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Research article

The effects of apigenin on lipopolysaccharide-induced depressive-like behavior in mice

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HIGHLIGHTS

- Apigenin reversed the LPS-induced behavioral abnormalities in mice.
- Apigenin inhibited cytokines expression the in the prefrontal cortex in LPS-treated mice.
- Apigenin significantly inhibited the expression of iNOS and COX-2 in the prefrontal cortex.
- Apigenin inhibited the NF-κB activation in the prefrontal cortex in LPS-treated mice.

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ABSTRACT

Increasing evidence shows that inflammation may contribute to the pathophysiology of depression. Apigenin, one type of natural flavone, has a number of biological actions including anti-inflammatory effects. Although it has potential antidepressant activity in a chronic mild stress model, the mechanisms of antidepressant effect for apigenin remain unclear. Here, we examined the effects of apigenin on lipopolysaccharide (LPS)-induced depressive-like behavior in male mice. A single administration of LPS (0.5 mg/kg, i.p.) increased the immobility time in the tail suspension test (TST) and reduced sucrose preference without changing spontaneous locomotor activity in open field test (OFT). Pre-treatment with apigenin (25, 50 mg/kg, i.p.) or fluoxetine (positive control drug, 20 mg/kg, i.p.) once daily for 7 consecutive days prevented the abnormal behavior induced by LPS. Apigenin or fluoxetine also effectively attenuated LPS-induced production of pro-inflammatory cytokines IL-1β (interleukin-1β) and TNF-α (tumor necrosis factor-α). Moreover, apigenin or fluoxetine significantly suppressed the inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression at both the mRNA and protein level via the modulation of nuclear factor-κB (NF-κB) activation in the prefrontal cortex. Additionally, apigenin (50 mg/kg, i.p.) or fluoxetine (20 mg/kg, i.p.) effectively reversed the depressive-like behavior induced by TNF-α (0.1 fg/site, i.c.v.) without altering the locomotor activity. These results demonstrate that apigenin exhibits antidepressant-like effects in LPS treated mice, partially due to its anti-inflammatory properties.

1. Introduction

Mounting evidence suggests that inflammation may play an important part in the pathophysiology of depression [1,2]. Patients who suffer from depression exhibit high levels of pro-inflammatory cytokines (i.e., IL-1β and TNF-α) in the blood and the cerebrospinal fluid [3]. On the other hand, both cytokines and lipopolysaccharide (LPS) could induce depressive-like behavior in animals [4,5]. In addition, antidepressants possess anti-inflammatory properties by decreasing the production of pro-inflammatory cytokines and increasing anti-inflammatory cytokine [6]. Furthermore, immunotherapy using IL-2 or interferon (IFN)-α increases the risk of the development of depression [7,8]. Taken together, these observations provide evidence that prevention of inflammatory disturbances could be a therapeutic target for psychiatric disease.

The peripheral administration of bacterial endotoxin LPS is a well-established model to investigate the link between activation

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of immune system and depressive symptoms [6]. The immunological challenges could induce depressive-like behavior by increase the immobility duration in the force swimming test and the tail suspension test, decrease the sucrose consumption [9], which can be prevented by antidepressant treatment. Besides, it has been demonstrated that the systemic administration of LPS induces time-dependent behavioral alterations, and depressive-like behavior should be observed 24 h later [10].

Apigenin (4′,5,7-trihydroxyflavone), widely found in fruits and vegetables, is a natural product belonging to the flavone class. It exhibits a variety of pharmacological properties, such as anti-oxidant [11,12], anti-inflammatory [13,14], and anti-tumor activities [15,16]. Apigenin was also found to exert antidepressant-like effects, for example, apigenin inhibited CMS-induced serotonergic alterations in stressed rats, indicating that this effects of apigenin might be related to the alteration of monoaminergic response [17].

Considering that apigenin has not been analyzed antidepressant-like effects in acute inflammation mice model, the present study was aimed to investigate the effects of apigenin on LPS-induced depressive behavior in mice. To understand whether the anti-depressive actions of apigenin are related to alteration of pro-inflammatory cytokines, we examined effects of apigenin treatment on the production of TNF-α, IL-1β and the levels of iNOS and COX-2 expression as well as the activities of NF-κB in the prefrontal cortex (PFC), which is the specific brain regions implicated in pathophysiology of depression [18]. In addition, we made an experiment to check whether the depressant-like behavior induced by TNF-alpha could be blocked with apigenin.

2. Materials and methods

2.1. Animals

Male ICR mice (Experimental Animal Center, Jiangsu Province), weighing 18–22 g, were housed in a room under standard laboratory conditions (temperature 25 ± 1 °C; a 12/12 h light/dark cycle; food and water provided libitum). All the experiments were carried out in agreement with related laws and approved by the local animal care committee.

2.2. Reagents

Fluoxetine hydrochloride was obtained from Changzhou Siyao Pharmaceuticals (Changzhou, PR China) Co., Ltd. LPS from *Escherichia coli* 055:B5 was purchased from Sigma–Aldrich (Product Number: L2880, St. Louis, MO, USA). Apigenin was purchased from Xi'an Xinyu Kailai Import & Export (Xi'an, China) Co., Ltd. The RNA extraction reagent was purchased from Vazyme Biotech (Nanjing, PR China) Co., Ltd. The primers were designed and synthesized by Sangon Biotech (Shanghai, China) Co., Ltd. Recombinant mouse TNF-α was obtained from R&D system Inc. (Minneapolis, MN, USA).

2.3. Drug treatments and experimental design

The mice were casually divided into 6 groups ($n = 10$ each group): control (received physiological saline, 10 ml/kg), apigenin (received apigenin 50 mg/kg), LPS (received physiological saline, 10 ml/kg), LPS-fluoxetine (received fluoxetine 20 mg/kg), and LPS-apigenin (received apigenin 25 and 50 mg/kg). All drugs were intraperitoneally administered once daily for 7 consecutive days. In the 7th day, LPS (0.5 mg/kg) was intraperitoneally injected into mice 30 min after drug administration. 24 h after LPS challenge, tail suspension test and sucrose preference test were performed to evaluate the behavioral activity. After behavioral measurements, the mice were sacrificed and brains were quickly dissected out. Then the prefrontal cortex was isolated and stored at −80 °C until detection.

2.4. Behavioral assessments

2.4.1. Tail suspension test (TST)

The test was carried out in accordance with the previous reported methodology [19]. Each mouse was suspended for 6 min, and the total immobility period was measured during the final 4 min. Mice were considered immobile only when they were passively suspended and remained completely motionless.

2.4.2. Sucrose preference test (SPT)

In order to evaluate the anhedonia behavior induced by LPS, the test was performed as described previously [20]. Before the test, mice were housed individually and accommodated to the two bottle test choice, one with water, the other with sucrose solution [1%, w/v]. 1 h after LPS administration, mice were deprived of water and food. 23 h later mice were given access to two bottles containing water and 1% sucrose solution for 1 h. The sucrose preference (SP) was measured as the SP(%) = sucrose consumption/(sucrose consumption + water consumption).

2.4.3. Open field test (OFT)

In order to estimate possible effects of drug treatment on spontaneous locomotor activity, the animals were submitted to the OFT according to the previous paper [21]. Each mouse was placed individually into the center of the arena and permitted free exploration. The number of crossing, rearing and grooming were registered during a period of 6 min. The instrument was cleaned with 5% ethanol after each trial.

2.5. ELISA

The level of TNF-α and IL-1β in the prefrontal cortex (PFC) were determined using commercially available ELISA assays (R&D system Inc Minneapolis, MN, USA), on the basis of the instructions supplied by the manufacturer and quantified by a microplate reader (450 nm). The results were shown as picograms per milliliter.

2.6. Quantitative real-time PCR (QPCR)

Total RNA from PFC was extracted using Trizol reagent in line with the manufacturer's instructions. RNA was reverse transcribed to cDNA by an EasyScript® First-Strand cDNA Synthesis SuperMix for QPCR. Real-time PCR analysis was performed in a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad USA). TransStart® Green qPCR SuperMix UDG was applied to real-time PCR analysis. The primers: iNOS: forward (5′-GTGGGGCGCCCCAGGCACC A-3′); reverse (5′-CTTCCTTAATGTCACGCACGATTTC-3′); COX-2: forward (5′-TGGGTGTGAAAGGAAATAAGGA-3′); reverse (5′-GAAGTGCTGGGCAAAGAATG-3′); β-actin: forward (5′-GTGGGGCGCCCCAGGCACCA-3′); reverse (5′-CTTCCTTAATGTCACGCACGATTTC -3′).

2.7. Western bloting analysis

Protein samples from PFC were homogenized and analyzed according to the procedure as our previously described [22]. The total protein concentrations were quantified by bicinchoninic acid assay kit (Beyotime Institute of Biotechnology Co., Ltd., Shanghai, China). Equal amount of protein were separated by SDS-PAGE and transferred to PVDF membranes. After incubated with primary rabbit polyclonal antibody for iNOS (1:400), COX-2 (1:400) (Santa Cruz Biotechnologies, CA, USA), phospho-NF-κB p65 (1:500), NF-κB