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Effects of peony glycosides on mice exposed to chronic unpredictable stress: Further evidence for antidepressant-like activity

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ABSTRACT

Ethnopharmacology relevance: Peony, the processed root of *Paeonia lactiflora* Pall. (Ranunculaceae), is a component herb of many traditional formulae for the treatment of depression-like disorders.
Aim of the study: The present study aimed to investigate whether the total glycosides of peony (TGP) could prevent depression induced by chronic stress.
Materials and methods: Mice were subjected to an experimental setting of chronic unpredictable stress (CUS). The effect of TGP treatment on CUS-induced depression was examined by measuring behavioral and neurochemical parameters of depression and the antioxidant status of brain tissue.
Results: CUS-induced depression, as indicated by a significant increase in immobility time in the tail suspension test, was associated with increases in the activities of monoamine oxidases, depletion of reduced glutathione, and an increase in malondialdehyde level, in mice brains. TGP treatment alleviated the extent of CUS-induced depression and the associated impairment of antioxidant status in the mouse brain.
Conclusion: The results suggest that TGP alleviates depression induced by chronic unpredictable stress. The antidepressant-like activity of TGP is probably mediated by inhibition of monoamine oxidases and the attenuation of oxidative stress in mouse brain.

1. Introduction

Depression is a chronic mental disorder clinically characterized by a pervasive low mood, loss of interest or pleasure in daily activities, low self-esteem, and a high suicidal tendency (Hankin, 2006; Perahia et al., 2009). The disease, which is common incidence worldwide, affects the quality of life of many people, and has become a major cause of suicidal death (Cukrowicz et al., 2009). It is believed that chronic stress is a crucial factor involved in depression onset and relapse (Bidzinska, 1984; Sheline, 2000; Lee et al., 2002). An animal model of chronic unpredictable stress (CUS)-induced depression has been developed to simulate the pathogenesis of depression in humans. CUS was found to induce long-term behavioral disturbances and neurochemical changes resembling the symptoms of clinical depression (Willner, 1997; Luo et al., 2008). The model has been developed by Katz and Schmaltz (1980) and Katz et al. (1981), and has been used in a number of studies (Zhou et al., 2007; Luo et al., 2008; Piato et al., 2008).

Antidepressant drugs are widely available in the pharmaceutical market (Bouvier et al., 2003; Shen and Liang, 2007). However, because multiple pathogenic factors are involved in depression, many synthetic antidepressant drugs show low response rates and even produce adverse side-effects such as cardiotoxicity, hypertensive crisis, sexual dysfunction, and sleep disorder, in depressed patients (Park et al., 2007). Therefore, it is desirable to seek antidepressants in naturally-occurring herbs; such materials are expected to show fewer side-effects. Peony, the processed root portion of *Paeonia lactiflora* Pall. (Ranunculaceae) is a component of many Chinese medicinal formulae prescribed for the treatment of depression-like disorders (Zhang et al., 1998; Xie and Wang, 2005). The total glycoside fraction of peony (TGP) has been shown to possess anti-inflammatory, antioxidant, immunomodulatory, antithrombotic, and neuroprotective properties, and glycosides such as paeoniflorin and albiflorin were found to be biologically active ingredients (Zhou et al., 1994; Liu et al., 2001; Dong et al., 2003; Yang et al., 2006; Xu et al., 2007). Previous studies in our laboratory have demonstrated the antidepressant-like effect of an ethanol extract and TGP in animals housed in a normal environment (Mao et al., 2008a,b). Chronic stress is relevant to the pathogenesis of depression in humans, but it is still unknown whether TGP can prevent chronic stress-induced depression. Therefore, the present study examined the effect of TGP treatment on mice exposed to

Abbreviations: CUS, chronic unpredictable stress; GSH, glutathione; MAO, monoamine oxidase; MDA, malondialdehyde; TGP, total glycosides of peony.

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CUS. TGP was given daily, before each stressor, for 24 days. The prophylactic effect of TGP on depression formation was evaluated using behavioral and neurochemical parameters. Given that free radicals play an important role in the pathogenesis of neuropsychiatric disorders (Bilici et al., 2001; Khanzode et al., 2003), we also investigated whether the antidepressive effect of TGP was related to antioxidant properties by measuring reduced glutathione (GSH) and malondialdehyde (MDA) levels in brain tissues of control and CUS-exposed mice, without or with TGP treatment.

2. Materials and methods

2.1. Drugs and chemical reagents

TGP (a light yellow-to-brown powder) were supplied by Ningbo Liwah Pharmaceutical Co. Ltd. (Zhejiang, China). The preparation method has been described previously (Zhao et al., 1997). The product was analyzed by high-performance liquid chromatography as previously described (Department of Health, Hong Kong SAR, 2008) and shown to contain 30% (w/w) paeoniflorin and 10% (w/w) albiflorin (Fig. 1), consistent with previous studies (Q. Wang et al., 2005; Xu et al., 2007). A voucher sample (TGP071024) has been deposited in the School of Chinese Medicine, The Chinese University of Hong Kong. Clomipramine, from Beijing Novartis Pharmaceutical Co. Ltd. (Beijing, China), was used as a positive control. The chemicals 5-hydroxytryptamine, β-phenylethylamine, thiobarbituric acid, and 5,5′-dithiobis-2-nitrobenzoic acid were obtained from Sigma–Aldrich (St. Louis, MO). All other reagents and solvents were of analytical grade.

2.2. Animals

Male ICR mice weighing 20–25 g were obtained from the Laboratory Animal Services Center of the Chinese University of Hong Kong. Animals were maintained on a 12-h light/dark cycle at a regulated temperature (22 ± 2 °C) and humidity (50 ± 10%) and fed a standard diet and water ad libitum. Animals were allowed to acclimatize for 7 days before use in experiments. Animal care procedures were conducted in accordance with guidelines of the National Research Council of USA (National Research Council, 1996).

2.3. CUS

Mice were randomly assigned into six groups of eight individuals: control, CUS plus vehicle (water), CUS plus TGP (40 mg/kg), CUS plus TGP (80 mg/kg), CUS plus TGP (160 mg/kg), and CUS plus clomipramine (20 mg/kg), and both TGP and clomipramine were given intragastrically 30 min before each stressor once every day for 24 days. The CUS procedure was performed as described by Zhong et al. (2006), with a slight modification. Briefly, CUS consisted of a variety of unpredictable stressors, namely, 24-h food deprivation, 24-h water deprivation, 6-min cold swimming (at 8 °C), 1-min tail pinch (1 cm from the end of the tail), 2-h restraint, 24-h soiled cage (200 mL water in 100 g sawdust bedding), and overnight illumination. One of these stressors (in random order) was given every day for 24 days. Control (unstressed) animals were undisturbed except for necessary procedures such as routine cage cleaning.

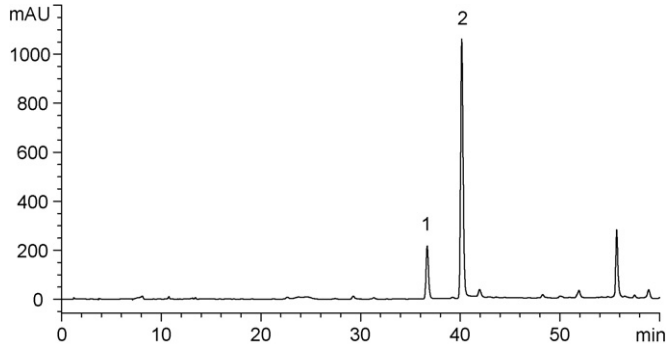

Fig. 1. High-performance liquid chromatography of total glycosides of peony: 1, albiflorin; 2, paeoniflorin.

2.4. Behavioral tests

The person who was responsible for recording the data of behavioral tests was blinded to the treatment groups.

2.4.1. Tail suspension test

The tail suspension test was carried out before CUS exposure and at the end of the 24-day CUS exposure. The test was performed as described by Steru et al. (1985). Briefly, mice were suspended 5 cm above the floor using adhesive tape, placed approximately 1 cm from the tip of the tail. The total duration of immobility (seconds) was recorded during a test period of 6 min. Mice were considered immobile only when they hung passively in a completely motionless state.

2.4.2. Open-field test

The open-field test was carried out before CUS exposure and at the end of 24-day CUS exposure. The test was performed as described by Aragão et al. (2006). The open-field apparatus consisted of a square wooden arena (30 cm × 30 cm × 15 cm), with black inner walls. The floor of the wooden arena was divided equally into 25 squares marked by black lines. In a test, each mouse was placed individually into the center of the arena and allowed to explore freely. The number of line crossings and rearings were recorded during a test period of 3 min. The apparatus was cleaned with detergent and dried after each occupancy.

2.5. Biochemical analysis

2.5.1. Tissue sample collection

Twenty-four hours after completion of behavioral tests, mice were sacrificed and whole brains were rapidly harvested. Each brain was washed with cold sterile physiological saline and stored at −80 °C until use for biochemical analysis.

2.5.2. Monoamine oxidase (MAO) assay

Brain MAO activity was measured following the procedure previously described by Yu et al. (2002) and Zhou et al. (2006), with slight modifications. Briefly, brain tissue was homogenized in 10 volumes of cold sodium phosphate buffer (10 mM, pH 7.4) containing 320 mM sucrose, at 4 °C for 30 s, using a Teflon-glass homogenizer. The homogenate was centrifuged at 600 × *g* for 10 min at 4 °C to remove nuclei and cell debris. The mitochondrial fraction was obtained by further centrifugation at 15,000 × *g* for 30 min at 4 °C and the pellet was resuspended in buffer. Protein content was determined by the Lowry method (Lowry et al., 1951), using bovine serum albumin as standard. Mitochondrial fractions were diluted to a protein concentration of 1 mg/mL. The MAO assay mixture contained 400 μL of mitochondrial protein in phosphate buffer. Either 5-hydroxytryptamine (4 mM) or β-phenylethylamine (2 mM) was added as specific substrates for monoamine oxidase A (MAO-A) or monoamine oxidase B (MAO-B), respectively. The final volume of each reaction mixture was 3 mL. Mixtures were incubated at 37 °C for 60 min, followed by the addition of HCl (600 μL, 1 M). Reaction products were extracted into 4 mL butyl acetate or cyclohexane, respectively. The organic phases were collected and