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Antidepressant-like effects of the saponins extracted from *Chaihu-jia-longgu-muli-tang* in a rat unpredictable chronic mild stress model

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ABSTRACT

Chaihu-jia-longgu-muli-tang (CLM) has been used for treating depressive disorders for thousands of years in China. In the present study, we investigated the antidepressant-like effect of the saponins extracted from CLM (SCLM) in rats subjected to unpredictable chronic mild stress (UCMS). The ameliorative effect of SCLM on symptom of depression through behavior tests including: sucrose preference test, open-field test and forced-swimming test was investigated. In addition, high performance liquid chromatography with electrochemical detection (HPLC–ECD), immunohistochemical staining analysis and RT-PCR were applied to explore the mechanisms underlying the antidepressant-like effects of SCLM. It was observed that administration of SCLM (70, 140 mg/kg) reversed the depressive-like behaviors, restored the reduction in the levels of monoamine neurotransmitters and up-regulated the expression of brain-derived neurotrophic factor (BDNF) in UCMS-treated rats. These findings confirmed the antidepressant-like effects of SCLM in UCMS model of rats.

1. Introduction

Depression is one of the most common mental disorders, with high prevalence and mortality, resulting in massive socioeconomic burden [1]. It is characterized by chronic depressed mood, the inability to experience pleasure, loss of interest in usual activities, feelings of worthlessness, and suicidal tendencies [2–4]. Up to now, chemical antidepressants including tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), selective serotonin and norepinephrine reuptake inhibitors (SSRIs, SNRIs, respectively), have been widely available in the pharmaceutical market [5]. However, many of antidepressants often produce side-effects such as sedation, cognitive impairment, sexual dysfunction etc. [6,7]. Therefore, research for new antidepressants with greater effectiveness without any (or with lower) adverse effects is still desirable [8].

Chaihu-jia-longgu-muli-tang (CLM, *saiko-ka-ryukotsu-borei-to* in Japanese) was originally recorded in "Treaties on Febrile Disease", which was written one thousand years ago. In China, CLM has been extensively used for treating neuropsychiatric disorders, such as depressive illness [9]. In our previous studies, we found that the saponins extracted from CLM (SCLM) were primary derived from *Radix Bupleuri*, *Radix Ginseng Poria*, *Fructus Zizyphi jujubae* and were the major constituent of the concoction. In addition, SCLM exerted antidepressant-like effects in the behavioral despair models of depression including tail suspension test (TST) and forced swimming test (FST) in mice. The SCLM at 35,

Abbreviations: CLM, *Chaihu-jia-longgu-muli-tang*;SCLM, saponins of *Chaihu-jia-longgu-muli-tang*;FST, forced swimming test;UCMS, unpredictable chronic mild stress;SP, sucrose preference;PFC, prefrontal cortex;DG, dentate gyrus;BDNF, brain-derived neurotrophic factor;HPLC–ECD, high performance liquid chromatography with electrochemical detection;FLU, Fluoxetine hydrochloride;DA, dopamine;DOPAC, 3, 4-dihydroxyphenylacetic acid; HVA, homovanillic acid;5-HT, serotonin;5-HIAA, 5-hydroxyindoleacetic acid;DHBA, 3, 4-dihydroxybenzylamine;TCAs, tricyclic antidepressants; MAOIs, monoamine oxidase inhibitors;SSRIs, selective serotonin reuptake inhibitors

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70, 140 mg/kg could also decrease the immobility time in FST [10]. The doses of SCLM (70, 140 mg/kg) used in this study were calculated according to the clinical dose and were effective for behavioral responses. But unfortunately, until now, the detailed mechanisms underlying the antidepressant-like effects of SCLM remain enigmas.

Recently, increasing evidence demonstrated that the levels of monoamine neurotransmitters and their metabolites were decreased by unpredictable chronic mild stress (UCMS) and reversed by the antidepressants [11–13]. Furthermore, there were accumulating studies proved that UCMS resulted in reducing the neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), expression in hippocampal and prefrontal cortex (PFC) [14–16]. Treatment with different kinds of antidepressants in naive animals increased neurotrophic factors expression in the brain [17,18].

In the present study, the unpredictable chronic stress model of rats, a well validated stress-related animal model of depression [19], was applied to induce biochemical and behavioral alterations and the ability of SCLM (70, 140 mg/kg, p.o., the effective doses for behavioral responses) to reinstate these alterations was studied. Besides that, the probable mechanisms of antidepressant-like activity were explored by analyzing the levels of monoamine neurotransmitters and BDNF expression in rats brain.

2. Materials and methods

2.1. Preparation of SCLM

2.1.1. Plant materials

Traditional Chinese medicines were purchased from Nanjing Herbal Materials Company (Nanjing, China) and were identified by Professor Minjian Qin, School of Chinese Materia Medica, China Pharmaceutical University, based on their microscopic and macroscopic characteristics. The voucher specimens were conserved at the herbal herbarium of China Pharmaceutical University.

2.1.2. Sample preparations

According to the research of Zhu [10], SCLM was prepared with slight modifications: the plant materials (about 3 kg) were mixed and decocted three times with distilled water, the combined extracts were centrifuged at 2400 rpm for 10 min, then the supernatant was concentrated to 3 l. Subsequently, the solution eluted through macroporous absorption resin (5 l), first with distilled water (10 l), then with 30% ethanol (20 l) successively. The combined elutes of 30% ethanol were concentrated by a rotary evaporator under reduced pressure and finally dried in vacuo to obtain the total saponins (34 g extract, yield 1.2%, w/w). Prior to treatment with animals, SCLM was dissolved and diluted with distilled water.

2.1.3. HPLC analysis

For HPLC analysis, 0.5 mg SCLM was dissolved in 1 ml of 30% methanol and filtered through 0.2 μm nylon membrane prior to injection into HPLC. The saikosaponin a standard solution was prepared by dissolving 0.2 mg saikosaponin a in 1 ml of 30% methanol and filtered through nylon membrane.

HPLC analysis was carried out using Agilent 1100 series HPLC systems linked to both diode array and multiple wavelength detectors (Agilent, USA). Samples were separated using an Agela C18 column (4.6 mm × 150 mm, i.d. 5 μm, Agela Technologies, USA) which was maintained at 25 °C. The mobile phase was used elution with CH_3CN–H_2O (0.1% H_3PO_4) in a gradient of 15:85 to 55:45 (v/v) within 80 min. For each run, 20 μl of sample solution was injected. The solvent flow was 1 ml/min, and the detection wavelength was 204 nm. The ChemStation software was used to control the instruments and for data acquisition and processing.

2.2. Animals

Adult male Sprague Dawley rats weighing 180–220 g were purchased from Experimental Animal Centre of China Pharmaceutical University. The animals were group housed in poly-propylene cages under standard experimental conditions of room temperature (21 ± 2 °C), humidity (50 ± 10%), light (12-h light/dark cycle, lights on at 7:00 a.m.) and had free access to food and water except when animals were subjected to deprivation stressors during the unpredictable chronic mild stress procedure. Animals were allowed to have a period of acclimation before any experimentation. All animal experiments were approved by the Animal Care and Use Committee of the China Pharmaceutical University and complied with the Declaration of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.3. Drug and reagents

Fluoxetine hydrochloride (FLU, a serotonin reuptake inhibitor) was obtained from Sigma (St. Louis, USA). Saikosaponin a was purchased from the National Institute for the Control of Biological and Pharmaceutical Products (Beijing, China). The rabbit anti-BDNF was obtained from Santa Cruz Biotechnology (CA, USA). Histostain-SP kits were purchased from Maixin Technology Company (Fujian, China). Dopamine (DA), 3, 4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), norepinephrine (NE), serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and internal standard 3, 4-dihydroxybenzylamine (DHBA) were purchased from Sigma and were dissolved in the mobile phase. Trizol reagent was from Gibco BRL (NY, USA), RT-PCR kit was from MBI Fermentas (Burlington, Canada).

2.4. Unpredictable chronic mild stress procedure

The UCMS procedure was performed as described by Willner [20] and Moreau [21] with some modifications. The stressed groups were subjected to the following stressors for four weeks: 23 h food deprivation immediately followed by 1 h of access to restricted food; 23 h water deprivation immediately followed by 1 h exposure to an empty bottle; continuous overnight illumination; cage tilt (45°); group housing; soiled cage overnight (200 ml of plain water into the sawdust bedding); stroboscopic illumination (120 flashes/min, 1 h); white noise. Table 1 shows the timing and length of all stressors in the first week and the procedure was repeated with unpredictable sequence from 1 week to the other. Prior to the other experiments, rats were trained to consume 1% (w/v) sucrose solution and divided into five