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Ferulic acid inhibits neuro-inflammation in mice exposed to chronic unpredictable mild stress

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ABSTRACT

Ferulic acid is a hydroxycinnamic acid that widely presents in plant cell wall components. It has been demonstrated that ferulic acid can attenuate depressive-like behaviors in both forced swimming test and tail suspension test. Considering that depression is an inflammatory related mental disease, our present study was aimed to investigate the role of ferulic acid in the regulation of microglia activation, pro-inflammatory cytokines, nuclear factor kappa B (NF-κB) and nucleotide binding and oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome in mice exposed to chronic unpredictable mild stress (CUMS). Our results firstly showed that decreased sucrose preference and increased immobility time were completely reversed by administration with ferulic acid and fluoxetine for four weeks. Then, we found that CUMS significantly caused interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) up-regulation, microglia, NF-κB signaling and NLRP3 inflammasome activation in the prefrontal cortex. On the contrary, these activated inflammatory response induced by CUMS were reversed by ferulic acid and fluoxetine as well, suggesting that anti-inflammatory related mechanism was involved in the antidepressant-like effects of ferulic acid in stressed mice.
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1. Introduction

Depression is a common but serious mood disorder with a very high prevalence in the general population. Its pathophysiology was elucidated from the aspects of monoaminergic systems since 1950s. This finding gave us a new understanding for depression treatment, which finally promoted the development of antidepressants. However, during the past several years, this monoamine deficiency hypothesis has been challenged [1]. Recently, accumulating studies have showed that inflammatory system was involved in the pathophysiology of depression [2]. Clinical researches clearly indicated that higher secretion of pro-inflammatory cytokines was in the peripheral of depressed patients, as compared with that in the healthy population [3]. This phenomenon was also supported by preclinical studies, which found that depressive-like animals produced more pro-inflammatory cytokines, such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) levels both in serum/plasma or brain [4,5]. A postmortem brain study also showed an elevated gene expression of pro-inflammatory cytokines in frontal cortex of depressed patients [6].

As a widely present hydroxycinnamic acid in plant cell wall components, ferulic acid has been found to possess a lot of biological activities. Besides its classical antioxidant activity, ferulic acid can also prevent trimethyltin-induced cognitive deficits in mice [7], attenuate the symptom of Alzheimer's disease induced by chronic neuroinflammation and oxidative stress in rats [8] and protect rats brain after nerve injury induced by cerebral ischemia [9]. In addition to above bioactivities, some studies indicated that ferulic acid produced the antidepressant-like effects in tail suspension test or forced swimming test. These researches indicated that serotonergic and norepinergic systems, as well as PKA, CaMKII, PKC, MAPK/ERK and PI3K signaling pathways were involved in the antidepressant-like effects of ferulic acid [10–13]. On the other hand, previous study has shown that ferulic acid decreased pro-inflammatory cytokines in hepatotoxicity rats induced by formaldehyde [14]. More importantly, ferulic acid inhibited inflammation in the brain through reduction of NF-κB [15]. Treatment with ferulic acid prevents NLRP3 inflammasome in monosodium urate crystal-induced inflammation in rats [16]. However, it is still unclear whether the antidepressant-like effect of ferulic acid is mediated by inhibiting inflammatory system.

Therefore, considering that interaction between inflammatory activation and the pathophysiology of depression, our present study was aimed to investigate the possible anti-inflammatory effects of ferulic acid in mice exposed to chronic unpredictable mild stress (CUMS) procedure. Also fluoxetine was used as a positive control. To elucidate this property, we examined behavioral alterations and IL-1β both in serum and prefrontal cortex. Furthermore, to further confirm the underlying molecular mechanisms of anti-inflammatory mechanism, we also

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evaluated the mechanism of ferulic acid on NF-κB signaling in the prefrontal cortex.

2. Materials and methods

2.1. Animals

Male ICR mice (22 ± 2 g; 6 weeks old) were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. Animals were housed six per cage (320 × 180 × 160 mm) under a normal 12 h/12 h light to dark schedule with lights on at 07:00 a.m. Animals were allowed to adjust to the housing conditions before experiments began. Ambient temperature and relative humidity were maintained at 22 ± 2 °C and at 55 ± 5%, respectively. Throughout the study, animals were given standard chow and water ad libitum. All procedures were performed in accordance with the published guidelines of the China Council on Animal Care (Regulations for the Administration of Affairs Concerning Experimental Animals, approved by the State Council on 31 October 1988 and promulgated by Decree No. 2 of the State Science and Technology Commission on 14 November 1988), and were approved by the Committee of Animal care of Henan University of Traditional Chinese Medicine.

2.2. Drugs and reagents

Ferulic acid and caspase-1 primary antibody were purchased from Santa Cruz Biotechnology, Inc. Fluoxetine hydrochloride was purchased from Sigma-Aldrich Co., Ltd. NF-κB, p-NF-κB, NLRP3 and β-actin primary antibodies were purchased from Cell Signaling Technology. CD11b primary antibody was purchased from Abcam Inc. IL-1β ELISA kit was purchased from R&D systems Inc.

2.3. Drug administration

Animals were divided into responding groups after sucrose training: the Control-vehicle group, the CUMS-vehicle group, the CUMS-fluoxetine group (20 mg/kg of fluoxetine dissolved in 0.9% saline containing 0.3% carboxymethyl cellulose, p.o.) and the CUMS groups that received 20, 40 or 80 mg/kg ferulic acid (dissolved in 0.9% saline containing 0.3% carboxymethyl cellulose, p.o.). Vehicle groups were administrated with 0.9% saline containing 0.3% carboxymethyl cellulose. The drugs were administrated once a day for successive four weeks.

2.4. CUMS

The CUMS procedure was obtained and followed from previous literature [17]. Briefly, the weekly stress paradigm consisted of food and water deprivation, exposure to an empty water bottle, exposure to a soiled cage, light/dark succession every 2 h, space reduction, a 45° cage tilt, overnight illumination, and predator sounds. All stressors were applied individually and continuously throughout the day and night. The control animals were housed in a separate room and had no contact with the stressed animals. To prevent habituation and to ensure the unpredictability of the stressors, all stressors were randomly scheduled over a 1-week period and were repeated throughout the duration of the experiment. On the basis of their sucrose preference following 4 weeks of CUMS, both stressed and control mice were divided into matched subgroups.

2.5. Sucrose preference test

Before the test, mice were trained to adapt to a sucrose solution (1%, w/v): two bottles of sucrose solution were placed in each cage for 24 h and then one bottle of sucrose solution was replaced with water for 24 h. After the adaptation, the mice were deprived of water and food for 12 h. After 24 h, the weights of the consumed sucrose solution and water were recorded. Sucrose preference was calculated using the formula as described below: Sucrose preference = Sucrose consumption / (Water and Sucrose consumption) × 100%.

2.6. Tail suspension test

The tail suspension test was conducted as previously described [18], with some modifications. Briefly, mice were individually suspended by tail with a clamp (1 cm from the tip of the end) in a box (25 × 25 × 30 cm^3) with the head 5 cm from the bottom. Testing was carried out in a darkened room with minimal background noise. A mouse was suspended for a total of 6 min, and the duration of immobility was recorded during the final 4 min interval of the test. Mice were considered immobile only when they hung passively and completely motionless. The test sessions were recorded by a video camera and scored by a competent observer blind to treatment.

2.7. qPCR

Total RNA was isolated from the prefrontal cortex using Trizol reagent according to the manufacturer's instructions. Total RNA was then reverse transcribed into cDNA using M-MLV reverse transcriptase kit. Real-time PCR reactions were performed by a SYBR green PCR kit in ABI step one plus system. The primers were synthesized by Sangon Biotech Co., Ltd. β-Actin was used to normalize gene expression.

2.8. Protein extraction and western blotting

Mice were sacrificed by decapitation after the tail suspension test. Whole brains were rapidly removed and chilled in an ice-cold saline solution. Prefrontal cortex tissues were dissected on a cold surface and were immediately frozen in liquid nitrogen. The tissue samples were stored at −80 °C until assay.

Tissue samples were homogenized in modified RIPA buffer followed by centrifugation at 12,000 ×*g* for 20 min at 4 °C and the resulting supernatant was collected. The protein concentration in the supernatant was determined by a Bradford protein assay using bovine serum albumin as a standard. The proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred to PVDFs membranes. The membranes were blocked with 5% (*w*/*v*) non-fat dried milk in Tris-buffered saline containing 0.1% Tween 20 (TBST) and were incubated with the following antibodies in TBST containing 5% milk. After incubation, the membranes were washed with TBST and were then incubated with a horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG secondary antibody in 5% non-fat dried milk in TBST. After washing, the immunocomplexes were detected using Chemiluminescence Imaging System. The images were subsequently subjected to densitometric analysis.

2.9. ELISA analysis for IL-1β cytokines

IL-1β levels in both serum and prefrontal cortex were measured by ELISA kit according to the manufacture's datasheet. For the serum samples, whole blood samples were collected and centrifuged (3000 ×*g* at 4 °C for 10 min) to get the serum.

2.10. Statistical analyses

All data are expressed as the mean ± SEM. The data were analyzed using a one-way ANOVA followed by a Tukey's post-hoc test. A value of $P < 0.05$ was considered to be statistically significant for analysis.

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