The Effects and Mechanisms of Mitochondrial Nutrient α-Lipoic Acid on Improving Age-Associated Mitochondrial and Cognitive Dysfunction: An Overview

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OVERVIEW

The Effects and Mechanisms of Mitochondrial Nutrient α-Lipoic Acid on Improving Age-Associated Mitochondrial and Cognitive Dysfunction: An Overview

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Abstract We have identified a group of nutrients that can directly or indirectly protect mitochondria from oxidative damage and improve mitochondrial function and named them "mitochondrial nutrients". The direct protection includes preventing the generation of oxidants, scavenging free radicals or inhibiting oxidant reactivity, and elevating cofactors of defective mitochondrial enzymes with increased Michaelis–Menten constant to stimulate enzyme activity, and also protect enzymes from further oxidation, and the indirect protection includes repairing oxidative damage by enhancing antioxidant defense systems either through activation of phase 2 enzymes or through increase in mitochondrial biogenesis. In this review, we take α-lipoic acid (LA) as an example of mitochondrial nutrients by summarizing the protective effects and possible mechanisms of LA and its derivatives on age-associated cognitive and mitochondrial dysfunction of the brain. LA and its derivatives improve the age-associated decline of memory, improve mitochondrial structure and function, inhibit the age-associated increase of oxidative damage, elevate the levels of antioxidants, and restore the activity of key enzymes. In addition, co-administration of LA with other mitochondrial nutrients, such as acetyl-L-carnitine and coenzyme Q10, appears more effective in improving cognitive dysfunction and reducing oxidative mitochondrial dysfunction. Therefore, administrating mitochondrial nutrients, such as LA and its derivatives in combination with other mitochondrial nutrients to aged people and patients suffering from neurodegenerative diseases, may be an effective strategy for improving mitochondrial and cognitive dysfunction.

Keywords Alpha-lipoic acid · Cognitive function · Mitochondrial dysfunction · Oxidative damage

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Introduction

Cognitive function declines with age. Increasing evidence shows that mitochondrial dysfunction due to the oxidation of lipids, proteins, nucleic acids plays an important role in brain aging and age-related neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis, and Huntington's disease, are age-associated. Mitochondrial decay may be a principal underlying event in aging, including brain aging [1–7] and is also associated with in the onset and development of neurodegenerative diseases [8–14].

We have identify a group of mitochondrial targeting nutrients and named them as "mitochondrial nutrients", which can (1) Preventing the generation of oxidants; (2) Scavenging oxidants or Inhibiting oxidant reactivity; (3) Repairing oxidative damage to lipids, proteins/enzymes, and RNA/DNA by enhancing antioxidant defense systems, and (4) Cofactor function: Elevating cofactors of defective enzymes (increased Km) in mitochondria to stimulate enzyme activity, and also protect enzymes from further oxidation. Mitochondrial dysfunction could possibly be reversed in aged animals by feeding them the mitochondrial nutrients. Thus, neurodegenerative diseases, such as AD and PD may be delayed or ameliorated by treatment with mitochondrial nutrients which could delay or repair mitochondrial damage, thus improve mitochondrial function.

Clinical trials to determine whether micronutrients will delay aging and improve memory in the elderly or will prevent or treat AD and PD have been promising. A few reviews have summarized the effects of different nutrients and antioxidants on aging and neurological diseases including PD and AD [15–22]. In the present review, we survey the recently published literature to alpha-lipoic acid (LA) and its derivatives on age-associated cognitive and mitochondrial dysfunction and on oxidative damage in the nervous system.

LA is a mitochondrial nutrient

Alpha-lipoic acid is a coenzyme involved in mitochondrial metabolism. The reduced form of LA, dihydrolipoic acid, is a powerful mitochondrial antioxidant [23–26]. It recycles other cellular antioxidants, including coenzyme Q (CoQ), vitamins C and E, glutathione (GSH), and chelates iron and copper [23–26]. LA readily crosses the blood-brain barrier and is accepted by human cells as a substrate where it is reduced to dihydrolipoic acid by nicotinamide adenine dinucleotide (NADH)-dependent mitochondrial dihydrolipoamide dehydrogenase [23].

Alpha-lipoic acid plays a fundamental role in mitochondrial metabolism. Biologically, it exists in proteins where it is linked covalently to a lysyl residue as a lipoamide. The mitochondrial E3 enzyme, dihydrolipoyl dehydrogenase, reduces lipoate to dihydrolipoate at the expense of NADH. Lipoate is also a substrate for the NADPH-dependent enzyme GSH reductase [24, 27, 28]. In recent years, LA has gained considerable attention as an antioxidant [26, 28, 29]. The reduced form of LA, dihydrolipoic acid, reacts with oxidants such as superoxide radicals, hydroxyl radicals, hypochlorous acid, peroxyl radicals, and singlet oxygen. It also protects membranes by reducing oxidized vitamin C and GSH, which may in turn recycle vitamin E. Administration of LA is beneficial to a number of oxidative stress models such as diabetes, cataract, HIV activation, neurodegeneration, and radiation injury in animals. Furthermore, LA functions as a redox regulator of proteins such as myoglobin, prolactin, thioredoxin, and NF-κB transcription factor [26–28]. LA has neuroprotective effects in neuronal cells. One possible mechanism for the antioxidant effect of LA is its metal chelating activity [30]. LA can increase ambulatory activity, and partially restore age-associated mitochondrial decay in liver and heart [31–33].

Based on our definition for mitochondrial nutrients, we consider LA satisfies all of the criteria. Therefore, LA is a mitochondrial nutrient and it is also one of the mostly studied mitochondrial nutrients on mitochondrial function in cellular and animal models related to brain aging and neurodegeneration.

Aging is associated with mitochondria dysfunction

Mitochondria provide energy for basic metabolic processes, produce oxidants as inevitable by products, and decay with age impairing cellular metabolism and leading to cellular decline. Mitochondrial membrane potential, respiratory control ratios, and cellular oxygen consumption decline with age, and oxidant production increases [4, 34, 35]. Oxidative damage to DNA, RNA, proteins, and lipid membranes in mitochondria may be involved. Mutations in mitochondrial genes compromises mitochondria by altering components of the electron transport chain, resulting in inefficient electron transport and increased superoxide production [4, 36]. The resulting oxidative damage to mitochondria compromises their ability to meet cellular energy demands. Mitochondrial enzymes are especially susceptible to inactivation by superoxide and hydroxyl radicals, as these oxidants are generated in mitochondria [37]. Oxidized proteins accumulate with age [38] which cause mitochondrial inefficiencies leading to more oxidant formation. Mitochondrial membrane fluidity also declines with age [39, 40], which may lead to deformation of membrane proteins and cause mitochondrial dysfunction. The significant age-related loss of cardiolipin, a phospholipid that occurs primarily in the mitochondrial inner membrane, may be in part because of greater oxidative damage or reduced biosynthesis. Loss of cardiolipin, coupled with oxidation of critical thiol groups in key proteins, adversely affects transport of substrates and cytochrome *c* oxidase activity [41] necessary for mitochondrial function. These changes could directly impact the ability of mitochondria to maintain their membrane potential.

LA improves age-associated cognitive dysfunction and neurodegenerative diseases

Alpha-lipoic acid shows improvement on cognitive function in normal old mice. LA improved longer-term memory of aged female NMRI mice in the habituation in the open field test and also alleviated age-related *N*-methyl-D-aspartic acid (NMDA) receptor deficits (B_{max}) without changing muscarinic, benzodiazepine, and alpha 2-adrenergic receptor deficits [42].

Alpha-lipoic acid shows improvement on cognitive function in senescence accelerated mice. The senescence accelerated prone mouse strain 8 (SAMP8), at the age of 12-months-old, exhibits age-related deterioration in memory and learning, has increased levels of beta-amyloid and oxidative damage to proteins and lipids. Chronic administration of LA improved cognition of 12-month-old SAMP8 mice in both the T-maze footshock avoidance paradigm and the lever press appetitive task without inducing non-specific effects on motor activity, motivation

to avoid shock, or body weight, and reduced oxidative damage to proteins and lipids [43]. The LA treatment also significantly increased the expressions of three brain proteins (neurofilament triplet L protein, alpha-enolase, and ubiquitous mitochondrial creatine kinase) and significantly decreased specific carbonyl levels of three brain proteins (lactate dehydrogenase B, dihydropyrimidinase-like protein 2, and alpha-enolase) in the aged SAMP8 mice [44, 45].

Alpha-lipoic acid shows improvement on cognitive function in chemical-induced aging accelerated mice. Chronic systemic exposure of D-galactose to mice induced a spatial memory deficit, an increase in cell karyopyknosis, apoptosis and caspase-3 protein levels in hippocampal neurons, a decrease in the number of new neurons in the subgranular zone in the dentate gyrus, a reduction of migration of neural progenitor cells, and an increase in death of newly formed neurons in granular cell layer [46]. The D-galactose exposure also induced an increase in peripheral oxidative stress, including an increase in malondialdehyde, a decrease in total anti-oxidative capabilities, total superoxide dismutase (SOD), and GSH peroxidase activities [46]. A concomitant treatment with LA ameliorated cognitive dysfunction and neurodegeneration in the hippocampus, and also reduced peripheral oxidative damage by decreasing malondialdehyde and increasing total anti-oxidative capabilities and total SOD, without an effect on GSH peroxidase [46].

Alpha-lipoic acid shows improvement on hippocampal-dependent memory deficits of Tg2576 mice, a transgenic model of cerebral amyloidosis associated with AD. LA-treated Tg2576 mice exhibited significantly improved learning and memory retention in the Morris water maze task and significantly more context freezing compared to untreated Tg2576 mice [47].

Alpha-lipoic acid shows improvement on cognitive function in X-irradiation-induced memory impairment in mice. Whole body X-irradiation of mice substantially impaired the reference memory and motor activities of mice and treatment with LA prior to irradiation significantly attenuated such cognitive dysfunction [48]. LA pretreatment also exerted a significant protection against radiation-induced increase in oxidative damage to proteins and lipids in mice cerebellum [48]. LA pre-treatment also inhibited radiation-induced deficit of total, nonprotein and protein-bound sulfhydryl contents of cerebellum and plasma ferric reducing power. In addition, LA treated mice showed an intact cytoarchitecture of cerebellum, higher counts of intact Purkinje cells and granular cells in comparison to untreated irradiated mice [48].

Alpha-lipoic acid shows effect on improving diabetic peripheral and cardiac autonomic neuropathy [49, 50]. LA prevents cognitive impairment and oxidative stress induced by intracerebroventricular streptozotocin in rats. Intracerebroventricular streptozotocin induced cognitive impairment in rats, which is characterized by a progressive deterioration of memory, cerebral glucose and energy metabolism, and oxidative stress while LA treated rats showed significantly less cognitive impairment as compared to the vehicle treated rats [51].

Alpha-lipoic acid prevents the development of multiple sclerosis. LA dose-dependently prevented the development of clinical signs in a rat model for multiple sclerosis, acute experimental allergic encephalomyelitis. LA has a protective effect on encephalomyelitis development not only by affecting the migratory capacity of monocytes, but also by stabilizing the blood-brain barrier [52].

Alpha-lipoic acid has also been studied as a treatment for Alzheimer-type dementia. A dose of 600 mg LA, given daily to nine patients with AD and related dementias for about 1 year, led to a stabilization of cognitive functions in the study group, as shown by constant scores in two neuropsychological tests [53]. Though the study was small and not randomized, it suggests that treatment with LA is a possible neuroprotective therapy option for AD and related dementias.

Possible mechanisms of LA on cognition: improving mitochondrial function and reducing oxidative damage

Various mechanisms of the LA effects on improving cognitive function have been suggested, including the improvement of memory-related signaling pathways, reducing oxidative stress, improving mitochondrial function etc.

Alpha-lipoic acid may restore the activity of acetylcholinesterase and Na^+, K^+-ATPase. The activity of acetylcholinesterase was found to be significantly decreased in the cerebral cortex, cerebellum, striatum, hippocampus, and hypothalamus in aged rats while administration of LA reversed the decrease in the activity in the discrete brain regions [54]. In aged rats, the level of lipofuscin was increased, and the activity of Na^+, K^+-ATPase was decreased. Administration of LA to aged rats led to a duration-dependent reduction in lipofuscin and elevation of enzyme activity, respectively, in the cortex, cerebellum, striatum, hippocampus, and hypothalamus of the brain [55].

Treatment with LA protected cortical neurons against cytotoxicity induced by beta-amyloid or hydrogen peroxide and induced an increase in the level of Akt, an effector immediately downstream of phosphatidylinositol kinase, suggesting that the neuroprotective effects of the LA are partly mediated through activation of the protein kinase B (PKB)/Akt signaling pathway [56].

The glutamate receptors mediate excitatory neurotransmission in the brain and are important in memory acquisition,

learning, and implicated in some neurodegenerative disorders [9, 57, 58]. This receptor family is classified in three groups: NMDA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate-kainate, and metabotropic receptors. Excessive activation of the NMDA receptor leads to a large influx of calcium into neurons and subsequent generation of oxidants and oxidative stress by the stimulation of phospholipase A_2 [10, 59, 60]. Increased intracellular calcium may cause mitochondrial dysfunction, which can result in localized oxidant formation within mitochondria and an inability to handle free calcium [9, 61]. Intact mitochondrial function appears to be essential for neuronal resistance to excitotoxic insults. It is believed that the reduced levels of ATP that accompany with abnormal mitochondrial function are insufficient to drive the ion pumps that maintain neuronal membrane polarization. With depolarization of the neuronal membrane, the magnesium that normally blocks the NMDA receptor ion channel is extruded, and ambient extracellular levels of glutamate may become lethal via NMDA receptor mechanism. Based on this mechanism, it seems likely that LA may play its memory improving effect by enhancing mitochondrial function, scavenging free radicals to decrease oxidative damage, or increasing the levels of the antioxidants GSH and ascorbate to enhance the antioxidant defense. We have examined the effects of LA on neurotoxin- or oxidant-induced toxicity in HT4 cells and HT22 cells. The HT4 cell line was constructed by McKay et al. in 1989, and derived from mouse neuronal tissue. Morimoto and Koshland have shown that HT4 cells possess NMDA receptors [62]. The HT22 cell line is a subclone of HT4. HT22 (the immortalized mouse cell line) lacks ionotropic glutamate receptors and responds to oxidative glutamate toxicity with a form of programmed cell death that is distinct from classical apoptosis.

We have found that dose-dependent cell injury in HT4 and HT22 cells is caused by glutamate (an excitotoxin), thapsigargin (an apoptosis inducing agent), hydrogen peroxide (a typical oxidant), homocysteic acid (a cysteine uptake inhibitor), diethyl maleate (a proxidant which depletes intracellular GSH), apomorphine (a memory impairing agent), SIN-1 (a generator of peroxynitrate), and 6-hydroxydopamine (an oxidant generator in brain) [63]. Mitochondrial dysfunction was a key event in the excitotoxicity-independent component of neuronal cell death. Reactive oxygen species accumulation and GSH depletion were prominent in glutamate-treated cells [64]. LA showed effects on reducing most of glutamate- and oxidants-induced cell death, decreasing oxidative damage, increasing antioxidant defense, and improving mitochondrial function [63]. These results, together with previous studies [64–67] suggest that LA is an effective neuroprotective agent for ameliorating excitotoxins and oxidants-induced and age-associated neurodegeneration.

Iron may play a role in cognitive dysfunction and LA may act as iron chelators to prevent oxidant generation. It was found that the cerebral iron levels in 24 to 28-month-old rats were increased by 80% relative to 3-month-old rats and the iron accumulation correlated with a decline in GSH and the GSH/GSSG ratio [68]. LA treated old rats showed a decrease in the cerebral iron and an improvement of the antioxidant status and thiol redox state, compared to the untreated old rats [68], confirming that LA is a potent chelator of divalent metal ions [30] in the brain.

Beside it direct antioxidant activities, such as free radical scavenging and iron-chelating, another mechanism of the protective effect of LA, like the thiol-reactive compound sulforaphane [69] and electrophillic neurite outgrowth-promoting prostaglandin compounds [70], is mediated through induction of the phase 2 enzyme response through the transcription factor Nrf2, which binds to antioxidant response element to activate the transcription of phase 2 enzymes, consequently, enhances the antioxidant defense system (Fig. 1). Phase 2 enzymes (e.g., hemeoxygenase 1, NAD(P)H:quinone oxidoreductase 1, and gamma-glutamate cysteine ligase) are part of the elaborate system for protection against the toxicity of reactive oxygen and nitrogen species that are constant dangers to the integrity of mammalian DNA [69]. Induction of phase 2 enzymes, which neutralize reactive oxygen species, appears to be an effective means for achieving protection against a variety of carcinogens and other oxidative damage in animals and humans [71]. LA was shown to induce Nrf2 and higher glutamylcysteine ligase activity

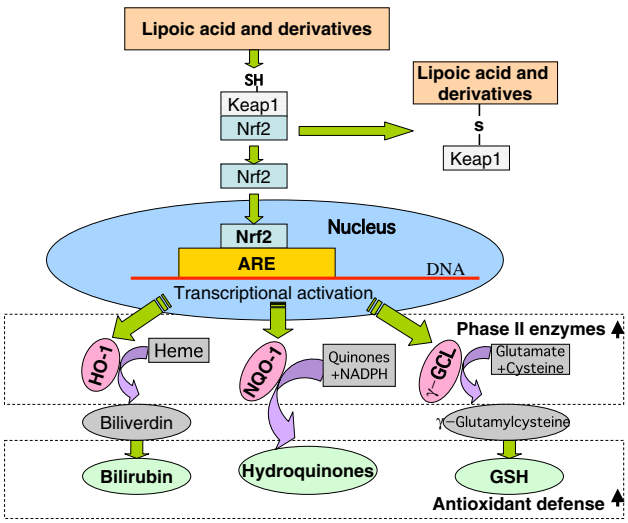

Fig. 1 Possible mechanism of neuroprotective effects afforded by α-lipoic acid and its derivatives through the Keap1/Nrf2 transcriptional activation of phase2 enzymes and subsequent antioxidant defense system. *HO-1* hemeoxygenase-1, *NQO-1*, NAD(P)H quinine oxidoreductase-1, and *γ –GCL* γ-glutamyl cysteine ligase. (adapted from Ref. [70])

[72]. This mechanism appears an indirect mitochondrial protection because the induced phase 2 enzymes reduce cytosolic oxidative stress and enhance the cellular antioxidant defense, thus indirectly relieves oxidative stress to mitochondria.

Mitochondrial function is related to mitochondrial content, which can be affected by exercise and environmental factors [73]. The key regulator of mitochondrial biogenesis include peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1alpha), AMPK-activated protein kinase (AMPK), calcium/calmodulin-dependent protein kinase IV, and nitric oxide [73–77]. Stimulation of PGC-1alpha could suppress neurodegeneration [78] while repression of PGC-1alpha could lead to mitochondrial dysfunction and neurodegeneration [79]. Recently, we have found that LA or its combination with ALC could stimulate PGC-1alpha and other key regulators, leading to an increase in mitochondrial biogenesis and an improvement in mitochondrial function in cellular systems and also in animals (unpublished). Therefore, stimulation of neuronal mitochondrial biogenesis is possibly one of the important mechanisms for LA to improve mitochondrial function, thus, ameliorate cognitive dysfunction in aging and age-related neurodegenerative diseases (Fig. 2).

Combination of LA and other mitochondrial nutrients or compounds is more effective in improving on cognitive function

It was suggested that a combination of non-steroidal anti-inflammatory drugs and appropriate levels and types of micronutrients might be more effective than the individual agents in the prevention and in the treatment of AD [80]. Based on epidemiologic, laboratory and clinical studies, we propose that using optimal combinations of LA and other mitochondrial nutrients to target mitochondrial dysfunction may provide an effective strategy in delaying aging, preventing, and treating cognitive dysfunction, including AD and PD.

Combinations of a number of nutritional cofactors have been tested in different mitochondrial disorders for additive or synergistic effects, including riboflavin + carnitine to improve muscle weakness and exercise capacity in complex I deficient myopathy; riboflavin + nicotinamide to improve encephalopathic symptoms and nerve conduction; vitamin K_3 + ascorbate to clinically improve exercise capacity in patients with complex III defect; CoQ + vitamin K_3, ascorbate, thiamin, riboflavin, and niacin to reduce mortality in mitochondrial myopathy and encephalomyopathies [18]; and carnitine + choline and caffeine to reduce body fat and serum leptin concentrations [81].

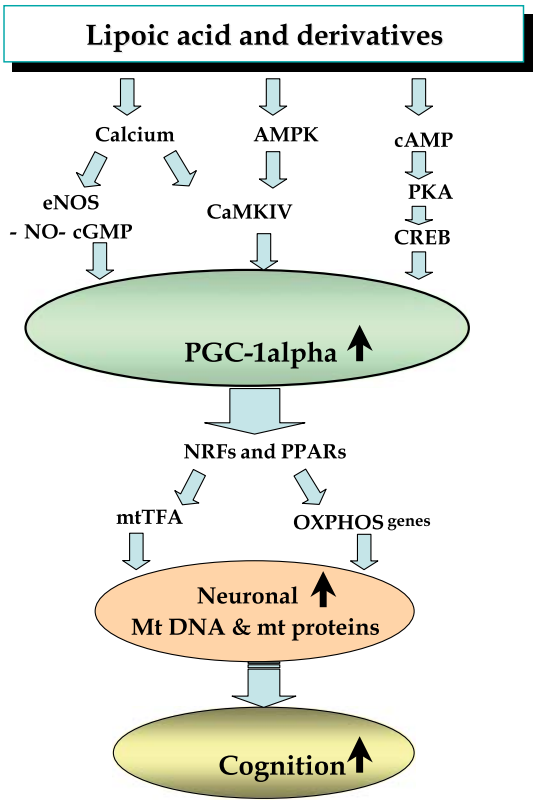

Fig. 2 Signal pathways of mitochondrial biogenesis that are possibly regulated by α-lipoic acid and its derivatives. *AMPK* AMP-activated protein kinase, *NRF* Nuclear respiratory factor, *NO* Nitric oxide, *NOS* nitric oxide synthase, *CaMKIV* Calcium/calmodulin-dependent protein kinase IV, *mtTFA* mitochondrial transcription factor A, *PKA* protein kinase A, *CREB* cAMP-responsive element binding protein, *PPARs* Peroxisome proliferator-activated receptors, *PGC-1α* Peroxisome proliferator-activated receptor-γ coactivator-1α (adapted from Ref. [73])

Alpha-lipoic acid and vitamin E have shown synergistic effects against lipid peroxidation by oxidant radicals in several pathological conditions such as a thromboembolic stroke model in rats for neurological functions, glial reactivity and neuronal remodeling [82]. We observed that LA + acetyl-L-carnitine (ALC) was more effective than when used LA and ALC individually to ameliorate the decay of mitochondria in old rats, possibly they play different roles in restoring mitochondrial function, including the complementary effect of LA on ALC by inhibiting oxidative stress [83–85].

We first examined the effects of ALC and LA as well as their combination on memory with the Morris water maze test [86], and with the Skinner box test (Fixed-Interval performance in the peak procedure) [87]. Old rats showed an age-associated decline in spatial memory in the Morris water maze test and the treatments with ALC, LA or their combination improved the age-associated spatial memory decline. ALC, LA, or their combination improved the spatial memory by (1) reducing the time to find the hidden

escape platform; (2) increasing the time at the platform position and the % time in the quadrant where the escape platform was formerly contained during the 60-s transfer (no platform) test; and (3) reducing the time to find the visible escape platform. ALC showed a greater effect than LA. The combination of ALC and LA showed a synergistic effect on reversing the decay of spatial memory in old animals [84]. The Skinner box test reflects the internal clock and memory. Old rats had a much lower response rate, and LA increased the response rate in old rats. While ALC does not show any effect (comparing the ALC group to the old controls), LA seems to slightly increase the peak rate in old rats. The combination of LA and ALC showed a larger and significant increase in response rate and also peak rate in old animals compared to LA alone, suggesting a synergistic action of LA and ALC [84].

The memory improving effect of LA + ALC was found to be accompanied by a decrease in lipid peroxidation, protein oxidation, oxidative RNA/DNA damage, and mitochondrial dysfunction in the brain of rats [83–85]. These results strongly suggest that LA + ALC improve cognitive dysfunction by improving mitochondrial dysfunction and decreasing oxidative damage in the brain. Similar results on reducing oxidative damage have been obtained by administrating LA and carnitine to rats by Muthuswamy et al. [88]. Aged rats had a significant decline in the antioxidant status and increase in lipid peroxidation, protein carbonyl, and DNA protein cross-links as compared to young rats in the cerebral cortex, striatum, and hippocampus; Co-supplementation of LA and carnitine was effective in reducing brain regional lipid peroxidation, protein carbonyl and DNA protein cross-links and in increasing the activities of enzymatic antioxidants in aged rats to near normalcy [88].

In a cellular oxidative stress model, LA + ALC is found to mediate pro-survival signaling mechanism in aldehyde-induced oxidative toxicity. 4-Hydroxy-2-nonenal (HNE) is a highly reactive product of lipid peroxidation of unsaturated lipids, and induces oxidative toxicity as a model of oxidative stress-induced neurodegeneration. Pretreatment of primary cortical neuronal cultures with ALC and LA significantly attenuated HNE-induced cytotoxicity, protein oxidation, lipid peroxidation, and apoptosis, also led to elevated cellular GSH and heat shock protein (HSP) levels, and activation of phosphoinositol-3 kinase (PI3K), PKG, and ERK1/2 pathways [89].

Alpha-lipoic acid may be more active and effective when combined with CoQ because dihydrolipoic acid (the reduced form of LA), which is soluble in the aqueous compartment, has been found to reduce CoQ to ubiquinol by the transfer of a pair of electrons, thereby increasing the antioxidant capacity in biomembranes [90]. Packer et al. [25] have suggested that LA can recycle most of the antioxidants, including CoQ, ascorbic acid, GSH, and thioredoxin. It may be useful to add LA to other antioxidants to recycle the oxidized antioxidants in vivo.

A mixture with LA and other antioxidants and mitochondrial enzyme cofactors could slow age-dependent cognitive decline in dogs. A mixture of LA, carnitine, and other antioxidants vitamin E, ascorbic acid, and also spinach flakes, tomato pomace, grape pomace, carrot granules, and citrus pulp, was fed to aged beagle dogs for 2 years. At 1 and 2 years, the mixture fed group with a behavioral enrichment showed more accurate learning than the other aged groups. Discrimination learning was significantly improved by behavioral enrichment. Reversal learning was improved by both behavioral enrichment and mixture feeding [91]. Oxidative stress biomarkers, i.e. protein carbonyls, 3-nitrotyrosine (3-NT), and the lipid peroxidation product, 4-hydroxynonenal (HNE), were decreased by the mixture feeding, the behavioral enrichment, and the combination of both when compared to control, with the most significant effects found in the combination of the mixture feeding and behavioral enrichment [92]. The combined treatment significantly reduced the specific protein carbonyl levels of glutamate dehydrogenase [NAD (P)], glyceraldehyde-3-phosphate dehydrogenase (GAPDH), alpha-enolase, neurofilament triplet L protein, glutathione-S-transferase (GST) and fascin actin bundling protein, significantly increased the expression of Cu/Zn SOD, fructose-bisphosphate aldolase C, creatine kinase, glutamate dehydrogenase, and GAPDH, and also significantly increased the enzymatic activities of GST and total SOD [92].

In one study, LA administration has been found to increase oxidative stress in rat brain. Kayali et al. [93] found that LA administration caused an increase in protein carbonyl and nitrotyrosine levels and a decrease in total thiol, non-protein thiol, and lipid hydroperoxide levels in the brain tissue of aged rats. The authors assumed prooxidative effects of LA in the brain tissue of aged rats may be due to the prooxidant effects of LA [93]. However, the results should be further confirmed with more convincing parameters and methods.

Development of more potent LA derivatives for treating neurodegenerative diseases

One active direction in LA studies is to develop LA derivatives with more potent effect or with more functions or specificities. Dr Packer and co-workers [94] have developed a positively charged water soluble LA amide analog, 2-(*N*,*N*-dimethylamine) ethylamido lipoate HCl and named it LA-+. Compared to LA, LA-+ was found to be more effective in [1] protecting cells against glutamate induced neurotoxicity; (2) preventing glutamate induced loss of intracellular GSH, and (3) disallowing increase of intracellular peroxide level following the glutamate

challenge [94]. LA-+ was also shown to provide protection against HNE-induced inactivation of pyruvate dehydrogenase in the mitochondria [95] and to inhibit NO production in RAW 264.7 macrophages [96]. Therefore, the authors considered that LA-+ was a potent protector of neuronal cells against glutamate-induced cytotoxicity and associated oxidative stress.

Alpha-lipoic acid has been connected with melatonin. The conjugate was named melatoninolipoamide [97]. It was found that the melatonin moiety of the conjugate reacts preferably with oxidizing radicals and the LA moiety exhibits preferential reaction with reducing radical. Using γ-radiation induced lipid peroxidation in liposomes and hemolysis of erythrocytes as a model, the radioprotection ability was compared with those of melatonin and LA and the results suggest that the conjugate can be explored as a probable radioprotector [97].

In order to target the Au nanoparticles (AuNP) to folate receptor positive tumor cells via receptor-mediated endocytosis, a polyethyleneglycol (PEG) construct with LA and folic acid coupled on opposite ends of the polymer chain was synthesized [98]. The folate-PEG grafted AuNPs was shown to have a selective uptake by KB cells, a folate receptor positive cell line that overexpress the folate receptor. This is helpful for developing methods that use targeted metal nanoparticles for tumor imaging and ablation [98].

For PD treatment, one big problem is the pro-oxidant effect associated with L-Dopa therapy. In order to overcome this problem, a series of multifunctional codrugs, obtained by joining L-Dopa and dopamine with (R)-LA, was synthesized and evaluated as potential drugs with antioxidant and iron-chelating properties [99]. It was found that there are potential advantages of using some of these codrugs rather than L-Dopa in treating PD due to their "in vivo" dopaminergic activity and a sustained release of the parent drug in human plasma [99].

Conjugated LA and γ -linolenic acid improves functional deficits in the peripheral and central nervous system in streptozotocin-diabetic rats. As we know, diabetes mellitus can lead to functional and structural deficits in both the peripheral and central nervous system. A 12-week-treatment with the conjugate in streptozotocin-diabetic rats showed that the conjugate treatment improved long-term potentiation in the hippocampus [100].

Conclusions and perspectives

Alpha-lipoic acid is used to improve age-associated decline of cognitive function, and also to treat or prevent peripheral neuropathy and cardiac autonomic neuropathy, insulin resistance in type II diabetes, retinopathy and cataract, glaucoma, HIV/AIDS, cancer, liver disease, Wilson's disease, cardiovascular disease, and lactic acidosis caused by inborn errors of metabolism, and also Alzheimer type dementia. Though further investigations are needed for studying molecular and cellular mechanisms and specially, more clinical trials on LA supplementations, evidence has demonstrated that LA has beneficial effects and show promising applications for delaying, preventing, and repairing mitochondrial decay, improving cognitive function, and preventing/ameliorating age-related degenerative diseases.

Future directions on LA and cognitive studies should be in the following two areas: (1) To confirm the effects on cognitive function from animals with well-designed, randomized, and controlled clinical trials in aged people and patients suffering from neurodegenerative diseases, and (2) To develop LA derivatives with more functions and specificities for treating neurodegenerative diseases.

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