**Polygala japonica Houtt.** reverses depression-like behavior and restores reduced hippocampal neurogenesis in chronic stress mice

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**ABSTRACT**

*Polygala japonica Houtt.* (PJ) has been reported have positive effect on the nerves system including depression, but the underlying mechanism is needed to be understood. Here we show that PJ counteracts behavioral effects induced by chronic restraint, a model of depression mimicking exposure to stress, through adult hippocampal neurogenesis (AHN) enhancement. Chronic stress increased the immobility time in the tail suspension test (TST) and forced swim test (FST) and decreased the time in the center of elevated plus maze (EPM) relative to controls. Moreover, chronic stress also induced the cognitive deficit in novel object recognition test, object location test and Barnes maze. These behavioral alterations were accompanied by the decreased AHN. Treatment with PJ reversed the behavioral and AHN alterations. We also found that PJ had no significant effect on cell proliferation and neuronal differentiation in dentate gyrus (DG) of the hippocampus, but it inhibited the apoptosis of the newborn neurons by activation of bcl-2 and phospho-erk1/2 and increased the number of the newborn neurons. Our results demonstrate that administration of PJ to chronic stress mice alleviates depression-like behaviors and normalizes the deficit in hippocampal neurogenesis with inhibiting newborn neuron apoptosis.

**1. Introduction**

Depression is a leading cause of the disability and mortality in our society [1]. Low mood with reduced pleasure from reward and greater anxiety are hallmarks for the depression disorder [2]. And about 20% population was affected by this disorder during the lifetime of the individual [3]. In addition to the disability and mortality, this disorder is linked with coronary artery disease and type 2 diabetes [4]. Although various antidepressant drugs are available to patients, nearly one-third of patients are ineffectue because of severely side effects [5]. In addition, as many as 30% of patients with major depressive disorder fail to respond to current treatments [6]. There is a great need to find new antidepressant agents for treatment of depression.

Depression is linked with abnormal hippocampal structure and function that decreases the hippocampal neuroplasticity [7]. Specifically, it decreases adult hippocampal neurogenesis, a process that new granule cell neurons are born and incorporated into the dentate gyrus (DG) of the hippocampus throughout life [2]. Human diagnosed with depression and depressive-like behavioral rodents have decreased neurogenesis. Antidepressant treatment in rodents increases neurogenesis, and ablation the neurogenesis can prevent the antidepressant efficacy [8]. On the contrary, increasing DG neurogenesis alone is sufficient to attenuate the depressive-like behaviors under stress-like condition [9]. These finding suggest that there is a causal link between DG neurogenesis and antidepressant efficacy.

The usage of natural products for treatment of various diseases has expanded rapidly over the past decades. Nearly 80% population of the world resorts to natural products as a primary medicine resource because of their less toxic effects and side effects [10]. *Polygala japonica Houtt.* (PJ), a member of Polygala L. family, is a traditional medicine for treatment of variety of ailments, including anti-inflammatory, antibacterial, sedative and nootropic agent [11]. Saponins, flavonoid, and xanthones are the major bioactivity compound isolated from PJ [12–21]. It has been reported that several saponins of PJ can improve the learning and memory in mice [22]. In addition, xanthones exhibits neurotrophic activity on PC12 cells and cultured cortical neurons [18,23]. Furthermore, it has been demonstrated that saponins and flavonoids are effective in reducing immobility during a forced swim test in a mouse model of depression [24].

In present study, we report that the aqueous extract of PJ can alleviate the depressive-like behavior via stimulating the adult DG neurogenesis. Furthermore, we show evidences that PJ enhances the adult DG neurogenesis is because of inhibiting the apoptosis of the newborn neurons via increasing the activity of the BCL2 and phosphate of ERK in DG.

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2. Materials and methods

2.1. Animals and stress exposure model

Subjects were 6 weeks old male C57BL/6 mice. Mice were housed on a 12 h light/dark cycle in clear plastic cages with free access to food and water in controlled temperature (24–26 °C). All behavioral experiments were conducted at the 9:00 a.m.–16:00 p.m., and all experiments were conformed to the Guiding Principles (NIH publication 85–23, revised in 1985) for the Care and Use of Animals and were approved by the institutional animal care committee of Nanchang University.

2.2. Aqueous extraction from Polygala japonica Houtt.

Polygala japonica Houtt. was bought from the local vendor (Jiangxi, China). The voucher specimen (272400) is deposited in the Institute of Materia Medici, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing [15]. The dry roots of PJ were boiled for 1 h, then filtered by filter paper (0.45 um) to get the supernatant. Ethanol was added into the supernatant to 80% and stirred overnight. The supernatant was collect after centrifuge at 10,000 rpm for 5 min and lyophilized. The lyophilized powder was stored in −20 °C.

2.3. Ultra-High-Performance Liquid Chromatography Quadrupole Time of Flight Mass Spectrometry (UPLC-QTOF-MS) analysis

Analysis of the aqueous PJ was performed using ultra-high-performance liquid chromatography (UPLC, Shimadzu, Japan) equipped with hybrid quadrupole-TOF LC/MS/MS Mass spectrometer: Triple TOF 5600 + (AB SCIEX, USA). Chromatographic separation was carried out on a Shim-pack GIST C18 (35 °C; 2.1 mm × 75 mm, 2 μm). The mobile phase consisted of H2O (0.1% formic acid; solvent A) and acetonitrile (ACN; solvent B). The optimized UPLC elution conditions were: 0–10 min, 10% B; 10–12 min, 90% B; 12–13 min, 90% B; 13–18 min, 10% B. The flow rate was 0.2 mL/min. The column eluate was directed to Mass spectrometry using the positive ion mode (m/z M + H+*) for the compounds. The first order MS scan range was from 50 to 1500 m/z and the two stage MS scan range was from 20 to 1500 m/z. Nitrogen settings for the curtain plate (CUR), the nebulizer (GS1) and the heater (GS2) were respectively 33, 50 and 50 psi. All data were acquired and processed by Analyst and Peakview software (AB SCIEX, USA).

2.4. Experimental design

The mice were divided into 4 groups: control, stress, stress with PJ treatment (P-S) and stress with fluoxetine (Aladin, Shanghai, China) treatment (F-S). Both PJ and fluoxetine were dissolved in saline before injection (PJ concentration: 600 μg/ml, fluoxetine concentration 200 μg/ml). Except mice in control group, all mice were daily restrained into a cylinder-shaped tube (10 cm long and 2.5 cm in diameter) for 4 h over 21 consecutive days. After the restraint, for the control and stress group, mice were daily intraperitoneal injected saline (2.5 ml/kg/day); for (P-S) group, mice were daily intraperitoneal injected PJ (2.5 ml/kg/day); for (F-S) group, mice were daily intraperitoneal injected fluoxetine (2.5 ml/kg/day). All behavioral experiments started on day 22 after 21 consecutive days-injection.

2.5. Behavioral measurements

2.5.1. Force swimming test (FST)

Each mouse was placed into a 20 cm diameter glass cylindrical beaker that was filled with warm water (25–30 °C) and allowed to swim freely for 6 min. All the experiments were recorded by video. We analyzed the immobility time in last 4 min.

2.5.2. Tail suspension test (TST)

Each subject was suspended at a hook with tape for 6 min and recorded by video. The immobility time was analyzed in last 4 min.

2.5.3. Elevated plus maze (EPM)

EPM is an assay widely used to evaluate the anxiety-related behavior in rodents [25]. The apparatus (Med Associates, USA, ENV-564A) is consisted of two open arms (35 × 5 cm) and two close arms (35 × 5 cm) with 18 cm high wall. The maze was raised to a height of 60 cm above the floor and placed in a dimly lit room (20 lx) illuminated with one 28 W red fluorescent lamb. In the beginning of the experiment, each mouse was placed at the junction of open arms and closed arms facing to same open arms. The activities of mice were recorded for 10 min by video. The time spent in open arms, closed arm and central time was analyzed.

2.5.4. Object location test (OLT)

The experiments were conducted in a 50 × 50 cm square arena with 45 cm walls and Illumination in the center of the arena was ∼150 lx. All movements of each mouse were recorded by video (Med Associates, USA). At the beginning of the experiment, each mouse was placed at the middle of the arena. Two identical objects were put in two corners respectively. Mouse was allowed freely explored the arena and objects for 10 min and returned to their home cage for 5 min. After cleaned the arena and objects with 75% ethanol, we randomly moved one object to another unoccupied corner of the arena. The mouse was placed at the middle of the arena and allowed to freely explored the field and objects again for 10 min. Object exploration was defined as the nose to the object at a distance ≤2 cm or the forepaws on the objects. The time spent in exploring the object was manually recorded in a blind manner. Exploration percent (novel location exploration time/total exploration time × 100%) were calculated.

2.5.5. Novel object recognition test (NORT)

The experiments were conducted in the same arena of OLT. Each mouse was placed at the corner of a square arena with two identical objects placed in two corners respectively. Mouse could freely explore the field and objects for 10 min and returned to the home cage for 5 min. After cleaned the arena and objects with 75% ethanol, we randomly replaced one familiar object by a novel object. The mouse was placed into the arena and explored the field again for 10 min. All movements of each mouse were recorded by video (Med Associates, USA), the time spent in exploring the object was manually recorded in a blind manner. Exploration percent (novel object exploration time/total exploration time × 100%) were calculated.

2.5.6. Barnes maze

The barn maze procedure was similar to the previous work [26]. The apparatus consisted in a white circular platform (diameter: 50 cm) with 18 equally spaced holes located 2 cm from the border. Beneath one hole, we provided one black box where mouse could hide from the light. During the training session, the mouse was placed at the platform center and explored the target hole (with the black box) by itself for 3 min. A mouse which failed to find the black box was guided into the hole leading to the box and allowed to remain in the hole for 1 min as a “dark reward”. The mouse which success to find the black box was allowed to remain in the hole for 1 min as a “dark reward”. The mice were trained with two trials per day for 7 days. Probe trial was done at the day 14 of the experiment. No training occurred between Days 8 and 13. All experiments were recorded by video (Taimeng, Chengdu, China) and the videos scored by Barnes maze analysis system (BMT-100, China). The parameters, primary latency, time spent in areas, and errors was scored. Primary latency was defined as the time for mouse to make the first contact with the target hole in probe trial. Errors were defined as the number of holes mouse visited before the first contact with the target hole.
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