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Antidepressant-like effects of Xiaochaihutang in a neuroendocrine mouse model of anxiety/depression

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ABSTRACT

Ethnopharmacological relevance: Hyperactivity of hypothalamic-pituitary-adrenal (HPA) axis is often observed in the pathophysiology of depression. Antidepressant therapy can restore hippocampal neurogenesis to rescue the HPA axis regulation defects. Xiaochaihutang (XCHT), a famous Chinese herbal formula, has been used clinically in depressive disorders in China. Our previous studies have demonstrated XCHT improved depressive-like behaviors in chronic unpredictable mild stress rat, but the underlying mechanisms of XCHT on hippocampal neurogenesis and the HPA axis were still unclear.

Materials and methods: We used chronic corticosterone (CORT)-induced mouse model of anxiety/depression to investigate antidepressant-like effects of XCHT by several physical and behavioral testing, including body weight, coat state, open field test, elevated plus maze, tail suspension test and forced swimming test. The integrity of negative feedback function on HPA axis was assessed by the dexamethasone (DEX) suppression test. In addition, Ki-67 and doublecortin (DCX) were performed to assess hippocampal cell proliferation and neurogenesis by immunohistochemistry. Chemical profile of active constituents in brain after oral administration of XCHT was revealed by ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS).

Results: Our results showed that oral administration of XCHT (2.3, 7 and 21 g/kg) for 30 days remarkably normalized chronic CORT-induced the slowness in weight gain, the deterioration in coat state, the escape behavior in open field test and elevated plus maze, and the increase of immobility time in tail suspension test and forced swimming test. Moreover, XCHT significantly reversed chronic CORT-induced the reduction of DEX-induced plasma corticosterone/c-Fos suppression and Ki-67/DCX positive cells. Finally, a total 13 potential active constituents in brain were identified by UPLC-MS/MS after oral administration of XCHT, including 10 prototype components and 3 metabolites.

Conclusions: Our findings showed that XCHT could remarkably alleviate chronic CORT-induced anxiety/depression-like behaviors, which were probably attribute to promoting hippocampal neurogenesis and remodeling the integrity of the negative feedback loop on HPA axis. The constituents identified in brain might contribute to understanding the therapeutic basis of XCHT on depression.

1. Introduction

When all living organisms are threatened by certain physical and psychological events, one of the major physiological responses is to activate the neuroendocrine systems, especially the hypothalamic-pituitary-adrenal (HPA) axis (de Kloet et al., 2005; Pariante and Lightman, 2008). Corticotrophin releasing factor (CRF) is produced from the paraventricular nucleus (PVN) to induce adrenocorticotropic hormone (ACTH) release from the pituitary, which in turn stimulates glucocorticoids (GCs) from the adrenal (Lucassen et al., 2014). GCs can easily cross blood-brain barrier, enter into the brain and exert multiple functions. Depression patients was frequently accompanied with hyperactivity of HPA axis, and previous studies indicated that negative feedback of HPA axis was impaired in major depression patients

Abbreviations: XCHT, Xiaochaihutang; TST, tail suspension test; FST, forced swimming test; HPA, hypothalamic-pituitary-adrenal; CORT, corticosterone; DCX, doublecortin; DEX, dexamethasone; CRF, Corticotrophin releasing factor; ACTH, adrenocorticotropic hormone; GCs, glucocorticoids

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(Swaab et al., 2005; Willner et al., 2013). This impairment might be caused by high levels of GCs and weakness of hippocampal control (Guidotti et al., 2013; Snyder et al., 2011). Indeed, chronic stress or high levels of GCs could decrease the production and the survival of the new hippocampal neurons (de Kloet et al., 2005; Pariante and Lightman, 2008). More and more researches focused on hippocampal neurogenesis based on the fact that antidepressant therapy could restore hippocampal neurogenesis to rescue the HPA axis regulation defects (Surget et al., 2011). In addition, ablation of hippocampal neurogenesis weakened antidepressant effect and stress response regulation of HPA axis (David et al., 2009; Snyder et al., 2011). Hence, hippocampal neurogenesis and neurogenesis-dependent regulation of HPA axis played an important role in the pathophysiology of depression and might be targets of antidepressant drug action.

Xiaochaihutang (XCHT), one of famous Chinese herbal formulas, was first described in “Shang Han Lun” two thousand years ago for the treatment of Shaoyang symptom, including influenza and emesis (Bao et al., 2004). XCHT consists of seven herbs: Radix Bupleuri (Latin: *Bupleurum chinense* DC.), Radix Scutellariae (*Scutellaria baicalensis* Georgi), Ginseng (*Panax ginseng* C.A. Meyer), Rhizoma pinelliae (*Pinellia ternata* (Thunb.) Breit.), Radix glycyrrhizae (*Glycyrrhiza uralensis* Fisch.), Rhizoma zingiberis recens (*Zingiber officinale* Rosc.) and Fructus jujubae (*Ziziphus jujuba* Mill). So far, XCHT has been successfully used in depressive disorders in China (Jia et al., 2009; Li and Gao, 1996), and preclinical research indicated that XCHT alleviated stress response and prednisolone-induced adrenal gland suppression (Amagaya and Ogihara, 1990; Iwama et al., 1986). Our previous studies demonstrated that XCHT significantly increased BDNF, NGF and their receptors (TrkB and TrkA) expression in the hippocampus (Su et al., 2014a), and these neurotrophic factors might contribute to hippocampal neurogenesis, but the underlying mechanisms among XCHT, hippocampal neurogenesis and neurogenesis-dependent regulation of HPA axis were unclear. Moreover, it was demonstrated that the fixed combination of Radix scutellariae, Ginseng and Radix glycyrrhizae was the core in compatibility of XCHT, and our studies revealed active constituents in plasma after oral administration of XCHT (Zhang et al., 2015). However, the unknown components absorbed in brain and accurate action mechanisms on antidepressant therapy still need to further research.

In this study, we used several physical and behavioral tests to investigate antidepressant-like effects of XCHT by CORT-induced mouse model of anxiety/depression. Potential mechanisms were assessed by the integrity of negative feedback function on HPA axis and hippocampal neurogenesis. The active constituents in brain after oral administration of XCHT were revealed by UPLC-MS/MS. Our findings wish to provide new perspectives for understanding the relationships among XCHT, hippocampal neurogenesis and neurogenesis-dependent regulation of HPA axis in depression and revealing the potential therapeutic basis of XCHT.

2. Materials and methods

2.1. Preparation of XCHT

XCHT consisted of seven Chinese herbs was showed in Table 1. Seven herbs were purchased from Tongrentang Chinese Pharmaceutical Co. Ltd. (Beijing, China), identified by Dr. Jiuzhi Yuan (School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, China), and confirmed by National institutes for food and drug control according to the Pharmacopeia of China. The dried herbs were boiled three times and the decoctions were mixed and filtered, then lyophilized and stored in a desiccator. All manufacturing processes and quality were standardized based on our previous study (Su et al., 2014b). The yield of powdered extract was about 25.2% (w/w).

Table 1
Recipe of Xiaochaihutang (XCHT).

Ping Yin	English name	Latin name	Weight
Chai Hu	Radix Bupleuri	*Bupleurum chinense* DC.	12 g
Huang Qin	Radix Scutellariae	*Scutellaria baicalensis* Georgi	9 g
Ren Shen	Ginseng	*Panax ginseng* C.A. Mey.	9 g
Ban Xia	Rhizoma Pinelliae	*Pinellia ternate* (Thunb.) Breit.	9 g
Gan Cao	Radix Glycyrrhizae	*Glycyrrhiza uralensis* Fisch.	6 g
Shen Jiang	Rhizoma Zingiberis recens	*Zingiber officinale* Rosc.	6 g
Da Zao	Fructus Jujubae	*Ziziphus jujube* Mill.	4 pieces (9 g)

2.2. Quality control of XCHT

Our lab has established a standardized method for the quality control of XCHT (Wang et al., 2015; Zhang et al., 2015). The amounts of active ingredients, such as Liquiritin, Baicalin, Wogonoside, Baicalein, Wogonin, Oroxylin A and Saikosaponin A, have been determined by ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and stable contents have been secured (Wang et al., 2015). Briefly, the UPLC-MS/MS system consisted of a Waters ACQUITY™ ultra performance liquid chromatography (UPLC) system (Waters Corp., Milford, USA) coupled to a Micromass Quattro Micro™ API mass spectrometer. The analytes were qualified on a BEH C_{18} column (100 mm×2.1 mm, i.d. 1.8 μm) with the column temperature maintained at 20 °C. The gradient mobile phase and MS analysis were showed in Supplemental material. Data were acquired and processed using MassLynx 4.1 software. The contents of main components of XCHT were Liquiritin (159 μg/g), Baicalin (1210 μg/g), Wogonoside (623 μg/g), Baicalein (0.906 μg/g), Wogonin (5.86 μg/g), Oroxylin A (16 μg/g) and Saikosaponin A (68.1 μg/g).

2.3. Animals

Adult male C57BL/6J mice weighing 18–22 g were supplied by the Experimental Animal Centre of Shenyang Pharmaceutical University. Animals were maintained on standardized environmental conditions (22 ± 2 °C, 12 h light/dark cycle with light on at 8:00 a.m.) with free access to food and water and housed six per cage. All experiments were carried out according to the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of China. All anesthetization was performed under sodium pentobarbital, and all efforts were made to minimize suffering.

2.4. Drug treatment

The procedure and dose of corticosterone (CORT) administration was performed as previously described (Wu et al., 2013). Mice were randomly divided into two parts (16 for the control and 80 for the CORT part). For the CORT part, the mice were split into 5 treatment groups (the model group, XCHT (2.3, 7, 21 g/kg) groups and FLU (20 mg/kg) group, n=16/group). In CORT group, mice were injected subcutaneously with CORT (40 mg/kg, TCI Development Co., Ltd, Japan) dissolved in sesame oil between 8:00 a.m. and 10:00 a.m. for 35 days. XCHT (2.3, 7, 21 g/kg) and fluoxetine (FLU, 20 mg/kg, Lilly S.A.) were dissolved in distilled water and given by gastric gavages 30 min prior to the corticosterone injection for 35 days. The selected doses for XCHT administration were based on the fact that XCHT was prescribed at a daily dose of 51 g of the fixed combination of herbs in clinical practice (Su et al., 2014b). We converted human dose into mouse dose in accordance with body surface area principle and chose 7 g/kg as clinical equivalent dose (70 kg of Human and 0.02 kg of mouse at a