MICRONUTRIENTS AND BRAIN HEALTH

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23 Acetyl-l-Carnitine and Ferulic Acid Action in Aging and Neurodegenerative Diseases

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23.1 INTRODUCTION

In 1956, Harman proposed the “free radical theory of aging,” which suggested that free radicals and/or reactive oxygen species (ROS) contributed to the loss of molecular and cellular function in organisms over time (Harman 1956). This loss of function in aging is due in part to ROS exposure causing an imbalance in cellular homeostasis because the organism is not able to sufficiently scavenge free radicals. Harman’s theory has more recently been accepted as the “oxidative stress theory of aging” (Muller et al. 2007) and has been linked to disorders including cancer, atherosclerosis, stroke, and diabetes (Mariani et al. 2005). Oxidative stress also has been implicated in several neurodegenerative disorders such as Alzheimer’s disease (AD), Parkinson’s disease (PD), and amyotrophic lateral sclerosis (ALS) (Markesbery 1997). Because oxidative stress can affect cellular processes such as metabolism, structural integrity, inflammation, and apoptosis (Terman et al. 2006), its link with many human diseases is not surprising. As a result, many researchers have investigated the molecular mechanisms underlying oxidative stress in various model systems of aging and age-related disorders (Humphries et al. 2006; Muller et al. 2007) in order to find clues that lead to the development of therapeutic molecules. While the development/synthesis
of novel compounds that are able to combat oxidative stress is necessary, there is much interest in the application of naturally occurring compounds that may also offer beneficial protection.

Under physiological conditions, cellular ROS levels are regulated by antioxidant molecules and antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). An overabundance of ROS renders cellular materials vulnerable to oxidative modifications that adversely can affect the structure and function of proteins, lipids, carbohydrates, and nucleic acids and can eventually lead to cell death (Smith et al. 1994; Mark et al. 1997; Markesbery 1997; Lovell et al. 2001; Butterfield 2002; Butterfield et al. 2002a). Although oxidative stress can occur in any cell, neuronal oxidative stress is particularly detrimental in part because brain cells do not divide or regenerate. Moreover, the brain is highly susceptible to oxidative insult because it consumes a vast amount of oxygen, has high levels of polyunsaturated fatty acids and redox-active transition metal ions, and contains low levels of antioxidant enzymes (Markesbery 1997; Butterfield et al. 2001; Butterfield et al. 2002a; Butterfield et al. 2002b; Floyd et al. 2002). Therefore, antioxidant compounds that are able to cross the blood-brain barrier (BBB) and increase endogenous brain antioxidant levels are desirable because they may provide neuroprotection against oxidative damage in aging and neurodegenerative disorders. Examples of such compounds include feric acid (FA), FA derivatives, and acetyl-l-carnitine (LAC), all of which are naturally occurring molecules. This review will focus on literature reports that have investigated the use of FA and its derivatives and LAC as means to suppress or halt the oxidative damage present in aging and age-related neurodegenerative conditions.

23.2 FA IN AGING AND NEURODEGENERATIVE DISEASES

FA [3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid], a derivative of cinnamic acid, is found in many edible plants, vegetables, and fruits (Graf 1992). Therapeutically, significant attention has been given to FA because it is a naturally occurring antioxidant molecule. The trans isomer predominates in plants, where FA is typically esterified and covalently bound to sugars, glycoproteins, polyamines, and other cell wall components (Graf 1992). This molecule is synthesized from phenylalanine and tyrosine via the shikimate pathway, in which both amino acids are converted to p-coumaric acid, which is then hydroxylated to caffeic acid. Caffeic acid is then methylated to form FA, with methionine serving as the methyl donor. The structure of FA contains a phenol whose hydroxyl group is para-substituted to propenoic acid (Figure 23.1); the antioxidant capacity of FA lies in its ability to trap free radicals in an extensive pi-conjugated network. Radicals, such as hydroxyl radical (-OH), encounter FA and immediately abstract an H atom from the

![Structure of FA](image)

**FIGURE 23.1** Structure of FA.
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![Chemical Structures]

**FIGURE 23.2** Diagram of ROS scavenging by FA.

Phenolic -OH to make a phenoxy radical; the remaining unpaired electron on the oxygen atom is capable of resonating throughout the multiple conjugated sites of FA (Figure 23.2). Because of its radical scavenging properties, FA is sometimes referred to as a “chain-breaking” antioxidant. Toxic events such as oxidation of lipids, DNA, RNA, and proteins are mediated by free radical reactions that damage cellular components and, in turn, produce more free radical species that propagate until the “chain” is broken. FA intercepts ROS and other reactive radicals, thereby halting insidious modifications and preventing other downstream consequences.

It has been suggested that the neurotherapeutic capabilities of FA are partially hampered by the negative charge that exists on the carboxylic oxygen atom at physiological pH, as well as relatively low lipophilicity (Scapagnini et al. 2004). The presence of the BBB, an epithelial cell layer connected by tight junctions, prevents the passage of most peripherally localized materials into the brain space. Exogenous small molecules, lipophilics, and molecules with transport systems are the select species allowed to enter the brain. Therefore, derivatives of FA such as ferulic acid ethyl ester (FAEE) may be more neuroprotective because the ethyl ester moiety decreases the overall polarity of the molecule and neutralizes the negative charge, thus increasing its permeability at the BBB. FAEE is also reported to be a superior scavenger of -OH and superoxide (O$_2^-$) relative to FA (Scapagnini et al. 2004). However, *in vivo* studies in which FA was peripherally administrated to mice report both behavioral and neurobiochemical improvements when mice were subjected to insults, such as intracerebroventricular injection of amyloid beta (Aβ) (Yan et al. 2001; Cho et al. 2005) or buthionine...
sulfoximine (BSO) (Mamiya et al. 2008). Studies of this nature indicate potential neurological gains from FA that are similar to FA derivatives. Therefore, the applications of both FA and its derivatives for providing potential protection from detriments associated with aging and neurodegeneration are discussed below.

23.2.1 Cellular Protection from Oxidative Damage by FA and FA Derivatives

The capability of FA to combat ROS has been well characterized (Scott et al. 1993; Kanski et al. 2002; Ōgiwara et al. 2002; Hsieh et al. 2005; Joshi et al. 2006). FA has been shown to scavenge ·OH, peroxynitrite, hypochlorous acid, 1,1-diphenyl-2-picrylhydrazyl, and oxidized low-density lipoprotein (Scott et al. 1993; Kanski et al. 2002; Hsieh et al. 2005). FA can also scavenge nitric oxide (NO) and O$_3^-$ (Ogiwara et al. 2002). Treatment of synaptosomes with Fe$^{2+}$ and H$_2$O$_2$ (reagents for Fenton hydroxyl production) and 2,2-azobis(2-amidino-propane) dihydrochloride (AAAPH) (a source for peroxyl and alkoxyl radicals) resulted in reduced free radicals and oxidative stress products when mice were pretreated in vivo with FAEE (Joshi et al. 2006). FeSO$_4$ administration to neurons caused increased levels of thiobarbituric acid substances, a measure of lipid peroxidation, which were attenuated with FA (Zhang et al. 2003). Intracerebroventricular injection of BSO, an inhibitor of glutathione (GSH) synthesis, enhanced protein carbonyl levels in mice brains, and this elevation was prevented by subcutaneous administration of FA (Mamiya et al. 2008). Also, FA suppressed malondialdehyde levels, another marker of lipid peroxidation, in rat brain homogenates (Sharma 1976). Several studies investigating the effects of radiation-mediated oxidative stress report increased protection of lipids, antioxidants, and DNA by FA (Hsieh et al. 2005; Maurya et al. 2005, 2006; Prasad et al. 2006; Srinivasan et al. 2006).

Although oxidative stress is not specific to a single neurological condition, many studies investigating the effects of FA on neurodegenerative disease pathogenesis focus on AD. AD is characterized behaviorally by cognitive dysfunction and memory impairment and pathologically by brain-localized senile plaques and neurofibrillary tangles. Amyloid β peptide (Aβ) is the major component of senile plaques. Aβ associates into oligomers/fibrils and causes oxidative stress by direct oxidation of biomolecules and by producing large amounts of ROS/reactive nitrogen species (RNS), possibly due to the methionine residue at position 35 (Varadarajan et al. 2001). In vitro, FA in a dose-dependent manner inhibited Aβ fibril formation and also destabilized preformed fibrils (Ono et al. 2005). FAEE scavenged Aβ(1–42)-generated ROS and protected against Aβ(1–42)-induced oxidative damage in rat primary neuronal cell culture (Sultana et al. 2005). FAEE injected intraperitoneally into rodents also protected synaptosomes from oxidative stress induced by Aβ(1–42) (Perluigi et al. 2006). The benefits of FA as an AD therapeutic also extend to regulation of acetylcholine levels, which are markedly decreased in AD brain (Davies et al. 1976; Coyle et al. 1983; Wevers et al. 2000). Orally administered FA significantly elevated choline acetyltransferase activity, an acetylcholine synthesizing enzyme, in trimethylin-induced cognitively deficient mice, in addition to improving memory (Kim et al. 2007). It has also been reported that decreased cortical acetylcholine levels were attenuated by FA in Aβ(1–42)-treated mice (Yan et al. 2001).

Besides scavenging free radicals, FA and FAEE have a hormesis effect by promoting increased levels of antioxidant defense molecules. FAEE induces the expression of heme-oxygenase 1 (HO-1) at both protein and mRNA levels (Scapagnini et al. 2004; Sultana et al. 2005; Joshi et al. 2006; Perluigi et al. 2006). HO-1 is an inducible enzyme that catalyzes the breakdown of heme to carbon monoxide (CO) and biliverdin. Biliverdin is reduced to bilirubin, which at low levels is an excellent antioxidant (Poon et al. 2004). The protective properties of HO-1 are manifested in the antioxidant and anti-inflammatory capabilities of these products (Stocker et al. 1987; Clark et al. 2000; Poon et al. 2004). The mechanism by which FAEE up-regulates HO-1 is unclear, but is hypothesized to be through the Nrf-2 pathway (Scapagnini et al. 2004). In addition to HO-1, FAEE also up-regulates heat shock proteins (HSPs), namely HSP72 (Sultana et al. 2005; Perluigi et al. 2006) and HSP70.
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(Joshi et al. 2006). HSP72, an inducible member of the HSP70 family, is a chaperone protein used to refold denatured proteins. HSP72 has been shown to protect against stroke and ischemia (Lee et al. 2001; Kelly et al. 2002; Zheng et al. 2008). Also, heat shock proteins have been reported to protect central nervous system (CNS) cells against apoptosis and necrosis in vitro (Poon et al. 2004). In hepatocytes subjected to gamma radiation, FA significantly increased antioxidant levels of GSH, vitamins A, E, and C, ceruloplasmin, and uric acid (Srinivasan et al. 2006). FA also increased diminished activities of antioxidant enzymes such as SOD, GPx, and CAT (Srinivasan et al. 2006); similar findings were reported in diabetic rats treated with FA (Balasubashini et al. 2004). The return to baseline antioxidant levels may be due to ROS scavenging by FA, so as to preserve the levels of these molecules for normal cellular function (Srinivasan et al. 2006). Likewise, the generated ROS may damage antioxidant enzymes, thus lowering activity levels. FA restored decreased activities of SOD, GPx, and CAT, again possibly due to direct oxidant scavenging (Srinivasan et al. 2006).

23.2.2 FA Offers Protection from Inflammation

As previously described, the beneficial effects of FA and its derivatives are derived from an excellent ability to combat cellular oxidative stress. However, FA also has anti-inflammatory capabilities (Chawla et al. 1987; Ozaki 1992; Fernandez et al. 1998; Akihisa et al. 2000), partly due to the fact that inflammation is associated with large amounts of ROS and in part due to the suppression/up-regulation of key species in this cascade. Inflammation is a bodily defense mechanism generated in response to a pathogen; inflammation is characterized by activation of immune cells, cytokines, chemokines, and acute phase molecules that aid in the removal of the insult. The anti-inflammatory ability of FA appears to be fourfold: (1) scavenging preformed ROS that can contribute to inflammation; (2) scavenging ROS produced by inflammation; (3) suppression of key proteins/molecules contributing to inflammation; and (4) enhancing proteins/molecules that are inhibitory to inflammation. Neuroinflammation is implicated in several neurodegenerative conditions, namely PD, AD, ALS, Creutzfeldt-Jakob disease, multiple sclerosis (MS), and Pick’s disease (Klegeris et al. 2007).

Gliai cells, such as microglia and astrocytes, largely mediate inflammation in the CNS. Upon activation, these cells produce cytokines, chemokines, and other proinflammatory agents for action against toxic antigens; however, they also produce large amounts of ROS/RNS, thereby contributing to oxidative stress and its consequences. Reactive agents such as lipopolysaccharide (LPS) and Aβ can trigger glial cell activation, which leads to the release of species such as NO and H2O2, capable of killing neighboring cells (Block et al. 2007). Factors released from dying cells can in turn activate more microglia, creating a toxic cycle if not properly regulated (Block et al. 2007). Activated microglia and astrocytes are implicated in conditions such as AD, PD, MS, and HIV/AIDS. Long-term administration of FA to mice centrally treated with Aβ(1-42) suppressed immunoreactivity of OX-42, a microglial active marker (Kim et al. 2004). Another study found microglial activation to be suppressed with FA, and more so with a nitric oxide releasing FA derivative (Wenk et al. 2004). Increases in glial fibrillary acidic protein (GFAP), a marker of glial cell activation, and proinflammatory cytokine interleukin 1β (IL-1β) by Aβ were successfully suppressed with FA, and also with sodium ferulate (SF) in a separate study (Yan et al. 2001; Jin et al. 2005). Along with endothelial nitric oxide synthase (eNOS) and 3-nitrotyrosine (3NT), astrocytic IL-1β immunoreactivity was also decreased in mice treated with FA and Aβ(1-42) compared with mice treated only with Aβ(1-42) (Cho et al. 2005). In addition to its inflammatory contributions, IL-1β has downstream effects related to apoptosis (see following section).

Inducible nitric oxide synthase (iNOS) is a protein directly involved in both oxidative/nitrosative stress and inflammation. iNOS produces NO from arginine and is only expressed in response to immunological stimuli (Bredt et al. 1994; Sessa 1994). Active microglia are capable of activating iNOS, thereby producing large amounts of RNS/ROS. Overproduction of NO can cause nitration of cellular materials, leading to dysfunction. Treatment of neurons with Aβ(1-42) caused an increase
in iNOS levels, which was attenuated by FAEE administration (Sultana et al. 2005). Similar results were obtained using Fe$^{2+}$/H$_2$O$_2$ or AAPH as the oxidant species (Joshi et al. 2006). Accordingly, both of these reports show increased 3-NT levels after treatment with Aβ(1–42), as well as Fe$^{2+}$/H$_2$O$_2$ and AAPH that are abrogated with FAEE administration. This suggests that prevention of nitrosative stress is possibly due to lowered iNOS expression (Sultana et al. 2005; Joshi et al. 2006), thereby supporting the duality of FAEE to act as both an antioxidant and anti-inflammatory molecule.

### 23.2.3 FA Offers Protection Against Apoptosis

Another downstream result of oxidative stress is cell death. Preemptive treatment with antioxidants such as FA to antagonize inflammation and/or oxidative stress is a way to minimize signs of apoptosis and synapse loss in degenerative conditions. As mentioned previously, IL-1β increases with subject exposure to Aβ. Interestingly, increases in IL-1 β levels by Aβ(25–35) were accompanied by increases in the p38 mitogen-activating kinase (p38 MAPK) pathway (Jin et al. 2005). This led to increased caspase 3 levels and activity and to an increased Fas ligand expression, which is indicative of cell death; these trends were attenuated by SF treatment (Jin et al. 2005). SF has also been reported to abrogate Aβ(1–40)-induced apoptosis (Jin et al. 2006). Caspase 3 activation and activation of proapoptotic genes p53 and p21$^{waf1/cip1}$ decreased in neurons subject to Fe$^{2+}$-induced oxidative damage by FA treatment (Zhang et al. 2003). Mitochondrial release of cytochrome c is an initiating step in the apoptotic pathway, and this initiating activity is reportedly decreased when bound to and stabilized by FA (Yang et al. 2007). Similarly, FAEE decreased release of cytochrome c triggered by Aβ(1–42), and restored the reduction in synaptosomal phospholipid asymmetry caused by this peptide, further supporting the notion that FA compounds are protective against Aβ-induced apoptosis (Mohammad Abdul and Butterfield 2005). Excitotoxic events such as increased intracellular Ca$^{2+}$ can also lead to apoptotic cell death. Yu et al. (2006) reported evidence of SF as a competitive N-methyl-d-aspartate (NMDA) receptor antagonist and hence neuroprotectant most likely by preventing Ca$^{2+}$ influx ([Ca$^{2+}$]) and subsequent cell death.

### 23.3 LAC in Aging and Neurodegenerative Diseases

LAC (γ-trimethyl-β-acetylbutyrobetaine) is the acetyl ester of the precursor molecule carnitine, a nonessential amino acid (Figure 23.3). Carnitine is synthesized from methionine and lysine amino acids in the brain, liver, and kidney. The acetyl moiety can be added to carnitine from the acetyl group on coenzyme A (CoA) with the aid of LAC transferase in order to form LAC. LAC is located

![Structure of LAC](http://example.com/lacstructure.png)

**FIGURE 23.3** Structure of LAC.
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in the inner mitochondrial matrix and is involved in β-oxidation/fatty acid metabolism, cellular energy production, neuronal maintenance and repair, regulation of mitochondrial enzymes, and buffering of toxic acyl-CoA metabolites (Calabrese et al. 2006b). In addition, LAC assists in the uptake of acetyl-CoA into the mitochondria, increases acetylcholine production, and promotes phospholipid synthesis of proteins and membranes (Calvani et al. 1999). The acetyl moiety also increases the permeability of carnitine allowing it to readily cross the BBB and transport fatty acids across mitochondrial membranes. Thus, LAC is a crucial molecule involved in the maintenance of normal mitochondrial function. Recent evidence suggests that, overall, LAC increases mitochondrial ATP production and provides protection against oxidative attack (Beal 2003; Mazzio et al. 2003).

While the molecular mechanisms of LAC treatment are discussed in this review, it is noteworthy to mention the other beneficial aspects that have been reported upon LAC administration. For example, LAC has been shown to induce synaptic long-term potentiation in aged animals (Ando et al. 2002) and to influence attention, learning, and memory in rodents (Spagnoli et al. 1991). In AD patients, LAC has been reported to improve memory function and cognition and/or to slow brain deterioration (Spagnoli et al. 1991; Nikitovic et al. 1998; Alam et al. 2003; Balogun et al. 2003; Poon et al. 2004). This is the case even in AD patients younger than 61 years old (Calabrese et al. 2000; Calabrese et al. 2002) and demonstrates the behavioral/symptomatic benefits of LAC administration in AD.

23.3.1 Restoration of Mitochondrial Function by LAC

Mitochondria are key to maintaining proper organism function and to organism survival. Approximately 90% of the cell’s ATP is generated from the mitochondria, thus its term the “energy powerhouse” of the cell. However, the constant metabolism of oxygen by the mitochondria also generates ROS and thus can contribute to oxidative stress and cause cellular damage. This effect can be significantly enhanced upon impairments to mitochondrial functional integrity (Kidd 2005). In aging, AD, PD, Down’s syndrome, MS, ALS, Huntington’s disease, and other neurodegenerative disorders, one of the commonly suggested mechanisms of disease pathogenesis involves mitochondrial dysfunction (Kidd 2005); consequently, treatments that either maintain or improve mitochondrial function are necessary. LAC in combination with the mitochondrial molecule, α-lipoic acid, has been reported to reverse mitochondrial damage associated with aging (Ames et al. 2004). A double-blind placebo or LAC study in AD patients revealed that LAC improved metabolic measures determined by magnetic resonance spectroscopy, suggesting renewal of neuronal membranes and energy stores in the cortex (Pettegrew et al. 1995).

In the N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD mouse model, LAC provided protection against dopaminergic neuron loss in the substantia nigra (Bodis-Wollner et al. 1991). The potential of LAC as a neuroprotective agent against neurotoxic insults, such as MPTP, were also demonstrated in animal models of PD and AD, in which LAC controlled the mitochondrial acyl-CoA/CoA ratio, peroxisomal oxidation of fatty acids, and production of ketone bodies (Virmani et al. 2005). Furthermore, in MS patients, LAC treatment decreased nitrosative end products found in the cerebrospinal fluid and increased GSH levels (Calabrese et al. 2003). Damage to mitochondrial enzymes in the hypoxic brain region of stroke patients was partially attenuated by LAC treatment through decreases in hypoxic damage and enhancement of cell survival (Corbucci et al. 1992). Overall, substantial evidence exists for the use of LAC as a potential treatment against mitochondrial damage and dysfunction in aging and neurodegenerative disorders.

23.3.2 Cellular Protection from Oxidative Damage by LAC

As mentioned above for FA, naturally occurring antioxidants have the potential to provide significant protection against oxidative damage. LAC provides neuroprotection against oxidative attack by either mediating oxidative damage as an antioxidant molecule (in which the mechanisms are not completely
understood) or by influencing the levels of other antioxidant and stress response-related molecules. Our laboratory recently reported that combined pretreatment of cortical neurons with LAC and α-lipoic acid reduces 4-hydroxy-nonenal (HNE) toxicity, protein oxidation, lipid peroxidation, and apoptosis (Abdul and Butterfield 2007). In addition, this combined antioxidant treatment increases cellular GSH, HSP levels (i.e., HO-1 and HSP72), decreases iNOS levels, and activates the PI3K, PKG, and ERK1/2 pathways, which are important prosurvival pathways for neuronal survival (Abdul and Butterfield 2007).

Similar results were also obtained utilizing a senescent rat model of aging. Calabrese et al. (2006a) demonstrated that senescent rats treated with LAC for four months caused an induction in HO-1, HSP70, SOD2, and GSH levels. Oxidatively modified levels of protein carbonyls and HNE-bound proteins (e.g., marker of lipid peroxidation) were also reduced in LAC-treated senescent rats and LAC was reported to prevent age-related mitochondrial respiratory chain complex expression (Calabrese et al. 2006a). In addition, astrocytes treated with LAC induced HO-1 in a dose- and time-dependent manner, which was correlated with an increase in HSP60 and the redox-sensitive transcription factor, Nrf2 (Calabrese et al. 2005). Pretreatment of astrocytes isolated from rat with LAC before lipopolysaccharide and interferon γ exposure (e.g., molecules that induce inflammation and nitrosative stress) prevented protein nitration, disturbances to mitochondrial respiratory chain complex activity, and antioxidant status (Calabrese et al. 2005). These reports provide additional evidence that LAC is beneficial for maintaining proper mitochondrial function and providing protection against oxidative damage. Furthermore, LAC appears to be influential in the expression of endogenous antioxidants (e.g., GSH, SOD-2) and in the so-called vitagenes (e.g., HO-1, HSP70), which are crucial for maintenance and repair functions in the brain (Calabrese et al. 2006b; Poon et al. 2004). Taken together, these pathways influenced by LAC antioxidant supplementation provide a potential means for delaying aging, reducing the risk of age-related disorders, and treating the symptoms associated with neurodegenerative disorders, such as AD.

Insights into specific proteins and brain regions that are protected by LAC treatment have been revealed by immunochemical and redox proteomics analysis of aged rats in our laboratory (Poon et al. 2006). LAC reduced age-associated protein oxidation in protein carbonyls of aged rats in cortex (CX), substantia nigra (SN), septum (SP), hippocampus (HP), and cerebellum (CB) regions of the brain (Poon et al. 2006). Significant increases in protein-bound HNE levels were only observed in CX, SP, and HP of aged rats and were attenuated with LAC administration. Aged rats were also observed to have elevated 3-NT-modified protein levels in SN and HP, which were reduced with LAC. Redox proteomics analysis of HP in aged rats relative to young rats revealed that significant increases to protein carbonylation levels of hemoglobin, cofolin 1, and β-actin proteins in aged rats were reduced after LAC treatment (only the reduction in cofolin 1 was statistically significant). LAC treatment in aged rats also restored the levels of mitochondrial aconitase, inositol monophosphatase, α-enolase, creatine kinase B chain, and tubulin α-1 chain to similar levels of young rats. In the CX brain region, LAC administration reduced the carbonyl levels associated with eight proteins, including heat shock cognate protein 70, β-actin, and peroxiredoxin 1. It should be noted that these changes were not statistically significant. However, LAC administration in aged rats restored the levels of F-actin capping protein β-subunit, Rab GDP dissociation inhibitor β, and ubiquitin to levels similar to young rats (Poon et al. 2006). The pathways associated with these altered proteins and oxidatively modified proteins in aged rats include antioxidant, mitochondria function, and plasticity. This provides further evidence and is consistent with the notion that LAC may be a suitable candidate for treatment in aging and age-related neurodegenerative diseases in order to reduce oxidative stress, mitochondrial decline, and improve learning and memory deficits that are a result of declines to neuronal plasticity.

23.3.3 Restoration of Cholinergic Functions by LAC

The structural similarity of LAC to acetylcholine potentially allows LAC to have cholinergic benefits such as stabilizing cholinergic neurotransmission (Virmani et al. 2004) and modifying acetylcholine production in the CNS (Carta et al. 1993). Cholinergic neuronal loss is associated with
AD (Davies et al. 1976; Coyle et al. 1983; Wevers et al. 2000), such that primary therapies for AD patients are based on cholinesterase inhibitors (e.g., Aricept®). The exact mechanism by which LAC provides protection in AD brains is not clearly understood, however; several reports provide insights about key pathways. For example, in aged rats and rat models of the aging CNS, LAC increases choline acetyltransferase (ChAT) activity and nerve growth factors (NGF) (Piovesan et al. 1994; Tagliatala et al. 1994). In streptozotocin-treated rats (e.g., a diabetic rodent model), LAC attenuated ChAT activity (Prickaerts et al. 1995). Reductions in ChAT activity, if not attenuated by interventions such as LAC treatment, can result in low levels of acetylcholine, which is important in maintaining normal neurotransmission (Ojika 1998).

23.3.4 LAC OFFERS PROTECTION AGAINST APOPTOSIS

Similar to FA, LAC also has beneficial aspects that are utilized in apoptotic pathways.

In a rat model of peripheral neuropathy, LAC prevents apoptosis induction, reduces cytosolic cytochrome c levels, and impairs caspase 3 protease activity, suggesting that LAC prevents regulated cell death (Di Cesare Mannelli et al. 2007). Scorziello et al. (1997) utilized a derivative of LAC, LAC amide (ST857), to pretreat cerebellar granule cells that were exposed to Aβ (25-35). ST857 improved cell survival, rescued cells from Aβ-induced neurotoxicity, and reduced modified glutamate-induced [Ca2+]i levels (Scorziello et al. 1997). Increased intracellular Ca2+ levels can cause mitochondrial swelling, which results in caspase activation and ultimately leads to apoptotic neuronal cell death. LAC has been reported to improve neuronal survival in cerebellar granule cells possibly by increasing aspartate uptake (e.g., a marker of maturation of glutamatergic neurons) and maintaining functional NMDA receptors (Rampello et al. 1992).

23.3.5 NEUROTROPHIC ACTIONS PROVIDED BY LAC

LAC has also been reported to enhance NGF receptors (Kidd 2005). Growth factors generated in brain cells require similar levels of growth factor receptors in order to maintain a healthy state. Declines in NGF binding capacity have been reported in the HP and basal forebrain regions of aged rats (Angelucci et al. 1988). LAC-treated aged rats had twice the level of NGF binding capacity when compared with non-LAC-treated aged controls (Angelucci et al. 1988). Growth factors and their corresponding receptors have been of much interest as potential therapeutics against neurodegeneration (Kidd 2005); however, when administered peripherally, NGF does not cross the BBB. Thus, LAC because of its ability to cross the BBB may also be a potential therapeutic related to increasing NGF receptors.

23.4 SUMMARY

Investigations of FA and its derivatives and LAC have revealed their tremendous cellular protective benefits and have provided evidence in support of the oxidative stress theory of aging and its relation to age-related disorders. Much of the potential of FA to fight disease is manifested in its ability to combat oxidative stress and its downstream consequences, such as damage to cellular materials, inflammation, and cell death. LAC also fights oxidative stress and cell death in addition to mitochondrial damage, cholinergic dysfunction, and neurotrophic mediation. Both compounds are able to influence protein expression in antioxidant and stress-related pathways, which are key to maintaining redox balance in the cell and ultimately brain. FA and LAC administration appear to be potential therapeutic antioxidant interventions to treat conditions predicated by oxidative stress; for example, aging, age-related neuronal conditions like AD and PD, and other age-related conditions such as diabetes. Because FA and LAC are naturally occurring compounds, they can be easily incorporated into clinical trials in aging and neurodegenerative studies. As the search for the cure/treatment of human disease continues, FA, LAC, and other antioxidants deserve substantial attention as potential therapeutics and neuroprotectants.
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REFERENCES


Acetyl-l-Carnitine and Ferulic Acid Action in Aging and Neurodegenerative Diseases


Joshi, G., M. Perluigi, R. Sultana, R. Agrippino, V. Calabrese, and D. A. Butterfield, In vivo protection of synaptosomes by ferulic acid ethyl ester (FAEE) from oxidative stress mediated by 2,2-azobis(2-amidino-propane)dihydrochloride (AAPH), or Fe(2+)/H(2)O(2): Insight into mechanisms of neuroprotection and relevance to oxidative stress-related neurodegenerative disorders. Neurochem Int, 48(4), 318–27. 2006.


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