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The protective role of DL-α-lipoic acid in biogenic amines catabolism triggered by Aβ amyloid vaccination in mice

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Abstract

The major pathological consequence of Alzheimer disease (AD) is accumulation of β-amyloid (Aβ) peptide fibrillar plaque in the brain and subsequent inflammatory reaction associated with the surrounding cells due to the presence of these aggregates. Inflammation is the major complication associated with Aβ peptide vaccination. Aβ peptide activated T-helper cells are shown to enhance the existing-inflammatory conditions in the brain and other organs of AD patients. Hence systematic studies on potential approaches that will prevent inflammation during the vaccination are highly desired. DL-α-Lipoic acid (LA), an antioxidant with known function as cofactor in mitochondrial dehydrogenase reactions, will be a good candidate to annul the oxidative damage due to vaccination triggered inflammation. For the first time, levels of principal neurotransmitters and their major metabolites in hippocampus and neocortex regions of brain are quantified to find out the level of inflammation. We have used high performance liquid chromatography with electro chemical detection (HPLC–EC) for monitoring neurotransmitter levels. We have shown a significant ($p < 0.05$) reduction of 5-hydroxytryptamine (5-HT), dopamine (DA) and norepinephrine (NE) in the systemic inflammation induced (SI), vaccinated (VA) and inflammation induced vaccinated (IV) mice. Nevertheless their metabolites such as 5-hydroxyindole acetic acid (5-HIAA) and homovanillic acid (HVA) are significantly ($p < 0.05$) increased when compared with control. Interestingly, antioxidant LA treated mice with systemic inflammation (IL), vaccinated (VL) and inflammation induced vaccinated (IVL) mice exhibited enhanced level of 5-HT, DA and NE and the concentration of 5-HIAA and HVA gradually returned to normal. These results suggest a possible new way for monitoring and modifying the inflammation and thereby preventing Aβ vaccination mediated tissue damage.

Keywords: β-Amyloid fibril; Inflammation; Antioxidant; 5-Hydroxytryptamine; Dopamine; Norepinephrine; Neocortex; Hippocampus; Oxidative stress; Inflammation

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenarative disease that involves aggregation and deposition of misfolded proteins such as β-amyloid (Aβ) peptide [1,39] leading to activation of glia (gliosis) prior to the deposition of amyloid plaques and formation of neurofibrillary tangles in degenerated neurons that ascend from the basal forebrain to cortical and hippocampal areas [19]. The search for AD pathogenic factors is hindered by the lack of diagnostic certainty in life and by the heterogeneity of the disease [40]. Recent reports suggest that perturbations in systems using the excitatory amino acid L-glutamate (L-Glu) may underlie such pathogenic mechanisms of AD [22,23].

Along with L-Glu, biogenic amines viz., 5-hydroxytryptamine (5-HT), dopamine (DA), and norepinephrine (NE) have been identified as the principal transmitters mediating fast excitatory synaptic responses in the vertebrate central nervous system (CNS) and have widespread influence on the cortex, basal ganglia, limbic

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areas and spinal cord. They play an important role in regulation of brain functions: memory, mood, movement, behavior and autonomic functions such as blood pressure regulation. These functions are impaired in Alzheimer's disease (AD) due to loss of the catecholamines (CA) neurons, which regulate the brain functions [45]. The major and well-documented concept in neurodegenerative disorder is the deficiency of the cholinergic system due to the progressive loss of cholinergic presynaptic neurons situated in the basal fore brain that leads to structural and functional damage of monoaminergic systems [7,8]. The neurodegenerative disease condition can be induced by different insults that have been related to systemic oxidative stress such as inflammatory stimulus [47].

Lemere et al. [26] demonstrated that intranasal immunization with freshly solubilized Aβ 1–40 reduced cerebral amyloid burden in PDAPP mouse (PDAPP mouse is a model of familial Alzheimer's disease in which mice express the human V717F mutation in the amyloid precursor protein (APP) gene under the control of the platelet derived (PD) growth factor-beta promoter). The Antibodies formed against Aβ might stimulate Aβ clearance by stimulating Fc-receptor-mediated phagocytosis of Aβ by microglia [4]. In addition antibodies against Aβ might act as an artificial chaperone for extra cellular amyloid-β and prevent aggregation of amyloid β by binding to epitopes [13]. However, phase II clinical trial with the immunization of AD patients with amyloid peptides has recently ended prematurely because of cerebral hemorrhage due to inflammation observed in several patients [11]. Neocortex and hippocampus are susceptible to neurodegeneration in response to an external insult such as infection and/or oxidative stress that leads to disruption of the neuronal circuitry of the hippocampus and neocortex cerebral areas [12,20].

In the past decade an extraordinary progress has been made to understand the toxicity of Aβ peptide. Presently, compounds targeting Aβ vaccination hold much promise as therapy for AD. Therapies that decrease Aβ production, enhance its degradation or cause its clearance from CNS are the two major therapeutic ventures being pursued by various group of researchers. One of the most promising approaches towards the Aβ clearance is the use of immunotherapies, including vaccination with Aβ fragments [46]. However, inflammation is the limiting factor in therapy due to the sudden increase in the soluble Aβ peptide in the peripheral and brain tissues on vaccination.

DL-α-Lipoic acid (LA) and its reduced form, dihydrolipoic acid (DHLA) are natural potent antioxidants and cofactors of mitochondrial decarboxylation enzymatic reactions and essential for an adequate ATP production through citric acid cycle [36]. They are involved in different multi enzyme complexes such as pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, branched chain α-ketoacid dehydrogenase and glycine decarboxylase complex [37]. LA, a low molecular substance which is absorbed and able to cross blood brain barrier [32] in animals, and preliminary human studies indicate that LA has neuroprotective effects on Aβ mediated cytotoxicity and may be effective in a number of neurodegenerative diseases [33].

The antioxidant therapy along with Aβ vaccination reduces the inflammation associated with Aβ vaccination. Our preliminary biochemical and histological investigation shows that LA therapy reduces the oxidative vulnerability of Aβ vaccination and that it is a good candidate in inflammatory AD models where active immunization is administered [35]. This study was focused on the quantitative analysis of central nervous system neurotransmitters (DA, NE and 5-HT) and its metabolites (HVA and 5-HIAA) in neocortex and hippocampus by the aid of isocratic RP–HPLC system with electrochemical detection. We have used one of the important antigenic fragment of $A\beta_{25–35}$ as peptide vaccination candidate which exhibit all the biological activity of the full length Aβ [16,43] and is used by other researchers to study the neuronal toxicity and oxidative vulnerability extensively [18].

2. Materials and methods

Six- to eight-week-old Swiss strain male mice weighing 25–30 g, were obtained from Tamilnadu Veterinary and Animal Sciences University, Madhavaram, Chennai, Tamilnadu, India. The animals were maintained in the Poly acrylic cages under hygienic conditions at ambient room temperature (28–30 °C) on a 12 h light/dark cycle. The animals had free access to water. They were fed with commercial pellet diet (Hindustan Lever Ltd., Bangalore, India) ad libitum. All experimental conditions were in accordance with the NIH guidelines for the use and care of laboratory animals and following approval by the animal ethical committee of Central Leather Research Institute.

2.1. Experimental design

The animals were divided in to seven groups, consisting of six each and were subjected to the following treatments:

2.1.1. Control group

Mice were given physiological saline (0.5 ml) subcutaneously.

2.1.2. Systemic inflammation group (SI)

Mice were given single subcutaneous injection of 0.5 ml 2% silver nitrate. The dosage was fixed on the basis of information available from the report of Lindhorst et al. [27].

2.1.3. Aβ vaccinated group (VA)

Mice were given a single intraperitoneal injection of $A\beta_{25–35}$ (25 μg) dissolved in 0.5 ml of sterile deionized water which was previously incubated at 37 °C for 24 h before administration. The dosage was fixed on the basis of information available from the report of Spooner et al. [42].

2.1.4. Systemic inflammation along with Aβ vaccinated group (IV)

Mice were given single intraperitoneal injection of $A\beta_{25–35}$ after 24 h of subcutaneous injection of 2% silver nitrate.

2.1.5. Systemic inflammation and LA therapy group (IL)

Mice were given single subcutaneous injection of 0.5 ml 2% silver nitrate followed by daily intraperitoneal injection of LA (100 mg kg^{-1} body weight) for 4 days. The dosage was fixed as per our earlier investigation [35].

2.1.6. Aβ vaccinated and LA therapy group (VL)

Mice were given a single intraperitoneal injection of $A\beta_{25–35}$ followed by daily intraperitoneal injection of LA after every 24 h for 4 days at the prescribed dose levels mentioned earlier.

2.1.7. Systemic inflammation along with Aβ vaccinated and LA therapy group (IVL)

Mice were administered with $A\beta_{25–35}$ (i.p.) after 24 h of subcutaneous injection and of 2% silver nitrate and subsequently treated with LA daily for 7 days at the dose level prescribed earlier.

2.2. Chemicals and treatment

DL-α-LA was dissolved in 6.7 mM Tris hydrochloride at 45 °C and neutralized to pH 7.4 with 0.1 M sodium hydroxide at 37 °C. The cooled solution was filtered through a 0.25 μm Millipore membrane [29]. The filtered solution was administered intraperitoneally to the experimental animals. Control animals received 0.5 ml of physiological saline. The standards for 5-HT, DA, NE, 5-HIAA and HVA were purchased from Sigma chemical company (St. Louis, MO, USA).

2.3. Synthesis and purification of $A\beta_{25–35}$

$A\beta_{25–35}$ peptide was synthesized by solid phase synthesis and purified by RP–HPLC as described earlier [10]. The peptide was characterized by MALDI–TOF Mass spectrometry (Theoretical molecular weight 1060.4 Da; experimental molecular weight 1060.2 Da). Scanning was performed from mass/charge (m/z) 500–2000. At least 10 scans were summed before transformation mass of the peptides. The peptide was then characterized by ^{1}H-NMR (data not shown). The $A\beta_{25–35}$ fibrils were formed by solubilizing the peptide in sterile de-ionized water (1mg/ml) and incubated for 24 h at 37 °C [31].

2.4. Sample preparation

Sample preparations were carried out according to the procedure of Kim et al. [24]. Dissected out hippocampus and neocortex were weighed before homogenizing at 4 °C with perchloric acid (0.1 M) in Teflon/glass homogenizer. The homogenate was centrifuged at 4 °C for 20 min at 20,000 × *g*. The supernatant was collected and filtered through 0.2 μm Acrodisc filter (Pall Gelman, USA) before injecting into the HPLC system using Hamilton syringe for the estimation of neurotransmitters.

2.5. Preparation of standard solution

Standard solutions of DA, 5-HT, NE, HVA, 5-HIAA and dihydroxy benzylamine (DHBA, internal standard) each 2.5 ng/ml concentrations were prepared in 0.17 M perchloric acid, which was previously filtered and degassed. These solutions were stored at −70 °C and freshly prepared every 4 weeks. The prepared standard solution was filtered through 0.2 μm Acrodisc filter (Pall Gelman, USA) before use. Different concentrations were injected by maintaining the volume of injection at 20 μl and a standard graph was prepared in order to quantify sample values after authenticating the retention time of individual neurotransmitters. Percent recovery was also calculated and accordingly the sample values were adjusted. The entire protocol, which includes storage, sample preparation and standards preparation, involved only polypropylene disposable wares.

2.6. Mobile phase

Citric acid (mol. wt. 210.14; 32 mM; 13.448 g), Na_2PO_4 (mol. wt. 141.96; 3.54 g), EDTA (mol. wt. 372.24; 0.037 g) and octyl sodium sulphate (Sigma, USA, 0.236 g) were added to 2 l of triple distilled water and mixed well. The pH of above solution was adjusted to 4.2. To this 265 ml of methanol was added and the entire solution was filtered using 0.2 μm nylon filter (Pall Gelman, USA) and then degassed just before use.

2.7. HPLC–EC detection of neurotransmitters

HPLC–EC detection of standard for DA, 5-HT, NE, HVA and 5-HIAA was made and we maintained the following working conditions: isocratic elution; mobile phase (32 mM citrate buffer in methanol with EDTA and octyl sodium sulphate); Phenomenex column (Reverse phase C_{18}, particle size 3 μm, 250 mm × 4.6 mm); Flow rate 1 ml/min; Glassy carbon working electrode (+0.49 V versus Ag/AgCl electrode). The level of neurotransmitters is expressed as ng/g wet tissue.

3. Statistical analysis

Data are expressed as mean ± S.E.M. for six animals in each group. Statistical analysis of variance (ANOVA) followed by the Tukey's test was applied to determine the significant differences among the groups. *p* values less than 0.05 were considered significant.

Table 1
Changes in the level of biogenic amines and metabolites in the neocortex and hippocampus regions after inflammatory stimulus following LA therapy

Parameters	Neocortex		Hippocampus	
	1st day	4th day	1st day	4th day
5-HT				
Control	1506 ± 91	1567 ± 86	1388 ± 69	1439 ± 74
SI	1050 ± 47*	1151 ± 48*	1041 ± 52*	1049 ± 46*
IL	1214 ± 42*	1486 ± 66	1151 ± 61*	1337 ± 52
DA				
Control	1933 ± 89	2060 ± 68	1844 ± 66	1891 ± 90
SI	1449 ± 57*	1545 ± 72*	1383 ± 74*	1394 ± 71*
IL	1680 ± 91*	1944 ± 94	1614 ± 60*	1907 ± 65
NE				
Control	227 ± 21	248 ± 17	995 ± 48	1021 ± 84
SI	140 ± 19*	178 ± 14*	646 ± 35*	765 ± 38*
IL	162 ± 18*	234 ± 25	861 ± 40	964 ± 90
5-HIAA				
Control	756 ± 75	804 ± 70	45 ± 6	53 ± 5
SI	1067 ± 62*	1031 ± 65*	81 ± 5*	83 ± 6*
IL	895 ± 48*	819 ± 59	63 ± 4*	46 ± 9
HVA				
Control	933 ± 78	987 ± 71	854 ± 62	913 ± 60
SI	1266 ± 60*	1380 ± 89*	1292 ± 80*	1196 ± 58*
IL	1130 ± 55*	955 ± 68	1095 ± 75*	1027 ± 74

DA: dopamine, LA: lipoic acid, HVA: homovanillic acid, 5-HT: 5-hydroxytryptamine, 5-HIAA: 5-hydroxyindoleacetic acid, NE: norepinephrine.
* Significance ($p < 0.05$) as compared to control group; values are mean ± S.E.M.; ng/g wet tissue wt.

Table 2
Changes in the level of biogenic amines and metabolites in the neocortex and hippocampus regions in the mice after Aβ vaccination following LA therapy

Parameters	Neocortex		Hippocampus	
	1st day	4th day	1st day	4th day
5-HT				
Control	1506 ± 91	1567 ± 86	1388 ± 69	1439 ± 74
VA	878 ± 73*	920 ± 69*	902 ± 77*	969 ± 65*
VL	1127 ± 65*	1497 ± 78	1092 ± 55*	1368 ± 91
DA				
Control	1933 ± 89	2060 ± 68	1844 ± 66	1891 ± 90
VA	1256 ± 79*	1337 ± 75*	1198 ± 84*	1268 ± 71*
VL	1413 ± 65*	1952 ± 57	1314 ± 50*	1740 ± 82
NE				
Control	227 ± 21	248 ± 17	995 ± 48	1021 ± 84
VA	137 ± 12*	154 ± 14*	646 ± 55*	663 ± 36*
VL	173 ± 13*	232 ± 14	802 ± 49*	930 ± 68
5-HIAA				
Control	756 ± 75	804 ± 70	45 ± 6	53 ± 5
VA	1088 ± 57*	1112 ± 61*	94 ± 8*	89 ± 9*
VL	972 ± 46*	865 ± 42	72 ± 6*	56 ± 7
HVA				
Control	933 ± 78	987 ± 71	854 ± 62	913 ± 60
VA	1259 ± 40*	1298 ± 67*	1287 ± 58*	1302 ± 58*
VL	1174 ± 51*	948 ± 45	1145 ± 49*	957 ± 65

DA: dopamine, LA: lipoic acid, HVA: homovanillic acid, 5-HT: 5-hydroxytryptamine, 5-HIAA: 5-hydroxyindoleacetic acid, NE: norepinephrine.
* Significance ($p < 0.05$) as compared to control group; values are mean ± S.E.M.; ng/g wet tissue wt.

4. Results

HPLC–EC technique was employed to measure biogenic amines and their metabolites of control and experimental mice. The endogenous content of monoamines (5-HT) and catecholamines (DA, NE) was found to be heterogeneously distributed in the neocortex and hippocampus of saline treated control animals (Tables 1–3). Among the biogenic amines examined in the neocortex area of control mice, the level of DA was found to be at highest concentration followed by 5-HT and NE. Although similar trend was shown in hippocampal area, the level of NE was approximately four-fold higher as compared to neocortex region of control mice. In case of metabolites of biogenic amines, the neocortex region of control mice showed was higher level of HVA than its counterpart in the hippocampal area, however, this increase is only marginal. Interestingly, the concentration of 5-HIAA in the hippocampus region of control mice was nearly 15-fold lower than the observed level of 5-HIAA in neocortex region.

5. The efficacy of LA on the biogenic amines in systemic inflammation induced mice

Recently, we demonstrated that SI induced by silver nitrate leads to accumulation of reactive oxygen species (ROS) and lipid peroxidation (LPO) in the lymphocytes, hepatocytes, astrocytes of mice [17]. In the current study, the efficacy of LA therapy in SI induced mice was assessed by the level of biogenic amines. The neurochemical responses in the neocortex and hippocampus during SI are depicted in Table 1. Result shows a significant ($p < 0.05$) depletion in the level of 5-HT, DA, and NE in the neocortex and hippocampus of SI group when compared to control. In contrast, their metabolites HVA and 5-HIAA were significantly ($p < 0.05$) higher as compared to control group. Treatment with LA significantly ($p < 0.05$) reduced the biogenic amines catabolism triggered by SI in the neocortex and hippocampus after 4th day.

6. The efficacy of LA on biogenic amines in Aβ immunized mice

Fu et al. reported that catecholamines exacerbate oxidative stress induced by Aβ with mitochondrial dysfunction and perturbed calcium homeostasis [14]. In the present study, we monitored the protective effects of LA on biogenic amines catabolism induced by Aβ. Result shows that Aβ significantly ($p < 0.05$) impair the levels of DA, NE and 5-HT with an increase in their metabolites in the neocortex and hippocampus on 1st day as shown in Table 2. However, LA therapy restored Aβ depleted biogenic amines catabolism on 4th day.

Table 3
Changes in the level of biogenic amines and metabolites in the neocortex and hippocampus regions after Aβ vaccination along with systemic inflammatory stimulus following LA therapy

Parameters	Neocortex		Hippocampus	
	1st day	7th day	1st day	7th day
5-HT				
Control	1506 ± 91	1567 ± 86	1388 ± 69	1439 ± 74
IV	756 ± 67*	812 ± 55*	694 ± 56*	735 ± 66*
IVL	1096 ± 58*	1472 ± 72	1084 ± 71*	1350 ± 61
DA				
Control	1933 ± 89	2060 ± 68	1844 ± 66	1891 ± 90
IV	968 ± 65*	1036 ± 57*	921 ± 70*	922 ± 82*
IVL	1218 ± 74*	1897 ± 96	1018 ± 62*	1795 ± 69
NE				
Control	227 ± 21	238 ± 17	995 ± 48	1021 ± 84
IV	114 ± 14*	120 ± 15*	490 ± 31*	511 ± 49*
IVL	157 ± 20*	217 ± 22	730 ± 43*	964 ± 55
5-HIAA				
Control	756 ± 75	804 ± 70	45 ± 6	53 ± 5
IV	1610 ± 56*	1640 ± 69*	96 ± 14*	102 ± 9*
IVL	1302 ± 64*	875 ± 77	74 ± 8*	60 ± 5
HVA				
Control	933 ± 78	987 ± 71	854 ± 62	913 ± 60
IV	1863 ± 118*	1918 ± 69*	1905 ± 106*	1986 ± 95*
IVL	1324 ± 65*	948 ± 65	1250 ± 59*	984 ± 77

DA: dopamine, LA: lipoic acid, HVA: homovanillic acid, 5-HT: 5-hydroxytryptamine, 5-HIAA: 5-hydroxyindoleacetic acid, NE: norepinephrine.

* Significance ($p < 0.05$) as compared to control group; values are mean ± S.E.M.; ng/g wet tissue wt.

7. The efficacy of LA on biogenic amines in the mice after systemic inflammation and Aβ immunization

In an earlier work, we reported that Aβ immunization with inflammation induces accumulation of ROS and LPO in lymphocytes, hepatocytes, astrocytes of mice [35]. In the present study, Aβ immunization in inflammation induced mice was used to evaluate the role of LA through the changes in aminergic system. The level of biogenic amines was significantly ($p < 0.05$) low in the neocortex and hippocampus of IV and IVL groups mice when compared with control on 1st day (Table 3). Regarding the level of 5-HIAA in the neocortex region of IV and IVL groups, there was an increase of approximately 2.0- and 1.7-fold, respectively, on 1st day. While the level of 5-HIAA returned to normal on 7th day after LA therapy in this region, the level of 5-HIAA in IV group remained significantly high. A similar trend was noticed in hippocampal region of groups IV and IVL mice as well. It was also observed that IV and IVL group showed 1.9- and 1.4-fold increase in HVA on 1st day, however, LA therapy successfully brought the level to normal on 7th day while the level of HVA remain almost similar in IV group in the neocortex region. A similar trend was noticed in the level of HVA in hippocampal region after LA therapy.

8. Discussion

Systemic inflammation activates inflammatory cells to produce a variety of proinflammatory and cytotoxic factors, including the cytokines, tumor necrosis factor-α, free radicals like nitric oxide (NO) and ROS. These proinflammatory cytokines are transported to various tissues through the blood stream [6], including the brain parenchyma and circumventricular sites, which lack a functional blood brain barrier [21]. These factors lead to loss of the dopaminergic neuronal function [28]. This may be a possible reason for the significant changes observed in the present study in the catabolism of biogenic amines in the neocortex and hippocampus of the inflammation-induced mice. The present study shows that on treatment with LA, biogenic amines level was normalized. It has been demonstrated that LA acts as an antioxidant because it exhibits properties, like (i) ability to scavenge reactive species including hydroxyl radicals, superoxide anion, alkoxy radicals [44], and peroxide radical [38], (ii) inhibition of the activation of nuclear factor kappa B (NFkB), a DNA-binding protein [25] and NO [30], (iii) regeneration of other antioxidants such as glutathione (GSH), vitamin C and E from their radical or inactive forms and (iv) metal chelating activity [5,34]. These might be the possible reasons for reduction of biogenic amines catabolism in LA treated mice.

AD brains are densely surrounded by reactive astrocytes. These astrocytes participate in the inflammatory responses by producing proinflammatory cytokines and free radicals [15] thus enforcing the progression of cellular oxidative imbalance that contributes to astrocyte activation [9]. The possible mechanism in AD is the accumulation of such free radicals during inflammation [2]. An earlier study has reported that injection of Aβ causes the loss of cholinergic neurons up to 4 months post-injection [41]. Non-cognitive impairments such as depression or aggressive behavior involved in AD are primarily influenced by the serotonergic system [3]. During the Aβ injection the biogenic amines were abnormally catabolized leading to enhanced oxidative vulnerability. A fenton type reaction may be involved in this process causing DNA damage, which ultimately leads to neuronal death. LA is capable of scavenging the free radicals by regenerating antioxidants (Vitamins C and E), and increasing cellular glutathione content.

Our results highlights that the biogenic amine catabolism was significantly higher in the IV group when compared with SI and VA group. On treatment with LA, biogenic amines catabolism was normalized like that of control group on 7th day. This provides compelling evidence in favor of those deleterious effects, which took longer time to restore to normalcy. The mechanism of action of LA may be through its antioxidant action by scavenging free radicals generated during autoxidation of DA, 5-HT and NE.

Our findings suggest that the external inflammatory stimuli on aminergic metabolism are not only due to loss of synapses or degradation of particular neurons but are caused by dysfunction in biosynthesis and degradation of

monoaminergic neurotransmitters. Neurotransmitters and neuropeptides from the CNS are known to influence the functions of the immune system but the extent of their contribution to the enhancement of immune reactivity needs to be examined in SI, VA, and IV group mice. Further studies are required in this line to elucidate the significance of these neurochemical changes on neural circuitry underlying the central effects of immune system. Our findings reveal that the supplementation of LA would represent a viable therapeutic approach to diminish oxidative stress in the CNS and thereby modulating the levels of neurotransmitters in hippocampus and neocortex regions of IL, VL and IVL group mice. Therefore LA is an excellent drug of choice in inflammatory AD models where active immunization is performed. In conclusion, LA therapy in parallel with Aβ immunization may serve as a potent therapeutic agent.

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