox-active transition metals, thus inhibiting the formation of hydroxyl radicals and also scavenges reactive oxygen species (ROS), thereby increasing the levels of reduced glutathione. In addition, LA down-regulates the expression of redox-sensitive pro-inflammatory proteins including TNF and inducible nitric oxide synthase. Furthermore, LA can scavenge lipid peroxidation products such as hydroxynonenal and acrolein. In human plasma, LA exists in an equilibrium of free and plasma protein bound form. Up to 150 μM, it is bound completely, most likely binding to high affinity fatty acid sites on human serum albumin, suggesting that one large dose rather than continuous low doses (as provided by "slow release" LA) will be beneficial for delivery of LA to the brain. Evidence for a clinical benefit for LA in dementia is yet limited. There are only two published studies, in which 600 mg LA was given daily to 43 patients with AD (receiving a standard treatment with choline-esterase inhibitors) in an open-label study over an observation period of up to 48 months. Whereas the improvement in patients with moderate dementia was not significant, the disease progressed extremely slowly (change in ADAScog: 1.2 points=year, MMSE: −0.6 points=year) in patients with mild dementia (ADAScog<15). Data from cell culture and animal models suggest that LA could be combined with nutraceuticals such as curcumin, (−)-epigallocatechin gallate (from green tea) and docosahexaenoic acid (from fish oil) to synergistically decrease oxidative stress, inflammation, Aβ levels and Aβ plaque load and thus provide a combined benefit in the treatment of AD.

Contents

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1. Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder that gradually destroys a patient's memory and ability to learn, make judgments, communicate with the social environment and carry out daily activities. In the course of the disease, short-term memory is affected first, caused by neuronal dysfunction and degeneration in the hippocampus and amygdala. As the disease progresses further, neurons also degenerate and die in other cortical regions of the brain [1]. At that stage, sufferers often experience dramatic changes in personality and behaviour, such as anxiety, suspiciousness or agitation, as well as delusions or hallucinations [2]. AD prevalence in the different age groups is 1% (65–69 years), 3% (70–74 years), 6% (75–79 years), 12% (80–84 years), and 25% (85 and over).

AD is further characterized by two major neuropathological hallmarks. The deposition of neuritic, β-amyloid peptide-containing senile plaques in hippocampal and cerebral cortical regions of AD patients is accompanied by the presence of intracellular neurofibrillary tangles that occupy much of the cytoplasm of pyramidal neurons. Inflammation, as evidenced by the activation of microglia and astroglia, is another hallmark of AD. Inflammation, including superoxide production ("oxidative burst"), is an important source of oxidative stress in AD patients [3,4]. The inflammatory process occurs mainly around the amyloid plaques and is characterized by pro-inflammatory substances released from activated microglia and astroglia [5]. Cytokines are prominent molecules in the inflammatory process, including IL-1β, IL-6, M-CSF and TNF-α [6].

Besides morphological alterations, AD is associated also with a markedly impaired cerebral glucose metabolism as detected by reduced cortical [^{18}F]-desoxyglucose utilization in positron emission tomography of AD patients [7].

2. The cholinergic deficit in Alzheimer's disease

Alzheimer's disease patients show a progressive neuronal cell loss that is associated with region-specific brain atrophy. In particular, the cholinergic projection from the nucleus basalis of Meynert to areas of the cerebral cortex is the pathway that is very early and most severely affected in brains from Alzheimer patients [8]. Loss of basal forebrain cholinergic neurons is demonstrated by reductions in number of cholinergic markers such as choline acetyltransferase, muscarinic and nicotinic acetylcholine receptor binding, as well as levels of acetylcholine (ACh) itself [9]. These changes are highly correlated with the degree of dementia in AD. ACh is derived from choline and acetyl-CoA, the final product of the glycolytic pathway. Pyruvate derived from glycolytic metabolism serves as an important energy source in neurons. Therefore, the inhibition of pyruvate production e.g. by glucose depletion, is considered a crucial factor that leads to acetyl-CoA deficits in AD brains. Based on the findings that a) AD patients have reduced levels of the enzyme choline acetyltransferase and the neurotransmitter ACh compared to healthy elderly people and b) ACh is hydrolyzed by acetylcholine esterase (AChE), acetylcholine esterase inhibitors were the first drug class successfully introduced for the treatment of Alzheimer's patients.

3. Alzheimer's disease — current treatment strategies

At the present moment, only symptomatic treatments with acetylcholine esterase inhibitors are approved for mild to moderate forms of AD. Only one neuroprotective treatment strategy (using the NMDA receptor antagonist memantine) is used in clinical practice, and is approved for moderate to severe forms of AD. The need for a "cholinergic + pro-energetic + neuroprotective" therapy for early stage AD is urgent, and we have proposed that LA is a promising candidate for such treatment [10].

4. LA — a multimodal drug for the treatment of ad

4.1. Possible modes of action of LA interfering with AD specific degeneration

In vitro and *in vivo* studies suggest that LA also acts as a powerful micronutrient with diverse pharmacologic and antioxidant properties [11]. LA naturally occurs only as the *R*-form (RLA) but pharmacological formulations have extensively used a racemic mixture of RLA and *S*-lipoic acid (SLA) in the past before stereoselective synthesis methods became available.

In brief, LA has been suggested to have the following anti-dementia/anti-AD properties:

A) To increase acetylcholine production by activation of choline acetyltransferase,
B) To increase glucose uptake, supplying more acetyl-CoA for the production for acetylcholine,
C) To chelate redox-active transition metals, inhibiting the formation of hydrogen peroxide and hydroxyl radicals,
D) To scavenge reactive oxygen species (ROS), increasing the level of reduced glutathione,
E) To scavenge reactive oxygen species (ROS), down-regulating inflammatory processes,
F) To scavenge lipid peroxidation products and
G) To induce the enzymes of glutathione synthesis and other antioxidant protective enzymes.

These diverse actions suggest that LA acts by multiple mechanisms, both physiologically and pharmacologically, many of which are only now being explored. It has been initially proposed that the reduced form of LA, DHLA, is responsible for many of its pharmacological benefits. However, more and more evidence suggests that many of the "antioxidant" effects of LA *in vivo* are mediated by an indirect effect, in which LA acts as a pro-oxidant and activates the transcription factor Nrf2 which in turn up-regulates the expression of phase II detoxification enzymes as well as antioxidant proteins including glutathione-*S*-transferases, NAD(P)H:quinone oxidoreductase-1, gamma-glutamyl-cysteine synthase, ferritin, and heme oxygenase-1 [12,13].

4.2. LA — an activator of choline acetyltransferase

Haugaard and Levin have demonstrated that dihydrolipoic acid (DHLA), the reduced form of LA, which is formed from LA by reduction by the PDH complex, strongly increases the activity of a purified preparation of choline acetyl transferase [14]. In a further publication, the authors show that removal of DHLA by dialysis from purified choline acetyl transferase (and also from extracts from rat brain and heart as well as rabbit bladder tissue) causes complete disappearance of enzyme activity, and that addition of DHLA but not that of reduced ascorbic acid or reduced nicotinamide adenine dinucleotide restores activity towards normal [15]. The authors conclude that DHLA serves an essential function in the action of this enzyme and that the ratio of reduced to oxidized LA plays an important role in acetylcholine synthesis. From these data the authors further conclude that DHLA: a) may act as a coenzyme in the choline acetyl transferase reaction or b) is able to reduce an essential functional cysteine residue in choline acetyltransferase, which cannot be reduced by any other physiological antioxidant, including reduced glutathione [15].

4.3. LA — a potent metal chelator

There is now compelling evidence that β-amyloid peptide (Aβ) the main component of amyloid plaques in the AD brain, does not spontaneously aggregate, but that there is an age-dependent reaction with excess brain metal ions (copper, iron and zinc), which induces the peptide to precipitate and form plaques. Furthermore, the abnormal combination of Aβ with Cu or Fe ions induces the production of hydrogen peroxide from molecular oxygen [16], which subsequently produces the neurotoxic hydroxyl radical by the Fenton or Haber–Weiss reactions. Because LA is a potent chelator of divalent metal ions *in vitro*, it was investigated whether feeding RLA could lower cortical iron levels and improve antioxidant status. Results show that cerebral iron levels in old animals fed LA were lower when compared to controls and were similar to levels seen in young rats. These results thus show that chronic LA supplementation may be a means to modulate the age-related accumulation of cortical iron content, thereby lowering oxidative stress associated with aging [17]. Since amyloid aggregates have been shown to be stabilized by transition metals such as iron and copper, it was speculated that LA could also inhibit the formation of aggregates or maybe even be able to dissolve existing amyloid deposits. Fonte et al. successfully re-solubilized Aβ with transition metal ion chelators, and showed that LA enhanced the extraction of Aβ from the frontal cortex of APP overexpressing transgenic mice, suggesting that, like other metal chelators, it could reduce amyloid burden in AD patients [18]. In another published study, the effects of LA were tested on the Tg2576 mouse, a transgenic model of cerebral amyloidosis associated with AD. Ten-month-old Tg2576 and wild type mice were fed a LA-containing diet for 6 months and assessed the influence of this diet on memory and neuropathology. The authors could demonstrate that the LA-treated Tg2576 mice exhibited significantly improved learning, and memory retention in the Morris water maze task compared to untreated Tg2576 mice. However, assessment of brain soluble and insoluble beta-amyloid levels and nitrotyrosine levels revealed no differences between LA-treated and untreated Tg2576 mice. These data suggest that chronic dietary LA can reduce hippocampal-dependent memory deficits of Tg2576 mice without affecting beta-amyloid levels or plaque deposition [19]. A potential side effect of a long-term therapy with high doses of a metal chelator such as LA could be its inhibition of metal containing enzymes such as insulin degrading enzyme or superoxide dismutase. Suh et al. investigated whether LA and DHLA remove copper or iron from the active site of enzymes, using Cu,Zn superoxide dismutase and the iron-containing enzyme aconitase. They found that even at mM concentrations neither LA nor DHLA altered the activity of these enzymes [20].

4.4. LA — an anti-inflammatory antioxidant and modulator of redox-sensitive signalling

AD is characterized by a chronic inflammatory process around amyloid plaques, characterized by the activation of micro- and astroglia and increased levels of radicals and pro-inflammatory cytokines such as inducible nitric oxide synthase (iNOS), IL-1 β, IL-6 and TNF-α [6]. AD patients also show increased cytokine levels (e.g. IL-1 β and TNF) in the CSF, with TNF being a good predictor for the progression from mild cognitive impairment to AD. Recently, much attention has been paid to ROS as mediators in signalling processes, termed "redox-sensitive signal transduction". ROS modulate the activity of cytoplasmic signal transducing enzymes by at least two different mechanisms: oxidation of cysteine residues or reaction with iron–sulphur clusters. One widely investigated sensor protein is the p21ras protein [21]. Activation of Ras by oxidants is caused by modification of a specific cysteine (Cys118). Ras interacts with PI3K-kinase, protein kinase C, diacylglycerol kinase, and MAP-kinase-kinase-kinases, regulating expression of IL-1, IL-6 and iNOS. LA can scavenge intracellular free radicals (acting as second messengers), down-regulate pro-inflammatory redox-sensitive signal transduction processes including NF-κB translocation, and thus attenuates the release of more free radicals and cytotoxic cytokines [22,23]. In addition, LA induces a scope of cellular antioxidants and phase 2 enzymes including catalase, reduced glutathione, glutathione reductase, glutathione-*S*-transferase, and NAD(P)H:quinone oxidoreductase-1 [24]. Glutathione (GSH) significantly declines during aging, and γ-glutamylcysteine ligase (GCL) is the rate-controlling enzyme in GSH synthesis [25]. With age, both the expression of the catalytic (GCLC) and modulatory (GCLM) subunits of GCL and the overall enzyme activity decline by approximately 50%. Because nuclear factor erythroid2-related factor 2 (Nrf2) governs basal and inducible GCLC and GCLM expression by means of the antioxidant response element (ARE), Suh et al. hypothesised that aging results in dysregulation of Nrf2-mediated GCL expression. The authors observed an approximately 50% age-related loss in total and nuclear Nrf2 levels, which suggests attenuation in Nrf2-dependent gene transcription. However, when old rats were treated with RLA, nuclear Nrf2 levels in old rats increased and, consequently, higher GCLC levels and GCL activity were observed as early as 24 h after injection of RLA [26].

4.5. LA — a carbonyl scavenger

Cell and mitochondrial membranes contain a significant amount of arachidonic acid and linoleic acid, precursors of lipid peroxidation products, 4-hydroxynonenal (HNE) and 2-propen-1-al (acrolein) that are extremely reactive. Acrolein decreases PDH and KGDH activities by covalently binding to LA, a component in both the PDH and KGDH complexes, most likely explaining the loss of enzyme activity. Acrolein, which is increased in AD brains, may be partially responsible for the dysfunction of mitochondria and loss of energy found in AD brain by inhibition of PDH and KGDH activities, potentially contributing to the neurodegeneration in this disorder [27]. In a further study, levels of lipid peroxidation, oxidized glutathione, non-enzymatic antioxidants and the activities of mitochondrial enzymes were measured in liver and kidney mitochondria of young and aged rats before and after LA supplementation. In both liver and kidney, a decrease in the activities of mitochondrial enzymes was observed in aged rats. LA supplemented aged rats showed an increase in the levels of lipid peroxidation and the activities of mitochondrial enzymes like isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, NADH dehydrogenase and cytochrome *C* oxidase. The authors conclude that LA reverses the age-associated decline in mitochondrial enzymes and, therefore, may lower the increased risk of oxidative damage that occurs during ageing [28].

4.6. LA — a stimulator of glucose uptake and utilization ("insulinomimetic")

Increased prevalence of insulin abnormalities and insulin resistance in AD may contribute to the disease pathophysiology and clinical symptoms. Insulin and insulin receptors are densely but selectively expressed in the brain, including the medial temporal regions that support the formation of memory. It has recently been demonstrated that insulin-sensitive glucose transporters are localised to the same regions, and that insulin plays a role in memory functions. Collectively, these findings suggest that insulin contributes to normal cognitive functioning and that insulin abnormalities may exacerbate cognitive impairments, such as those associated with AD [29]. This view is further supported by the finding that higher fasting plasma insulin levels and reduced CSF-to-plasma insulin ratios, suggestive of insulin resistance, have also been observed in patients with AD. When AD patients were treated with insulin in a glucose clamp approach (plasma level raised to 85 μU/mL), a marked enhancement in memory was observed, whereas normal adults' memory was unchanged [30]. As described before, AD is associated also with a markedly impaired cerebral glucose metabolism in affected regions. Impaired glucose uptake (partially mediated by insulin resistance) in vulnerable neuronal populations compromises production of acetylcholine but also renders neurons vulnerable to excitotoxicity and apoptosis. There is plenty of evidence that LA can ameliorate insulin resistance and impaired glucose metabolism in the periphery in type II diabetes mellitus. One study examined the beneficial effects of LA on glucose uptake using soleus muscles derived from non-obese, insulin-resistant type II diabetic Goto–Kakizaki (GK) rats, a well-known genetic rat model for human type II diabetes. In this model, chronic administration of the LA partly ameliorated the diabetes-related deficit in glucose metabolism, protein oxidation as well as the activation by insulin of the various steps of the insulin signalling pathway, including the enzymes Akt/PKB and PI-3 kinase [31]. In a further study, the incorporation of ^{14}C-2-deoxyglucose (2DG) into areas of basal ganglia was investigated in rats treated acutely or for 5 days with RLA or SLA. Following acute administration, RLA was more effective than SLA in increasing ^{14}C-2DG incorporation. For example, in substantia nigra, acute administration of RLA caused an approximately 40% increase in ^{14}C-2DG incorporation while SLA was without effect. However, the effects observed were dependent on basal ^{14}C-DG incorporation in different rat strains. Following sub-acute administration, the pattern of change in ^{14}C-2DG incorporation was altered and now both isomers were equally effective. The effects of RLA were largely maintained with increasing animal age but the ability of the *S*-isomer to alter ^{14}C-2DG incorporation was lost by 30 months. The authors conclude that RLA has the ability to increase glucose utilization *in vivo*, which may be relevant to the treatment of neurodegenerative disorders [32].

Based on this and similar studies it is quite conceivable that LA might increase glucose uptake in insulin-resistant neurons and thus provide more glycolytic metabolites including acetyl-CoA for these neurons. Since acetylcholine synthesis depends on the availability of acetyl-CoA, provided from glucose breakdown, and insulin, which controls the activity of acetylcholine transferase, LA might additionally be able to directly increase the concentration of the substrate acetyl-CoA for acetylcholine synthesis [33].

4.7. Protection of cultured neurons against toxicity of amyloid, iron and other neurotoxins by lipoic acid

Neurotoxicity of Aβ the major component of the senile plaques, contributes to neuronal degeneration in AD by stimulating formation of free radicals. Zhang et al. have investigated the potential efficacy of LA against cytotoxicity induced by Aβ (30 μM) and hydrogen peroxide (100 μM) in primary neurons of rat cerebral cortex and found that treatment with LA protected cortical neurons against cytotoxicity induced by both toxins [34]. In a similar study, Lovell et al. investigated the effects of LA and DHLA in neurons (hippocampal cultures) treated with Aβ (25–35) and iron/hydrogen peroxide (Fe/H_2O_2) [35].

In a further study, Müller and Krieglstein have tested whether pre-treatment with LA can protect cultured neurons against injury caused by cyanide, glutamate, or iron ions. Neuroprotective effects were only significant when the pre-treatment with LA occurred for >24 h. The authors conclude that neuroprotection occurs only after prolonged pre-treatment with LA and is probably due to the radical scavenger properties of endogenously formed DHLA [36].

In summary, data from these studies suggest that pre-treatment of neurons with LA before exposure to Aβ or Fe/H_2O_2 (or application of DHLA) significantly reduces oxidative stress and increases cell survival. Concomitant application of Aβ or Fe/H_2O_2 with LA can temporarily increase oxidative stress, because the reduction of LA by the pyruvate dehydrogenase complex consumes reducing equivalents and inhibits energy production.

4.8. Protective effects of LA against age-related cognitive deficits in aging rodents

Protective effects of LA against cognitive deficits have been shown in several studies in aged rats and mice. In one study, a diet supplemented with RLA was fed to aged rats to determine its efficacy in reversing the decline in metabolism seen with age. Young (3–5 months) and aged (24–26 months) rats were fed for 2 weeks. Ambulatory activity, a measure of general metabolic activity, was almost three-fold lower in untreated old rats vs. controls, but this decline was reversed in old rats fed RLA [37]. In a combination treatment study, the effects on cognitive function, brain mitochondrial structure, and biomarkers of oxidative damage were studied after feeding old rats a combination of acetyl-L-carnitine (ALCAR) and/or RLA. Spatial memory was assessed by using the Morris water maze; temporal memory was tested by using the peak procedure (a time-discrimination procedure). Dietary supplementation with ALCAR and/or RLA improved memory, the combination being the most effective for two different tests of spatial memory and for temporal memory. The authors suggest that feeding ALCAR and RLA to old rats improves performance on memory tasks by lowering oxidative damage and improving mitochondrial function. Feeding the substrate ALCAR with RLA restores the velocity of the reaction (K(m)) for ALCAR transferase and mitochondrial function. The principle appears to be that, with age, increased oxidative damage to protein causes a deformation of structure of key enzymes with a consequent lessening of affinity (K(m)) for the enzyme substrate [38].

Similar experiments were performed in the senescence accelerated prone mouse strain 8 (SAMP8) which exhibits age-related deterioration in memory and learning along with increased oxidative markers, and provides a good model for disorders with age-related cognitive impairment. In one study, the ability of LA (and also NAC) to reverse the cognitive deficits found in the SAMP8 mouse, was investigated. Chronic administration of LA improved cognition of 12-month-old SAMP8 mice in the T-maze footshock avoidance paradigm and the lever press appetitive task. Furthermore, treatment of 12-month-old SAMP8 mice with LA reversed all three indexes of oxidative stress. These results provide further support for a therapeutic role for LA in age- and oxidative stress mediated cognitive impairment including that of AD [39].

4.9. Clinical trials with LA in Alzheimer's disease patients

Although LA has been used for the treatment of diabetic polyneuropathy in Germany for more than 30 years, no epidemiological study has taken advantage of this large patient population and

investigated whether the incidence of AD in the LA-treated patients is lower than in the untreated diabetic and/or untreated non-diabetic population. Therefore, the first indication for a beneficial effect of LA in AD and related dementias came from a rather serendipitous case study. In 1997, a 74-year old patient presented herself at the Department of Medical Rehabilitation and Geriatrics at the Henriettenstiftung Hannover with signs of cognitive impairment. Diabetes mellitus and a mild form of polyneuropathy were her main concomitant diseases. With clinical criteria of DSM-III-R, deficits in the neuropsychological tests, a MRI without signs of ischemia and a typical SPECT showing a decreased bi-temporal and bi-parietal perfusion, early stage AD was diagnosed. A treatment with acetylcholinesterase inhibitors was initiated. The patient received 600 mg LA each day for treatment of her diabetic polyneuropathy. Since 1997, several re-tests have been performed, which showed no substantial decline of the cognitive functions. Therefore, the diagnosis of mild AD was re-evaluated several times, but the diagnostic features did not change and the neuropsychological tests showed an unusually slow progress of her cognitive impairment. This observation inspired an open pilot trial at the Henriettenstiftung Hospital in Hannover. 600 mg LA was given once daily (in the morning 30 min before breakfast) to nine patients with probable AD (age: 67±9 years, MMSE score at first visit/start of AChI therapy: 23±2 point) receiving a standard treatment with acetylcholinesterase inhibitors over an observation period of 337±80 days. The cognitive performance of the patients before and after addition of LA to their standard medication was compared. A steady decrease in cognitive performance (a 2 point/year decrease in scores in the mini-mental state examination (MMSE) and a 4 point/year increase in the AD assessment scale, cognitive subscale (ADAScog) was observed before initiation of the LA regimen. Treatment with LA led to a stabilization of cognitive function, demonstrated by constant scores in two neuropsychological tests for nearly a year [40]. This study was continued finally including to 43 patients which were followed for an observation, period of up to 48 months. In patients with mild dementia (ADAScog<15), the disease progressed extremely slowly (ADAScog: +1.2 points=year, MMSE: −0.6 points/year), in patients with moderate dementia at approximately twice the rate [41]. However, this study was small and not randomized. In addition, patients were diagnosed with “probable” AD, and the diagnosis was (in nearly all cases) not confirmed by a neuropathological post-mortem analysis. Therefore, a double blind, placebo-controlled phase II trial is urgently needed before LA can be recommended as a therapy for AD and related dementias.

4.10. Pharmacokinetics of LA

The correlation of pharmacokinetic (PK) [rate and extent of absorption, distribution, metabolism, and rates and extent of elimination] parameters with therapeutic efficacy provides important basic data for the rational design of preclinical and clinical studies. The PK of a compound may pose a limitation to its clinical use if pharmacological concentrations cannot be achieved or maintained long enough to achieve a therapeutic response.

To date, it has not been possible to positively correlate the PK and pharmacodynamics (PD) of LA [42] indicating that the therapeutic effects may be more dependent on C_{max} and the AUC than time to maximum concentration (T_{max}), elimination half-life (T 1/2) or the mean residence time (MRT) in plasma. This contention is supported by the recent PK study with multiple sclerosis patients where the therapeutic response (reductions in MMP-9 and sICAM) was positively correlated with C_{max} [43]. Several PK studies utilizing 600 mg rac-LA (P.O. and IV); the most frequent dose and form of LA used clinically to date and the dose used in the AD study (Section 4.9) have been reported. The mean values from eight human PK studies utilizing 600 mg rac-LA, indicate the concentration where therapeutic effects of LA begin is equal to C_{max} of 4–5 μg/mL (~20–25 μM) and AUC equal to 2.85 μg h/mL [42,44,45]. Recently, the PK profile of RLA administered as an aqueous solution of NaRLA was reported in a study with 12 healthy subjects [46]. The average dose was 8.25 mg/kg, generating a mean C_{max} of 16.03 μg/mL (range: 10.6–33.8 μg/mL), median T_{max}=15 min (range: 10–20 min), and mean AUC of 441.59 μg min/mL (7.36 μg h/mL).

Using the mean C_{max} and the mean AUC from 8 published PK trials, for 600 mg rac-LA and the NaRLA study, average plasma C_{max} levels for RLA are 4 times higher and the bioavailability (AUC) 3 times greater for RLA than rac-LA [46] indicating that NaRLA is the preferred dosage form for chronic administration. It should be possible to achieve plasma concentrations comparable to those utilizing 600 mg rac-LA with 150 mg RLA (as NaRLA P.O. solution) which is generally well tolerated during chronic treatment (Carlson, unpublished).

It has been suggested that the optimal therapeutic range may be higher and results more pronounced at plasma concentrations of 10–20 μg/mL (~50–100 μM) of the natural enantiomer, RLA [42]. Based on tolerability of LA, the upper limit of the human therapeutic concentration range is ~50 μg/mL (~250 μM) [47]. The use of 600 mg rac-LA for treatment of diabetic complications achieved a rational basis by demonstration of a reduction in the dose necessary to produce a similar improvement in the glucose challenge and insulin clamp tests [48]. Additionally, several clinical trials have indicated that this dose is effective for treatment of diabetic neuropathy and produced fewer side effects than 1200 or 1800 mg [49]. Intravenous load doses of rac-LA beginning at 600–1000 mg for 10 days were reduced to 500 mg to achieve the same result (improved glucose metabolic clearance rate and improved insulin sensitivity index [ISI]) as P.O. doses of 600–1200 mg daily for 4 weeks. The P.O. treatment led to similar reductions in the effective doses [42].

A preliminary evaluation was made on the bioavailability of R-DHLA in human plasma. R-DHLA, when administered as such produced insignificant increases in RLA or R-DHLA levels and is therefore not bioequivalent to RLA. This indicates that for R-DHLA to have clinical efficacy it must be administered by IV or obtained by endogenous reduction after administration of RLA. Any therapeutic potential of R-DHLA must consider its short biological half-life since it is rapidly metabolized [50].

The three forms of LA (RLA, SLA and rac-LA) produce different PK values when administered as sodium salts. Recently, NaRLA was compared to Na-rac-LA and NaSLA in humans using a simple three period crossover design. The mean values for C_{max} and AUC show that RLA values are considerably greater than either rac-LA or SLA (RLA C_{max}=15.67 μg/mL [78 μM], RLA AUC=6.86 μg h/mL; SLA C_{max}=6.43 μg/mL, SLA AUC=3.89 μg h/mL; rac-LA C_{max}=6.37 μg/mL; rac-LA AUC=2.69 μg h/mL). The time to maximum concentration (T_{max}) reveals SLA>RLA>rac-LA (18.33, 13.33, 10.00 min) and the elimination half-life (T 1/2) SLA>rac-LA>RLA (28.87, 20.7, 12.5 min). The diminished peak plasma concentration of rac-LA dosages compared with RLA and SLA suggests that the presence of SLA, in the racemate, may limit the overall bioavailability of RLA as well as a stereoselective transporter favoring RLA (Carlson, unpublished data).

Future clinical trials should attempt to correlate specific markers known to be up- or down-regulated in AD with the known PK profiles of LA.

4.11. Combination treatment of LA with nutraceuticals

Since AD is a multifactorial disease, it has been suggested that rather a combination than a single drug treatment might be most beneficial for AD patients. Among many suggested add-on treatments to LA, nutraceuticals with antioxidant and anti-inflammatory properties might quite be promising candidates [51]. Nutraceuticals may be broadly defined as any food substance that is considered to offer health or medical benefits [52,53]. Given that plant foods are derived from biological systems, they contain many compounds in addition to

traditional nutrients that can elicit biologic responses, and are also termed phytonutrients. One of the largest groups of phytonutrients that may confer beneficial health effects are the polyphenols [54]. Over the past decade, polyphenols, which are abundant in fruits and vegetables, have gained recognition for their antioxidant properties and their roles in protecting against chronic diseases such as cancer and cardiovascular diseases [55,56]. Consequently, diet is now considered to be an important environmental factor in the development of late-onset AD [57]. Polyphenols are therefore beginning to attract increasing interest with numerous epidemiological studies suggesting a positive association between the consumption of polyphenol-rich foods and the prevention of diseases. A recent epidemiological study reported that consumption of fruit and vegetable juices (high in polyphenols) greater than 3 times a week resulted in a 76% reduction in the risk of developing probable Alzheimer's disease over a nine-year period [58]. Another epidemiological study of 1010 subjects aged 60–93 reported that individuals who consumed curry (containing curcumin) "often" and "very often" had significantly better cognitive test scores as measured via the mini-mental state exam [59]. As antioxidants, polyphenols may protect cells against oxidative damage, thereby limiting the risk of AD associated to oxidative stress. Converging epidemiological data also suggests that a low dietary intake of omega-3 ($n-3$) essential fatty acids is a candidate risk factor for AD [60,61]. Docosahexaenoic acid (DHA) is one of the major $n-3$ fatty acids in the brain where it is enriched in neurons and synapses. DHA is associated with learning-memory and is also required for the structure and function of brain cell membranes. In the AD brain DHA is known to be decreased [62,63], while people who ingest higher levels of DHA are less likely to develop AD [61,64]. As many western diets have been reported to be deficient in DHA and also low in polyphenolic content, supplementation with DHA and polyphenols may offer potential preventative treatments for AD. Several of these nutraceuticals/phytonutrients (e.g., (−)-epigallocatechin gallate (EGCG) from green tea, curcumin from the curry spice turmeric and the omega-3 DHA from fish oils) have shown promising results, when used as single therapies in animal studies.

EGCG has previously been shown to prevent neuronal cell death caused by Aβ neurotoxicity in cell cultures [65,66]. A study by Rezai-Zadeh et al. [67] reported that EGCG reduced Aβ generation *in vitro* in neuronal-like cells and primary neuronal cultures from Tg2576 mice, along with promotion of the non-amyloidogenic α-secretase proteolytic pathway. To validate these findings they treated 12-month-old Tg2576 mice with 20 mg/kg EGCG via intra-peritoneal injections for 60 days, and showed decreased Aβ levels and plaque load in the brain, along with promotion of the α-secretase pathway [67]. Recent studies have since shown that EGCG promotes the processing of APP to the non-amyloidgenic α-secretase pathway, via PKC-dependant activation [66,68]. These previous data raise the possibility that dietary supplementation with EGCG may provide a potential preventative treatment for AD, by decreasing Aβ levels and plaque load via promotion of the non-amyloidogenic α-secretase pathway.

Curcumin has been reported to be several times more potent than Vitamin E as a free radical scavenger [69], and there is also increasing evidence showing that curcumin can inhibit Aβ aggregation [70]. In a study by Lim et al., curcumin was tested for its ability to inhibit the combined inflammatory and oxidative damage in Tg2576 transgenic mice. In this study Tg2576 mice aged 10 months old were fed a curcumin diet (160 ppm) for 6 months. Their results showed that the curcumin diet significantly lowered the levels of oxidised proteins, interleukin (IL)-1β, the astrocyte marker, glial fibrillary acidic protein (GFAP), soluble and insoluble Aβ, and also plaque burden. They found that the reduction in GFAP was localised such, that increased activity was shown in areas around plaques, showing a stimulatory effect of curcumin on the phagocytosis of plaques by microglia [71]. Following on from this work, Yang et al. [70] evaluated the effect of feeding a curcumin diet (500 ppm) in 17-month-old Tg2576 mice for 6 months. When fed to the aged Tg2576 mice with advanced amyloid accumulation, curcumin resulted in reduced soluble amyloid levels and plaque burden. These data raise the possibility that dietary supplementation with curcumin may provide a potential preventative treatment for AD, by decreasing Aβ levels and plaque load via inhibition of Aβ oligomer formation and fibrilisation, along with decreasing oxidative stress and inflammation.

The interest in dietary DHA supplementation has arisen from the view of helping to protect from neuronal degeneration and therefore prevent neurological diseases such as AD. Converging epidemiological data suggests that a low dietary intake of $n-3$ polyunsaturated fatty acids (PUFA) is a candidate risk factor for AD [72]. In the AD brain DHA is known to be decreased [62,63], while people who ingest higher levels of DHA are less likely to develop AD [61,73,74]. A recent study by Florent et al. [75] demonstrated that DHA provided cortical neurones *in vitro* a higher level of resistance to the cytotoxic effects induced by soluble Aβ oligomers. Lukiw et al. [76] also demonstrated that DHA decreased $A\beta_{40}$ and $A\beta_{42}$ secretion from aging human neuronal cells. A study by Calon et al. [77] showed that a reduction of dietary $n-3$ PUFA in Tg2576 transgenic mice resulted in a loss of post-synaptic proteins and behavioural deficits, while a DHA-enriched diet prevented these effects. Other studies have shown that DHA protects neurones from Aβ accumulation and toxicity and ameliorates cognitive impairment in rodent models of AD [78,79]. A recent study by Cole and Frautschy [80], showed that DHA supplementation in Tg2576 transgenic mice aged 17 months markedly reduced Aβ accumulation, oxidative damage and also improved cognitive function. Thus, dietary supplementation with DHA may also provide a potential preventative treatment for AD via prevention of cognitive deficits and decreased Aβ accumulation and oxidative stress.

These nutraceuticals have all been demonstrated to have varying mechanisms of action, relating to decreasing cognitive deficits, oxidative stress, inflammation and Aβ levels. Therefore, combination therapies of these nutraceuticals containing, polyphenols (EGCG and curcumin), $n-3$ essential fatty acids (DHA) and lipoic acid have the potential to provide nutritional supplement therapies for the prevention of AD-pathology and cognitive impairments, as LA has been previously demonstrated in Tg2576 mice to prevent cognitive deficits [19].

The use of LA in addition to other nutraceuticals would provide the aim of preventing cognitive deficits in combination with the benefits of curcumin, EGCG and DHA to decrease oxidative stress, inflammation, Aβ levels and Aβ plaque load.

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