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Chinese herbal medicines promote hippocampal neuroproliferation, reduce stress hormone levels, inhibit apoptosis, and improve behavior in chronically stressed mice

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ABSTRACT

Ethnopharmacological relevance: An efficacious antidepressant without unwanted side effects is need urgently at present. This study aimed to investigate whether treatment with four Chinese herbal medicines (CHMs), namely *Radix Astragali, S*aposhnikovia *divaricate* (SD), *Eucommia ulmoides* Oliv. bark (EU), and *Corydalis yanhusuo* W. T. Wang (*C. yanhusuo*), could reverse the effects of chronic mild stress (CMS) in a depression-like mouse model and the potential mechanism(s) of their action.

Materials and methods: *In vitro* study, the proliferation of NSCs was assessed using the MTS assay. *In vivo* study, chronic mild stress (CMS) was used in mice for 14 days to establish a depression-like mouse model. Plasma corticosterone levels were assessed by UPLC coupled to a triple-quadrupole mass spectrometer. The forced swim test (FST) was used to assess the effects of the four CHMs on depression. BrdU incorporation and TUNEL staining were used to assay hippocampal precursor cell proliferation rate and apoptosis.

Results: The CHMs included *Radix Astragali*, EU, *C. yanhusuo*, and SD were shown to promote neuroproliferation *in vitro*. *In vivo* study, oral administration of these four CHMs for 14 days reversed the elevated plasma corticosterone levels, body weight loss, decrease in proliferation of hippocampal precursor cells; they also inhibited hippocampal cell apoptosis, and exhibited an antidepressant-like effect in a depression-like mouse model induced by CMS.

Conclusions: Our study indicates that each of these CHMs has the potential to ameliorate depression. The possible mechanisms of action include modulation of the HPA axis, reduction in stress hormone levels, inhibition of apoptosis, and promotion of hippocampal neuronal plasticity and neurogenesis.

1. Introduction

Depression is a serious emotional disorder, with a lifetime prevalence as high as 21% in the general population of some developed countries (Wong and Licinio, 2001). Chronic exposure to stress and stressful life events may lead to development of major depression (Pittenger and Duman, 2008). Exposure to chronic stress may not only induce depression-like behavior, but also result in various structural and functional changes in the brain, including neural damage (Harvey et al., 2006). The hippocampus provides negative control over the hypothalamic-pituitary-adrenal (HPA) axis (Jankord and Herman, 2008), and is highly sensitive to

Abbreviations: BDNF, Brain-derived neurotrophic factor; BrdU, Bromodeoxyuridine; CHM, Chinese herbal medicine; CMS, Chronic mild stress; EGF, Epidermal growth factor; EU, Eucommia ulmoides Oliv. bark; FGF, Fibroblast growth factor; FST, Forced swimming test; GDNF, Glia-derived neurotrophic factor; GR, Glucocorticoid receptor; HPLC, High-performance liquid chromatography; HPA, Hypothalamic-pituitary-adrenal; IHC, Immunohistochemistry; MTS, 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; NGF, Nerve growth factor; NSC, Neural stem cell; Rf, Retention factor; SD, Saposhnikovia divaricate; SGZ, Subgranular zone; SNRI, Serotonin norepinephrine reuptake inhibitor; TLC, Thin-layer chromatography; UPLC, Ultra-performance liquid-chromatography

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stress (Mirescu and Gould, 2006). Chronic stress suppresses cell proliferation in the hippocampal subgranular zone (SGZ) (Mirescu and Gould, 2006), and leads to activation of the HPA axis (Schloesser et al., 2009). In contrast, chronic chemical antidepressant treatments increase cell proliferation or even enhance survival of the newborn neurons in the SGZ (Malberg and Duman, 2003). Moreover, the behavioral effects of certain antidepressants also depend on SGZ neurogenesis (Santarelli et al., 2003). Certain antidepressants can reverse the decrease in adult hippocampal cell proliferation induced by inescapable stress. These findings suggest that neurogenesis and newly generated neuronal cells of adult hippocampus are involved in the pathogenesis of depression, and may also be the target of drugs used for treating depression (Malberg and Schechter, 2005).

There is a significant unmet need in options available to treat depression. With the requirement for greater therapeutic efficacy and reduced adverse effects, research on new antidepressants, as well as their action and mechanisms in traditional medicine has gained importance. The concept of multi-targeted drugs or multi-component therapy is gaining increased attention because many diseases (such as hypertension or epilepsy) are best treated by multi-drug or multi-target therapies (Efferth and Koch, 2011; Zimmermann et al., 2007). Traditional medicine, such as Chinese herbal medicines (CHMs) have been re-evaluated and are important as a resource of bioactive molecules with therapeutic effects and for designing multi-targeted drugs (Wang et al., 2012).

There is an urgent need for more sensitive animal models and *in vitro* methods to screen new antidepressant compounds and identify key molecules for clinical trials in major depression (Kulkarni and Dhir, 2009). Cultured neural stem cells (NSCs) can be used as an *in vitro* screening model to evaluate the effects of a drug on neurogenesis. NSCs are multipotent, capable of self-renewal, and could serve as a convenient model at the cellular level for developing therapeutic medicines (Ma et al., 2009). The chronic mild stress (CMS) model has been shown to cause lower consumption of sucrose (sweet food) reflecting anhedonia (the loss of interest or pleasure) in these animals, and anhedonia is one of the two core symptoms required for diagnosis of a major depressive episode in humans.

We have evaluated the efficacy of 90 CHMs, including 56 single herbs and 34 CHM formulae on promoting NSC proliferation using a cultured cell line. The three most efficacious were the Ligusticum chuanxiong, *Scutellaria baicalensis* and *Phellodendron amurense* as shown by their ability to: (1) stimulate proliferation of NSCs *in vitro*, (2) promote hippocampal neuroproliferation *in vivo*, (3) modulate the HPA axis, and (4) show antidepressant-like effects in a depression-like mouse model following oral administration for 14 days (Pao et al., 2012). Our previous preliminary data suggested that four other single herbs, namely, *Radix Astragali*, *S*aposhnikovia *divaricate* (SD), EU, and Corydalis *yanhusuo* W. T. Wang (*C. yanhusuo*) had substantially promoted proliferation of NSCs. Therefore, in the current study, their antidepressant-like properties as well as their effect on neurogenesis were investigated both *in vitro* and *in vivo*.

2. Materials and methods

2.1. CHMs

CHMs were bought from a GMP pharmaceutical company (Sun Ten Pharmaceutical Co., Ltd., Taipei, Taiwan). The dry powder provided was prepared from a decoction of the raw herb in hot distilled water, and concentrated by instant spray-drying and low-temperature vacuuming. The voucher specimen numbers of *Radix Astragali*, SD, EU, and *C. yanhusuo* are 70,643, 150,701, 20,609, and 30,605, respectively. The raw herbs were accumulated from various locales in China. Identification of the voucher specimens was performed at the herbarium of the Brion Research Institute (BRI) in Taiwan. The species were confirmed by microscopic identification. Phytochemical analysis of the extracts and classification of herbal parts were based on the Pharmacopoeia of the People's Republic of China (Pharmacopoeia Commission of People's Republic of China, 2010).

2.2. In vitro study

2.2.1. Mouse NSC line

The mouse NSC line (mNSC 9601) was cultured in a mixture of serum-free Dulbecco's modified Eagle medium and F-12 nutrient, and was kindly contributed by Dr. Shih-Hwa Chiou (Institute of Clinical Medicine, National Yang-Ming University, Taiwan)).

2.2.2. Preparation of CHMs for the in vitro study

The four CHMs were further extracted before the *in vitro* study. Each was dispensed such that the final concentration of each CHM was the same. The extract process was conducted to simulate the physiological conditions under which a CHM is digested in humans following oral administration. CHMs were dissolved in saline at a concentration of 100 mg/25 ml. After adjusting the pH to 1.2 with 1 N HCl, the solution was vortexed for 5 min at 37 °C to mimic physiological digestion in the stomach; the pH of the above solution was further adjusted to 6.8 with 1 N NaOH and vortexed for 5 min at 37 °C to simulate the intestinal environment. The four CHM solutions were then centrifuged at 3000 rpm for 10 min. The supernatant was filtered through a 0.22 mm filter. Serial dilutions were performed and the solutions were finally diluted with medium to a concentration of 1:100.

2.2.3. NSC proliferation activity assay in vitro

The proliferation of NSCs was assessed using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. Trypsin was added to neurosphere-like NSCs to dispersed cells, and viable NSCs were seeded at a density of 4×10^3 cells per well on 96 well plates. The CHM extracts were diluted with culture medium. Different extracts concentrations of 0.04, 0.4, 4, and 40 mg/ml was added to each well and incubated for 48 h. A 20 μl MTS reagent was added to each of the 96 well plates and incubated for 3 h in an atmosphere of 5% CO_2 at 37 °C. MTS assay was performed at 490 nm with Microplate reader (Thermo Electron Co.). Each sample was analyzed in triplicate.

2.3. In vivo study

2.3.1. Animals

Male BALB/c mice (10 weeks of age) were housed in polypropylene cages under standard laboratory conditions (12-h light/dark cycle; 22 °C, 55% humidity; food and water available *ad libitum*). All animal procedures were in conformity to the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Animal Care Committee of the National Defense Medical Center (Taipei, Taiwan). The animals (a total of 61) were randomly assigned to either the control or the stressed group after two weeks arrival. Animals in the control group (n=10) were left undisturbed in their cages in a separate room and received an intraperitoneal (i.p.) injection of 300 μl saline and oral administration of 200 μl saline daily. The stressed group was randomly divided into CMS (n=10), CMS-fluoxetine (positive control, n=9), and four CMS-CHM groups namely *Radix Astragali* (n=8), SD (n=8), EU (n=8), and *C. yanhusuo* (n=8). Mice in the stressed groups were exposed to different mild stressors for