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Essential oil of *Perilla frutescens*-induced change in hippocampal expression of brain-derived neurotrophic factor in chronic unpredictable mild stress in mice

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ABSTRACT

Ethnopharmacological relevance: *Perilla frutescens* (Perilla leaf), a traditional Chinese medicinal herb, has been used for centuries to treat various conditions including depression. A previous study of the authors demonstrated that essential oil of *Perilla frutescens* (EOPF) attenuated the depressive-like behavior in mice.

Aim of the study: This study was undertaken to explore the dynamic change of behaviors and brain-derived neurotrophic factor (BDNF) expression induced by chronic unpredictable mild stress (CUMS), and improved by EOPF.

Materials and methods: Four separate CUMS experimental groups (1-week, 2-week, 3-week and 4-week treatment) were treated with EOPF (3 mg/kg and 6 mg/kg, p.o.) or fluoxetine (20 mg/kg, p.o.), followed by sucrose preference, locomotor activity, immobility and hippocampal BDNF measurement.

Results: EOPF, as well as fluoxetine, restored the CUMS-induced decreased sucrose preference and increased immobility time, without affecting body weight gain and locomotor activity. Furthermore, CUMS (3 or 4-week) produced a reduction in both BDNF mRNA and protein expression in the hippocampus, which were ameliorated by EOPF (4-week) and fluoxetine (3 or 4-week) treatment.

Conclusion: These results presented here show that BDNF is expressed depending on length of CUMS procedure and EOPF administration. And this study might contribute to the underlying reason for the slow onset of antidepressant activity in clinic.

1. Introduction

Depression, one of the major causes of disability worldwide, is a mood or affective disorder caused by many factors, from the psychological, social, and environmental to the genetic, and metabolic (World Health Organisation (WHO), 2007). The mechanisms underlying depression as well as antidepressant activity are still not fully understood. Stress is well known to be one of the most important factors responsible for depressive disorders (Zhu et al., 2012). Recently, a novel hypothesis has emerged suggesting a role for neurotrophin in the pathogenesis of depression and in its treatment (Sen et al., 2008). For example, preclinical and clinical studies have demonstrated that stress and depression resulted in neurogenesis impairment and down-expression of neurotrophin in the brain (Tanis et al., 2007).

Brain-derived neurotrophic factor (BDNF), one of the most extensively neurotrophins studied in relation to depression, has been shown to promote neuronal survival, differentiation, function, and plasticity (Huang and Reichardt, 2001), suggesting that BDNF plays a key role in the pathophysiology of depression (Castren and Rantamaki, 2010). In addition, a growing number of clinical and experimental evidence reports that alterations in BDNF levels are associated with the mechanisms of action underlying the favorable therapeutic activity of antidepressant drugs (Schmidt and Duman, 2010; Thompson Ray et al., 2011). As a result, BDNF has been considered as a possible target for antidepressants.

Perilla frutescens (Perilla leaf), a traditional Chinese medicinal herb, has been used for centuries to treat various conditions including depression. A previous study of the authors demonstrated that essential oil of *Perilla frutescens* (EOPF) treatment decreased the immobility time in the forced swimming test (FST) via monoaminergic systems (Yi et al., 2010), suggesting that EOPF might be useful for the prevention of depression. However, the antidepressant-like mechanism involved in the aspect of BDNF remains unknown. Given the importance of BDNF in the pathophysiology of depression and antidepressant treatment, the aim of the present study was to determine how duration of treatment influences hippocampal BDNF levels in mice exposed to chronic unpredictable mild stress (CUMS) procedure.

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2. Materials and methods

2.1. Animals

Male ICR mice (22–26 g; 4 weeks old) were purchased from Laboratory Animal Centre, Fujian Medical University, Fujian Province, PR China. Animals were housed 8 per cage (320 cm × 180 cm × 160 cm) under a normal 12-h/12-h light/dark schedule with the lights on at 07:00 a.m. and had free access to tap water and food pellets. Ambient temperature and relative humidity were maintained at 22 ± 2 °C and at 55 ± 5%, and given a standard chow and water ad libitum for the duration of the study. The animals were allowed 1 week to acclimatize themselves to the housing conditions before the beginning of the experiments. All procedures were performed in accordance with the published guidelines of the China Council on Animal Care (Regulations for the Administration of Affairs Concerning Experimental Animals, approved by the State Council on October 31, 1988 and promulgated by Decree No. 2 of the State Science and Technology Commission on November 14, 1988).

2.2. Reagents

Fluoxetine hydrochloride was purchased from Changzhou Siyao Pharmaceuticals Co., Ltd (Changzhou, PR China). All primers used in this study were designed and synthesized by Sangon Biotech Co. Ltd. (Shanghai, PR China). Trizol reagent was purchased from Invitrogen (Carlsbad, USA). Reverse transcriptase Moloney Murine Leukemia Virus (M-MLV) used for cDNA synthesis was from Promega Corporation (Madison, USA). All other reagents used in RT-PCR were purchased from Sangon Biotech Co. Ltd. (Shanghai, PR China).

2.3. Plant material and preparation of EOPF

The best quality commercial Perilla leaf [*Perilla frutescens* (L.) Britt (Labiatae)] was purchased from Fujian Pharmaceutical CO., LTD, PR China, which was collected in Zhejiang province of China and authenticated by Cheng-Fu Li, Department of Pharmacy, Xiamen Hospital of Traditional Chinese Medicine, PR China (voucher specimen number HU/CE-10251).

EOPF was prepared from 250 g of *Perilla frutescens* using supercritical equipment (Zeng et al., 2003; Yi et al., 2010). The extract was collected for 100 min under the condition of 45 °C and 30 MPa (3.1% w/w yield).

As described by Ling, the main constituents of EOPF extracted by supercritical fluid are L-perillaldehyde, limonene, *trans*-caryophyllene, selinene, santalene and bergamotene (Ling, 2005).

2.4. CUMS procedure

The procedure of CUMS was performed as described previous (Mao et al., 2009; Schweizer et al., 2009), with some modifications. Briefly, the weekly stress regime consisted of food and water deprivation, exposure to an empty bottle, soiled cage, light/dark succession every 2 h, space reduction, 45° cage tilt, overnight illumination and predator sounds (Table 1). All stressors were applied individually and continuously, day and night. Control animals were housed in separate room and had no contact with the stressed groups. They were deprived of food and water for the 24 h preceding sucrose preference test (SPT), but otherwise food and water were freely available in the home cage.

2.5. Drug treatments and experimental design

Four separate experiments (1-week, 2-week, 3-week and 4-week treatment) were carried out and in each case a total of 40 mice were used. In each experiment, animals were divided into five treatment groups as follows: one vehicle-control (0.9% physiological saline), one vehicle-CUMS (0.9% physiological saline), one CUMS-fluoxetine (20 mg/kg) and two CUMS-EOPF treatments (3, 6 mg/kg). Thus, different groups of mice, 8 animals per group, were used for drug treatment and for each experiment. All treatments were administered by oral (p.o.) gavage in a volume of 10 ml/kg body weight. Drugs were administered successively for 9, 16, 23 or 30 days, respectively (Fig. 1). The treatment protocols of dose and administration route used for EOPF and fluoxetine was adopted according to the literature and our previous study (Mao et al., 2009; Yi et al., 2010). Body weights of all animals were weighed at the beginning and end of the separate experiment.

2.6. SPT

SPT was carried out at the end of 1-week, 2-week, 3-week or 4-week CUMS exposure. The test was performed as described previously (Mao et al., 2009). Briefly, before the test, mice were trained to adapt to sucrose solution (1%, w/v): two bottles of sucrose solution were placed in each cage for 24 h, and then one bottle of sucrose solution was replaced with water for 24 h. After the adaptation, mice were deprived of water and food for 24 h. SPT was conducted at 9:30 a.m. in which mice were housed in individual cages and were free to access to two bottles containing 100 ml of sucrose solution (1% w/v) and 100 ml of water, respectively. After 24 h, the volumes of consumed sucrose solution and water were recorded and sucrose preference was calculated as sucrose preference (%) = sucrose consumption (ml)/[sucrose consumption (ml) + water consumption (ml)] × 100%.

2.7. Open-field test (OFT)

The mice were treated with EOPF or fluoxetine 60 min before the exposure to the OFT on 8th, 15th, 22nd or 29th day, in order to assess possible effects of drug treatment on spontaneous locomotor activity. The apparatus consisted of a wooden box measuring 40 cm × 40 cm × 30 cm, with the floor divided into 25 equal squares (8 cm × 8 cm). The numbers of squares crossed with all

Table 1
CUMS procedure.

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Food and water deprivation	09:30→	09:30					
Exposure to an empty bottle		09:30–10:30					08:30–09:30
Soiled cage		10:30→	10:30		20:30→	20:30	
Light/dark succession every 2 h			10:30–20:30				09:30–21:30
Space reduction			20:30→	08:30			21:30→
45° cage tilt				08:30–20:30		20:30→	08:30
Overnight illumination				20:30→	08:30		
Predator sounds					08:30–20:30		

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