Antidepressant-like effects of Sanyuansan in the mouse forced swim test, tail suspension test, and chronic mild stress model

Shuo Yan, Zi-Li You, Qiu-Ying Zhao, Cheng Peng, Gang He, Xiao-jun Gou, Bin Lin

School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu, China
Key Laboratory of Medicinal and Edible Plants Resources Development of Sichuan Education Commission, Chengdu University, Chengdu, China
Key Laboratory of Systematic Study and Exploitation Utilization of Tradition Chinese Medicine Resources, Pharmacy College, Chengdu University of Traditional Chinese Medicine, Chengdu, China

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Abstract Natural products have been widely reported as effective therapeutic alternatives for treatment of depression. Sanyuansan is a compound recipe composed of ginseng total saponins, fish oil, and valeriana. The aims of this study were to validate whether Sanyuansan has antidepressant-like effects through acute behavioral tests including the forced swimming test (FST), tail suspension test (TST), locomotor activity test, and chronic mild stress (CMS) mice model of depression. C57BL/6 mice were given oral administration of 30 mg/kg imipramine, Sanyuansan, and saline, respectively. The acute behavioral tests including the TST, FST, and locomotor activity test were done after the administration of drugs for consecutively three times (24 hours, 1 hour, and 0.5 hour prior to the tests). Furthermore, the sucrose preference and the serum corticosterone level of mice in the CMS model were examined. Sanyuansan only at 900 mg/kg markedly reduced immobility time in the TST compared with the saline-treated group of mice. Sanyuansan at doses of 225 mg/kg, 450 mg/kg, and 900 mg/kg significantly reduced immobility time of mice in the FST. Sanyuansan reversed the CMS-induced anhedonia and hyperactivation of the hypothalamus—pituitary—adrenal axis. In addition, our results showed that neither imipramine nor Sanyuansan at any dosage increased spontaneous motor activity. These results suggested that Sanyuansan induced...
significant antidepressant-like effects in mice in both acute and chronic animal models, which seemed unlikely to be attributed to an increase in locomotor activities of mice, and had no sedative-like effects.

Introduction

Depression is a major contributor to the global burden of disease and disability. According to the World Health Organization survey, the relatively high lifetime prevalence of depression ranges from 2% to 15% around the world [1]. It costs $150 billion per year, according to the latest survey in the United States [2]. Several physiological systems are involved in the pathophysiology of depression, such as the alteration of monoamine system, dysregulation of the hypothalamus–pituitary–adrenal (HPA) axis, and imbalanced cytokine system [3–5]. So far, treatment of depression entirely depends on antidepressants, which, based on serendipitous findings made in the 1950s, act via the monoamine neurotransmitters, such as serotonin or noradrenaline. Only about 50% of patients show full remission in response to these antidepressants [6]. Hence, there is a pressing need for more effective and better tolerated antidepressants.

Sanyuansan is a compound recipe composed of ginseng total saponins (GTSs), fish oil, and valeriana, which is widely used as a tonic food in China. In traditional Chinese medicine, ginseng is used for treatment of various diseases of the neuropsychological system, including mood disorders, learning and memory deficit, and cerebral ischemia [7,8]. The major pharmacological active ingredients of ginseng are triterpenoid saponins, which are responsible for its effects on the central nervous system [9]. Omega-3 polyunsaturated fatty acids are composed of alpha-linoleic acid, eicosapentenoic acid, and docosahexenoic acid, mainly found in fish oil. Several epidemiological investigations showed a negative correlation between consumption of omega-3 fatty acid and the prevalence and severity of depression. In addition, recent clinical studies also provided evidence about the benefits of omega-3 fatty acid in the treatment of depressive disorders [10–13]. Omega-3 supplementation in rodents’ diet exhibited antidepressant-like effects in chronic studies [14,15], associated with an increase in the hippocampal volume, an overexpression of brain-derived neurotrophic factor, and an increase in the number of newborn cells [16]. Valeriana is traditionally used for the treatment of epilepsy, insomnia, neurosis, and sciatica [17]. In clinical investigations, extract of valeriana has been shown to alleviate symptoms of depression [18]. Moreover, it has been used in the treatment of anxiety and depression in combination with other herbs [19–21].

In the present study, we investigated the antidepressant-like effects of Sanyuansan in acute and chronic mice models of depression.

Materials and methods

Animals

All 137 male C57BL/6 mice weighing 18–22 g were purchased from the Institute of Experimental Animals (Chengdu, China). The animals were housed in groups under controlled conditions (23.5 ± 1.5°C, under a 12-hour light–dark cycle with lights on at 8:00 AM, food and water provided ad libitum). The animals were allowed to adapt to laboratory conditions for at least 1 week before the experiments started. All experiments were performed during the light phase of the light–dark cycle. All animal handling procedures were performed in compliance with the Principles of Laboratory Animal Care (NIH publication No. 80-23, revised 1996) and the PR China legislation for the use and care of laboratory animals. All possible steps were taken to avoid animals’ suffering at any stage of the experiments.

Drug preparation and administration

Fish oil (25% omega-3) and imipramine (99%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). GTSs was purchased from Sigma–Aldrich (Wuhan, China). Sanyuansan was composed of GTSs, omega-3, and valeriana in a ratio of 2:3:1 (w/w/w), suspended with 0.1% carboxymethylcellulose.

In acute behavioral tests, animals were randomly divided into five groups (n = 21 per group) and orally administered with imipramine (30 mg/kg), low dose Sanyuansan (225 mg/kg), medium dose Sanyuansan (450 mg/kg), high dose Sanyuansan (900 mg/kg), and saline, respectively. These doses were chosen based on earlier reports that GTSs, omega-3, valeriana, and imipramine were effective in producing physiological and behavioral effects on rodents. All drugs and water were given at a volume of 20 mL/kg for three times at 24 hours, 1 hour, and 0.5 hour prior to each behavioral test.

In the chronic mild stress (CMS) experiment, animals were randomly divided into four groups (n = 8 per group): Control (saline), CMS + Vehicle (CMS + saline), CMS + Sanyuansan (CMS + Sanyuansan 900 mg/kg), and CMS + IMI (CMS + imipramine 30 mg/kg). These doses were
chosen according to the acute behavioral tests results. All drugs and water were given at a volume of 20 mL/kg once per day during the CMS experiment.

**Acute behavioral tests**

We conducted three acute behavioral tests: the tail suspension test (TST), the forced swimming test (FST), and the locomotor activity measurement. These three tests were each performed on seven mice of each drug/dose group.

**TST**

The TST was conducted as previously described by Steru et al. [22]. Briefly, 30 minutes after the last drug administration, animals were suspended by the tail from a ledge with adhesive tape (approximately 1 cm from the tip of the tail). The distance between the tip of the tail of the mouse and the floor was approximately 30 cm. Each animal was partitioned to avoid interference during the test. Immobility was defined as the absence of movement for 6 minutes. Each mouse in the test was recorded by a videocamera and scored by a blinded experimenter.

**FST**

The test was performed using the method described by Porsolt et al. [23]. Briefly, 30 minutes after the last drug administration, animals were placed in an open cylindrical container (total volume, 2500 mL; height, 20 cm; diameter, 14 cm) filled with 10 cm of water (25°C). Immobility was defined as mouse ceasing struggling, remaining floating motionless in water, and making only movements necessary to keep its head above water. The duration of immobility in the last 4 minutes of the (total) 6 minutes of swimming time was recorded with a videocamera and scored by a blinded experimenter.

**Locomotor activity measurement**

Locomotor activity was measured with a 36-point infrared ray passive sensor system (model No. ZZ-6; Taimeng Tech Ltd., Chengdu, Sichuan, China). Thirty minutes after the last drug administration, animals were placed in a chamber of locomotor activity measurement device to adjust to the environment for 1 minute, then recorded and scored by with infrared ray passive sensor system for 10 minutes.

**CMS experiment protocol**

The CMS procedure followed a fixed weekly schedule of commonly used mild stressors such as cold water swimming (4°C, 5 minutes), overnight illumination (10 W LED, 15 Hz, 12 hours), wet cages (200mL water in sawdust bedding, 12 hours), cage tilt (keeping the cage tilted 45° without bedding), restraint stress (2 hours), tailed clipped (5 minutes), empty water bottle (2 hours), and deprivation of food and water (22 hours) with animals of the CMS group. Single housed CMS mice received one stressor once per day for 3 weeks consecutively.

**Sucrose preference test**

The sucrose preference test was used to evaluate the anhedonia state of CMS mice [24]. The test involved 22 hours of food and water deprivation, followed by presentation of 2% sucrose and water for 2 hours. Intake was measured by comparing the bottle weight prior to and after the test [25]. The sucrose preference test was composed of training and testing trials. The training test also served as the baseline test prior to the start of the CMS protocol. The testing trial was performed 24 hours after the 3-week CMS protocol was completed. Sucrose preference index was calculated as the ratio of the consumed sucrose solution to the total amount of liquid consumed in 2 hours.

**Determination of serum corticosterone level**

Mice were anesthetized with sodium pentobarbital (100 mg/kg, intraperitoneally), and cardiac blood was collected just prior to sacrifice. Samples were centrifuged for 15 minutes at 1300g within 30 minutes of collection and were stored frozen (−80°C). Corticosterone (CORT) was assayed in serum using an ELISA kit (Biovalue Inc., China).

**Statistical analysis**

Data were expressed as the mean ± standard error of the mean for the indicated analyses. Behavioral data were analyzed using a one-way analysis of variance. Following the analyses of variance, Dunnett's post hoc tests were used. For all analyses, a p value of <0.05 was considered statistically significant.

**Results**

**Effects of Sanyuansan on immobility time in TST**

Sanyuansan at 900 mg/kg and imipramine at 30 mg/kg markedly reduced immobility time compared with the saline group [F(4, 30) = 8.423, p < 0.01; Figure 1]. Post hoc analysis found that Sanyuansan at 900 mg/kg (p < 0.05) decreased immobility time significantly less than imipramine did (p < 0.01) in the TST, suggesting that Sanyuansan was less sensitive than imipramine in the TST. Sanyuansan at 900 mg/kg showed significant antidepressant-like effects in the TST.

**Effects of Sanyuansan on immobility time in FST**

The effects of oral administration of Sanyuansan on immobility time in the mouse FST are shown in Figure 2. Sanyuansan (at 225 mg/kg, 450 mg/kg, and 900 mg/kg) and imipramine (at 30 mg/kg) all significantly reduced immobility time compared with saline-treated mice [F(4, 30) = 4.984, p < 0.05]. Post hoc analysis found that imipramine (p < 0.01) decreased immobility time but not as much as Sanyuansan did (p < 0.01) in the FST, suggesting that Sanyuansan was more sensitive than imipramine in the FST. Sanyuansan exhibited a slight but insignificant dose-dependent decrease...
in immobility. The results indicated that Sanyuansan showed significant antidepressant-like effects in the FST.

**Effects of Sanyuansan on the locomotor activity**

Treatments with Sanyuansan at 225 mg/kg, 450 mg/kg, and 900 mg/kg did not cause any significant difference in time of either spontaneous activity or spontaneous standing (Figure 3A and B). On the contrary, mice treated with imipramine at 30 mg/kg showed markedly reduced time of spontaneous activity \(F(4,30) = 4.255, p < 0.01\) and spontaneous standing \(F(4,30) = 3.457, p < 0.01; \text{Figure 3A and B}\). The results indicated that Sanyuansan did not affect the spontaneous activity of mice.
Effects of Sanyuansan on the anhedonia in CMS mice

The effects of 3 weeks of oral administration of Sanyuansan and imipramine on anhedonia in CMS mice are shown in Figure 4 with the sucrose preference index. In the baseline phase, the sucrose preference index did not differ significantly among all the groups \( F(3, 28) = 0.993, p > 0.05; \) data not shown. After 3 weeks of CMS, significant differences were observed among the groups \( F(3, 28) = 32.526, p < 0.01 \).

The post hoc results revealed that 3 weeks of CMS significantly reduced mice sucrose preference (Control vs. CMS + Vehicle), and oral administration of Sanyuansan at 900 mg/kg and imipramine at 30 mg/kg both significantly ameliorated the sucrose preference reduction in CMS mice compared with the CMS + Vehicle group.

Effects of Sanyuansan on serum CORT level in CMS mice

The effects of 3 weeks of oral administration of Sanyuansan and imipramine on the serum CORT level in CMS mice are shown in Figure 5. After 3 weeks of CMS treatment, significant differences of CORT were noted among the groups \( F(3, 18) = 11.638, p < 0.01 \).

Figure 3. Effects of drugs on locomotor activity. The mice (7 for each group) were orally administered saline, low dose Sanyuansan (225 mg/kg), medium dose Sanyuansan (450 mg/kg), high dose Sanyuansan (900 mg/kg), and imipramine (30 mg/kg), respectively. The drugs were administered at 24 hours, 1 hour, and 0.5 hour prior to the locomotor activity test. Results are expressed as mean (±SEM) of the times of (A) spontaneous activities and (B) spontaneous standings. The differences were analyzed using one-way ANOVA followed by a post hoc Dunnett’s test. For statistical significance, **\( p < 0.01 \) when compared to the saline group. ANOVA = analyses of variance; SEM = standard error of the mean.
The post hoc results revealed that the 3-week CMS treatment significantly elevated the mice serum CORT level (Control vs. CMS + Vehicle), and oral administration of Sanyuansan at 900 mg/kg and imipramine at 30 mg/kg both significantly reduced the serum CORT level in CMS mice compared with the CMS + Vehicle group.

Discussion

In the present study, we investigated the antidepressant-like effects of Sanyuansan, composed of GTSs, omega-3, and valeriana, through acute mouse behavioral models of despair tasks, TST and FST, which are well-established screening paradigms for the antidepressants and CMS mice model of depression, one of the most valuable and credible rodent model to study depression in animals and mimicking human depressive symptoms [24,26]. In the acute models, Sanyuansan produced a significant reduction in immobility time. In the CMS model, Sanyuansan reversed the anhedonia state and hyperactivation of the HPA axis induced by CMS. These effects were comparable with that of the reference antidepressant imipramine. Unlike imipramine,
Sanyuansan did not affect the spontaneous activities in the locomotor activity test. These results suggested that Sanyuansan did have antidepressant-like effects, and its antidepressant-like effects were of different pharmacologic mechanism from that of imipramine.

The behavioral tests TST and FST are sensitive, believable, and useful measurement in the evaluation of antidepressant drugs. Both tests placed mice in an inescapable situation, and the antidepressant-like activity is expressed by the decrease in immobility duration. In our study, oral administration of Sanyuansan and imipramine showed different anti-immobility capacity in the TST and the FST. In the TST, only Sanyuansan at 900 mg/kg significantly decreased immobility time, although at a less effective level than imipramine. In the FST, each dose of Sanyuansan showed more anti-immobility capacity than imipramine. These different behavioral results might be attributed to the following factors. First, variability of the two animal models in response to certain antidepressants indicates potentially different pharmacological mechanisms mediating performance in these tests. Second, the spectrum of pharmacological sensitivity of the two models is different. Detske et al. and Cryan et al. reported that swimming behavior in the FST is modulated by serotonergic neurotransmission, whereas climbing is mediated by norepinephrinergic neurotransmission. Norepinephrinergic drugs and the mixed serotonin–noradrenaline reuptake inhibitor are more effective than serotonin reuptake blockers in the TST. Moreover, imipramine induces a significant reduction of immobility at lower doses in the TST (4 mg/kg) than in the FST (30 mg/kg). Based on the different performance of the two drugs between the TST and the FST, we speculated that Sanyuansan might have different effects on the monoamine system compared with imipramine.

It has been reported that psychotonics, which are clinically ineffective as antidepressants, also show anti-immobility effects in the TST and FST by stimulants. In order to detect the possibility of a false positive result in anti-immobility effects, we also performed a locomotor activity test to the mice. Our results showed that neither imipramine nor Sanyuansan at any dosage increased spontaneous activity, suggesting that the antidepressant-like effects of Sanyuansan in the TST and FST was unlikely to be attributed to the false positives. It is of interest to note that imipramine significantly decreased locomotor activities including times of spontaneous activity and spontaneous standing, whereas Sanyuansan did not. Several established antidepressants such as fluoxetine, desipramine, and citalopram are reported to decrease the locomotor activity, possibly because of their sedative effect.

The CMS model is considered to be the most valid and reliable rodent model to study depression in animals and mimicking human depressive symptoms. Stress was thought to be one of the key contributors to depression, so the pattern that CMS induced depressive symptoms was relevant to the causes of depression. According to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition classification, presence of anhedonia, a key symptom of depression, is a basis of depressive disorder diagnostics. Therefore, we investigated the antidepressant-like effects of oral administration of Sanyuansan in the CMS model. The results of the study were in line with the previous finding that chronic exposure to mild stressors caused a decrease in the sucrose preference index in mice, indicating the anhedonia state of mice. Our result revealed that the anhedonia state induced by CMS was normalized by oral administration of Sanyuansan at 900 mg/kg. Moreover, the effect of Sanyuansan was comparable to that of imipramine, suggesting that Sanyuansan showed an antidepressant-like effect in the CMS model.

The HPA axis is one of the responders to stress. In the face of persistent stress, continued activation of the HPA axis could lead to a final common pathway, that is, failure in maintaining homeostasis. Moreover, hypercortisolemia had been one of the most reproducible biological findings in major depression. Therefore, we detected the level of serum CORT in CMS mice to assess the hypercortisolemia state. Our results were consistent with the previous finding that CMS elevated the serum CORT level in mice, indicating hyperactivation in the HPA axis of CMS mice. Furthermore, drug treatment showed that hyperactivation of the HPA axis induced by CMS was reversed by Sanyuansan at 900 mg/kg and imipramine at 30 mg/kg.

Generally, Sanyuansan reduced immobility in the TST and the FST but did not affect general locomotor activity in acute behavioral tests and normalized anhedonia and hyperactivation of the HPA axis in the CMS model, which means that it has an antidepressant-like effect but is not sedative.

In summary, Sanyuansan produced a significant antidepressant effect in acute and chronic mice models of depression. In contrast to imipramine, however, Sanyuansan did not affect the spontaneous activities in the locomotor activity test, suggesting that Sanyuansan did have antidepressant-like effects, and its antidepressant-like effects involved a different pharmacologic mechanism from that of imipramine.

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References


