Possible Mechanisms for the Role of Dietary Supplements in Management of Alzheimer’s Disease in Aged Rats

1Aziza B. Shalby, 2Magdy N. Ashour and 1Hanaa H. Ahmed

1Hormones Department, National Research Centre, Dokki, Cairo, Egypt.
2Medical Biochemistry Department, National Research Centre, Dokki, Cairo, Egypt.

ABSTRACT

Alzheimer’s disease (AD) is now the most common cause of dementia in the elderly. This study aimed to explore new markers for AD as total homocysteine (tHcy), insulin, insulin like growth factor-1 (IGF-1), interleukin-1β (IL-1 β) and tumor necrosis factor-α (TNF-α); to determine the modulatory effects of vitamin E (VE), acetyl-L-carnitine (ALC) and α-lipoic acid (LA) on the investigated parameters and to evaluate the possible mechanisms for the role of these nutraceutical in regression of AD that is induced in female rats. Our results revealed that brain acetylcholine esterase (AChE) activity and tHcy levels were significantly increased in AD model. Folic acid, vitamin B12 levels and Na+/K+ ATPase activity were markedly reduced. Plasma insulin and IGF-1 levels were noticeably decreased while plasma TNF-α and IL-1β concentrations were significantly increased, indicating that abnormal inflammatory response is associated with AD. Treatment by VE, ALC or LA restored the above mentioned parameters to nearly normal levels comparable to those achieved by donepezil. These findings indicate that tHcy, insulin, IGF-1, IL-1β and TNF-α may be considered as new biomarkers for AD. The present study sheds light on the potential restoring effects of VE, ALC and LA in AD model with special focus on the possible mechanisms. Our study provides evidence for the importance of dietary supplements in delaying the progression of age-related neurodegenerative diseases.

Key words: Alzheimer's disease, biomarkers, Vitamin E, Acetyl-L-carnitine, α-lipoic acid.

Introduction

Alzheimer’s disease (AD) is the most prevalent neurodegenerative disorder, afflicting 10% of the population over the age of 65 and 50% of the population over the age of 85 (Zhang et al., 2011). AD pathology is characterized by the progressive accumulation of senile plaques (consisting of amyloid β-peptide, Aβ) and neurofibrillary tangles (consisting of aggregates of the microtubule-associated protein tau) (Readnower et al., 2011).

Amyloid β-peptide (Aβ) stimulates the glial and microglial production of interleukins and other cytokines, leading to an ongoing inflammatory cascade and contributing to synaptic dysfunction and loss, which lead to neuronal death. Inflammatory pathways involving interleukin and cytokine signaling might suggest potential targets for intervention and influence the development of novel therapies to circumvent synaptic and neuronal dysfunction ultimately leading to AD neurodegeneration (Weisman et al., 2006). Proinflammatory cytokines may trigger or accelerate ongoing neurodegenerative processes in mice (Cunningham et al., 2009) and high levels of tumor necrosis factor-α receptors are present in the cerebrospinal fluid of patients with mild cognitive impairment (Buchhave et al., 2008).

Epidemiological data have indicated an association between plasma insulin and insulin like growth factor-1 and the prevalence of neurological disorders (Torres-Aleman, 2008 and Jacobsen et al., 2010). Both insulin and IGF-1 modulate neuronal growth, survival, differentiation, metabolism, gene expression, protein synthesis, cytoskeletal assembly and synapse formation. In addition, they regulate myelin production as well as they are involved in higher brain functions including cognition. Correspondingly, impaired signaling through insulin/IGF-1 adversely affects a broad range of neuronal and glial functions (Carro and Torres-Aleman, 2004). Furthermore, epidemiology and clinical investigations have demonstrated that the elevated plasma homocysteine
has also been implicated as a strong and independent risk factor for the development of dementia and AD (Seshadri et al., 2002 and Ho et al., 2010). Other studies, however, found no association between tHcy and AD (Nilsson et al., 2002 and Mizrahi et al., 2003). Moreover, AD is also associated with a loss of cholinergic neurons resulting in profound memory disturbances and irreversible impairment of cognitive function (Fodale et al., 2006).

Aluminum (Al) neurotoxicity is not caused by a single alteration, but it is probably a result of adverse effects at multiple cellular levels. Studies have shown that Al interacts with the cholinergic system by altering cholinergic projection functioning and also by intensifying its inflammation (Gulya et al., 1990). Al potentiates the ability of iron salts to promote reactive oxygen species (ROS) formation. In addition, Al has been reported to enhance peroxidative damage to lipids and proteins and decreases antioxidant enzyme status (Walton, 2007). AlCl3 has potential to get accumulated in the brain (Good et al., 1992) which was earlier correlated with the degenerative changes. Therefore, Al-induced AD in rat is the most commonly utilized animal model to mimic human AD-like symptoms (Ogasawara et al., 2003, Lukiw et al., 2005 and Garcia et al., 2010).

A growing body of research indicates that nutritional deficiencies contribute to age-related cognitive decline, including AD. Several studies have demonstrated that cognitive performance is subject to dietary compromise and that key dietary supplementation can alleviate, and in some cases, reverse the impact of dietary deficiencies on cognitive performance (Chan et al., 2008). This finding suggests the potential importance of nutritional intervention as therapeutic approaches before definitive diagnosis. Dietary supplements such as lipoic acid and acetyl-L-carnitine possess potent antioxidants and neuroprotectants. They reduce oxidative damage and improve cognitive performance in murine brain (Suchy et al., 2009). Moreover, vitamin E, as a powerful antioxidant, is important for the maintenance of neuronal integrity and brain function. Epidemiological studies indicate that a high intake of vitamin E may contribute to prevention of age-related neurodegenerative disease (Morris et al., 2005). However, most of the studies conducted so far have been limited to clinical surveys, prospective studies and autopsy-based research. Therefore, there is a need for experimental in vivo data.

The current study had triple purposes, firstly, it aimed to explore new biomarkers for AD in rat model, secondly, it was designed to determine the modulatory effects of superior antioxidants, vitamin E, α-lipoic acid and acetyl-L-carnitine on the studied markers and thirdly, to evaluate the possible role of these nutraceuticals in the regression of AD compared with donepezil, a well known acetylcholinesterase inhibitor (ACHEI), as a reference drug.

Materials and Methods:

I) Materials:

A) Chemical, Drug and Nutraceuticals:

i) Aluminum chloride (AlCl3) was purchased from BDH Laboratory Supplies Poole, BH15, TD, England.
ii) Donepezil was manufactured by Pfizer, Egypt, S.A.E. Cairo, A.R.E. under authority Pfizer INC. U.S.A.
iii) Vitamin E was produced by Pharco Pharmaceuticals, Alexandria, Egypt.
iv) Acetyl-L-carnitine (ALC) was purchased from Sigma Company USA.
v) Alpha-lipoic acid (LA) was provided by Eva Pharma for Pharmaceutical and Medical Appliances Company, Cairo, Egypt.

B) Experimental Animals:

Aged female Sprague Dawley rats (14-16 months) weighing 250-300g were obtained from the Animal House Colony of the National Research Centre, Cairo, Egypt and acclimated in a specific pathogen free barrier area where the temperature (25±1°C). Rats were controlled constantly with 12h light/dark cycle. They were individually housed with adlibitum access to standard balanced diet and water. Animals received human care in compliance with the guidelines of the Ethical Committee of Medical Research of National Research Centre, Egypt.

i) Experimental Design:

Rats were randomly assigned into six groups, each had eight rats. First group included rats that were orally administered vehicle (saline) for seven months and served as normal control. Second group was orally provided with AlCl3, in a dose of 100 mg/kg b.wt/day for four months (Dave et al., 2002), then with vehicle for three
months and served as positive control or AD group. Third group in which rats were given AlCl₃ daily for four months then they were orally treated with 2mg/kg/day of AChEI (donepezil) for other three months (Janowsky et al., 2004). Fourth group included rats which were given AlCl₃ for four months then they were orally treated with vitamin E 150 mg/kg/day (McDaid et al., 2005) for 3 months. Fifth group had rats given AlCl₃ for four months then they were orally administered acetyl-L-carnitine 100mg/kg/day (Binienda et al., 2004) for 3 months. The sixth group included rats that were orally administered AlCl₃ for four months then they were orally treated with α - lipoic acid 100mg/kg/day (Aguirre et al., 1999) for 3 months.

At the end of experimental period (seven months), fasting blood samples were collected from retro-orbital venous plexus under light anesthesia, in heparinized tubes, and then centrifuged under cooling at 800xg for 15 min. Plasma was separated and stored at -80°C until analysis of insulin, insulin like growth factor, tumor necrosis factor, and interleukin-1β levels.

After blood collection, brains were rapidly dissected, thoroughly washed with isotonic saline, and dried. Each brain was mid-saggitally divided into two portions. The first portion was fixed in formalin buffer for histopathological investigation. While the second portion was weighed and homogenized immediately to give 10% (w/v) homogenate in ice-cold medium containing 50 mM tris HCl and 300mM sucrose. The homogenate was centrifuged under cooling at 800xg for 10 min. The supernatant (10%) was used for determination of AChE, and Na⁺/K⁺-ATPase activities, folic acid, vitamin B₁₂, total homocysteine and total protein concentrations.

II) Methods:

i) Biochemical Analyses:

For evaluation of plasma insulin concentration, DRG insulin ELISA kit was used (Frier et al., 1981). Also, plasma IGF-1 level was estimated using DRG highly sensitive colorimetric sandwich ELISA Kit (Blum et al., 1993) after acidification and neutralization of samples by 1N HCl and 1N NaOH, respectively. Plasma levels of tumor necrosis factor-α and interleukin-1 beta were estimated using TNF-α and IL-1β ELISA kits (Idriss and Naismith, 2000 and Dinarello, 1994) respectively. Besides, brain cholinesterase activity was determined colorimetrically using Quimica Clinica Aplicada S.A. kit according to the method described by Blawen et al. (1983). While brain Na⁺/K⁺ ATPase activity was evaluated chemically by measuring the released inorganic phosphorus from the hydrolysis of ATP colorimetrically, according to the method of Tsakiris et al. (2004). Furthermore, quantitative determinations of brain cyanocobalamine (vitamin B12) and folic acid concentrations were carried out by radioimmunoassays (RIAS) kit with the use of Co⁵⁷ and I¹²⁵, respectively (Simul TRAC-SNB; ICN Pharmaceutical, Orangeburg, NY according to Kubasik et al., (1979) procedure. Finally, ELISA technique was used for the evaluation of brain total homocysteine level using Frantzen (1998) method and total protein concentration in the brain homogenate was estimated according to the method of Lowry et al. (1951).

ii) Histopathological Investigation:

Brain samples were fixed in buffered formalin solution for one week, then, were washed in running tap water for 24 hours, dehydrated in ascending series of alcohol (50-90), then in absolute alcohol. The samples were cleared by xylene and immersed in paraffin. The tissues were mounted in blocks and left at 4°C until the time to be used. The paraffin blocks were sectioned at 5 µm thickness and mounted on clean glass slides. The ordinary haemotoxylin and eosin stain was used according to the method of Drury and Wallington (1980).

Statistical Analysis:

Data were presented as mean ± standard error. Statistical differences were assessed by using F-test (one way analysis of variance-ANOVA) using MSTATC version 4 program. Duncan's multiple range tests were used to compare between means of treatments. Probability P<0.05 was considered significant.

Results:

Brain Concentrations of Acetylcholinesterase, Na⁺/K⁺ATPase, Folic Acid, Vitamin B₁₂, and Homocysteine in Control, AD and Nutraceuticals- Treated Groups:

The data in Table (1) revealed that aluminum-administered rats showed significant alteration in brain levels of each of the above mentioned parameters which were modulated by nutraceuticals treatment. Brain AchE
Table 1: Effect of dietary supplements on some biochemical parameters in the brain of AD rat model.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Acetylcholine Esterase (AchE) U/mg protein</th>
<th>Na+/K+ATPase μmol/pH/mg protein</th>
<th>Folic acid mg/mg protein</th>
<th>Vitamin B12 α (tHcy) Pg/mg protein</th>
<th>Homocysteine μmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>518± 29.0 b</td>
<td>7.1± 0.37 b</td>
<td>1.6± 0.08 b</td>
<td>110.3± 2.6 b</td>
<td>5.4± 0.14 b</td>
</tr>
<tr>
<td>Positive control (AD)</td>
<td>906±14.0 b</td>
<td>3.6±0.15 b</td>
<td>0.8±0.04 a</td>
<td>76.7± 2.6 a</td>
<td>10.1±0.01 a</td>
</tr>
<tr>
<td>AD+AChEI</td>
<td>542±18 b</td>
<td>4.3±0.08 a</td>
<td>1.1±0.06 a</td>
<td>84.9±2.2 a</td>
<td>8.2±0.1 a</td>
</tr>
<tr>
<td>AD+vitamin E</td>
<td>709.2±17.6 b</td>
<td>5.1±0.33 a</td>
<td>1.2±0.03 a</td>
<td>92.5±2.3 a</td>
<td>6.5±0.2 a</td>
</tr>
<tr>
<td>AD+ ALC</td>
<td>687± 25.5 a</td>
<td>4.9±0.5 a</td>
<td>1.2±0.05 a</td>
<td>87.9±3.8 a</td>
<td>7.1±0.2 a</td>
</tr>
<tr>
<td>AD+ LA</td>
<td>700±24 a</td>
<td>4.6±0.03 a</td>
<td>1.0±0.02 a</td>
<td>84.6±2.2 a</td>
<td>6.8±0.1 a</td>
</tr>
</tbody>
</table>

Data are presented as mean ± S.E.

Mean values at the same column sharing the same superscript letters are not significantly different (P< 0.05).

*Significant change at p < 0.05 in comparison with control group. **Significant change at p < 0.05 in comparison with AD group.

Activity was markedly increased (906±14.0) in Al-administered group as compared to the control group (518±29.0). The observed 1.75-fold increase in brain AchE represents the first demonstration of Al-intoxication-induced neurodegeneration characteristic to AD in aged rats. Moreover, Na+/K+ATPase activity was significantly decreased (3.6±0.15) in Al-administered group vs (7.1±0.37) for normal control group. It is obvious that 50.7% reduction in ATPase activity is another indicator for Al-induced neurotoxicity. Furthermore, brain folic acid and vitamin B12 levels were significantly reduced (0.8±0.04 and 76.7±2.6) with concomitant marked increase in tHcy level (10.1±0.01) in Al-administered group when compared to control values (1.6±0.08, 110.3±2.6 and 5.4±0.14, respectively). It is conceivable that folic acid and vitamin B12 deficiencies resulted in hyperhomocysteinemia as both are important for tHcy to be changed into methionine.

Treatment of AD group with donepezil (AChEI), vitamin E, acetyl-L-carnitine or α-lipoic acid significantly restored AchE activity (542±18, 709.2±17.6, 687±25.5 and 700±24, respectively) as compared to that of untreated AD group (906±14.0).

Donepezil (AChEI) showed the most pronounced effect, followed by vitamin E, then ALC and finally α-lipoic acid. Na+/K+ATPase was significantly increased in brain AchE by the various treatments (4.3±0.08, 5.1±0.33, 4.9±0.5 and 4.6±0.03) respectively as compared to that (3.6±0.15) in AD group. Moreover, folic acid concentration was significantly increased in donepezil, vitamin E, ALC and LA treatments and reached 1.1±0.06, 1.2±0.03 and 1.2±0.05 and 1.0±0.02, respectively as compared to that (0.8±0.04) in AD group. Besides, vitamin B12 level was markedly increased by the four treatments to be 84.9±2.2, 92.5±2.3, 87.9±3.8 and 84.6±2.2, respectively as compared to that of AD group (76.7±2.6). It is obvious that vitamin E showed the most pronounced effect. By contrast, tHcy concentration was significantly reduced in the different treated groups 8.2±0.1 for donepezil, 6.5±0.2 for vitamin E, 7.1±0.2 for ALC, and 6.8±0.1 for LA as compared to that in AD group (10.1±0.01), indicating that tHcy was relatively transformed to the methionine by the different treatments.

### Plasma Levels of Insulin, Insulin like Growth Factor-1, Tumor Necrosis factor-α and Interleukin-1β in Control, AD and Nutraceuticals Treated Groups.

Insulin concentration was significantly decreased in AD group (8.7±0.30) versus (10.4±0.49) in the normal control group (Table 2) which might result from reduced IGF-1 level (8.9±0.24) in the same group relative to the control value (10.6±0.49). Proinflammatory cytokines, TNF-α and IL-1β were significantly increased in AD group (94.4±0.75 and 2.9±0.06, respectively) versus (81.7±0.95 and 1.61±0.02, respectively) for the normal control group, indicating that there was an abnormal inflammatory response associated to the pathogenesis of AD. On the other hand, various dietary supplement treatments increased insulin level but the increase was significant only with either vitamin E (10.2±0.42) or ALC (9.7±0.18) as compared with that of AD group (8.7±0.30). Moreover, IGF-1 level was significantly elevated in the treated groups with either donepezil (9.9±0.31), vitamin E (10.7±0.37) or ALC (9.9±0.15) compared with that of AD group (8.9±0.24). However, TNF-α level was significantly depleted by the different treatments (89.4±1.1 for donepezil, 89.4±0.56 for vitamin E, 86.3±0.62 for ALC and 91.1±0.66 for LA) versus 94.4±0.75 for AD group. Similarly, IL-1β level was significantly reduced in all treated groups (2.5±0.03 for donepezil, 2.1±0.04 for vitamin E, 2.3±0.06 for ALC and 2.4±0.03 for LA) compared to (2.9±0.06) for AD group. The reduction of TNF-α and IL-1β in all treated groups indicated reduced inflammation.

### Histopathological Investigation:

Photomicrograph of brain section of normal control rats is presented in (Fig.1). It showed highly active nerve cells having huge nuclei with relatively pale stain. The nuclear chromatin and prominent nucleoli were
Table 2: Effect of dietary supplements on some biochemical parameters in plasma of AD rat model.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Insulin U/ml</th>
<th>Insulin like growth factor (IGF-1) ng/ml</th>
<th>Tumor necrosis factor – α (TNF- α) pg/ml</th>
<th>Interleukin-1β (IL-1β) pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>10.4±0.49b</td>
<td>10.6±0.49b</td>
<td>81.7±0.95b</td>
<td>1.61±0.02b</td>
</tr>
<tr>
<td>Positive control(AD)</td>
<td>8.7±0.30a</td>
<td>8.9±0.24a</td>
<td>94.4±0.75a</td>
<td>2.9±0.06a</td>
</tr>
<tr>
<td>AD+AChEI</td>
<td>9.6±0.35</td>
<td>9.9±0.31b</td>
<td>89.4±1.1ab</td>
<td>2.5±0.03ab</td>
</tr>
<tr>
<td>AD+vitamin E</td>
<td>10.2±0.42b</td>
<td>10.7±0.37b</td>
<td>89.4±0.56ab</td>
<td>2.1±0.04ab</td>
</tr>
<tr>
<td>AD+ ALC</td>
<td>9.7±0.18b</td>
<td>9.9±0.15b</td>
<td>86.3±0.62ab</td>
<td>2.3±0.06ab</td>
</tr>
<tr>
<td>AD + LA</td>
<td>9.4±0.21ab</td>
<td>9.7±0.36b</td>
<td>91.1±0.66ab</td>
<td>2.4±0.03ab</td>
</tr>
</tbody>
</table>

Data are presented as mean ± S.E.
Mean values at the same column sharing the same superscript letters are not significantly different (P< 0.05)
*Significant change at p < 0.05 in comparison with control group. aSignificant change at p < 0.05 in comparison with AD group.

Microscopic investigation of brain section of AD rats showed necrosis of the brain and the nerve cells and their nuclei lost normal structure and outlines (Fig. 2).

Microscopic examination of brain section of AD rats treated with donepezil (Fig. 3), vitamin E (Fig. 4), acetyl-L-carnitine (Fig. 5) and α-lipoic acid (Fig. 6) showed many neurons with normal structure and some neurons appeared to be necrotic indicating partial regeneration of the neurons in the brain.

Fig. 1: Photomicrograph of brain section of control female rat showing highly active nerve cells having huge nuclei with relatively pale-stained. The nuclear chromatin and prominent nucleoli are dispersed. The surrounding relatively inactive supporting cells have small nuclei with densely-stained, condensed chromatin and no visible nucleoli (H & E X 400).

Fig. 2: Photomicrograph of brain section of female AD rat shows necrosis of the brain. The normal structure and the outlines of the nerve cells and their nuclei are lost. Some neuron appear like ring shape (H & E X 400).
Fig. 3: Photomicrograph of brain section of female AD rat and treated with donepezil shows many neurons with normal structure and some neurons appeared to be necrotic (H & E X 400).

Fig. 4: Photomicrograph of brain section of female AD rat and treated with vitamin E shows many normal neurons. Some neurons accumulate abnormal filament forming flame-like shape (H & E X 400).

Fig. 5: Photomicrograph of brain section of female AD rat and treated with L-carnitine shows the appearance of normal neurons. Some neuron accumulate abnormal filament forming flame shape (H & E X 400).
Fig. 6: Photomicrograph of brain section of female AD rat and treated with $\alpha$-lipoic acid shows the normal structure of the neurons that appear more or less as control one (H & E X 400).

Discussion:

Aluminum (Al) exposure has been reported to be a risk factor for Alzheimer's disease, although the role of Al in the etiology of Alzheimer's disease remains controversial (Yumoto et al., 2009). It is well established that aluminum is a neurotoxic agent inducing production of free radicals in the brain. The accumulation of free radicals may represent the main cause of the neurodegenerative events such as Alzheimer's disease (Garcia et al., 2010). Moreover, exposure to high levels of aluminum leads to formation of amyloid plaques and intraneuronal neurofilamentous aggregates that are tau positive in human brain (Bharathi et al., 2008; Walton and Wang, 2009).

Our emerging data revealed that chronic administration of Al produced significant elevation of AChE activity while the activity of Na$^+$/K$^+$ ATPase was markedly inhibited in brain tissue as compared to the normal control. These findings are in consistent with the previous studies of Yu (2003) and Virgilia et al. (2007).

Donepezil, (potent AChEI) used for treatment of AD, is thought to have a neuroprotective effect in AD patients. Because a deficit in cholinergic neurotransmission is a major feature in AD, and Aβ accumulation is a main causative phenomenon-induced neuronal cell injury in AD (Kimura et al., 2005), donepezil has been proposed to have a bifunctional role to alleviate neuronal injury in AD patients. This could be achieved not only by improving cholinergic function, but also by inhibiting progressive neurodegeneration in AD. Therefore, treatment of AD rats with donepezil in the current study showed marked decrement in AChE activity which is the main target for this drug. The considerable improvement in the other tested biochemical parameters could be attributed to the inhibitory action of donepezil on Aβ itself.

Na$^+$/K$^+$-ATPase activity has been found to be decreased in AD patients platelet membrane. This might result from subconformational changes in the protein (Fu et al., 2009). Moreover, it has been demonstrated that the increased nitrite radicals levels plays a role in lowering Na$^+$/K$^+$-ATPase activity (Arianna et al., 2006). As a consequence of decreasing nitrite radicals by vitamin E, Na$^+$/K$^+$-ATPase activity could be increased. Vitamin E could inhibit the release of proinflammatory cytokine IL-1β via inhibition of 5-lipoxygenase pathway (Devaraj and Jialal, 1999). Vitamin E therapy has been shown to decrease the release of TNF-α in addition to IL-1β (Ayasolla et al., 2004). Chen (2004) reported that vitamin E affected brain cholinesterase configuration. Insulin/IGF-1 levels were elevated due to vitamin E supplementation in the current study. This could be explained via reducing oxidative stress, thus improving pancreatic β cells function and increasing insulin production with consequent improvement in IGF-1 level.

Our data revealed that brain homocysteine (tHcy) level was significantly increased while vitamin B$_{12}$ and folic acid concentrations were markedly declined as a result of supplementation with vitamin E. These findings are in agreement with those of Li et al. (2008) and Ho et al. (2010). Elevated tHcy levels have been shown to be associated with an increased risk for dementia and AD. Several reports indicated an inverse association between tHcy levels and simultaneously assessed cognitive function (Morris et al., 2001).

Carnitine is an essential intracellular constituent synthesized from peptide-bound lysine in liver, brain and kidney (Kaminska et al., 1993). It is involved in the beta oxidation of fatty acids by facilitating the transport of long chain fatty acids across the mitochondrial membrane. In the brain, L-carnitine and ALC have important roles in cerebral bioenergetics and in neuroprotection through different mechanisms including: (1) their...
antioxidant properties, (2) the modulation and promotion of synaptic neurotransmission, most notably cholinergic neurotransmission and (3) their ability to enhance neuronal metabolism in mitochondria.

Astrocytes, the immune cells in the brain, are able to produce large amounts of ketone bodies that are thought to supply adjacent neurons with easily transferable substrate for generation of energy. L-carnitine uptake mechanism is the rate-limiting step in astrocyte ketogenesis (Inazu and Matsumiya, 2008). Treatment with ALC has been found to be a powerful inhibitor for AChE (Svoboda et al., 2005). In addition, ALC is a potent antioxidant that prevents the decrease in endogenous antioxidant enzymes and scavenges free radicals (Binienda and Virmani, 2003). ALC acts via several mechanisms, inducing regeneration of injured nerve fibres, reducing oxidative stress, supporting DNA synthesis in mitochondria, and enhancing nerve growth factor concentrations in neurons (Vanotti et al., 2007). There is a possible mechanism for the role of ALC in improving the biochemical markers measured in the present study. The finding of significant modulation in plasma levels of insulin, IGF-1, vitamin B₁₂, folic acid and tHcy levels in ALC treated group is in agreement with that of Ahmed (2010).

Lipoic acid acts as a powerful micronutrient with diverse pharmacologic and antioxidant properties that might act to increase the production of ACh or as chelator of redox active metals or even to combat the accumulation of lipid peroxidation products (Holmquist et al., 2007). It also protects neuronal cells through cell signaling mechanisms including the extracellular signal related kinase pathways which are dysregulated in AD (Zhu et al., 2004). Modulating these pathways led to control the inflammation and increased Na⁺/K⁺ ATPase activity. Besides, LA targets the mitochondria, the most affected organelle responsible for AD, thus providing the strong protection (Siedlak et al., 2009). According to the anti-inflammatory effect of α-lipoic acid based on similar findings by Ahmed (2010), treatment of AD group with α-lipoic acid leads to modulation in plasma levels of insulin, IGF-1, vitamin B₁₂, folic acid and tHcy levels. Our present biochemical results were well confirmed by histopathological examination of the brain which showed that the treatments by vitamin E (Ayasolla et al., 2004), α LA (Zhang et al., 2001) or ALC (Gonzalez-Perez et al., 2002) showed marked reduction in the neurodegeneration with observable improvement in neuronal and regeneration features.

In summary, the present results further indicated the role of metabolic disturbance in the etiology of AD so that some bioindicators could be used as markers for AD such as tHcy, insulin, IGF-1, and proinflammatory-cytokines as TNF-α and IL-1β. In addition, this study potentially demonstrated the possible mechanisms for the role of some nutraceuticals as neuroprotectant agents in modulating the metabolic disturbance in AD disease model. Therefore, these dietary supplements could have a powerful value in the treatment of AD or even in the regression of this disease. The effects of the present three agents vitamin E, ALC and LA were comparable and equivalent in action and they have superior influence over the conventional donepezil drug.

Acknowledgment

The authors express deep appreciation to Dr Abdel Reazik Farrage, Assistant professor of histology and histochemistry, Department of pathology at the National Research Centre for his kind cooperation in the histopathological demonstrations involved in this research.

References


