Neuroprotective Epigenetic and DNA Damage Repairing Molecular Mechanisms of L-Carnitine and its Congeners Against Aging and Age-Related Neurodegenerative Diseases

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Abstract

Aging is an ubiquitous biological phenomena characterized by ever-increasing susceptibility to diseases due to increased oxidative stress (OS) and an ultimate severe non-repairable membrane molecular and mitochondrial damages coupled with energy (ATP) depletion. L-Carnitine (β-hydroxy-γ-trimethyl amino butyrate) and its congeners plays an essential role in mitochondrial ATP synthesis while being a powerful anti-inflammatory antioxidant and an organic, non-ionic bi-phasic osmolytes. L-carnitine exerts antimitogenic and genome stabilizing effects by increasing mitochondrial metabolism, anneals DNA-strand breaks and enhances genome stability by modulating histones and DNA-repairing enzymes. Poly (ADP-ribose) polymerase-1 (PARP-1) is an abundant nuclear enzyme and normally functions in DNA damage repair mechanism acts as a double-edged sword, which anneals mild repairable DNA damages, but extensive PARP-1 activation can promote cell death through processes involving energy depletion in severe OS. It has been reported that, severe oxidative stress-mediated extensive non-repairable DNA damage can over-activate PARP-1 and consumes enormous NAD+ and consequently ATP, culminating in cell dysfunction or necrosis by autocatalysis of PARP-1 and caspases. The DNA damage associated with OS known to activate DNA repair proteins, including PARP-1, an important biomarker of brain aging and age-related neurodegenerative diseases. This study delineates the neuroprotective epigenetic and DNA-repairing molecular mechanisms of the iron-chelating anti-inflammatory genome stabilizing antioxidant ergogenic aid L-Carnitine and its congeners against selected degenerative diseases such as Parkinson’s disease (PD), Alzheimer’s disease, Amyotrophic Lateral Sclerosis (ALS) and Multiple sclerosis (MS).

Keywords: Aging, Neurodegenerative diseases, Oxidative stress, DNA damage, Poly (ADP-Ribose) polymerase-1, Caspase, ATP, L-Carnitine, Iron-Chelating Anti-inflammatory Antioxidants.

Introduction

Aging is a ubiquitous biological phenomena, characterized by ever-increasing susceptibility to diseases and toxic effects of xenobiotic which leads to irreversible membrane-molecular-mitochondrial damages and ultimately death. Oxidative damage to neuronal molecules and increased accumulation of iron coupled with decreased antioxidant status in specific discrete brain areas are considered major pathological aspects of brain aging and age-related neurodegenerative diseases (NDDs) such as Parkinson’s disease (PD), Alzheimer’s disease (AD), Multiple sclerosis (MS), Amyotrophic lateral sclerosis (ALS) otherwise known as Motor Neuron Disease (MND) and Senile dementia (SD) and thus, special interest has been assigned to the therapeutic feature of nutritional antioxidants and iron chelators in neurodegenerative diseases. Considering the need to identify new alternatives to treat neurodegenerative diseases, this study of review delineates the neuroprotective epigenetic and DNA-damage-repair molecular mechanisms of L-carnitine.
(LC) and its congeners such as acetyl-L-carnitine (ALC) against aging and selected age-related neurodegenerative diseases.

**Aging, biomarkers of oxidative stress and antioxidants**

It has been documented that, malondialdehyde (MDA), nitrotyrosine, and oxo8dG/oxo8G (mainly RNA oxidation) in young and old rats support the proposition that accumulated oxidative damage to macromolecules such as lipid, protein, and nucleic acid may be a major contributor to cellular aging and the neurodegenerative diseases that accompany aging [1-11] which includes Parkinson’s disease, Alzheimer’s disease, Amyotrophic Lateral Sclerosis, Multiple sclerosis and Senile dementia.

Parkinson’s disease (PD) is the most common neurodegenerative movement disorder that is estimated to affect approximately 1% of the population older than 65 years of age [12-13]. PD is characterized by the progressive depletion of pigmented dopamine-containing neurons in the region known as the substantia nigra pars compacta and by the presence of intraneuronal aggregates called Lewy bodies (LBs), which are enriched in filamentous α-synuclein and other proteins that are often ubiquinated [14]. Approximately 80% of dopaminergic neurons in the substantia nigra are already irreversibly destroyed when the symptoms of PD becomes significantly visible.

Nevertheless, currently available pharmacological therapies are unable to arrest or to reverse the progression of this relentlessly progressive and severely debilitating condition in most of the age-related neurodegenerative diseases (NDDs). PD is currently an incurable disease, and the number of subjects afflicted with this disease is constantly increasing due to the increasing global geriatric population. Therefore, the need for newer and more effective agents is receiving a great deal of attention and, consequently, being subjected to extensive research. The vast amount of information gained regarding the etiopathogenesis of PD has fuelled numerous developments and vast range of investigated agents have demonstrated immense potential for preventing and eventually providing cure for this condition. Clinical and biochemical evidences suggest that PD involves multifactorial, oxidative neurodegeneration and that L-dopa therapy aggravates the oxidative burden. Strong evidence now exists to support an aberrant role for mitochondrial functions, as well as increased oxidative stress (OS) and an ultimate severe irreversible membrane-molecular and mitochondrial damages which culminates in energy depletion and genome instability, in the pathogenesis of PD [15].

If mitochondrial defects and oxidative damage play a role in the pathogenesis of PD, then one would suspect that agents that may improve mitochondrial function or exert iron-chelating antioxidative effects could be neuroprotective. There are several agents that are currently under investigation for their potential neuroprotective effects based on their capacity to modify mitochondrial dysfunction. These include quercetin, resveratrol, astaxanthin, creatine, coenzyme Q10, nicotinamide, lipoic acid, L-carnitine and acetyl-L-carnitine, etc.

**Carnitine synthesis and function: L-carnitine**

Carnitine (L-β-hydroxy-γ-N-trimethylaminobutyric acid) is a vitamin-like nonprotein nutraceutical primarily biosynthesized in the liver and kidney from the amino acids lysine and methionine, ascorbic acid, vitamin B₆ and niacin [16]. LC has been described as a conditionally essential nutrient for humans. Due to its chiral structure, carnitine has two stereoisomeric forms: D and L. However, only the L isomer is known to be essential for human and animal health and possess biological activity, while the other isomer is biologically inert. The main physiologic role of L-carnitine is involvement in fat and energy metabolism by mediating the transport of long-chain free fatty acids (LCFAs) across the mitochondrial membrane for β-oxidation [17] and ATP synthesis and exerts lipotropic / lipolytic effects in a variety of cardiovascular diseases including atherosclerosis and stroke [17]. Aside from this leading task, LC supplementation has been reported to be associated with several health benefits such as regulation of carbohydrate metabolism and insulin sensitivity, mitigation of lipid peroxidation (LPo) and oxidative stress (OS), synthesis of heat shock proteins (HSPs), enhancement of the immune system and cytoprotection [18-22]. The mitochondrial antioxidant/nutrient acetyl-L-carnitine (ALC), with its iron-chelating antioxidant energizing protective
activities and with its trophic effects, at optimal doses, can be an effective and safe prevention strategy for PD, offering the possibility of new and innovative therapeutic strategies for brain aging and several age-related NDDs. Acetyl-L-carnitine is a highly bioavailable bi-phasic antioxidant ergogenic aid molecule, it is thought to penetrate the brain barrier better than carnitine, and it is readily converted to carnitine as needed. It is known that ALC is rapidly metabolized in human subjects, with plasma half-lives of 4.2 hours [23], which is a non-toxic conditionally essential nutrient.

![Structure of L-Carnitine and Acetyl-L-Carnitine](image)

**L-Carnitine, Acetyl-L-Carnitine and Energy (ATP) Synthesis**

Acetyl-L-Carnitine (ALC) (β-trimethyl-γ-acetylbutyrrbetaine) is the acetyl ester of carnitine that plays a key role in the transport of fatty acids from cytosol into the mitochondrial matrix of β-oxidation [24-27]. Carnitine is a cellular component with a key role in energy metabolism control. Treatment with ALC has proved to be effective in the treatment of fatigue in a variety of chronic neurological diseases [28] including dementia and in chronic fatigue syndrome [29].

**L-Carnitine deficiency in aging**

Experimental data demonstrate an age-associated decrease of tissue levels of L-carnitine in animals, including humans, and an associated decrease in the integrity of the mitochondrial membrane [30-32]. It has been documented that nutritional deficiency associated with carnitine precursors and iron leads to systemic carnitine deficiency. Because carnitine levels and carnitine transport decline significantly with age and the beneficial effects of acetyl-L-carnitine (ALC) supplementation on mitochondrial function have been described [33-38].

**Pleotropic cytoprotective effects of L-carnitine and its congeners**

**L-Carnitine and free radical scavenging effects**

L-Carnitine and its acyl esters such as acetyl-L-carnitine, propionyl-L-carnitine act as an oxidant either having a primary antioxidant activity (inhibiting free radical generation, scavenging the initiating free radicals, and terminating the radical propagation reactions) or, more likely, functioning as a secondary antioxidant (repairing oxidized polyunsaturated fatty acids (PUFAs) esterified in membrane phospholipids [39-40]. L-carnitine protects the cell membrane and DNA against damage induced by oxygen free radicals (OFRs) and has a pivotal role in mitochondrial β-oxidation of long-chain fatty acids (LCFAs) that increase energy supply to the cell [41] in the form of ATP. Mitochondrial dysfunction may lead to incomplete detoxification of free radicals, which may lead to oxidative damage to macromolecules such as lipids, proteins and DNA. LC has free radical-scavenging activity and the ability to scavenge superoxide anion and inhibit lipid peroxidation, thereby conferring protection against damage induced by hydrogen peroxide (H₂O₂) [42].

**Antioxidant and cytoprotective effects of L-Carnitine and its congeners**

L-Carnitine has been described as a conditionally essential nutrient for humans. L-Carnitine facilitates entry of long-chain fatty acids into mitochondria for utilization as fuel and facilitates removal from
mitochondria of short-chain and medium chain fatty acids that accumulate as a result of normal and abnormal metabolism [43]. Experimental data demonstrate an age-associated decrease of tissue levels of L-carnitine in animals, including humans, and an associated decrease in the integrity of the mitochondrial membrane [44-45].

**L-Carnitine and ATP and neurodegenerative diseases**

Experimental evidences suggest that acetyl-L-carnitine boosts mitochondrial ATP production and helps to protect mitochondria against free radicals (FRs). This molecule is therefore of great interest for its wide clinical application in various neurological disorders, it has beneficial effects in preventing the loss of brain function which typically occurs during aging, and its neuroprotective benefits have been observed in the hippocampus, prefrontal cortex, substantia nigra and muscarinic receptor portions of the brain. These include iron-chelating effect, antioxidant activity, improve mitochondrial energetics, stabilization of intracellular membranes and cholinergic neurotransmission [46] and modulation of caspases nd PARP-1.

**L-Carnitine and α-Lipoic acid antioxidant effects**

Experimental studies indicate that α-lipoic acid and acetyl-L-carnitine play important and potentially synergistic roles in normal mitochondrial function, and that reduced levels of these compounds are associated with increased mitochondrial oxidant production [47-49]. L-Carnitine also has an ability to reduce production of oxygen free radicals (OFRs) [50]. The effects of α-lipoic acid and acetyl-L-carnitine on oxidative stress (OS) that contributes to the pathogenesis and cardiovascular complications of hypertension suggest that these compounds may be useful adjuncts in treatment [51] and enhances overall antioxidant status of cells.

**Genome stabilizing and anti-mutagenic effects of L-Carnitine and Acetyl-L-Carnitine**

Previous study demonstrated that, improvement in sperm chromatin quality was demonstrated by oral intake of some antioxidant agents such as L-carnitine [52]. L-carnitine and ALC, as antioxidant [53], may protect sperm plasma membrane with high level of unsaturated fatty acid content [54]. L-carnitine and LAC act as buffering system to adjust acetyl-CoA concentration [54]. ALC is considered as donor of acetyl group and can transfer its acetyl group to histone by acetyl transferase enzyme and by this way it can improve protamination [55]. It has been documented that, both L-carnitine and acetyl-L-carnitine enhances the chromatin quality and hence genome stability by annealing the DNA single strand breaks (DNA-SSBs) in human peripheral blood smears by augmenting the activity of the DNA repair enzyme poly (ADP-ribose) polymerase-1 (PARP-1) and cellular ATP levels [56]. Further L-carnitine ameliorates and repairs the DNA-strand breaks induced by the genotoxic agents such as acrylamide [57], exerts antimutagenic and genome stabilizing effects and cytoprotective anti-apoptotic effects.

**L-Carnitine and GSH**

Acetyl-L-Carnitine (ALC) is a small water-soluble peptide located in the mitochondria. It contains a carnitine moiety which is important for the oxidation of fatty acids and an acetyl moiety which is involved in maintenance of acetyl-CoA levels and can promote the production of the antioxidant glutathione [58-60] and acetylcholine.

**Anti-inflammatory and neuroprotective effects of L-Carnitine**

Acetyl L-carnitine has multiple roles in neuro-protection. ALC readily crosses the blood-brain barrier, where it stabilizes cell membranes, acts as an effective antioxidant and protects brain. CRP is a positive acute phase protein that is increased in inflammation. Inflammatory stimuli cause the release of cytokines like interleukin (IL-1), IL-6 and tumour necrosis factor-α (TNF-α), and these cytokines increase the synthesis and release of CRP [61]. CRP nd IL-6 levels may be decreased with carnitine treatment. There are a few reports supporting the hypothesis that treatment with L-carnitine has improved the chronic inflammation in haemodialysis patients in recent years [62-63]. L-carnitine was reported to down-regulate
cytokines such as IL-1, IL-6 and TNF-α and/or increase clearance of these cytokines in knee osteoarthritis [64] and a variety of inflammatory disorders.

**Iron-chelating antioxidant and chromatin stabilizing effects of L-Carnitine and its congeners**

ALC inhibits lipid peroxidation and xanthine oxidase activity in rat skeletal muscle [65]. ALC reduces lipid peroxidation and lipofuscin concentration in aged rat brain [66]. ALC also inhibits oxidant-induced DNA single-strand breaks (DNA-SSBs) [67]. ALC may possess a direct antioxidant activity as demonstrated in vivo [68-69]. Related compounds, such as L-propionyl L-carnitine and L-carnitine (LPC), have been shown to have antioxidant activity by chelating metals, and inhibiting the age-associated increase in lipid peroxidation [70-71]. An antioxidant role of L-propionyl L-carnitine has also been implicated in ischemia-reperfusion injury. L-Carnitine in rats prevented doxorubicin cardiotoxicity as monitored by echocardiography, release of myosin light chain-1, and aldehydic lipid peroxidation products [72]. ALC prevented oxygen radical-induced cell death in human diploid fibroblast cell lines, which was explained as due to increasing the activity of antioxidant enzymes and sustaining the activity of mitochondrial complex I-NADH ubiquinone reductase and complex IV-cytochrome oxidase of the electron transport chain [73]. In addition, ALC protected mitochondrial complex III ubiquinol cytochrome c reductase, perhaps as an iron chelator [74].

**L-Carnitine and antiapoptotic effects**

It has been documented that, L-carnitine and its congeners exerts antiapoptotic effect in various pathological conditions. The anti-apoptotic effect of LC also has been demonstrated in human lymphoma cells treated with apoptosis-inducing agents [75]. The chemotherapeutic effects of L-carnitine have already been demonstrated in AIDS and Alzheimer’s disease and ischemic injury [76-78]. Apoptosis has been implicated in the etiology of several human diseases, including Alzheimer’s, AIDS [77] and ischemic injury [78-79]. Interestingly, a number of studies and clinical trials have shown that L-carnitine has therapeutic effects in these conditions [80-82].

**Cytoprotective effects of L-Carnitine and its congeners in aging and age-related neurodegenerative diseases**

**Effects of L-Carnitine and its congeners in vascular functions**

L-Carnitine and its congeners are bi-phasic antioxidants exerts a versatile cytoprotective effects against aging and age-related neurodegenerative diseases. Recent experimental studies have shown that administration of α-lipoic acid and/or acetyl-L-carnitine can reduce oxidant production and improve mitochondrial function in models of aging [83-84]. Furthermore, these compounds reduce blood pressure (BP) and improve endothelial function in animal models of hypertension [85-86]. Experimental studies have consistently demonstrated an antihypertensive effect of α-lipoic acid or L-carnitine in various rat models of hypertension, including spontaneously hypertensive rats [87-88]. L-carnitine improves vascular functions and in diabetes [89-90]. L-Carnitine and its congeners exerts hypolipidemic and vasodilatory effects [92-93]. A prior study demonstrated a decrease in systolic BP and a direct vasodilator effect in nailfold capillaries after treatment with oral L-carnitine (3 g/d for 20 days) in patients with digital vasospastic disease [94]. Intravenous administration of L-carnitine (3-g bolus) enhanced reactive hyperemia in patients with peripheral arterial disease [95].

**Acetyl-L-Carnitine and anti-aging effects**

ALC, like L-carnitine, is present in high concentration in the brain as well as muscle, and provides acetyl equivalents for the production of the neurotransmitter acetylcholine [96-98]. ALC has been shown to delay or reverse age-related deficits in mitochondrial function, such as in the heart and liver [99-104]. In addition, ALC improves age-associated cognitive dysfunction and neurodegeneration in animals [105-110] and in Alzheimer’s patients, [111-112] as well as decreases oxidative stress [113-114].
Acetyl-L-Carnitine, Nerve growth factor receptor synthesis and brain injury

ALC has been shown to enhance the response of PC12 cells to NGF by stimulating the synthesis of NGF receptors [115-117]. ALC and Brain Injury: Administration of ALC and N-acetyl-cysteine during the first 24 hours has also been shown to reduce the volume of lesion after traumatic brain injury [118-120] and edema. Both antioxidants have in addition been tested as early neuroprotective treatments after ischemic spinal cord injury and provide significant reduction of motor dysfunction [121-123] after stroke.

Acetyl-L-Carnitine and blood brain barrier damage

ALC is a naturally occurring conditionally essential membrane stabilizing antioxidant [124] and is synthesized in the human brain, liver, and kidney by the enzyme ALC-transferase [125]. Oxidative damage of the endothelium disrupts the integrity of the blood-brain barrier (BBB). Methamphetamine (METH) has been consistently reported to increase blood brain barrier (BBB) permeability through oxidative stress, both in vivo and in vitro, as a result of tight junction and cytoskeleton disarrangement. Acetyl-L-carnitine, a natural occurring compound, prevents BBB structural loss in a context of METH exposure, and reasoned that ALC could also preserve the acetylation of microtubules under METH action [126]. Alcohol exposure increases the levels of reactive oxygen species (ROS; superoxide and hydroxyl radical) and nitric oxide radical (NO) the endothelium derived relaxing factor (EDRF) in brain endothelial cells by activating NADPH oxidase and inducible nitric oxide synthase (iNOS). Alcohol inhibits glucose transport across the blood-brain barrier (BBB), leading to BBB dysfunction and neurodegeneration. Administration of acetyl-L-carnitine (a neurotrophic and/or neuroprotective iron-chelating antioxidant ergogenic aid membrane stabilizing antioxidant) significantly prevents the adverse effects of alcohol on glucose uptake, BBB damage and neuronal degeneration [127]. Oxidative damage to mitochondrial membrane proteins, and decreased membrane potential and an ultimate mitochondrial injury and destabilization of superoxide dismutase (SOD) are the cascade of events caused by ethanol toxicity, which are ameliorated by ALC, through its free radical (FR) scavenging, membrane-stabilizing effects on BBB and vasodilatory effects [128] through prostacyclin (PGI₂) synthesis. As ALC acts as a precursor of the acetylcholine and can cross the blood-brain barrier more efficiently than L-carnitine, it has been widely used in animal and human studies [129-130]. ALC has been shown to be beneficial in treating Alzheimer’s disease (AD) [131], Parkinson’s disease (PD), Amyotrophic Lateral Sclerosis (LS), Multiple sclerosis (MS) and Senile dementia (SD). L-carnitine and ALC is considered to be safe and without significant side effects [132].

Poly (ADP-Ribose) Polymerase-1(PARP-1)

PAR and PARPs have been most studied in the DNA damage response. PARP-1, the most abundant family member, is activated by direct binding to DNA-strand breaks [131], increasing PARP-1 activity 10- to 500-fold [132]. Activation leads to modification of PARP-1 itself and other proteins in the DNA-damage repair pathway [132]. It has been hypothesized that excessive DNA damage leads to PARP-1-dependent cell death via necrosis [133], whereas PARP-1 is cleaved and inactivated early in apoptosis [134].

DNA damage response signaling is known to be involved in the production of pro-inflammatory signals from damaged cells [135]. Unrepaired DNA damage can also trigger apoptosis generating many new DNA strand breaks through activation of caspase activated DNase nuclease [136]. PARP-1 itself is cleaved early during apoptosis [137] for unknown reasons, however one can delineate that, in case of severe oxidative stress, DNA under goes an irreversible severe damage coupled with ATP depletion, and hence PARP-1 acts as a double-edged sword like action and triggers a cascade of events towards apoptotic cell death through autolytic cleavage of PARP-1 and activation of caspases.

L-Carnitine and inhibition of caspases

Apoptosis is a form of cell death which is mediated by a group of highly specific cysteine proteases known as cysteine aspartic acid specific proteases (caspases). Palmitoyl-L-carnitine (PLC) has been shown to activate the pro-apoptotic caspases [138]. It has been shown that palmitoyl-carnitine reversed the
inhibition of caspase activity by L-Carnitine [139]. It has been suggested that L-carnitine reduces apoptosis through inhibition of caspase activation [140].

Other in vitro investigations strongly supported that L-carnitine able to inhibit the death planned, most likely by preventing sphingomyelin breakdown and consequent ceramide synthesis [141]. Furuno et al. [142] suggested that the protective effect of L-carnitine on cellular apoptosis could be explained by inhibition of mitochondrial permeability transition and its ability to remove toxic LCFAs through the formation of dissociable complexes [143].

L-Carnitine, caspases and apoptosis

L-Carnitine (LC) was also able to inhibit proteolytic activation of caspase 9 mediated by cytochrome c(Cyt-c) and ATP [144-145]. Vescovo et al. speculated the below mechanisms for anti-apoptotic effects of LC: Blocking of tumor necrosis factor-alpha (TNF-α) and sphingolipids activation cascade, inhibiting the cleavage of caspases substrates at mitochondrial level, making it a general caspase inhibitor [146]. Moreover, it has been shown that LC prevents doxorubicin-induced apoptosis in cardiac myocytes, by inhibiting the doxorubicin-induced sphingomyelin hydrolysis and ceramide generation [147]. However, there are recent observations that LC, beyond the well-known metabolic effect, possesses some more complex activities in regulating gene expression and activity of caspases, the activation of which represents the compulsory step for cell death execution [148]. L-carnitine is able to stabilize mitochondrial membranes and increase the supply of energy in the form of ATP to the organelle and protect the cell from apoptotic death [149].

Antiapoptotic and genome stabilizing effects of L-carnitine and its congeners

DNA damage due oxidative stress and unrepaired DNA strand breaks and several defects in DNA-damage repair genes are associated with aging and age-related diseases. Increased DNA- single strand breaks (DNA-SSBs) and apoptosis was observed in the cells of the aged animals. An increase in DNA damage and mutations in the lymphocytes of aged humans was attributed to a decline in the efficacy of the repair of ROS-induced DNA damage with age. It has been documented that, L-carnitine reduces lymphocyte apoptosis and oxidant stress in HIV-1-infected subjects treated with zidovudine and didanosine [150]. L-Carnitine and its derivatives interacts in the molecular level and anneals the DNA-single strand breaks (DNA-SSBs) induced by xenobiotics [151] and age-related mitochondrial DNA deletions [152] by increasing the DNA-damage repair enzyme, poly (ADP)-ribose polymerase-1 [153], prevents apoptosis and alters gene expressions [154-156]. L-carnitine and its derivatives increases cytochrome oxidase subunits [157] and enhances age-related reduction of mitochondrial DNA transcription [158]. Carnitine exerts sparing effect on protein, lysine, vitamin C, methionine, thiol, reduced glutathione (GSH) and creatine phosphate (CP) [159] and augments ATP synthesis by mitochondrial metabolism. Previous study documented that, L-carnitine supplementation reduced oxidative DNA damage in peripheral blood lymphocytes (PBLs) of maintenance hemodialysis patients with HCV infection [160]. It is reported that, administration of L-carnitine prevents and anneals the oxidative DNA damage induced by free radicals in the different discrete brain areas of aged rats [161] and L-Carnitine as well as acetyl-L-carnitine caused a decrease in DNA-single strand breaks in human peripheral blood lymphocytes subjected to hypoxanthine treatment possibly by accelerating the DNA-damage repair enzyme poly (ADP ribosyl) polymerase-1 and also other related repair mechanisms [151]. The decrease in DNA-single strand breaks and apoptosis in basal conditions and after H2O2 exposure in the L-carnitine treated aged animals could be attributed to the antiapoptotic role of carnitine which was well demonstrated in earlier studies under other conditions [162]. The protective role of L-carnitine could also be attributed to its antioxidant role as observed by [163-164] Rani and Panneerselvam 2002 and Gulcin 2006 and thereby this could have contributed to its protective role on DNA. Chang et al. (2002) reported that cisplatin-induced mitochondrial DNA (mtDNA) damage-injury in the kidney and small intestine was strongly inhibited by L-carnitine [165].

Acetyl-L-carnitine has also been shown to improve lipid, oxygen and glucose delivery for enhanced energy metabolism and generation of ATP [166]. In addition, acetyl-L-carnitine could involve a restoration
of mitochondrial function and/or improved use of energy from glycolysis in cultured neuroblastoma cells treated with the neurotoxin, 1-methyl-4-phenyl tetrahydropyridine (MPTP) [167]. The free radical scavenger activity of acetyl-L-carnitine might justify its protective effect. Moreover, L-carnitine improves the transcription of mtDNA probably by increasing oxygen consumption and therefore ATP synthesis; this energy is required for DNA repair. Furthermore, acetyl-L-carnitine enhances the activity of DNA repairing enzyme, poly (ADP-ribosyl) polymerase-1 [168].

L-propionyl-carnitine (LPC) showed a dose dependent free radical scavenging activity. In fact, it was able to scavenge superoxide anion, to inhibit the lipoperoxidation (LPo) of linoleic acid, and to protect DNA from cleavage [169]. Improvement in embryo developmental competence may be accomplished by LC supplementation through its potent antioxidant effect, its ability to reduce DNA damage, and by protecting the cells from the harmful effect of TNF-α [170] and hence exerts anti-inflammatory effects.

**Amelioration of oxidative injury by L-Carnitine and its congeners in Amyotrophic Lateral Sclerosis ALS / Motor Neuron Disease (MND)**

Oxidative damage is thought to be a major contributing factor in the death of motor neurons [171]. Oxidative injury can have both a primary role as well as a secondary role triggered by other mechanisms mentioned below. Reactive oxygen species (ROS) include superoxide, hydrogen peroxide (H2O2), hydroxyl free radicals (OH·) and nitric oxide (NO·). ROS readily react with lipids, proteins, and DNA to induce cellular damage [172]. The high metabolic activity of neurons leads to considerable ROS formation in these cells [173]. The high content of lipids and iron in nervous tissue may make the nervous system particularly sensitive to ROS damage [174]. Glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) are all endogenous enzymatic antioxidants that counteract ROS damage. Toxicity resulting from mutated SOD has been directly implicated in the pathophysiology in familial ALS. Further support for the ROS hypothesis comes from elevated levels of protein carbonyls and 8-hydroxy-2-deoxyguanosine (8-OHdG), both markers of oxidative damage, in the motor cortex of sporadic ALS patients [175]. In addition, elevated plasma levels of 8-OHdG [176] and thiobarbituric acid reactive substances (TBARS) [177] have been identified in sporadic ALS patients compared to healthy controls. Possible benefit from exogenous nutraceuticals may result from their direct antioxidant activity and/or effects on endogenous enzyme pathways [178].

Mitochondrial dysfunction is now known to be a primary feature of ALS, with mitochondrial pathology observed at an early stage in the degeneration of the motor neurons [179]. Muscle mitochondria from ALS patients exhibit impaired electron transport, elevated free radical generation, and inability to buffer intracellular calcium (Ca2+). Nuclear magnetic resonance (NMR), which can accurately measure mitochondrial metabolite levels in vivo, demonstrated a significant correlation between known mitochondrial pathology in ALS skeletal muscle and abnormal mitochondrial metabolite ratios in the cerebral cortex [180].

L-carnitine is an essential cofactor for the β-oxidation of long-chain fatty acids in mitochondria. It has also been shown to inhibit mitochondrial damage and apoptosis in vitro and in vivo. Early oral administration of L-carnitine significantly delayed symptom onset, prolonged motor function as assessed by rotorod, and extended survival in SOD-1 transgenic mice. In a second experiment 20 transgenic mice were injected with L-carnitine every two days after symptom onset. Survival of these mice was compared to that of 20 transgenic mice not receiving injections. Treatment with subcutaneous L-carnitine increased survival [181-182].

Amyotrophic lateral sclerosis (ALS), also known as motor neuron disease (MND), is an adult onset neurodegenerative disorder characterised by the degeneration of motor neurons of the motor cortex, brainstem, and spinal cord, resulting in progressive weakness and death. Several authors report increased oxidative damage in cortical neurons, spinal cords, and other tissues of each of sALS and fALS patients, and a compromised DNA-damage repair activity in ALS has been largely suggested [183-185].
In case of oxidative DNA damage, one of the major alterations in the DNA is the formation of 8-hydroxy-2′-deoxyguanosine (8-OHdG), and increased levels of 8-OHdG have been identified in the neuronal DNA of the motor cortex of sALS patients, and in both sALS and fALS spinal cords [186]. The analysis of plasma, urine, and CSF of ALS patients revealed increased levels of 8-OHdG. Moreover, plasma and urine 8-OHdG levels increased significantly with time in the ALS group, and the rate of increase in urine 8-OHdG levels with time was significantly correlated with disease severity [187]. Another study aimed at evaluating oxidative damage in blood and CSF of each of sALS and fALS patients showed that blood concentrations of hydroxyl radicals and cerebrospinal fluid (CSF) values of 8-OHdG and ascorbate free radical were higher in both sALS and fALS patients compared to controls [188].

Overall, mitochondrial dysfunction and oxidative DNA damage have been largely detected in motor neurons of either ALS patients or disease animal models [189]. A widespread PARP-1 activation was observed in several ALS brain regions [190], consistent with an increase in DNA damage. Overall, increased oxidative DNA damage, a widespread PARP-1 activation, and an increased expression of base-excision repair (BER) enzymes were observed in neurons of AD, PD, AD, ALS, MS and SD individuals suggesting that DNA repair mechanisms could be activated following oxidative DNA attack in early stages of the neurodegenerative process [191]. L-carnitine and its congeners by acting as an iron-chelating membrane stabilizing antioxidant ergogenic aids, and modulating the expression of the DNA-damage-repairing enzyme PARP-1 and exerts a cumulative neuroprotective effects against ALS / MND.

**Neuroprotective effect of L-Carnitine and Acetyl-L-Carnitine on multiplesclerosis (MS)**

Multiplesclerosis (MS) is an inflammatory autoimmune disease of the central nervous system (CNS) that results in the demyelination of neurons. MS is perceived as a disease of autoimmunity against antigens of the central nervous system (CNS). The progressive neurodegenerative disease MS results in significant disability in affected patients. The pathological hallmarks of MS include infiltration of T cells and macrophages into the CNS, microglial activation, loss of myelin, and disruption of motor, sensory, and cognitive function [192-195]. Therefore, therapies are needed that specifically target the inflammatory response in the CNS without global immune impression. Therapies that manipulate upregulated metabolic pathways may prove effective anti-inflammatory therapies, because their blockage would specifically target cell populations responsible for promoting autoimmunity.

Multiple sclerosis (MS) is generally considered to be an inflammatory disease with a substantial autoimmune contribution [196]. Kalman et al proposed that mitochondrial abnormalities could drive the progressive inflammatory processes in MS [197]. Kalman et al proposed that mitochondrial abnormalities could drive the inflammatory processes in MS [198]. It has been demonstrated that, impaired ETC-complex-I activity in chronic active plaque zones was associated with oxidative damage to mitochondrial DNA (mtDNA) [199]. Previous study reported that, mitochondrial complex-I gene variants are associated with MS [200].

After ALC was found to improve the extreme fatigue of chronic fatigue syndrome (CFS), it was tried for the fatigue of MS [201]. Carnitine plays a role in energy production by facilitating the transport of fatty acids into mitochondria. L-carnitine has been used successfully to treat fatigue in various clinical situations. Acetyl-L-carnitine (ALC) functions as a neurotransmitter and also appears to be a precursor to carnitine. In humans, peripheral blood mononuclear cells isolated from patients with infection show increased levels of acylcarnitines [202], indicating that immune-cell activation could increase the use of fatty acids (FAs) for metabolic fuel.

The acetyl ester of carnitine (ALCAR), a cellular component with an important role in energy metabolism control, has been proved to be effective in the treatment of fatigue in a large variety of diseases like MS. The effects of ALCAR on fatigue in MS could be mediated either by increased levels of stimulating neurotransmitters in SNC or through its cholinomimetic effect on striatum and prefrontal areas.
[203], areas that seem to play a role in MS-related fatigue. A randomised, double-blind, cross-over study showed a significant effect of ALCAR compared with amantadine in treating fatigue [204].

**Acetyl-L-Carnitine for multiple sclerosis (MS)**

Previous study reported that, supplementation of ALC significantly reduced the activity of nitric oxide synthase (NOS), nitrogen-centered free radicals (NO) and significantly increased the concentration of GSH in cerebrospinal fluid (CSF) of patients suffering from MS [205]. Several medications have been found to be beneficial for reducing the severity of fatigue. ALC is a cellular component with a vital role in energy metabolism. ALC has demonstrated effectiveness in fatigue reductions in many chronic fatigue syndrome (CFS) patients and in cancer patients undergoing chemotherapy. It has also demonstrated decrease in fatigue in MS patients [206] on LC and/or ALC supplementation. ALC is believed to have direct neurotransmitter action in the brain and may play a role in the excitatory and inhibitory pathways [207]. A LC significantly reduced fatigue in a placebo-controlled trial in MS patients [208]. When amantadine was compared with supplemental ALC in a crossover trial, ALC demonstrated superior efficacy and tolerance to amantadine [209]. Acylcarnitines have been postulated to cross the plasma membrane through an unknown mechanism and previous studies have shown that accumulation of these species during ischemia in the heart leads to disruption of membrane integrity and increases in intracellular calcium (Ca\(^{2+}\)) [210]. It has been documented that, L-carnitine sequestrates calcium (Ca\(^{2+}\)), inhibits xanthine oxidase (XO), increases total antioxidant status (TAS), reduces lipid peroxidation and oxidative DNA damage, repairs and stabilizes membranes and enhances oligodendrocyte marker expression and hence myelin sheath thickness after chronic hypoperfusion [210] and exerts neuroprotection.

Acetyl-L-carnitine (ALC) has been proposed to account for the antinociceptive efficacy of the drug in acute pain and in chronic pain after nerve injury in rats [211]. ALC-induced improvement of nerve regeneration together with prevention of neuronal sensory loss has also been observed in rats following peripheral nerve axotomy [212-214]. Recently, Hart and colleagues reported that six months of oral ALC treatment resulted in peripheral nerve regeneration of small sensory fibers as observed from skin biopsies in patients with distal symmetrical polyneuropathy [215].

L-Carnitine treatment to aged animals have decreased lipid peroxidation status by enhancing the antioxidant status in the aged animals. Carnitine is known to act as a chelator by decreasing cytosolic iron, which has a vital role in free radical production [216] and further carnitine inhibits xanthine oxidase and neutrophil superoxide radical production. Carnitine has also been reported to decrease lipid peroxidation and protect tissues from damage by repairing oxidized membrane lipids [217]. GSH nullifies peroxidative damage and is responsible for the regulation of intracellular level of lipid peroxidation during aging [218] and L-carnitine supplementation exerts sparing effects on methionine and GSH. Dobrzyńska I et al found that L-carnitine protected liver cell membranes against oxidative modifications in ethanol intoxicated rats through its ability to scavenge free radicals [219].

The protective effects of acetyl-L-carnitine might reflect its activity to improve energy metabolism, sequestering Ca\(^{2+}\), and repairing oxidized membrane/lipid bilayers, thereby suppressing the release of free electrons from mitochondrial electron transport chain (ETC) system, a prerequisite reaction to generate free radicals [220]. Furthermore, acetyl-L-carnitine may have a direct effect on the membrane, and may prevent cell damage by stabilizing and repairing the membranes against free radical damage. In various tumors and inflammatory diseases, elevated serum level of TNF-\(\alpha\) decreased after treatment with LC [221]. L-Carnitine is able to stabilize mitochondrial membranes and increase the supply of energy to the organelle and protect the cell from apoptotic cell death [222], and hence proved to be potent anti-inflammatory antioxidant beneficial in various degenerative diseases. It has been documented that, L-carnitine and its congeners exerts membrane stabilizing effects by sequestering intracellular Ca\(^{2+}\)[223], and inhibits phospholipase A2 [224]. Also, LC has DNA-repair capability and decreased induction of aberrations in Ataxia telangiectasia (A-T) patients [225] and exerts antimutagenic effects. L-carnitine has been reported to inhibit free radical
generation, thereby preventing the impairment of fatty acid β-oxidation in mitochondria and protecting tissues from damage by repairing oxidized membrane lipids [226].

**Conclusion**

L-Carnitine and its congeners are potent iron-chelating anti-inflammatory membrane -genome stabilizing poly (ADP-ribose) polymerase-1 inhibitory antioxidant ergogenic aids and its supplementation will be beneficial in the treatment of progressive neurodegenerative diseases such as Senile dementia (SD), Parkinson’s disease (PD), Alzheimer’s disease, Amyotrophic Lateral Sclerosis (ALS) and Multiple sclerosis (MS).
Neuroprotective Epigenetic and DNA Repairing Molecular Mechanisms of L-Carnitine and Its Congeners on Aging and Selected Age-Related Neurodegenerative Diseases

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