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Research article

Neuroprotective effects of acetyl-L-carnitine on lipopolysaccharide-induced neuroinflammation in mice: Involvement of brain-derived neurotrophic factor

Filiz Kazak[a,*], Gul Fatma Yarim[b]

[a] Department of Biochemistry, Faculty of Veterinary Medicine, Mustafa Kemal University, Hatay, 31060, Turkey
[b] Department of Biochemistry, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, 55220, Turkey

HIGHLIGHTS

- Acetyl-L-carnitine administration ameliorated LPS-induced neuroinflammation.
- Acetyl-L-carnitine increased the concentration of BDNF in the brain.
- Acetyl-L-carnitine may have protective and therapeutic potential for inflammation-related neurodegenerative diseases.

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ABSTRACT

Neuroinflammation is the inflammation of nervous tissue that can lead to neurodegeneration. Brain-derived neurotrophic factor (BDNF) is a neurotrophin which affects growth, function and survival of neurons, enhances the stabilization of synapses, regulates synaptic function and branching of dendrites and axons. Brain-derived neurotrophic factor is believed to be involved in the pathophysiology of central nervous system (CNS) diseases associated with neuroinflamation. The aim of this study was to investigate new protective and therapeutic effect of acetyl-L-carnitine (ALCAR) in neuroinflammation. Acetyl-L-carnitine was administered into Swiss Albino mice as 100 mg/kg/day and 300 mg/kg/day for 5 days. Neuroinflammation was induced by lipopolysaccharide (LPS). Histopathological findings associated with ALCAR administration on neuroinflammation in the brain were determined. Moreover, the effects of ALCAR on BDNF concentration in the brain tissue was evaluated. The LPS administration showed higher microglial activation in the brain of LPS, 100A + LPS and 300A + LPS groups compared to that in the control ($p < 0.05$). In the 100A + LPS group, microglial activation was lower and BDNF concentration was higher than in the 300A + LPS group ($p > 0.05$). The findings suggest that the dose of ALCAR at 100 mg/kg/day i.p. may have a beneficial effect on LPS-induced neuroinflammation in mice. As a conclusion, ALCAR may be used as an optional neuroprotective and therapeutic agent to attenuate inflammatory damage in the CNS regarding BDNF, in a dose dependent manner.

1. Introduction

Neuroinflammation and microglial activation are two crucial components of the pathogenesis for many neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Multiple sclerosis, Huntington's disease and Amyotrophic lateral sclerosis [1–5].

Acetyl-L-carnitine (ALCAR), an acetyl ester of L-carnitine, is produced as a physiological peptide in the body and is also available in the diet, particularly in foods of animal origin [6]. It crosses the blood-brain barrier and is naturally found in the brain [7]. Studies [8,9] indicate that ALCAR protects neuronal function against oxidative stress and apoptosis in the central nervous system (CNS). Numerous researchers have investigated ALCAR's neuroprotective effects in neonatal hypoxia ischemia-induced brain injury [10], oxygen-glucose deprivation-induced neural stem cell death [11], inhibition of glial activation and oxidative stress [12]. The literature also suggests that ALCAR positively affects the regeneration of unmyelinated fiber in the sciatic nevre [13]. The neurotrophic

* Corresponding author.
E-mail address: filizkazak@mku.edu.tr (F. Kazak).

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effects of ALCAR on neuronal survival and regeneration depend on neurotrophic factors; exogenous ALCAR administration enhances nerve growth factor (NGF) activity and increases the extracellular signal-regulated kinase 1/2 phosphorylation, maintaining cell survival in the brain [14,15]. Acetyl-L-carnitine also exerts antidepressive effects that might be mediated by the PI3K/AKT/BDNF/VGF signaling pathway [16].

The second discovered neurotrophin in CNS, brain-derived neurotrophic factor (BDNF) is highly expressed and widely distributed in the brain [17,18]. It provides trophic support to neurons and plays a crucial role in various neurodevelopmental processes, such as development, maintenance and survival of the nervous system, synaptic plasticity and cognitive function [17–21]. Brain-derived neurotrophic factor demonstrates these functions by binding its receptor (tyrosine receptor kinase B) and activating the mitogen-activated protein kinase, the phospholipase C gamma and the phosphatidylinositol 3-kinase pathways (19). Based on these functions, researchers have proposed that dysregulation of BDNF signaling leads to the onset of neurodegenerative diseases and neuropsychiatric disorders [22–25].

Lipopolysaccharide (LPS), an endotoxin from the outer membrane of bacteria, is one of the widely-used animal models of peripherally induced neuroinflammation. It has been demonstrated that administration of LPS in mice induced microglial activation in the brain [26–28].

To our knowledge, no studies have reported on the effects of ALCAR in LPS-induced neuroinflammation in mice brains. Thus, the present study was designed to investigate the effects of ALCAR on glial cell activation in the brain of LPS-induced mice. The study models neuroinflammation by using ionized calcium binding adaptor molecule 1 (Iba1) immunohistochemical staining. We also examined BDNF's involvement in neuroprotective activity and the therapeutic effects of ALCAR.

2. Materials and methods

2.1. Animal model

A total of 40, 8–10 weeks old male Swiss Albino mice provided by Ondokuz Mayis University Experimental Animal Application and Research Center were used in this study. All experiments were performed in accordance with approved animal protocols and guidelines established by the Animal Experiments Local Ethics Committee of Ondokuz Mayis University for animal experiments (Approval no: 2014/19).

2.2. Experimental design

The mice were randomly divided into four groups containing an equal number of animals. Lipopolysaccharide from *Salmonella typhimurium* (Sigma, L6511) was dissolved in 0.5 ml aliquots of sterile physiological saline solution. Neuroinflammation was induced by a single intraperitoneal dose of 3 mg/kg LPS on the third day of the experiment, and saline was administered for 5 days (LPS group) [27]. Acetyl-L-carnitine (Sigma, O-Acetyl-L-carnitine hydrochloride, A6706) was applied intraperitoneally in two different doses at 100 mg/kg/day (100A + LPS group) and 300 mg/kg/day (300A + LPS group) for 5 days [29,30]. To investigate both the protective and therapeutic effect of ALCAR on LPS-induced neuroinflammation, a single dose of 3 mg/kg LPS was administered intraperitoneally to the 100A + LPS and 300A + LPS groups in the middle of the experiment (on the third day). The control group received saline (0.5 ml i.p.) once daily for 5 days. The mice then were sacrificed under ketamine and xylazine anesthesia, and brain tissue was removed on the sixth day of the experiment.

2.3. Immunohistochemical examinations

Histopathological examination determined which lesions were related to neuroinflammation and the effects of ALCAR in the brain tissue. We applied immunohistochemical staining for Iba1 to sections of brain tissue samples in order to determine the microglial activation of neuroinflammation [31,32]. For this purpose, we utilized a streptavidin-biotin immunohistochemistry kit and primer antibody developed against mouse Iba1. Ionized calcium binding adaptor molecule 1 immunopositive cell numbers were determined using a research microscope. To identify Iba-1-positive microglia, five random fields in the brain cortex were imaged at 20× with a light microscope (Nikon Eclipse E600, Nikon Instruments Inc., Tokyo, Japan), counting microglial cell bodies with clear morphology.

2.4. Measurement of BDNF in brain supernatants

The brain tissue was dissected and homogenized by sonication with a Sonics Vibra Cell VCX 130 (USA). Homogenates were immediately centrifuged (18,000 rpm) for 10 min at 4 °C, and the supernatant was removed and stored at −80 °C until analyzed for BDNF concentration. For all samples, we measured BDNF concentrations with an enzyme-linked immunosorbent assay (ELISA) kit (201-02-0014 SunRed, Shanghai, China).

Briefly, the kit uses a double-antibody sandwich ELISA to assay the concentration of mouse BDNF in samples. BDNF was added to monoclonal antibody Enzyme well which is pre-coated with mouse BDNF monoclonal antibody. BDNF antibodies were labeled with biotin, and combined with Streptavidin-HRP to form immune complex. Choromogen Solution A, B, and stop solution were added and the color of the liquid finally became yellow. The color absorbance of the microplate for ELISA analysis was measured at 450 nm in an ELISA instrument (Tecan Infinite F50, Austria). The results were calculated using the standard curve.

2.5. Statistical analysis

SPSS 15.0 package program was used for the statistical analysis of the data obtained in the study. Prior to the significance tests, all data were evaluated by Shapiro Wilk for normality from the parametric test hypotheses and by Levene test for the homogenity of variances. Kruskal Wallis test was used for the variables that did not provide parametric test assumptions. For the variables that did not provide parametric test assumptions, Mann Whitney *U* test was used for each comparison and Bonferroni correction was applied to the results. For all statistical evaluations, $p < 0.05$ was used as the significance criterion.

3. Results

In the brain tissue, the concentrations of BDNF in the control, LPS, 100A + LPS and 300A + LPS groups were 0.65 ± 0.08, 0.33 ± 0.08, 1.07 ± 0.22 and 0.64 ± 0.09 ng/mg protein, respectively. BDNF concentrations in the LPS group were found to be significantly lower than the control and 100A + LPS groups ($p < 0.05$); on the other hand, there were no significant differences in terms of BDNF concentrations in the brains of the 300A + LPS group and control group. In the 100A + LPS group, the BDNF concentration was higher than that in the 300A + LPS group ($p > 0.05$) (Fig. 1).

Immunohistochemical staining showed that Iba1 expression in the cerebral cortex of the LPS, 100A + LPS and 300A + LPS groups increased compared to the control group ($p < 0.05$). We also observed that the increased expression of Iba1 in the cerebral cor-