Psychiatry Research 240 (2016) 131–136

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Protective effect of liquiritigenin on depressive-like behavior in mice after lipopolysaccharide administration

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ARTICLE INFO

Article history:
Received 27 January 2016
Received in revised form
27 March 2016
Accepted 1 April 2016
Available online 14 April 2016

Keywords:
Liquiritigenin
Depression
BDNF/TrkB
Inflammation

ABSTRACT

Liquiritigenin (Liq), the main active ingredient of traditional Chinese medicine licorice, possesses anti-inflammatory and neuroprotective properties. The current investigation was designed to explore whether liquiritigenin could relieve lipopolysaccharide (LPS)-induced depression-like behavior in mice and the underlying mechanism. Liquiritigenin (7.5 mg/kg, 15 mg/kg) and fluoxetine (20 mg/kg) were pretreated intragastrically once daily for 7 consecutive days. LPS (0.5 mg/kg) was injected subcutaneously to establish the depression model 30 min after pretreatment on day 7. Interleukin (IL)-6 and tumor necrosis factor (TNF)-α levels in serum and hippocampus were detected by enzyme-linked immunosorbent assay (ELISA). Behavioral assessment was conduct 24 h post LPS injection. The expressions of p65NF-κB, IκBα, brain-derived neurotrophic factor (BDNF) and tropomyosin-related kinase B (TrkB) in hippocampus were determined by western blot. The obtained results showed that liquiritigenin effectively reduced the levels of pro-inflammatory cytokines and the expressions of p-p65NF-κB and p-IκBα. Furthermore, liquiritigenin preconditioning could down-regulate the immobility time in tail suspension test (TST), forced swimming test (FST) and up-regulate BDNF and TrkB contents in hippocampus. Thus, it is assumed that the antidepressant activity of liquiritigenin might be attributed to its anti-inflammatory property and BDNF/TrkB signaling pathway.

1. Introduction

The World Health Organization (WHO) reported that major depressive disorder (MDD) will be the leading cause of disability and have the highest burden of disease by the year 2030. Currently, the focus of researches treating depression was on 5-hydroxytryptamin system, hypothalamicpituitary-adrenal axis (Rohleder, Wolf et al., 2010), neurotrophic system and neurogenesis (Duman and Monteggia, 2006). Numberous clinical studies and animal experiments confirmed that depression led to nerve tissue atrophy and neuronal loss of emotion regulation zone in brain including hippocampus, amygdala, prefrontal cortex and insula. Neurotrophic factors, as such nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) are critical regulators of the formation and plasticity of neural networks (Huang and Reichardt, 2001) and several studies have highlighted the role of neurotrophic factors in depression, as well in actions of antidepressant treatment (Garcia et al., 2009; Trajkovska et al., 2009). Among those neurotrophin, BDNF neurons are widely distributed in the brain, especially in hippocampal dentate gyrus and cortical. Previous literatures supported the theory that pre-incubation of BDNF could significantly suppressed glutamate-induced excitotoxic insult in the hippocampal neurons (Almeida et al., 2005). TrkB (Jiang et al., 2005), which triggers multiple signaling cascades is also essential for cellular survival. Thus, it is speculated that liquiritigenin might exert antidepressant effect through BDNF/TrkB pathway.

Furthermore, pro-inflammatory cytokines may also play a vital role in the pathophysiology of depression. Recent research has suggested a central role of inflammatory processes in mediating this effect (Slavich and Irwin, 2014). What's more, episodes of depression can be induced with many inflammatory stimulus (Capuron et al., 2002; Bull et al., 2009) and alleviated with anti-inflammatory medication (Köhler et al., 2014). Lipopolysaccharide (LPS) is reported that it can elicit systemic inflammatory responses (Müller et al., 2015), and heightened inflammation commonly is

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http://dx.doi.org/10.1016/j.psychres.2016.04.002
0165-1781/

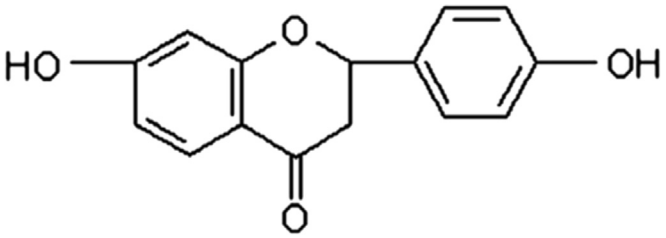

Fig. 1. Chemical structure of liquiritigenin.

associated with periods of depressive illness (Haapakoski et al., 2015; Strawbridge et al., 2015).So it is reasonable to infer that inflammation-related mediators and proteins are also implicated in the depression-like behavior (Maes, 2008).

Liquiritigenin (7,4′-dihydroxyflavanone, Fig. 1), a flavonoid extracted from the radix of Glycyrrhiza, is used clinically for the treatment of liver toxicity in vivo and in vitro (Huang and Reichardt, 2001; Kim et al., 2004; Kimet al., 2006), β-amyloid peptide (Aβ)-induced neurotoxicity in vivo and in vitro (Liu et al., 2009, 2010), ischemia, cancer and inflammation (Kim et al., 2008). However, the antidepressant function of liquiritigenin has not been reported. We extended our study by investigating the effects of liquiritigenin on LPS-induced depression-like behavior in mice and the related mechanism.

2. Materials and methods

2.1. Main reagents and kits

Liquiritigenin (purity 98%) was purchased from National Institutes for Food and Drug Control (Beijing, China). Fluoxetine hydrochloride, provided by Changzhou Siyao Pharmaceuticals Co., Ltd. (Changzhou, PR China), was dissolved in 0.03% sodium carboxymethyl cellulose (CMC-Na). LPS (Escherichia coli serotype 055: B5, No. L-2880, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in sterile, pyrogen-free physiological saline. TNF-α and IL-6 enzyme-linked immunosorbent assay (ELISA) kits were produced by Nanjing KeyGEN Biotech. CO., Ltd. (Nanjing, China). All antibodies were purchased from Cell Signaling Technology Inc (Beverly, MA, USA).

All other chemicals and reagents used for study were of analytical grade and were purchased from approved organizations.

2.2. Animals

50 male ICR mice were provided by the Experimental Animal Center in Jiangsu Province (Nanjing, China). All the mice were maintained at a constant temperature (22 ± 1 °C) under a 12 h light/12 h dark cycle environment and standard food and water were provided ad libitum. The experimental protocol was approved by an Institutional Review Committee for the use of Human or Animal Subjects or that procedures are in compliance with at least the Declaration of Helsinki for human subjects, or the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985), the UK Animals Scientific Procedures Act 1986 or the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.3. Experimental protocol

Mice were randomly assigned to five groups (n = 10) as follows: control group, model group, LPS + Flu (20 mg/kg) group, LPS + Liq (7.5 mg/kg) group and LPS + Liq (15 mg/kg) group. Mice were pretreated with liquiritigenin (7.5 mg/kg, 15 mg/kg) and fluoxetine (20 mg/kg) intragastrically once daily for 7 consecutive days. LPS (0.5 mg/kg) was injected subcutaneously to establish the depression model 30 min after pretreatment on day 7. The control group and model group were given equal volume normal saline. Mice were anesthetized 1.5 h posterior to LPS injection and blood samples were collected from orbit. Behavioral assessment was conduct 24 h after LPS challenge, and then mice were sacrificed to get hippocampus.

Mice were randomly assigned to five groups (n = 10) as follows: control group, model group, LPS + Flu (20 mg/kg) group, LPS + Liq (7.5 mg/kg) group and LPS + Liq (15 mg/kg) group. LPS + Flu (20 mg/kg) group were treated intragastrically with Flu at the doses of 20 mg/kg for 7 days. LPS + Liq (7.5 mg/kg) group and LPS + Liq (15 mg/kg) group were treated intragastrically with Liq at the doses of 7.5 and 15 mg/kg respectively for 7 days. Model group were given normal saline for 7 days. Then on the 7th day, except the control group, all groups were injected LPS (0.5 mg/kg) subcutaneously just 30 min after the Liq, Flu and saline were given. All the control animals were given normal saline in the same volume orally and subcutaneously, respectively. Behavioral tests were measured with 24 h after LPS injection. After behavioral tests, all the mice were sacrificed, the hippocampus were rapidly separated from the brain for biochemical assays and Western blot analysis.

2.4. Behavioral evaluation

2.4.1. Forced swimming test (FST)

The forced swimming test was conduct according to the conventional method described previously (Porsolt et al., 1977). Briefly, 24 h post LPS stimulation, every mouse was forced to swim for 6 min in an open cylindrical container (diameter = 14 cm, height = 20 cm) containing water up to a height of 12 cm at 25 ± 1 °C. The period of escape-oriented behaviors were considered as immobility time. The total immobility time was recorded during the last 4 min period by two independent observers blinded to the experiment. The water was changed after each group.

2.4.2. Tail suspension test (TST)

The tail suspension test was conduct according to the conventional method described previously (Steru et al., 1985). Briefly, 24 h post LPS stimulation, every mouse both acoustically and visually isolated was individually suspended using adhesive tape (approximately 2 cm from the end) for 6 min with 50 cm above the floor. The duration of immobility were measured for the last 4 min by two independent observers blinded to the experiment.

2.5. Cytokine measurement in serum

Blood samples were collected from carotid artery and centrifuged at 5000 rpm for 15 min The supernatant were collected for serum cytokines analysis. The levels of IL-6 and TNF-α were detected by ELISA kits according to the manufacturer's instructions.

2.6. Cytokine measurement in hippocampus

Mice were sacrificed after behavioral assessment. Hippocampus was harvested, minced and homogenized for proteins and cytokines analysis. The levels of IL-6 and TNF-α in hippocampus were detected by ELISA kits According to the standard protocol.

2.7. Western blot analysis

Minced and homogenized hippocampus in lysis buffer were centrifugated at 12,000g for 5 min at 4 °C to remove the debris. BCA protein assay kit (Beyotime, Nanjing, China) was used to measure the total protein concentration in supernatant. Proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred onto the polyvinylidene difluoride membrane. The membrane was incubated with primary antibody overnight at 4 °C blocked in skim milk, and then they were incubated with secondary antibody for 1 h at room temperature after washing with TBST three times. The blotted protein bands were developed and fixed by an ECL Advanced kit. The image analysis software was applied to quantify protein intensity.

2.8. Statistical analysis

The data were expressed as mean values ± SD. Comparison between groups were analyzed by *T*-test. All data were processed with Graphpad, while $p < 0.05$ was considered significant.

3. Result

3.1. Effect of liquiritigenin on behavioral assessments

3.1.1. Effects on immobility time in forced swimming test (FST)

The forced swimming test was performed 24 h post-LPS administration. LPS-induced depressive mice showed significant increases in immobility duration compared with the control group. Flu (20 mg/kg) as well as Liq (7.5 mg/kg, 15 mg/kg) notably shortened the immobility period in comparison with the model group (Fig. 2A).