Combination Therapy of Salvianolic Acid and Fluoxetine Improves the Cognitive Function of Rats with Chronic Stress-induced Depression

Lulu Yu[1,3], Cuixia An[1,3], Lina Jia[1,3], Yunpeng Li[1,3], Qianqian Chen[1,3], Fengya Zhen[1,3], Shuo Wang[1,3], Mingwei Wang[2,3]

■ OBJECTIVE: **To establish the beneficial effects of salvianolic acid and fluoxetine on the improvement of cognitive function and amelioration of depression-like symptoms of rats with chronic stress-induced depression.**

■ METHODS: **Ninety-nine male Sprague-Dawley rats were randomly divided into 5 groups—a control group with no stress challenge and 4 chronic stress groups. Rats assigned to chronic stress groups were exposed to stress for 3 weeks, and then were given placebo, fluoxetine (20 mg/kg), salvianolic acid (40 mg/kg), or combined fluoxetine and salvianolic acid. Body weight of each rat was recorded throughout the study. Sucrose preference test and water maze experiment were performed after chronic stress challenge and after drug treatment to assess the effect of drug treatments on depressive-like symptoms and cognitive function. The sucrose preference test was also performed before chronic stress exposure for baseline measurement.**

■ RESULTS: **Exposure of rats to chronic stress for 3 weeks significantly reduced body weight and sucrose preference values compared with the no stress control. The water maze experiment showed that chronic stress impaired the spatial learning of rats as well. Treatment of stress-challenged rats with fluoxetine and fluoxetine combined with salvianolic acid resulted in shorter training latency and longer time spent in the target quadrant during the exploration stage of the water maze experiment compared with placebo treatment. Effect of the combined regimen was found more obvious.**

■ CONCLUSIONS: **Combination therapy of salvianolic acid and fluoxetine could alleviate depression-like symptoms and cognitive deficit induced by chronic stress.**

INTRODUCTION

Depression is one of the common mental disorders, and is characterized by symptoms such as depressive mood, loss of interest, reduced mobility and physical activity. These symptoms severely impede patients' daily life and social interaction, and more unfortunately, epidemiologic surveys have indicated that the lifetime prevalence of depressions in a general population can reach 10%–15%.[1] The pathophysiology of depression is not fully understood; however, imbalance of neurotransmitter levels,[2] disturbances in the hypothalamic pituitary adrenal (HPA) axis,[3] and structural abnormality of brain[4] have been proposed as possible mechanisms contributing to disease development and progression. Antidepressants, developed based on the monoamine hypothesis of depression, represent one of the treatment options for patients with depression, but the response rate was only 60%–70%.[5] The treatment is also associated with side effects of different severity. It is, therefore, imperative to decipher novel disease mechanisms to identify more promising therapeutic targets.

Evidences accumulating from studies have implicated the activation of inflammatory response in depression, in addition to the aforementioned mechanisms. Inflammatory mediators, like interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-2 soluble receptor, and C-reactive protein, were found elevated in the etiology of depression. In addition, proinflammatory cytokines,

Key words
- Chronic stress
- Cognitive function
- Depression
- Salvianolic acid

Abbreviations and Acronyms
5-HT: 5-hydroxytryptamine
CMS: Chronic mild stress
HPA: Hypothalamic pituitary adrenal
IL: Interleukin
TNF: Tumor necrosis factor

From the [1]Department of Psychiatry, The First Hospital of Hebei Medical University and Institute of Mental Health, Hebei Medical University; [2]Department of Neurology, The First Hospital of Hebei Medical University; and [3]Hebei Brain Ageing and Cognitive Neuroscience Laboratory, Shijiazhuang, China

To whom correspondence should be addressed: Mingwei Wang, M.D., Ph.D. [E-mail: 13785162650@126.com]

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including IL-1, IL-6, and TNF-α, could act as depression-like behaviors, which were alleviated by treatment with anti-inflammatory regimens like Celecoxib,[6] St. John's wort,[7] and Minocycline.[8] The promoting effect of inflammatory cytokines on depression is attributed to their modulations on hormones and neurotransmitters. Inflammatory cytokines directly interacted with the receptors along the HPA axis and affected hormone release.[9] For neurotransmitter (5-HT), its extracellular level was reduced by interferon-γ, interferon-α, IL-1β, and TNF-α, inflammatory cytokines that increased 5-HT reuptake by elevating transporter proteins in the brain.[10,11]

Cognitive impairment is a key dysfunction of depression—its severity increases with the number of affective episode.[12] Although the mechanistic link between depression and cognitive impairment is yet to be elucidated, it is generally accepted that both share inflammation as a common etiology.[13] As such, alleviating inflammation holds promises as a novel therapeutic approach for the treatment of depression and cognitive impairment.

Salvianolic acids isolated from *Radix Salviae miltiorrhizae* (Danshen) are water-soluble compounds with well-known anti-inflammatory action.[14] Among major bioactive compounds of salvianolic acids, salvianolic acid B has drawn much attention because of its antidepressant-like effects. Treatment of mice with salvianolic acid B reduced the immobility time in forced swimming and tail suspension tests.[15] Salvianolic acid B improves also cognitive function. In studies on mice, the treatment with salvianolic acid B ameliorated memory dysfunction, which resulted from cerebral transient ischemia,[16] and improved spatial learning and memory abilities after traumatic brain injury.[17] Despite these important findings, whether salvianolic acid B treatment would improve chronic stress-induced depression-like symptoms and cognitive impairment remains to be addressed. To fill the knowledge gap, the present study is aimed to demonstrate the beneficial effects of salvianolic acid on relieving chronic stress-induced depression-like symptoms and improving cognitive functions.

METHODS

Animals

Ninety-nine male Sprague-Dawley rats (body weight 180–220 g) were obtained from Hebei Medical University Experimental Animal Center (certification no. 1411040). Rats (1 rat/cage) were housed in an air-conditioned facility (23 ± 2°C) in a 12-hour light/dark cycle with food and water ad libitum. All experiments strictly followed procedures approved by the ethnic committee of Hebei Medical University on animal care and use.

Rats were randomly assigned into 5 groups: 1) no chronic mild stress (CMS)+vehicle ($n = 22$), control rats with no CMS challenge; 2) CMS+vehicle ($n = 20$), CMS-challenged rats receiving no treatment; 3) CMS+fluoxetine ($n = 20$), CMS-challenged rats receiving fluoxetine (20 mg/kg); 4) CMS+salvianolic acid ($n = 20$), CMS-challenged rats receiving salvianolic acid (40 mg/kg); and 5) CMS+fluoxetine+salvianolic acid ($n = 17$), CMS-challenged rats receiving combined regimen of 20 mg/kg fluoxetine and 40 mg/kg salvianolic acid.

Experimental Drugs

Salvianolic acid for injection was provided by Tianjin Tasly Pharmaceutical Co. Ltd, Tianjin, China (approval no. Z20110011). Fluoxetine (BODFo-DQ), the positive control, was obtained from Tokyo Chemistry Industry (Tokyo, Japan). Salvianolic acid and fluoxetine was prepared with 0.9% sodium chloride injection to a final concentration of 4 mg/mL and 2 mg/mL, respectively. Physiologic saline (i.e., 0.9% sodium chloride injection) served as the negative control. Salvianolic acid and both controls were injected in a volume of 10 mL/kg in a daily basis for 3 weeks. Detailed schedule of drug administration and functional tests is depicted as in **Figure 1**.

Chronic Mild Stress

Rats were challenged with CMS after the procedures of a previous study[18] with slight adjustment. In brief, during a period of 6 weeks, every day rats of CMS-inflicted groups were exposed to 2 types of mild stressors including 1-hour cage shaking, 24-hour water deprivation, 24-hour wet bedding, reversed day and night cycle, 24-hour food deprivation, 24-hour tilted cage, 5-minute swimming at 45°C, 1-hour wrap restraint at 4°C, 1-minute tail clamp, and 1-hour wrap restraint. Rats of the control group received normal feeding and no stress.

Sucrose Preference Test

Anhedonia is the main symptom of depression, and reduced preference for sucrose is an important indicator for that.[19] The sucrose preference test was performed in two stages: 1) sugar adaptation phase, in which rats were given access to 1% sucrose water (wt/vol) for 24 hours, after that the sucrose water was replaced by plain water; and 2) sucrose preference test on the second day after sugar adaptation phase, rats were deprived of food and water for 10 hours. Rats then had the free choice of either drinking 1% sucrose solution or plain water for 2 hours. Water and sucrose solution intake were measured by weighing the bottles before and after the experiments. No food was given during the experiments. Sucrose preference value is calculated as the Volume of sucrose intake/(Sucrose intake + Plain water intake) × 100%.

Morris Water Maze

Morris water maze (Shanghai Xinruan Information Technology Co. Ltd, Shanghai, China) is a round metal tank (diameter, 2 m) with a black interior surface. The tank has no lid, and at 45 cm above the tank, markers made of different colors and shapes were placed to give visual cues for location. During the experiment, the tank was filled with water (19 cm deep, 25 ± 1°C) mixed with black dye. The test was divided into 2 stages: training stage (place navigation) and testing stage (space exploration).[20] The training stage lasted for 5 days. During the training phase, the water maze was divided into 4 quadrants. A platform (diameter, 12 cm) was placed in the middle of the fourth quadrant (northeast direction) 2 cm below the water. Each rat was trained 4 times a day, and each training session was 60 minutes.[21] Rats were placed facing the tank wall and then allowed to enter into the water from different directions in each training (see **Table 1** for the sequences of direction). The duration that the rat required to enter into the water with all 4 limbs and climb onto the platform (escape latency) was recorded. If the rat could not find