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Esculetin attenuates lipopolysaccharide (LPS)-induced neuroinflammatory processes and depressive-like behavior in mice

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HIGHLIGHTS

- Esculetin attenuates lipopolysaccharide (LPS)-induced neuroinflammatory processes.
- Esculetin attenuates lipopolysaccharide (LPS)-induced depressive-like behavior.
- Esculetin inhibited neuroinflammatory proteins.

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ABSTRACT

Esculetin is one of the major bioactive compounds of *Cichorium intybus* L. The main purpose of the present study was to investigate the effects and possible underlying mechanism of esculetin (Esc) on lipopolysaccharide (LPS)-induced neuroinflammatory processes and depressive-like behavior in mice. Mice were pretreatment with esculetin (Esc, 20, 40 mg/kg, intragastric administration) and a positive control drug fluoxetine (Flu, 20 mg/kg, intragastric administration) once daily for 7 consecutive days. At the 7th day, LPS (0.83 mg/kg) was intraperitoneal injection 30 min after drug administration. Higher dose (40 mg/kg) of esculetin and fluoxetine significantly decreased immobility time in TST and FST. There was no significant effect on locomotor activity in mice by the drugs. Esculetin significantly reduced LPS-induced elevated levels of pro-inflammatory cytokines including interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in serum and hippocampus. Esculetin attenuated inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) protein expression by inhibiting nuclear factor-κB (NF-κB) pathway in hippocampus. In addition, neuroprotection of esculetin was attributed to the upregulations of Brain derived neurotrophic factor (BDNF) and phosphorylated tyrosine kinase B (p-TrkB) protein expression in hippocampus. The obtained results demonstrated that esculetin exhibited antidepressant-like effects which might be related to the inhibition of NF-κB pathway and the activation of BDNF/TrkB signaling.

1. Introduction

Major depressive disorder (MDD) is the common debilitating mental disorder diminished quality of life, medical morbidity and mortality [19]. Nearly about 65% of individuals suffer from recurrent episodes of the disorder [22,26]. It was reported by the World Health Organization (WHO) that depression would be the second leading cause of disability after cardiovascular diseases [18]. However, the pathogenesis of this mental disease remains poorly understood. The concept of most modern theories of depression is the emotional stress which initiate cognitive disorders and possibly biological processes [2]. Accumulating evidence demonstrates that inflammation may play an important role in the pathophysiology of depression and be involved in the body's response to physical injury [24]. Moreover, former literature displayed that psychological stress could lead expand inflammatory reaction [13]. Recently, numerous studies have demonstrated the associations between inflammatory biomarkers and depressive symptoms, such as sad mood, fatigue, psychomotor retardation and social behavior

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withdrawal [36]. Patients who suffer from depression exhibit high levels of inflammatory cytokines in the blood [16]. The peripheral administration of lipopolysaccharide (LPS) is a well-established model to study behavioral and physiological responses under peripheral inflammation [31]. LPS, the component of Gram-negative bacteria outer membrane, can activate innate immune response and secrete inflammatory cytokines (Q. [20]). Intraperitoneal (i.p.) injection with LPS has been shown to induce several depressive-like behaviors in vivo, followed by a precise temporal profile with an earlier "sickness behavior" and "depressive-like behavior" manifesting at 24 h [10]. Various antidepressants, including serotonin and norepinephrine reuptake inhibitors (SNRIs) and selective serotonin reuptake inhibitors (SSRIs), possess anti-inflammatory effects. Therefore, anti-inflammatory substances can be used as alternative therapy for LPS-induced depression (R. [24]).

It is proposed that neurotrophins play critical roles in neuron survival, plasticity, neurogenesis and synaptogenesis. Brain derived neurotrophic factor (BDNF), a homodimeric protein, is mediated via the tyrosine kinase B (TrkB). BDNF is recognized as a factor which expressed in the nervous system, with the highest level in the hippocampus and frontal cortex [1]. Moreover, BDNF serves as an important mediator of neuroplasticity on the basis of its positive contribution on neurogenesis. In addition, mounting evidence suggests that BDNF is highly related to the pathophysiology of depression [40]. Reduced BDNF levels in serum in depressed patients are normalized after antidepressant treatment [3]. Several studies have demonstrated that inflammation affects the expression of BDNF in brain. In particular, it has been reported that the administration of pro-inflammatory cytokines or lipopolysaccharide causes a significant reduction of BDNF gene expression [27].

Esculetin (6,7-dihydroxycoumarin, structure shown in Fig. 1) is a natural coumarin compound isolated from various plant species, such as *Cichorium intybus* L., *Artemisia capillaris*, *Citrus limonia* and *Euphorbia lathyris* [30]. Previous studies showed that esculetin possesses multifarious pharmacological activities, including anti-oxidant [28], anti-inflammatory [6] and hepatoprotective effects [12]. In the present work, we hypothesized that esculetin might have protective effects on lipopolysaccharide (LPS)-induced depressive-like behavior in mice by decreasing neuroinflammation through BDNF/TrkB signaling cascades.

2. Materials and methods

2.1. Chemical reagents

Esculetin(purity 98%) was purchased from National Institutes for Food and Drug Control (Beijing, China). Fluoxetine (Flu) was provided by Simcare Drug Store (Nanjing, China). LPS from *Escherichia coli* serotype 0111: B4 was produced by Sigma–Aldrich (St. Louis, USA). IL-6, IL-1β and TNF-α enzyme-linked immunosorbent assay (ELISA) kits were purchased from Nanjing KeyGEN Biotech (Nanjing, China). The antibodies of p-TrkB, TrkB, IKKα, p-IKKα, IKKβ, p-IKKβ,iNOS, COX-2, IκBα, p-IκBα, NF-κBp65 and p-NF-κBp65 were obtained from Cell Signaling Technology (Danvers, USA). The antibody of BDNF was purchased from Abcam (Cambridge, USA). All other chemicals and reagents used for the study were analytical grade and were provided by approved organizations.

2.2. Animals

60 male ICR mice (18–22 g) were purchased from Comparative Medicine Centre of Yangzhou University (Yangzhou, China) and maintained at 23 ± 2 °C with a relative humidity of 55 ± 5% and a regular 12 h light/12 h dark cycle. The animals were housed in a specific pathogen-free (SPF) laboratory in the Animal Center of China Pharmaceutical University. All procedures were approved by the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

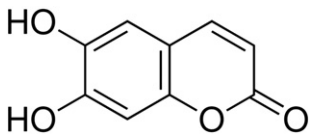

Fig. 1. Structure of esculetin (Esc).

2.3. Experimental protocol

The animals were provided with standard chow ad libitum in the duration of the study and allowed 1 week to adapt to the laboratory environment before experiments. Mice were randomly divided into six groups (n = 10): (1) Control; (2) Control + Esc (40 mg/kg); (3) Model; (4) Model + Flu (20 mg/kg); (5) Model + Esc (20 mg/kg); (6) Model + Esc (40 mg/kg). All drugs were intragastrically administered once daily for 7 days. In the 7th day, LPS (0.83 mg/kg) was intraperitoneally injected 30 min after drug administration. Blood samples from eye socket were collected 90 min after LPS injection and then centrifuged at 4500 rpm for 15 min. The serum were harvested and stored at −80 °C. Behavioral tests were measured 24 h after LPS injection. TST, FST and OFT were conduct in sequence, between two of which test were allowed to rest for 1 h. Afterwards, all the mice were sacrificed and the brains were harvested subsequently for Immunohistochemistry and protein quantification. All the experimental mice were subjected to behavioral tests, namely, TST, FST and OFT. Specifically, 3 of the mice described above were used for protein quantification, while another 3 of them were used for immunohistochemistry analysis.

2.4. Behavioral evaluations

2.4.1. Tail suspension test (TST)

The TST was conducted in accordance with previously reported methods [36]. Mice were individually suspended upside down by their tails for 6 min and the immobility time was recorded during the last 4 min. Mice were considered immobile when they hung passively and completely motionless. In the study, observers were blinded to the treatment group independently.

2.4.2. Forced swimming test (FST)

The FST was carried out as previously described with minor changes [25]. In brief, mice were individually placed into an acrylic cylinder (height: 25 cm, diameter: 15 cm) containing water (depth: 10 cm). Mice were considered immobile when they floated motionless and made the movement necessary to keep their heads above the water. Each mouse was forced to swim for 6 min and the immobility time was measured during the final 4 min. In the study, the observers were blinded to the group of the mice.

2.4.3. Open field test (OFT)

In order to assess possible effects of drug treatment on spontaneous locomotor activity, the animals were submitted to the open-field paradigm according to the previous paper [35]. Briefly, mice were individually placed into a wooden box (40 cm × 60 cm × 50 cm) with the floor of the arena divided into 12 equal squares. Each mouse was placed individually into the center of the arena and allowed to free exploration. The number of crossing and rearing were registered during a period of 6 min. The instrument was cleaned with 10% ethanol after each trial.

2.5. Determination of cytokines in serum and hippocampus by ELISA

The concentration of IL-6, IL-1β and TNF-α in serum and hippocampus were measured by ELISA kits on the basis of the manufacturer's instructions. The results were shown as picograms per milliliter (pg/ml) and picograms per milligram (pg/mg).