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Magnolol abrogates chronic mild stress-induced depressive-like behaviors by inhibiting neuroinflammation and oxidative stress in the prefrontal cortex of mice

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ABSTRACT

Magnolol, the main constituent of *Magnolia officinalis*, has been shown to produce antidepressant-like effect in rodents. Growing evidence shows that neuroinflammation, oxidative stress and neuroendocrine contribute to the pathogenesis of major depression. Here, the aim of this present study was to determine whether magnolol affected these systems in mice exposed to chronic mild stress (CMS). The ameliorative effect of magnolol on depressive-like symptoms was investigated through behavioral tests, including the classical sucrose preference and forced swimming tests. The behavioral evaluation showed that magnolol reversed the depressive-like deficits both in sucrose preference test and forced swimming test. The elevation of prefrontal cortex pro-inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) was decreased by magnolol. Consistently, the microglia activation by CMS was also alleviated by magnolol. In addition, the hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis induced by CMS was attenuated by magnolol. Moreover, the increased lipid peroxidation such as malonaldehyde (MDA) and decreased antioxidant defense enzymes including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) induced by CMS were also reversed by magnolol. These findings suggest that administration of magnolol could alleviate depressive-like behaviors in CMS mice that are mediated by suppressing neuroinflammation and oxidative stress in the prefrontal cortex.

1. Introduction

Depression is a major psychiatric disorder affecting as high as 21% of the general population in some developed countries, resulting in enormous personal suffering, as well as economic and social burdens. The mechanisms underlying depression are very complicated, and its pathophysiology has not been well characterized [1]. Among the many biological processes thought to be involved in the pathophysiology of depression, some studies have highlighted changes in neurotrophic, neuroinflammation, neuroendocrine and oxidative balance as important mechanisms underlying depression. Moreover, the success of some classic antidepressants has been attributed to the changes in neuroinflammation, oxidative stress and neuroendocrine [2].

The link between neuroinflammation and major depressive disorder is verified by the clinical investigation that there is a positive correlation between the depression and neuroinflammation [3]. The basic studies have deeply demonstrated that activation of Toll-like receptor 4 (TLR4) by stress or LPS causes the activation of nuclear factor kappa B (NF-κB) and nucleotide binding and oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome [4,5]. NLRP3 is responsible for the process from pro-Interleukin-1β (IL-1β) to mature IL-1β. The production of IL-1β then induce its own expression and the expression of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) [6]. Elevation of the pro-inflammatory cytokines subsequently activates the hypothalamic–pituitary–adrenal (HPA) axis by stimulating neurons of the paraventricular nucleus [7]. At the same time, elevation of the pro-inflammatory cytokines also leads to damage of oxidative/anti-oxidative systems [8].

Magnolol, the main constituent of *Magnolia officinalis*, has been shown to decrease immobility time in the forced swimming test and increase sucrose preference in the chronic mild stress (CMS), which indicates that magnolol possesses the antidepressant-like effects of [9]. Furthermore, previous studies also indicated that the antidepressant-like effects of magnolol might be related to normalize the hypothalamic-pituitary-adrenal hyperactivity and increase the monoamine neurotransmitters [9,10]. In addition, magnolol could also increase BDNF levels, promote neurogenesis and improve glial atrophy in the brain [11–13]. However, the mechanisms underlying the

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antidepressant response of magnolol via anti-inflammation have not been investigated in depth, although several previous studies indicated that magnolol produced anti-inflammatory activity in vitro and in vivo [14,15].

Here, we established a mouse model of depression induced by CMS and assessed the antidepressant-like effects of magnolol. Importantly, we aimed to elucidate the molecular mechanisms underlying the antidepressant-like response of magnolol by measuring the changes of pro-inflammatory cytokines and microglia activation in the prefrontal cortex. Besides, considering that hyperactivity of the HPA axis and oxidative stress are also involved in the pathophysiology of depression and induced by neuroinflammation, our study also evaluated whether the antidepressant-like effects of magnolol was mediated by HPA axis suppression and antioxidation in the peripheral or the prefrontal cortex.

2. Material and methods

2.1. Animals

Male Kunming mice (22–26 g, ten weeks old) were purchased from Fujian Medical University (Fuzhou, China). Four animals were housed per cage (320 × 180 × 160 mm). The mice were housed in a normal environment with a 12-h/12-h light/dark cycle, 22 ± 2 °C ambient temperature and 55 ± 5% relative humidity. During the whole procedure, these animals were fed with normal food and water. All procedures were approved and performed according to the standard guidelines of the China Council on Animal Care.

2.2. Reagents

Magnolol was purchased from Aladdin (Shanghai, China, > 98% by HPLC). Fluoxetine hydrochloride and DAPI was purchased from Sigma-Aldrich (St. Louis, USA). IL-1β, IL-6 and TNF-α ELISA kits were purchased from Boster (Wuhan, China). Adrenocorticotropic hormone (ACTH) and corticosterone ELISA kits were purchased from Cusabio (Wuhan, China). Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malonaldehyde (MDA) kits were purchased from Nanjing Jiancheng (Nanjing, China). The antibody for iba1 was purchased from Abcam (Cambridge, USA).

2.3. Chronic mild stress (CMS) procedure

The procedure of 8-week CMS using in the present study was performed as described previously [16]. Briefly, we used the following stressors to established depressive-like symptoms: deprivation with food and water, dirty cage, empty bottle exposure, irregular light/dark cycle, space reduction, noise, 45° cage tilt, sustained and illumination overnight. During the procedure, these stressors were applied continuously and were randomly scheduled to ensure the unpredictability and avoid the adaptation by mice.

2.4. Drug administration

The following subgroups were applied (n = 12): the Control-vehicle group, CMS-vehicle group, the CMS-magnolol group receiving 20 mg/kg or 40 mg/kg magnolol, and the CMS-fluoxetine group receiving 20 mg/kg fluoxetine. Saline with 0.3% carboxymethyl cellulose was used as vehicle, magnolol and fluoxetine were dissolved in vehicle and were administered in a volume of 10 mL/kg. Vehicle, magnolol and fluoxetine were orally administrated (p.o.) once daily for the last 4 weeks of the experiment. All these agents the doses chosen were based on the behavioral results and previous reports [11,13].

2.5. Sucrose preference test

The sucrose preference test was conducted after 4 weeks drug administration. Firstly, the mice were trained to adapt to two bottles sucrose solution (1%, w/v) for 24 h before the formal test. Then the mice were adapted to one bottle of sucrose solution and one bottle of water for another 24 h. Subsequently, the mice were deprived of water but not food for 12 h. One sucrose bottle and one water bottle were placed into the cage for 24 h. Finally, the sucrose preference was calculated by weighing the consumed sucrose solution and water.

2.6. Forced swimming test

The forced swimming test was conducted 24 h after the sucrose preference test according to the traditional method previously described by Porsolt et al. [17], with minor modifications. Briefly, a glass cylinder (20 cm in height, 14 cm in diameter) filled with 10-cm of water (25 ± 2 °C) was used for mice. All mice were placed into the water and forced to swim for 6 min. Only the last 4 min of the test was used for immobility time record. Water was replaced every session. The test were recorded and were scored by a blind observer.

2.7. Serum and brain samples collection

Mice were killed by decapitation after the forced swimming test. Blood samples were collected for serum separation. The separated serum samples were stored at −20 °C until assay. Whole brains were extracted from and the brain region of prefrontal cortex was carefully dissected and then stored at −80 °C until assay. Before the formal measurement, the tissues of prefrontal cortex were smashed with liquid nitrogen and sub-packed in tubes.

2.8. ELISA analysis for pro-inflammatory cytokines, ACTH and corticosterone

IL-1β, IL-6 and TNF-α levels in the prefrontal cortex were measured by ELISA kits following the manufacture's instruction.

ACTH and corticosterone levels in the serum were measured using the commercial ELISA kit following the manufacturer's instructions.

2.9. qPCR

Total RNA was isolated from the frozen hippocampus after adding Trizol reagent following the manufacturer's instructions. The concentration and purity of RNA were measured by Micro Nucleic Acid Analyzer. Then, the high quality of total RNA was reversely transcripted. Subsequently, real-time PCR reactions were performed using a SYBR Premix Ex Taq Kit. The IL-1β (forward 5′-TGCCACCTTTTGACA GTGATG-3′; reverse 5′-TGATGTGCTGCTGCGAGATT-3′), IL-6 (forward 5′-CCCCAATTTCCAATGCTCTCC-3′; reverse 5′-CGCACTAGGTTTGCCG AGTA-3′), TNF-α (forward 5′-GATCGGTCCCCAAAGGGATG-3′; reverse 5′-CCACTTGGTGGTTTGTGAGTG-3′), and internal control GAPDH (forward 5′-TGAGGCCGGTGCTGAGTATGT-3′; reverse 5′-CAGTCTTCT GGGTGGCAGTGAT-3′) primers were used. GAPDH was used as an internal reference to normalize gene expression.

2.10. Determination of SOD, GSH-Px and MDA

SOD and GSH-Px activities, as well as MDA levels in the prefrontal cortex were measured by commercial kits following the manufacturer's instructions. The SOD and GSH-Px activities was expressed as unit/mg protein of the tissue, the MDA levels were expressed as nmol/mg protein of the tissue.

2.11. Immunofluorescence

Mice were anesthetized and then sacrificed by intracardial perfusion with PBS and 4% paraformaldehyde, respectively. The brains were removed and postfixed with 4% paraformaldehyde for 24 h and were