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Baicalin exerts neuroprotective effects via inhibiting activation of GSK3β/NF-κB/NLRP3 signal pathway in a rat model of depression

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ABSTRACT

Chronic stress can provoke depressive-like behaviors through activation of inflammation and apoptosis, leading to a reduction of neurons. Antidepressant therapy may contribute to inhibiting inflammation responses and have neuroprotective effects. Baicalin (BA) has an antidepressant effect in the chronic unpredictable mild stress (CUMS) animal model and exerts anti-inflammation, anti-apoptosis, as well as neuroprotective effects in many central nervous system (CNS)-related diseases. But the effects of BA on neuroprotection, apoptosis, and neuroinflammation and the potential mechanisms in depression are unclear. Here, we focused on examining the therapeutic effects of BA in CUMS-induced depression rats and investigating the molecular mechanisms. Results showed that administration of BA improved depressive-like behaviors and significantly increased the levels of doublecortin (DCX), Neuron-specific enolase (NSE), and Brain-derived neurotrophic factor (BDNF) in hippocampus. Furthermore, administration of BA increased the cell survival by reducing the level of malondialdehyde (MDA) and increasing the level of superoxide dismutase (SOD). Finally, administration of BA significantly decreased CUMS-induced apoptosis and inflammatory cytokines (caspase-1 and IL-1β) in hippocampus. These responses were mediated by Glycogen synthase kinase-3 (GSK3) β/Nuclear factor-κB (NF-κB)/Nucleotide-binding domain, leucine-rich repeat, pyrin domain containing protein 3 (NLRP3) signal pathway. Taken together, these results indicate that BA could promote neuronal maturation and rescue neurons from apoptosis via inhibiting activation of GSK3β/NF-κB/NLRP3 signal pathway that is known to be associated with inflammation, thus exerting neuroprotective effects and preventing CUMS-induced depressive-like behaviors.

1. Introduction

Major depressive disorder (MDD), a mood disorder with a variety of symptoms, remains a significant cause of mortality. Several mechanisms are involved in its pathophysiology including monoamine deficiency, abnormalities in circadian rhythm, dysregulation of Hypothalamus-Pituitary-Adrenal (HPA) axis, changes in hormonal status and increased levels of inflammatory cytokines [1]. Although there are multitude antidepressant drugs, nearly one third of patients are refractory to antidepressant treatment [2]. Thus, it's necessary to develop effective treatment and further understand the mechanisms of depression.

Reduction of mature neurons and increases of neural apoptosis are two important factors that take part in the physiological and

Abbreviations: MDD, major depressive disorder; HPA, Hypothalamus-Pituitary-Adrenal; CNS, central nervous system; NSCs, neural stem cells; DG, dentate gyrus; CUMS, chronic unpredictable mild stress; BA, Baicalin; SPT, sucrose preference test; FST, forced swimming test; OFT, open field test; ELISA, Enzyme-linked immunosorbent assay; FLU, fluoxetine; DCX, doublecortin; NSE, Neuron-specific enolase; MDA, malondialdehyde; SOD, superoxide dismutase; SPR, sucrose preference ratio; PVDF, polyvinylidene difluoride; ECL, chemiluminescence; BDNF, Brain-derived neurotrophic factor; Bcl-2, B-cell lymphoma-2; Bax, Bcl2-associated x; GSK3, Glycogen synthase kinase-3; NF-κB, Nuclear factor-κB; IL-1β, Interleukin-1 beta; NLRP3, Nucleotide-binding domain, leucine-rich repeat, pyrin domain containing protein 3; CORT, corticosterone; OBX, olfactory bulbectomy; GSH-Px, glutathione peroxidase; GR, glucocorticoid receptor

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pathological processes of depression. Evidence supports the view that depression is accompanied by neurodegenerative processes [3,4]. There is also evidence that enhanced apoptosis is detected in the brain structures of rats tested in animal models of depression, such as repeated unpredictable stress [5]. Interestingly, Maes puts forward the hypothesis that all these changes in depression are caused by inflammation and neurodegeneration [6]. Moreover, a review of the relationship among inflammation, neurogenesis, and apoptosis in depression concludes that external stressors may provoke depression-like behaviors through activation of inflammatory, oxidative, apoptotic, and antineurogenic mechanisms [7]. These studies indicate that anti-inflammation, anti-apoptosis, and protection of neurons may be promising strategies for treating depression.

Baicalin (BA), a major polyphenol compound isolated from the dry root of *Scutellaria baicalensis* Georgi, showed a variety of biological effects in the central nervous and immune systems, and several studies have investigated the anti-inflammatory [8], anti-oxidant [9], and anti-apoptosis [10] properties of BA. Moreover, it is recently reported that BA can promote neurogenesis and attenuate emotional and olfactory dysfunctions in chronic corticosterone (CORT)-induced depression [11]. However, the effects of BA on neuroprotection, apoptosis, and neuroinflammation and the potential mechanisms in a chronic unpredictable mild stress (CUMS)-induced depression animal model are unclear. CUMS-induced depression animal model can mimic the core symptom of human depression and is more widely used to study the mechanism of antidepressants than other models of depression. So we used CUMS procedure to establish depression model in order to study the effects of BA on neuroprotection, apoptosis, and neuroinflammation, which is different from previous studies.

Therefore, the present study examined the therapeutic effects of BA in a CUMS-induced depression rat model and investigated the molecular mechanisms underlying the perspective of neuroprotection, apoptosis, and neuroinflammation. The antidepressant-like effects of BA were evaluated by sucrose preference test (SPT), the forced swimming test (FST), and the open field test (OFT). In addition, we assessed the expression of proteins for neuroprotection, apoptosis, and inflammation in the hippocampus with Western blot and Enzyme-linked immunosorbent assay (ELISA) kit.

2. Material and methods

2.1. Animals

A total of 60 adult male Sprague-Dawley rats (180–220 g) were purchased from Experimental Animal Center in Jiangsu Province (Nanjing, China) and allowed acclimating to the animal facility for 1 week prior to experiments. The rats were housed under a 12/12-hour light/dark cycle (7 am/7 pm) and regulated temperature conditions (22 ± 2 °C), with food and water freely available. All animal procedures were performed in accordance with the National Institute of Health (NIH publication No. 80-23, revised 1996) Guide and were conformed to the PR China legislation for the care and use of laboratory animals.

2.2. Chronic unpredictable mild stress (CUMS) procedure

The CUMS procedure was slightly modified from the published procedures described by Willner [12]. Specific details of the CUMS procedure were as follows: (1) food deprivation (24 h); (2) water deprivation (24 h); (3) overnight illumination; (4) cage tilt (45°); (5) soiled cage (200 ml water in 100 g sawdust bedding); (6) exposure to a foreign object; (7) light/dark perversion; (8) overhang (10 min); (9) strained in a bottle; (10) tail pinch (1 cm from the beginning of the tail); (11) oscillation; and (12) white noise. Each animal was exposed to two stresses per day individually. This paradigm was devised to maximize unpredictability, in that the stressors were applied in seemingly random order and at varying times. All procedures were carried out in isolated rooms adjacent to the housing room, requiring minimal handling or transport of the rats. Control rats were housed for the same period of time, and were handled daily for 30s in the housing room, but were not stressed. The CUMS procedure was applied for six weeks.

2.3. Drug and treatment

BA (purity > 97%) was purchased from Tianjin Silan Technology Co., Ltd. (Tianjin, P.R, China). Fluoxetine hydrochloride (FLU) (positive control drug) was obtained from Changzhou Siyao Pharmaceuticals Co., Ltd. (Changzhou, P.R, China). BA and FLU were dissolved in saline.

After the third week of the CUMS paradigm, when depressive-like behaviors occurred, drugs were administered once per day for three weeks. All drugs and vehicle (0.9% normal saline) were administered in a volume of 10 ml/kg of body mass via intragastric administration between 8:00 and 10:00 a.m. The control group was given the same volume of vehicle.

All rats were randomly assigned to five groups (n = 12/group) defined by control group, CUMS group, CUMS + FLU (10 mg/kg) group, and CUMS + BA (20 and 40 mg/kg) group. In the control group, animals did not receive the CUMS procedure and received only saline. The other groups were exposed to the CUMS procedure and received freshly prepared vehicle (normal saline), FLU (10 mg/kg), and BA (20 and 40 mg/kg). Behavioral tests were performed 1 h after the last drug administration on d42.

2.4. Behavioral studies

2.4.1. Sucrose preference test (SPT) and body weight measurement

The SPT was performed as a traditional method with minor modifications [13]. In brief, 72 h before the test, rats were singly housed with two identical bottles of sucrose solution to adapt sucrose solution (1%, w/v) for 24 h; then one bottle of sucrose solution was replaced with water (24 h); after the adaptation, laboratory rats were deprived of water and food (24 h). Sucrose preference test was conducted at 9:00 p.m. Each animal was singly housed in a freshly made home cage and provided with two bottles containing either tap water or 1% sucrose solution for 12 h. The sucrose preference ratio (SPR) was calculated as follows: SPR (%) = sucrose intake (g) / [sucrose intake (g) + water intake (g)] × 100%.

Body weight was measured between 15:00 and 17:00 on Monday every week to calculate the mean body weight gain during the entirety of the experiment.

2.4.2. Open field test (OFT)

In order to exclude false positive results of BA as a psychostimulant drug that reduces the immobility time in depression-related behavior in FST, the OFT was conducted before the FST. One hour after the treatment on d42, the OFT was performed to evaluate the locomotor activity of rats (crossing: horizontal movement scores reflect range of motion; standing: vertical movement scores reflect exploratory behaviors; and grooming). The open field apparatus was a four-sided 100 × 100 × 40 cm black metallic enclosure with a white open floor and was divided into 25 equal sectors by red lines. Each animal was placed individually into the center of arena, and was allowed to explore freely for 6 min. The crossing (entering into a new sector with four paws), standing (erecting on its hind legs), and grooming were documented by a trained technician who was blind to the experimental group during the last 4 min. After each trial, the open field apparatus was cleaned.

2.4.3. Forced swimming test (FST)

The FST was conducted using a method adapted with minor modifications [14]. During the FST, rats were individually subjected to a 6 min swim session in clear Plexiglass cylinders (50 cm height × 20 cm