Video Article

Evaluating the Anti-depression Effect of Xiaoyaosan on Chronically-stressed Mice

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Abstract

In addition to the standardized use of antidepressant medications and psychotherapy, the usage of traditional Chinese medicine has lead to an overall improvement of patients with major depressive disorder (MDD). Therefore, the purpose of this study was to establish the mouse depressive model, observe the behavior changes associated with chronic unpredictable mild stress (CUMS), and then evaluate the anti-depression effect of Xiaoyaosan. Mice were randomly divided into four groups: a control group, a model group, a treatment group with Xiaoyaosan, and a treatment group with fluoxetine. All mice were individually kept in cages, and depression was induced in the mice by exposing them to several designed manipulations of CUMS for 21 days, as described in the protocol. Mice in the control group and model group received 0.5 mL of distilled water, while mice in the treatment groups received either Xiaoyaosan (0.25 g/kg/day) or fluoxetine (2.6 mg/kg/day). The drugs used in the study were given intragastrically daily during the entire three weeks. To estimate the depressive-like behaviors, a series of parameters including the coat state, body weight, open field test score, and sucrose preference test score were recorded. Data analysis showed that behaviors of model mice were significantly changed compared to behaviors of mice in the control group, which were improved by the treatment of Xiaoyaosan and fluoxetine. The current findings demonstrated the anti-depression effects of Xiaoyaosan on the behaviors of CUMS-induced mice and revealed that compounds from the Xiaoyaosan prescription may be worthwhile for treating depression, considering their beneficial effects on depressive-like behaviors.

Video Link

The video component of this article can be found at https://www.jove.com/video/58276/

Introduction

MDD is a chronic, highly recurrent mental disease that presents with persistent low mood, depression, low self-esteem, mental retardation, loss of interest, sleep disorders, profoundly pessimistic or suicidal tendencies; it has caused great harm to human health and social stabilization[1]. It has become one of the world's primary cause of disability, and it may be one of the main leading causes of disease burden by 2030 according to the World Health Organization[2,3]. Currently, antidepressant medications and psychotherapy are considered to be the first choice to treat MDD. Nevertheless, an enormous number of patients diagnosed with MDD do not respond to continuous medication or therapy, and they are listed as having treatment-resistant depression[4].

Xiaoyaosan is a classic Chinese compound[5] that has been widely used to treat psychological diseases for thousands of years since the Song dynasty in China[6]. It is composed of 8 Chinese herbs: *Radix Bupleuri*, *Radix Paeoniae Alba*, *Radix Angelicae Sinensis*, *Poria*, *Rhizoma Atractylodis Macrocephalae*, *Radix Glycyrrhizae*, *Rhizoma Zingiberis Recens*, and *Herba Menthae*. Xiaoyaosan contains multiple constituents, has multiple targets, and utilizes multiple pathways[7]. Xiaoyaosan has been commonly shown to be a safe and effective formula for treating various diseases related to mental disorders, and modern studies have also focused on the anti-depression mechanism of this compound[8,9,10]. The pathogenesis of MDD is yet unclear, even though there are multifarious hypotheses, including the monoamine hypothesis, the neurotransmitter receptor hypothesis, the neuroendocrine function change hypothesis, the immune system abnormality hypothesis, *etc*. Several studies have shown that the antidepressant effect of Xiaoyaosan has a connection with the material bases of the above hypotheses[11,12], and its therapeutic action manifests as an improvement to depressive-like behaviors[13].

Moreover, with the prevalence of depression, modern society has suffered from reductions in the work force, early retirements, and expensive treatments[14]. Expensive treatment needs strict medical attention, which means a range of increased medical costs and precise anticipation of precise undesirable outcomes as well as side effects[15]. The Chinese herb Xiaoyaosan has accessible components, unique effects, and slight side effects. Its application can play a role in reducing the burden of modern medical treatment. Therefore, to provide a more theoretical basis for the application of Xiaoyaosan, an alternative clinical therapy should be offered for the treatment of depression, the incidence of which increases year by year, and should improve human health at the same time. This paper describes a protocol involving the use of Xiaoyaosan to prevent the depressive-like behaviors by establishing the depressive model of the mouse. The open field test and sucrose preference test were used to verify the antidepressant effects of Xiaoyaosan.

Protocol

This protocol was approved by the Animal Ethics Committee of Beijing University of Chinese Medicine and was in accordance with all guidelines for the Care and Use of Laboratory Animals of China. There was no accidental death situation during the experimental procedure, and no animals needed to be euthanized in this study.

1. Preparing Drugs for the Experiment

NOTE: The Chinese herbs were purchased and then authenticated by Dr. B. Liu of the Beijing University of Chinese Medicine. Xiaoyaosan contains the following eight traditional Chinese drugs in a ratio of 6:6:6:6:3:6:2:2: Radix Angelicae Sinensis, Radix Paeoniae Alba, Poria, Radix Bupleuri, Radix Glycyrrhizae, Rhizoma Atractylodis Macrocephalae, Herba Menthae and Rhizoma Zingiberis Recens,.

1. Prepare Xiaoyaosan as previously described[16].
   Note: We extracted these compounds in the Chinese medicine preparation room of the China-Japan Friendship Hospital, and the extraction yield was 18.8%.
2. Verify the quality of Xiaoyaosan by high-performance liquid chromatography-mass spectrometry analysis (LC-MS/MS)[17].

2. Preparing Mice for the Experimental Procedure

NOTE: For the experimental procedure, 60 healthy male C57BL/6J mice aged 12 weeks were selected, with each mouse weighing between 18 and 22 g.

1. Keep mice individually in cages in a typical Specific Pathogen Free (SPF) laboratory animal room (22 ± 1 °C, 12 h/12 h dark/light cycle, relative humidity: 30–40%) for one week before the CUMS procedure.
2. Randomly divide all mice into a control group, a model group, a Xiaoyaosan treatment group, or a fluoxetine treatment group according to their body weight. Each group has 15 mice.
3. Before initiation of the experiment, number the mice in each group on their tail using a marking pen.
4. Test all mice using a five-day sucrose preference test (refer to step 5) and an open field test (refer to step 6).

3. Induction of Depression in Mice

NOTE: To induce depression, the mice in the model group and two treatment groups underwent CUMS for 21 days[18]. The plan can be changed according to the practical situation, but the principle that the same stressor cannot be used continuously should be followed.

1. **Expose mice daily to any two of the following seven stressors for 21 consecutive days[19]. Our modeling plan is designed as follows:**
   1. On Monday, expose mice to food deprivation (empty the feed of each cage for 24 h, 8:00 a.m. to 8:00 a.m.) and water deprivation (remove the drinking bottle of each cage for 24 h, 8:00 a.m. to 8:00 a.m.).
   2. On Tuesday, expose mice to restraint stress (restrain mice in a small plastic tub for 3 h, 8:00 a.m. to 11:00 a.m.) and empty cages (remove the padding in each cage for 11 h, 8:00 a.m. to 19:00 p.m.).
   3. On Wednesday, expose mice to wet and soiled cages (pour water into each cage and keep wet for 24 h, 8:00 a.m. to 8:00 a.m.) and crowded cages (put five mice in one cage for 24 h, 8:00 a.m. to 8:00 a.m.).
   4. On Thursday, expose mice to restraint stress and ice-cold swimming[20,21] (force each mouse to swim in a clear glass aquarium with ice water for 5 min).
   5. On Friday, expose mice to wet and soiled cages and food deprivation.
   6. On Saturday, expose mice to restraint stress and crowded cages.
   7. On Sunday, expose mice to empty cages and ice-cold swimming.
2. Record the coat state and body weight weekly until the end of modeling (day 21). Calculate the total score of the coat state as the sum of scores from seven different body parts: head, neck, dorsal coat, ventral coat, forepaws, tail, and hind paws. A score of one indicates a well-groomed coat, and a score of zero indicates an unkempt coat[22].
3. Perform a five-day sucrose preference test (refer to step 5) and an open field test (refer to step 6) at the end of experiment.

4. Administration of Xiaoyaosan

NOTE: The dose of Xiaoyaosan (0.25 g/kg/day) was selected on the basis of its satisfactory efficacy as previously described[9].

1. During the experiment, use distilled water to prepare the Xiaoyaosan and fluoxetine suspensions, and make sure to use immediately.
2. Give 0.5 mL of distilled water to the mice in the control group and model group by intragastric administration every day before modeling. Meanwhile, give Xiaoyaosan (0.25 g/kg/day) or fluoxetine (2.6 mg/kg/day) to the mice in the two treatment groups.

5. Sucrose Preference Test

NOTE: No water or food deprivation was applied before the test. The protocol was performed as previously reported[23].

1. On the first two days, house the mice individually with two bottles filled with tap water.
2. On the next two days, give the mice two bottles filled with 1% sucrose solution.
3. On the last day, give the mice two bottles for 24 h, one with tap water and the other with 1% sucrose solution. To eliminate a side preference of the mice in drinking behavior, switch the position of the two bottles after 12 h.
4. Record the volume (mL) of both the consumed sucrose solution and tap water. Calculate the sucrose preference as follows: sucrose preference (%) = sucrose consumption/total liquid consumption × 100%.

6. Open Field Test

1. Perform the open field test in a dimly lit and quiet room with a four-sided wooden black lusterless box. Divide the floor into 25 equal squares by blue lines.
2. Clean the open-field box with 5% alcohol prior to the access of each mouse. Then, place the mouse in the center of the open field gently.
3. Suspend a high definition digital camera approximately 200 cm right above the field, and record the behavior of each mouse for 5 min.
4. Evaluate the mice behavioral video by the video-tracking system software and record the data of the total running distance and number of entries into the center zone.
5. Analyze the data of the total distance moved and number of entries into the center zone by statistical software.

Representative Results

Note that the results have been previously published by Dr. Xiu-Fang Ding, *et al.*[18]

Data were expressed as the mean ± standard error of the mean (SEM). A one-way ANOVA or a non-parametric test was used for general data based on the normality test and the test for homogeneity of variance. The LSD test was used for the comparisons between groups. In addition, the repeated measurement process of the general linear model (GLM) was used to conduct one-way ANOVA analysis for repeated measured data (coat score and body weight), and a multivariate analysis process of variance was adopted to make comparisons between groups at each time point (LSD method).

The physical state of CUMS-induced mice

After exposing mice to three weeks of CUMS for induction of depression, a gradual dystrophy of coat state in the model group was induced; the dystrophy did not present after two weeks of stress, but it worsened at the end of the CUMS procedure (three weeks), and the coat score significantly decreased compared to that of the control group ($F (3, 56) = 25.08$, $p = 0.000$). The coat state of mice was notably improved after continuous treatment with Xiaoyaosan or fluoxetine compared to the model group ($F (3, 56) = 25.08$, both $p = 0.001$, **Figure 1a**). According to the weekly body weight data of each group, a significant reduction in body weight was manifested by the third week ($F (3, 56) = 16.149$, $p = 0.000$, **Figure 1b**), while Xiaoyaosan or fluoxetine treatment significantly increased the body weight compared with the model group ($F (3, 56) = 16.149$, both $p = 0.001$).

The sucrose preference test

Before stress exposure, mice in all groups had a similar sucrose preference (baseline condition, **Figure 2a**). However, a significant drop in sucrose preference was observed after three weeks of stress procedure in model group ($F (3, 56) = 41.379$, $p = 0.000$, **Figure 2b**). Xiaoyaosan and fluoxetine treatment effectively reversed these changes and significantly increased the sucrose preference compared with model group ($F (3, 56) = 41.379$, both $p = 0.000$).

The open field test

Before stress exposure, all mice exhibited a similar behavioral state (baseline conditions. **Figures 2c** and **2d**). However, after stress exposure for three weeks, the total distance moved in five minutes significantly differed between the groups. The total distance moved by mice in the model group was significantly lower ($F (3, 56) = 4.216$, $p = 0.003$, **Figure 2f**) than that of the control group. Furthermore, the number of entries into the central zone was also significantly different between groups. The number of entries into the central zone of the model group significantly decreased ($F (3, 56) = 2.809$, $p = 0.013$, **Figure 2e**) compared with the control group. Fluoxetine treatment prevented these changes in locomotion trend and significantly improved the total distance and the number of entries into the central zone ($F (3, 56) = 4.216$, $p = 0.029$; $F (3, 56) = 2.809$, $p = 0.004$). A similar phenomenon was also observed in the Xiaoyaosan treatment group ($F (3, 56) = 4.216$, $p = 0.014$; $F (3, 56) = 2.809$, $p = 0.029$).

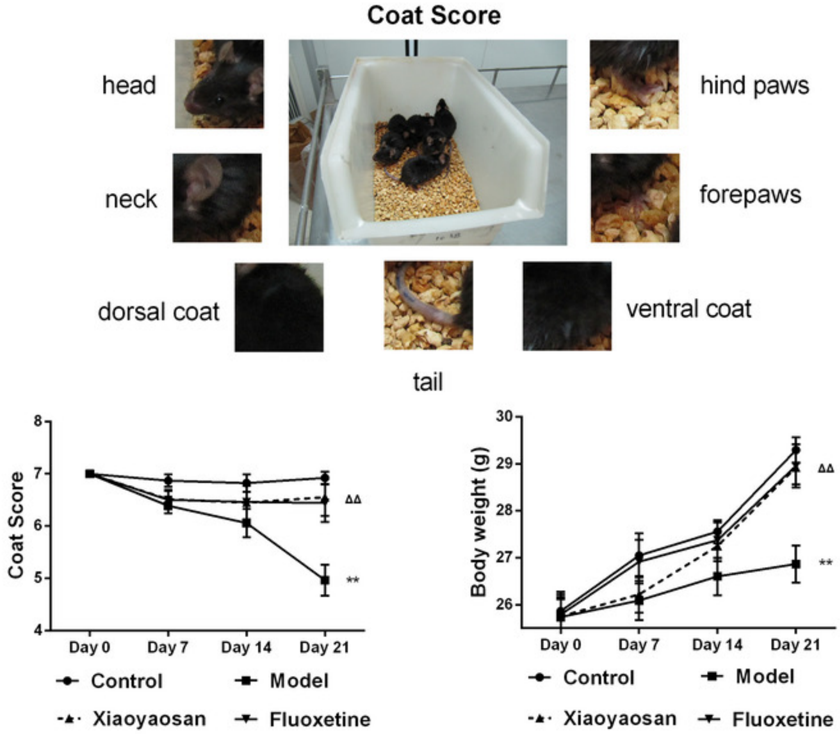

Figure 1: Effects of Xiaoyaosan on CUMS-induced changes in mice physical state. The mice coat state (a) and body weight (b) were measured weekly during the stress period. Data were expressed as the mean ± SEM, n = 15 per group. $^{**}p < 0.001$ versus control; $^{\Delta\Delta}p < 0.001$ versus the model. This figure has been modified from Ding, *et al.*[18] Please click here to view a larger version of this figure.

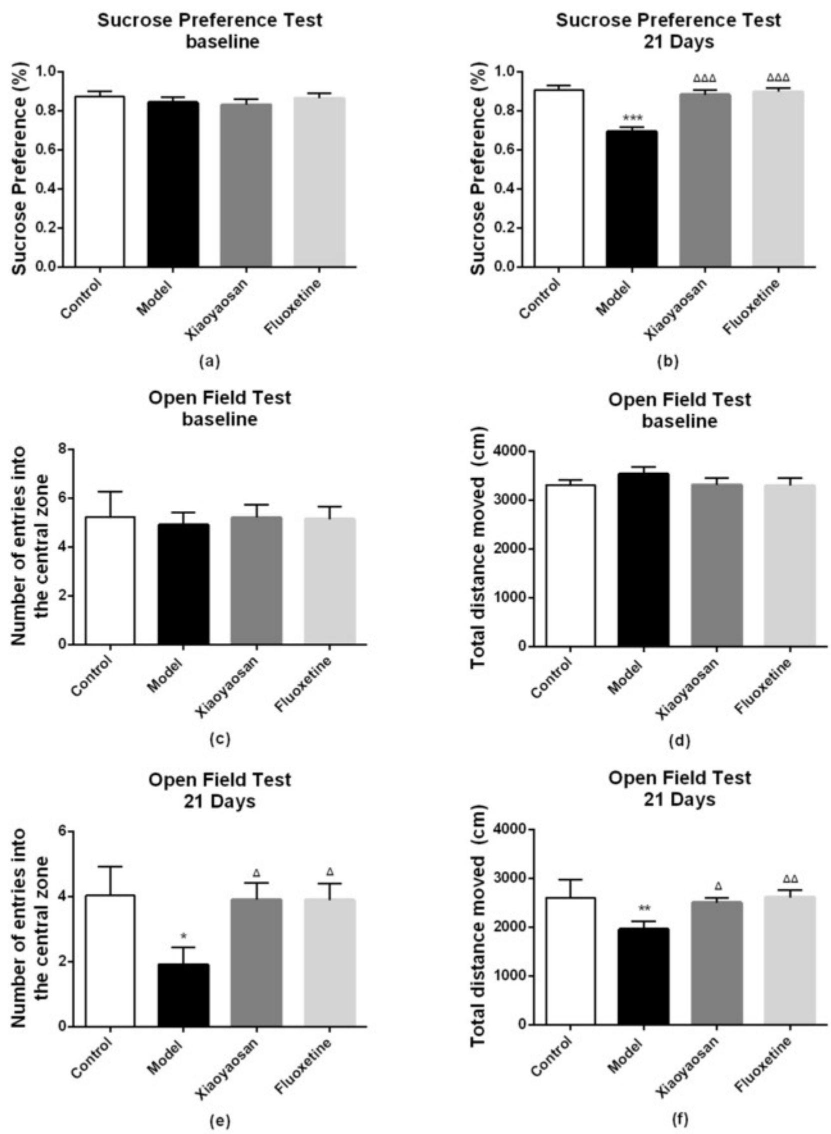

Figure 2: Effects of Xiaoyaosan on CUMS-induced depressive-like behaviors. A battery of behavioral tests was conducted, and the following parameters were measured: sucrose preference (**a, b**), number of entries into the center zone (**c, e**), and the total distance moved (locomotor activity) (**d, f**). Data were expressed as the mean ± SEM, n = 15 per group. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ versus control; $^{\Delta}p < 0.05$, $^{\Delta\Delta}p < 0.01$, and $^{\Delta\Delta\Delta}p < 0.001$ versus model. This figure has been modified from Ding, *et al.*[18] Please click here to view a larger version of this figure.

Discussion

The CUMS model used in this study is a common method to establish the stress diathesis model of depression[24]. Most manifestations of CUMS can be restored by antidepressant drugs, which indicated that this model method has a strong predictive validity[25]. By this method, animals receive chronic unpredictable stressful stimuli and develop core symptoms of major depression, including decreased place preference conditioning, anhedonia, and impaired emotion-like behaviors[26]. Here, in this protocol, the continuous usage of Xiaoyaosan or fluoxetine can improve the depressive-like behaviors of CUMS-induced model mice according to the results obtained in the experiment; the fluoxetine is as a positive control drug to prove the intervention effect of Xiaoyaosan.

The evaluation of coat score, unlike the open field test, forced swimming test, or novelty suppressed feeding test, is a measure that is not associated with depression in humans but is the most prevalent, reliable, and well-validated method for the depressive model of mouse[27]. In the present study, a gradual dystrophy of the coat state in the model group was induced and did not present after two weeks of stress but worsened at the end of the CUMS procedure (three weeks), while the body weight was not significantly different among groups after one week of stress exposure. However, the difference of body weight in each group began to emerge after modeling for two weeks, thus leading to a subsequent decrease by the end of three weeks. The results indicate that these changes in physical state of CUMS-induced mice could be reversed by both Xiaoyaosan and fluoxetine treatment, suggesting that Xiaoyaosan could improve the physical states of depressed mice.

To further validate the CUMS model and verify the effect of the compound used in this protocol, a sucrose preference test and an open field test were used to assess the possible depressive behaviors in CUMS-induced mice. The reduction of anhedonia in sucrose preference was used to evaluate the depressive-like state in rodents, while the open field test, as an animal psychological test, is commonly used to evaluate the locomotor function and emotionality of rodents[28]. The result showed that 3 weeks of stress exposure had an obvious influence on the sucrose preference of mice, and the anhedonia was reversed by both the Xiaoyaosan and fluoxetine treatments. The open field test is commonly used to assess locomotor function and emotionality in laboratory animals[29]. In this study, it was used to analyze depressive behaviors. The results of the open field test showed that the total distance moved by the mice in the model group was significantly lower than that of the control group. Furthermore, the number of entries into the central zone by the model group of mice was significantly lower than that of the treatment group of mice, which suggested that CUMS-induced mice displayed depressive-like behaviors. The study also showed that Xiaoyaosan and fluoxetine could ameliorate the changes presented in the open field test.

In agreement with the similar results discussed in previous studies, this paper showed that CUMS causes a decrease in sucrose consumption and locomotor activity. The continuous usage of Xiaoyaosan was effective for the prevention and treatment of CUMS-induced depression in mice. Many studies have examined depression, an advanced understanding of an animal model could be the key to clarifying the mechanism of depression[30]. The CUMS method described in this protocol can effectively display the depression state of mice. Unlike the chronic mild stress (CMS) or chronic immobilization stress (CIS) method[31,32], the key point of CUMS lies in the selection of unpredictable stressors and the rational application of model evaluation. To further confirm the method and result shown in this protocol, future work should include the selection of more stressors and evaluation methods for CUMS method and the detection of the longer-term effects of Xiaoyaosan for 28-48 days of CUMS.

Disclosures

The authors have nothing to disclose.

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