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The antidepressant-like effects of Chaihu Shugan San: Dependent on the hippocampal BDNF-TrkB-ERK/Akt signaling activation in perimenopausal depression-like rats

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ABSTRACT

Chaihu Shugan San (CSS), a traditional Chinese medicine formula, has been used to treat depression for hundreds of years. Recently, the antidepressant-like mechanism of CSS has been increasingly evaluated and demonstrated. However, there are few studies focused on the involvement of the neurotrophic system in mediating the antidepressant-like effects of CSS. Considering the high prevalence of perimenopausal depression around the world, the goal of the present study was to determine whether brain-derived neurotrophic factor (BDNF) signaling is required for the antidepressant-like effects of CSS in perimenopausal depressive-like rats. The results indicate that CSS reverses depressive-like behaviors and attenuates the downregulation of BDNF in the hippocampus of perimenopausal rats exposed to chronic unpredictable mild stress (CUMS). We found that the TrkB antagonist K252 not only blocks the effects of CSS on behavioral improvement but also abolishes the activation of CSS in BDNF-TrkB signaling. As a result, the downstream targets of BDNF signaling, such as the ERK and Akt pathways, are significantly inhibited by K252a. Furthermore, CSS increases hippocampal neurogenesis, while K252a fully prevents this action. In conclusion, the present results demonstrate that the activation of the hippocampal BDNF-TrkB-ERK/Akt signaling pathway is required for the antidepressant-like effects of CSS on the depressive-like state during perimenopause. Additionally, this study also demonstrates that neurogenesis is required for the effects of antidepressants in aging perimenopausal animals and provides fundamental evidence for the clinical application of CSS.

1. Introduction

Perimenopause is a transition stage that women go through before menopause. This stage is characterized by erratic fluctuations in hormone secretion, which causes abnormal mood swings in women. The link between depression and perimenopause has been confirmed previously in the clinic [1]. As a result, perimenopause has been recognized as a stage during which women are at high risk for the onset of depression [2]. Additionally, if women have a medical history of major depression before perimenopause, they are likely to be particularly vulnerable to suffering from depressive symptoms during this stage [3]. Although the depressive symptoms during perimenopause are far more complex than erratic fluctuations in hormone levels, the symptoms of perimenopausal depression are generally the same as those of major depression [4]. They commonly include cognitive impairment, lack of interest, hopelessness or helplessness. There is no doubt that treating depression during perimenopause requires antidepressants.

Chaihu Shugan San (CSS), a famous traditional Chinese medicine formula, was first recorded in the Jing Yue Quan Shu by the Doctor Jingyue Zhang five hundred years ago. The formula consists of *Bupleurum chinese DC* (Chai-hu), *Pericarpium citri reticulatae* (Chen-pi), *Ligusticum chuanxiong Hort* (Chuan-qiong), *Rhizoma cyperi* (Xiang-fu), *Fructus aurantii* (Zhi-ke), *Radix paeoniae alba* (Bai-shao) and *Glycyrrhiza uralensis Fisch* (Gan-cao) and can smooth the liver to promote the circulation of *Qi* according to the theory of traditional Chinese medicine. CSS has been widely used for treating perimenopausal-related

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symptoms previously [5]. It has also recently been introduced as a medication for perimenopausal depression. A previous study in our lab focused on the role of hormone secretion, and the results indicated that the enhancement of the estrogen receptor α/estrogen receptor β ratio was involved in the antidepressant-like mechanism of CSS in perimenopause [6]. However, it is still unknown whether brain-derived neurotrophic factor (BDNF) is involved in the mechanism of CSS. In the present study, we investigated the effects of CSS in a depressive-like animal model, chronic unpredictable mild stress (CUMS), by using rats in a perimenopausal state. This perimenopausal depression-like model was established by using aging female rats at the perimenopausal stage. The perimenopausal stage was determined by microscopic examination of vaginal smears for fifteen consecutive days. Then the rats with abnormal estrous cycle was induced by CUMS. We subsequently examined the expression of BDNF after CSS administration. Moreover, to confirm that the BDNF signaling pathway is required for the antidepressant-like effects of CSS in perimenopausal depression, we pretreated the rats with K252a, a TrkB antagonist, and measured the downstream pathways of BDNF signaling after CSS administration.

2. Materials and methods

2.1. Animals

Female Wistar rats (36-week-old and 360–410 g weight) were purchased from the Shanghai Slac Laboratory Animal Co. Ltd. (Shanghai, China). Animals were single-housed under a normal 12 h/12 h light to dark schedule with lights on at 07:00 a.m. The temperature and relative humidity were maintained at 22 ± 2 °C and at 55 ± 5%, respectively. During the entire experiment, animals had access to food and water unless otherwise specified. To monitor the perimenopausal state of the rats, we observed their estrous cycle through microscopic examination of vaginal smears. All procedures were approved and performed in accordance with the guidelines of Fujian University of Traditional Chinese Medicine.

2.2. Herbs and reagents

The recipe of CSS used in this experiment was based on our previous study [6]. All the herbs were purchased from the Luyan Drugstore in Xiamen and were authenticated by Cheng-Fu Li, Department of Pharmacy, Xiamen Hospital of Traditional Chinese medicine (Table 1).

K252a was purchased from Alomone Laboratories (Jerusalem, Israel). Fluoxetine, DAPI and anti-β-actin antibody were purchased from Sigma (St. Louis, USA). The anti-BDNF antibody was purchased from Santa Cruz (Santa Cruz, USA). The antibodies for TrkB, ERK1/2, phospho-ERK1/2, Akt and phospho-Akt were purchased from Cell Signaling Technology (Beverly, USA). The anti- phospho-TrkB antibody was purchased from Bioworld Technology (St. Louis Park, USA).). The anti-DCX antibody was purchased from Abcam (Cambridge, USA).

2.3. CSS preparation

The formula for CSS was extracted according to our previous study [6]. Briefly, the raw herbal ingredients of the CSS were mixed at a ratio of 4:4:3:3:3:3:1 and crushed into small pieces. The mixture was soaked in distilled water for 30 min, then extracted three times for 30 min at 100 °C. The filtrates were collected and condensed at 60 °C in a vacuum. The final yield of CSS was 20.38%.

Table 1
The recipe of Chaihu–Shugan–San (CSS).

Herb medicine	Ratio
Bupleurum chinese DC (Chai-hu)	4
Pericarpium citri reticulatae (Chen-pi)	4
Ligusticum chuanxiong Hort. (Chuan-xiong)	3
Rhizoma cyperi (Xiang-fu)	3
Fructus aurantii (Zhi-ke)	3
Radix paeoniae alba (Shao-yao)	3
Glycyrrhiza uralensis Fisch. (Gan-cao)	1

2.4. Drug administration

To evaluate whether BDNF is involved in the antidepressant-like effects of CSS, rats were randomly divided into the following eight groups after four sessions of sucrose training and baseline testing (two days per session): the Control-vehicle group, the Control-CSS groups, the Control-fluoxetine group, the CUMS-vehicle group, the CUMS-CSS groups, and the CUMS-fluoxetine group. Fluoxetine (10 mg/kg) and CSS (1 g/kg) were dissolved in water and were orally administered at a volume of 10 ml/kg body weight once daily for the last 4 weeks of the experiment. The dose of CSS used was based on our previous study [6]. Sucrose preference and forced swimming tests were performed after drug administration.

To assess whether BDNF-TrkB signaling is required for the antidepressant-like effects of CSS, rats were randomly divided into the following groups after four sessions of sucrose training and baseline testing (two days per session): the Control-vehicle group, the CUMS-vehicle group, the CUMS-CSS group, the CUMS-K252a group, and the CUMS-K252a-CSS group. K252a (25 μg/kg) was dissolved in 0.1% DMSO in saline and injected intraperitoneally, and CSS (2 g/kg) was dissolved in water and administered by oral gavage. K252a was administered once daily prior to CSS treatment for the last 4 weeks of the experiment. Sucrose preference and forced swimming tests were performed after drug administration.

2.5. CUMS

The animals were first trained to consume 1% sucrose solution and water for one day. After water deprivation for 12 h, a sucrose preference baseline test was performed four times for 1 h each session (once every two days). Abnormal animals were removed from the experiment if their sucrose preference was less than 50% during the three training tests. Then, the CUMS procedure was performed according to our recent study [6,7]. Briefly, throughout the entire procedure, we exposed the animals to several stressors, such as water deprivation, empty water bottle exposure, dirt exposure, overnight illumination, light/dark succession, cage tilt, space reduction, and predator sounds. All stressors were applied individually and were repeated throughout the duration of the experiment. To prevent habituation and to ensure the unpredictability of the stressors, all stressors were randomly scheduled over a 1-week period and were repeated throughout the duration of the experiment. The control animals were housed in a separate room and had no contact with the stressed animals.

2.6. Sucrose preference test

The sucrose preference test was briefly described in our recent study [7]. Before the formal sucrose preference test, all rats were given 1% sucrose solution for 24 h. Then, both sucrose solution and fresh water were made accessible to the rats for another 24 h. After being water deprived for 12 h, the formal sucrose preference was performed: the rats were given both 1% sucrose solution and fresh water for 1 h. Then, the sucrose preference was calculated based on the relative consumption of each.

2.7. Forced swimming test

The forced swimming test was performed after the sucrose preference test. The test was performed by placing a rat in a glass cylinder (46 cm in height, 20 cm in diameter) filled with 30 cm-high water