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Research paper

P2X7 as a new target for chrysophanol to treat lipopolysaccharide-induced depression in mice

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HIGHLIGHTS

- Chrysophanol inhibited P2X7 in LPS-induced depression.
- Chrysophanol inhibited NF-κB in LPS-induced depression.
- Chrysophanol mitigates lipopolysaccharide-induced depression.

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ABSTRACT

P2X7 receptor is a ligand gated ion channel found peripheral macrophages and microglia in the nervous system. The current study investigated the relationship between the activated P2X7 and depression for the first time. Chrysophanol (Chr) was examined for its protective effects against depression targeting P2X7. Chr (20 mg/kg, 40 mg/kg) and fluoxetine (20 mg/kg) were intragastrically treated once daily for 7 consecutive days. Lipopolysaccharide (LPS, 0.5 mg/kg) was intraperitoneally injected to develop depression model 30 min after drug administration on day 7. Behavioral tests were measured 24 h after LPS injection. Interleukin (IL)-6, IL-1β and tumor necrosis factor (TNF)-α levels in serum and hippocampus were measured by enzyme-linked immunosorbent assay (ELISA). The expressions of P2X7/NF-κB pathway-related proteins were assessed by western blot. The findings showed that Chr remarkably reduced the elevations of IL-6, IL-1β and TNF-α caused by LPS stimulation. The expressions of P2X7, p-IKKα, p-IKKβ, p-IκBα and p-NF-κBp65 were significantly decreased by Chr pretreatment. In addition, immobility time in tail suspension test (TST) and forced swimming test (FST) were reduced by Chr without affecting spontaneous locomotor activity in open filed test (OFT) and the preference for sucrose was also recovered in sucrose preference test (SPT) with Chr preconditioning. Thus, it is reasonable to speculate that Chr might exert antidepressant effect through inhibiting P2X7/NF-κB signaling pathway.

1. Introduction

Major depressive disorder (MDD) is a common debilitating disorder which can be triggered by chronic psychosocial stress. It was reported that depression affects 7% of the world's population [1]. Therefore, the development for new therapeutic drugs is imperative for patients with this diagnosis. Key symptoms of major depression are considered as depressed mood, boring, concentration deficits, anhedonia, fear, feelings of worthlessness, insomnia and weight loss [2]. During the past few decades, the monoamine, hypothalamus–pituitary–adrenal (HPA) system, neurotrophin and hippocampal neurogenesis hypothesis have received extensive attention in neurobiological studies of depression.

The traditional treatment for depression is focused on neurotrophic system, hypothalamicpituitary-adrenal axis, 5-hydroxytryptamin system and neurogenesis. In addition, inflammation is acknowledged as a critical pathophysiological mecha-

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nism of neuropsychiatric disorders. Previous research proposed that inflammation can induce the abnormities in CNS, including release of inflammatory cytokines, excitatory amino acids and nitroxidative species which may be responsible for cognition deficits and depressive-like behavior [3]. As the important constituent of the Gram-negative bacteria, LPS activates innate immune response and secrete inflammatory cytokines [4]. Inflammatory stimuli (like LPS) could activate indoleamine-2, 3-dioxigenase (IDO) enzyme and up-regulate 5-HT transporter (SERT) in the brain, which contributed to depressive-like behaviors. It has been suggested that excessive secretion of cytokines, such as IL-6, IL-1β and TNF-α, could be a potential causative factor for depression [5,6]. The purinergic P2X receptors, ligand-gated ion channels activated by 5′-triphosphate (ATP) and other related nucleotides, are constitutive of seven subunits that are regulated by different genes (P2X1–P2X7) [7]. P2X7 has been reported to be involved in the progression of neurodegenerative diseases, modulation of cell growth and inflammatory reaction [8–10]. As P2X7 receptor is implicated in inflammatory progression, it is reasonable to hypothesize that P2X7 receptors may play an important role in the pathology of depression. Herein, we conducted the investigation to verify whether Chr could relieve LPS-induced depression in mice targeting P2X7.

Nowadays, accumulating evidence reported the development of natural traditional Chinese medicine due to its abundant resources and less adverse effects [11,12]. Chrysophanol (Chr, 1, 8-dihydroxy-3-methy anthraquinone) belongs to the anthraquinone family and previous pharmaceutical literatures have documented that derivatives of anthraquinones possess anticancer [13] hepatoprotective [14] anti-microbial [15] and anti-inflammatory [16] properties. Recently, it has been reported that Chr is able to resist aging, improve learning and memory disorders [17]. Thus, the purpose of the present study was to explore whether Chr could protect mice against depression and explore its potential mechanism.

2. Materials and methods

2.1. Main reagents and kits

Chrysophanol (Chr, purity 98%) was purchased from National Institutes for Food and Drug Control (Beijing, China). Fluoxetine hydrochloride (Flu), provided by Changzhou Siyao Pharmaceuticals Co., Ltd. (Changzhou, PR China), was dissolved in 0.03% sodium carboxymethyl cellulose (CMC-Na). LPS (*Escherichia coli* serotype 055: B5, No. L-2880, Sigma–Aldrich, St. Louis, MO, USA) was dissolved in sterile, pyrogen-free normal saline. TNF-α, IL-1β and IL-6 enzyme-linked immunosorbent assay (ELISA) kits were obtained from Nanjing KeyGEN Biotech. CO., Ltd. (Nanjing, China). Primary antibodies against P2X7, p-IKKα, IKKα, p-IKKβ, IKKβ, p-IκBα, IκBα, p-NF-κBp65 and NF-κBp65 were produced by Cell Signaling Technology Inc (Beverly, MA, USA).

All other chemicals and reagents used for study were of analytical grade and were purchased from approved organizations.

2.2. Animals

50 male ICR mice were purchase from the Experimental Animal Center in Jiangsu Province (Nanjing, China) and maintained in the animal center at an ambient temperature (22 ± 1 °C) under a 12 h light/12 h dark cycle environment. Besides, standard food and water were provided *ad libitum*.

2.3. Experimental protocol

Mice were randomly assigned to five groups (with 10 in each group), namely, control group, model group, LPS + Flu (20 mg/kg) group, LPS + Chr (20 mg/kg) group and LPS + Chr (40 mg/kg) group. Chr (20 mg/kg, 40 mg/kg) and Flu (20 mg/kg) was intragastrically administered once daily for 7 consecutive days in advance. LPS (0.5 mg/kg) was subcutaneously injected to develop the depression model 30 min after drug administration on day 7. The control group was subcutaneously injected with sterile, pyrogen-free nomal saline. Behavioral assessment was conducted 24 h after LPS challenge and blood sample from orbit was immediately carried out after behavioral measurements. Hippocampi from both hemispheres were quickly and carefully removed by curved tweezers. The hippocampi were harvested in cryopreservation tubes and frozen at −80 °C until further assay.

2.4. Behavioral evaluation

2.4.1. Open field test (OFT)

The open-field apparatus consists of a square wooden arena (40 × 60 × 50 cm) with a dark surface covering the inside walls and it is divided into 12 equal squares. Mice were placed individually into the center of the area and allowed to acclimatize the environment freely before the test. During the test, the number of squares crossed, rearing and grooming behaviors were recorded for 4 min by two independent observers blinded to the experiment. After each animal was tested, the square arena was cleaned with a solution of 70% alcohol to eliminate odors for the next test.

2.4.2. Sucrose preference test (SPT)

Mice were subjected to the challenge as follows on day 4: (1) 1% sucrose solution (w/v) adaptation: two bottles of 1% sucrose solution were placed in each cage for 24 h, the 1% sucrose solution in one bottle was replaced with tap water for another 24 h, (2) water and food deprivation for 24 h, (3) the choice to drink for 12 h from two bottles filling with sucrose solution (1% w/v) and water respectively. In addition, the access to two bottles in the cage was the same and the positions of them were swapped 6 h later. The final consumption of each mouse was calculated by the following formula:

$$\text{SPT} = \left(\frac{\text{sucrose intake (g)}}{(\text{sucrose intake (g)} + \text{water intake (g)})}\right) \times 100$$

2.4.3. Forced swimming test (FST)

The forced swimming test was carried out according to the conventional method recorded previously [18]. Briefly, every mouse was forced to swim for 6 min 24 h post LPS exposure in an open cylindrical container (diameter = 14 cm, height = 20 cm) containing water 12 cm deep, maintained at 25 ± 1 °C. The period of escape-oriented behaviors were considered as immobility time. The total immobility time (mice remained completely motionless) during the last 4 min was blindly analyzed by two independent observers. The water was changed after each group.

2.4.4. Tail suspension test (TST)

The forced swimming test was carried out according to the conventional method recorded previously [19]. Briefly, 24 h post LPS stimulation, every mouse both acoustically and visually separated was individually hang up from tail with adhesive tape (approximately 2 cm from the end) for 6 min with 50 cm above the floor. The duration of immobility time (mice remained completely motionless) of the last 4 min was blindly analyzed by two independent observers.

2.5. Cytokine measurement in serum and hippocampus

Blood samples were collected from orbit and were centrifuged at 3500 rpm for 10 min to obtain the serum. Mouse hippocampus samples were homogenized in 0.9% normal saline (w:v 1:9) and