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Original article

Possible involvement of L-arginine–nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) signaling pathway in the antidepressant-like effect of Wuling mycelia powder in rat

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ABSTRACT

Context: Wuling mycelia powder is the dry powder of rare a fungi *Xyla ria* sp., Carbon species, with a long history of medicinal use in Chinese medicine. Recently it has shown a powerful antidepressant activity in clinic.

Objective: The present study explores the antidepressant activity of Wuling mycelia powder in chronic unpredictable mild stress (CUMS) rats and its possible involvement of L-arginine–nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) signaling pathway.

Materials and methods: Experiments were performed in the rat CUMS model. CUMS rats were treated with Wuling mycelia powder (0.5, 1.0 or 2.0 g/kg, i.g.) to test behavioral changes including the sucrose preference, the crossing number and food consumption. Further, L-arginine (substrate for nitric oxide) (750 mg/kg), 7-nitroindazole (a specific neuronal nitric oxide synthase inhibitor) (25 mg/kg), sildenafil (phosphodiesterase 5 inhibitor) (5 mg/kg) and methylene blue (direct inhibitor of both nitric oxide synthase and soluble guanylate cyclase) (10 mg/kg) were treated for 60 min before each test to detect the possible mechanism of antidepressant-like effect of Wuling mycelia powder.

Results: After 4 weeks of administration, both 1.0 or 2.0 g/kg Wuling mycelia powder suppressed the behavioral changes including the sucrose preference [$F(3, 31) = 50.87$, $p < 0.001$], the crossing number [$F(3, 31) = 68.98$, $p < 0.05$], and food consumption [$F(3, 31) = 19.04$, $p < 0.05$] in the CUMS rats. The antidepressant-like effect of Wuling mycelia powder was prevented by pretreatment with L-arginine and sildenafil. Pretreatment of rats with 7-nitroindazole and methylene blue potentiated the effect of Wulin mycelia powder.

Discussion and conclusion: Our findings demonstrate that Wuling mycelia powder has an antidepressant-like effect in the CUMS rats, and possible involvement of L-arginine–nitric oxide–cyclic GMP signaling pathway in its antidepressant effect.

1. Introduction

Wuling mycelia powder is the dry powder of a rare fungi, *Xyla ria* sp., carbon species, with a long history of medicinal use in Chinese medicine [1]. Wuling capsule, a compound traditional Chinese herbal medicine, of which the main ingredient is Wuling mycelia powder, has been used to improve the signs of insomnia and cognitive deficits in clinic for many years, and has shown a more powerful antidepressant activity in clinic recently [1,2].

Depression is a common and persistent psychiatric illness and presents a considerable social and economic burden [3]. The World Health Organization (WHO) predicted that depression would be the first leading cause of death worldwide by 2030. There are several hypothesises about pathogenesis of depression. L-arginine–nitric oxide–cGMP is an important signaling pathway that is reported to be involved in depression [4]. Nitric oxide, a messenger molecule in the brain, is synthesized from L-arginine by nitric oxide synthase (NOS), and has been implicated in neurotransmission, synaptic plasticity, learning, perception of pain, aggression and depression [5]. Recent evidences have shown that the reduction of NO levels within the hippocampus can induce antidepressant-like effects, thus implicating endogenous hippocampal NO in the neurobiology of stress and depression [6]. Several physiological actions of NO are mediated through its interaction with the heme

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iron of soluble guanylate cyclase [sGC], leading to enzyme activation and consequent increase in cGMP [7]. Recent studies have shown the possibility that the inhibition of NO synthase could be used as a strategy to enhance the clinical efficacy of serotonergic antidepressants [8].

Therefore, the present study attempted to investigate the antidepressant-like effect of Wuling mycelia powder in chronic unpredictable mild stress (CUMS) rats, and the possible involvement of L-arginine–nitric oxide–cGMP signaling pathway in mediating its antidepressant action.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (180–200 g) were obtained from the Laboratory Animal Center of Zhejiang University. Rats were kept in an air-conditioned room with a 12 h light/dark cycle with free access to food and water except when animals were subjected to deprivation stressors as described in CUMS. All experiments and procedures were carried out according to guidelines from the China Council on Animal Care and International Association for the Study of Pain Committee for Research and Ethical Issues (CONACYT 2nd Order).

2.2. Drugs and treatment

The following drugs were used: Wuling mycelia powder (Zhejiang Jolly Pharmaceutical Co., Ltd., China), fluoxetine (Eli Lilly and Co., USA), L-arginine (Sigma–Aldrich, St. Louis, MO, USA), 7-nitroindazole (Tocris Bioscience, Missouri, USA), methylene blue (Sigma–Aldrich, St. Louis, MO, USA), sildenafil (Sigma–Aldrich, St. Louis, MO, USA). All the drugs were dissolved in water except 7-nitroindazole, which was dissolved in few drops of Tween 80 and volume was made with distilled water (0.9% w/v). The doses of the drugs used were selected according to the previous studies [9].

In the antidepressant activity experiment of Wuling mycelia powder, rats were randomly divided into two groups (9 for the control and 45 for the CUMS group). For the CUMS group, the animals were isolated and treated simultaneously with CUMS. Two weeks later, the CUMS group was split into 5 treatment groups including the CUMS model group, Wuling mycelia powder groups (0.5, 1.0 or 2.0 g/kg, i.g.) and fluoxetine group (0.25 g/kg, i.g.) and each group contained 9 rats. And then all drugs were given once daily 30 min before the stress exposure for 4 weeks.

For studying the possible participation of the L-arginine–NO–cGMP signaling pathway in the antidepressant effect of Wuling mycelia powder, rats were randomly divided into the following groups (nine rats in each group): The CUMS group and the CUMS + Wuling groups (0.5, or 1.0 g/kg, i.g.) were treated the same as described above. The CUMS + nitric oxide modulators groups were injected with each drug (L-arginine 750 mg/kg, 7-nitroindazole 25 mg/kg, methylene blue 10 mg/kg or sildenafil 5 mg/kg, all i.p.) 60 min before each test. The CUMS + nitric oxide modulators + Wuling groups were given with Wulin mycelia powder once daily 30 min before the stress exposure during the last 4 weeks, and injected with nitric oxide modulators 60 min before each test.

3. Experimental procedure

3.1. CUMS procedure

The CUMS procedure was performed as described previously with minor modifications [10]. The CUMS treated rats were subjected to the following stressors in a random order every day for 6 weeks: 24 h food deprivation, 24 h water deprivation, exposure to an experimental room at 45 °C for 5 min, swimming in 4 °C cold water for 5 min, tail clamp for 2 min, foot shock for 2 min, noises for 3 h. The control group rats were left undisturbed except for necessary procedures such as routine cage cleaning.

3.2. Sucrose preference test

The sucrose preference test was carried out at the end of the 6-week CUMS exposure. The test was performed as described previously with minor modifications [10]. Briefly, before the experiment was carried out, all rats were exposed to a 1% (w/v) sucrose solution for 24 h to avoid neophobia. Then, two bottles, one containing 1% sucrose solution and the other containing tap water, were weighed and presented to each rat for 1 h. The position of the tap water bottle and sucrose solution bottle were randomly determined. Sucrose and water consumptions (g) were measured, and the sucrose preference was calculated using the equation:

$$\text{Surcrosepreference} = \frac{\text{sucroseconsumption}}{\text{waterconsumption} + \text{surcroseconsumption}} \times 100\%$$

3.3. Open-field test

The open-field test was performed at the end of 0, 2 and 6 weeks. The custom-made apparatus was a $100 \times 100 \times 40\,cm^3$ black metal cage whose bottom was divided into 25 equal sectors by white stripes. Each rat was gently placed in the central square and allowed to explore freely. The number of crossings during a test period of 5 min was recorded.

3.4. Food consumption test

The food consumption test was carried out 24 h after the final sucrose preference test. In this test, 50 g food was given to each isolated rat and the remaining food was weighed after 24 h, and the difference between the initial and final weight is the food consumption for the rat.

3.5. Measurement of NO levels

An indirect measurement of NO levels via the production of nitrite formed from its metabolism was performed to evaluate the effect of Wuling mycelia powder on NO levels. Animals were killed 60 min after administration of distilled water or Wuling mycelia powder (1.0 g/kg, i.g.), and hippocampi and cerebral cortices were rapidly removed. Briefly, one cerebral cortex or a pool of three hippocampi were mixed with 25% trichoroacetic and centrifuged at $1800 \times g$ for 10 min. The supernatant was immediately neutralized with 2 M potassium bicarbonate. Nitrate (NO^{3-}) was reduced to nitrite (NO^{2-}) by nitrate reductase. The total NO^{2-} in the incubation was measured by a colorimetric assay read at 540 nm, based on the Griess reaction. A standard curve was performed using sodium nitrate (0–80μM). Results were expressed as % of control (100%) [11].

3.6. Statistical analysis

Statistical analysis was carried out using SPSS 16.0 software for Windows (SPSS Inc., Chicago, IL, USA). All values were expressed as mean(s) ± S.E.M and the data was analyzed using One Way or Two Way Analysis of Variance (ANOVA) wherever appropriate. If any statistically significant change was found, *post-hoc* comparisons