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Genistein, a dietary soy isoflavone, exerts antidepressant-like effects in mice: Involvement of serotonergic system

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ABSTRACT

Genistein, a principal isoflavone property of soybeans, possesses multiple pharmacological activities such as neuroprotection. Recently, it was reported that genistein exerted antidepressant-like effects in animal models, but the mechanism of action remains ambiguous. The purpose of this study was to investigate the antidepressant-like effect of genistein in mice and explore the underlying mechanism(s), using two mouse models of depression, i.e. forced swim test (FST) and tail suspension test (TST). Chronic, but not acute (single dose), genistein treatment (5, 15 or 45 mg/kg, p.o., once per day for three weeks) exerted dose-dependently antidepressant-like effect in mice, concomitant with escalated levels of brain monoamines and suppressed monoamine oxidase (MAO) activity. Chemical depletion of brain serotonin by PCPA abrogated the antidepressant-like action of genistein, but it was not the case for ablation of NA by DSP-4. Moreover, the anti-depression by genistein was preferentially counteracted by co-administration of 5-HT_{1A} receptor antagonist WAY-100635, suggesting a pivotal role for 5-HT system coupled with 5-HT_{1A} receptors in mediating such genistein anti-depression. This point was further validated by the fact that genistein action was potentiated by co-treatment with 8-OH-DPAT, a selective 5-HT_{1A} receptor agonist. Collectively, these findings confirm that chronic genistein administration to mice engenders antidepressant-like efficacy evidenced by lessened behavioral despair. Serotonergic system that preferentially couples with 5-HT_{1A} receptors may be critically responsible for the present genistein anti-depression.

1. Introduction

Major depression is not only one of the most prevalent and debilitating psychiatric disorders globally, but also a serious society-economy problem (Greenberg et al., 2015). It afflicts appropriate 17% of individuals worldwide and causes purportedly 1 million people to committing suicide each year (Kessler et al., 2003). Despite severe impact of depressive disorders, clinically available antidepressants could not satisfy clinic need for palliating depressive symptoms in patients, mainly due to incomplete efficacy and undesirably adverse reactions such as sedation, sleep disturbance, sexual impotence and body weight gain (Covington et al., 2010). Thus, seeking improved treatments with a favorable risk vs. benefit profile against depression is an intriguing an urgent task.

While the etiology of depression is multifaceted and poorly understood, the most widely accepted hypothesis implicates a crucial and causal role for monoamine dysfunction (Jans et al., 2007; Krishnan and Nestler, 2010). Accordingly, antidepressants that enhance monoaminergic transmission constitute the majority of first-line drugs against anti-depression. These monoamine-target drugs include: tricyclic antidepressants (TCA), serotonin and noradrenaline reuptake inhibitors (SNRI), selective serotonin reuptake inhibitors (SSRI) and monoamine oxidative inhibitors

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(MAOIs). Therefore, the monoamine system still remains a crucial target for the development of novel antidepressants, albeit other targets such as NMDA receptors and cAMP phosphodiesterase deserve special attention (Mathew et al., 2008).

Genistein (4′,5,7-trihydroxy isoflavone, $C_{15}H_{10}O_5$, the chemical structure shown in Fig. 1A) is a natural isoflavone constituent found in soybean extract and is able to cross the blood-brain barrier in the mouse (Liu et al., 2008). It possesses a variety of pharmacological activities, including antioxidant, anti-inflammatory, anti-nociceptive, cancer chemo-preventive and neuro-protective activities (Qian et al., 2012; Roh et al., 2016; Valsecchi et al., 2008). Genistein is also a kind of phytoestrogen and this property confers its promise utility to alleviate menopause-related symptoms, such as depression. For example, genistein exhibited anti-depression profiles in osteopenic postmenopausal women (Atteritano et al., 2014) and ovariectomized rats (Kageyama et al., 2010). Subsequently, the antidepressant-like activity of genistein extended to general (non-ovariectomized) animals (Gupta et al., 2015), but the mechanism of action is still ambiguous up to now. Recently, it was reported that genistein inhibited monoamine oxidase (MAO) *in vitro* (Zarmouh et al., 2016), providing a clue to explore the mechanism behind genistein anti-depression. In fact, an early study also revealed that genistein regulated serotonergic activity in the hippocampus of ovariectomized rats (Kageyama et al., 2010), but the possible contribution of serotonergic receptors in the action induced by genistein is still not ascertained. Therefore, we hypothesized that the monoaminergic system may underpin the antidepressant-like action by genistein.

In this study, we first evaluated the antidepressant-like effect of genistein in mice, using the forced swim test (FST) and tail suspension test (TST). Subsequently, the mechanism underlying genistein anti-depression was explored with the focus on monoaminergic engagement. Finally, agonist/antagonist tests were performed to identify the subtype(s) of receptors implicated in the antidepressant-like effect by genistein.

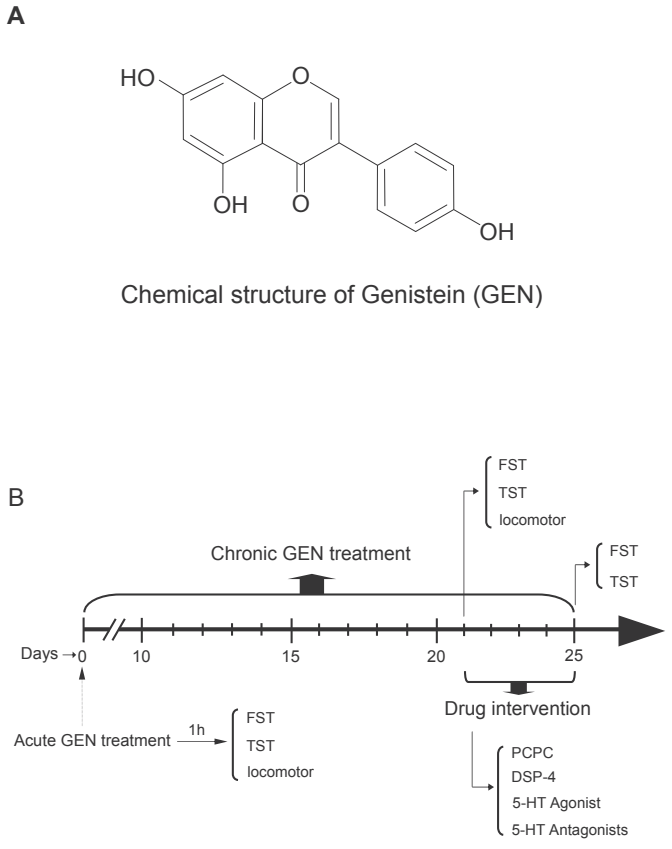

Fig. 1. Chemical structure of genistein (GEN) and schematic of experiments. (A) Chemical structure of genistein. (B) Schematic of experiments. For chronic treatment, genistein was orally administered to mice at the doses of 5, 15 and 45 mg/kg for three weeks. For mechanism-based experiments, co-administration of DSP-4, PCPA, 5-HT agonist (8-OH-DPAT) or 5-HT antagonists (WAY-100635, isamoltane, ritanserin, ondansetron) were performed from day 21–25. One hour after the last genistein dosing on day 21 or 25, the mice were submitted to behavioral tests (forced swim test, tail suspension test or locomotor activity test), immediately followed by decapitation for neurochemical and biochemical assays. For acute treatment, genistein was dosed to mice on day 0, followed by behavioral tests (forced swim test, tail suspension test or locomotor activity test) one hour later.

2. Methods

2.1. Animals

Male ICR mice (weighting 20–22 g upon arrival) were group-housed (4–5 per cage) with under stable temperature (22 ± 0.5° celcius) and relative humidity (60 ± 2%), and kept in 12 h light cycles (on at 07:00 a.m.) with food and water available *ad libitum*. Animals were habituated to the animal facilities for at least 5 days before any testing and behavioral tests were performed in blind respect to drug treatment. All experiments and animal handing were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the Ningbo University Committee on Animal Care and Use. All efforts were made to minimize the number of animals used and their suffering.

2.2. Drugs

The following drugs or agents were used in this study: Genistein, imipramine, N-(2-chloroethyl)-N-ethyl -2-bromobenzylamine hydrochloride (DSP-4), *p*-chlorophenylalanine (PCPA, an inhibitor of serotonin synthesis), 8-OH-DPAT (5-HT_{1A} receptor agonist), WAY-100635 (5-HT_{1A} receptor antagonist), isamoltane (5-HT_{1B} receptor antagonist), ritanserin (5-$HT_{2A/2C}$ receptor antagonist) and ondansetron (5-HT_3 receptor antagonist). Genistein was purchased from Ze Lang Biotechnology Co., Ltd. (Nanjing, China) with a purity more that 98% determined by HPLC analysis, and all other drugs were purchased from Sigma-Aldrich. All the drugs were dissolved or diluted in sterile saline with the exception of genistein and PCPA. Genistein was dissolved in saline containing 0.5% sodium carboxymethyl cellulose and PCPA was suspended in 0.5% gum acacia/physiological saline.

2.3. Experimental design and pharmacological treatment

The experimental procedures for pharmacological treatment were shown in Fig. 1B. For chronic treatment, genistein or vehicle was administered to mice (via gavage with a volume of 10 ml/kg) once per day (at 10:00 a.m.) for 21 consecutive days. After 3 weeks of genistein or vehicle treatment (day 21), the mice were repetitively co-administered with one of the 5-HT receptor antagonists or 8-OH-DPAT, 30 min following the genistein dosing from day 21 to day 25. For acute treatment, genistein or vehicle was administered to mice only once on day 0 (at 10:00 a.m., via gavage with a volume of 10 ml/kg). Behavioral tests were conducted on day 0 (FST, TST and locomotor test, for acute genistein treatment), day 21 (FST, TST and locomotor test, for chronic genistein treatment) and day 25 (FST and TST, for mechanism-oriented experiments), one hour following genistein dosing. For all these behavioral tests, the mice were challenged only once. The doses for genistein were used on the basis of our preliminary experiments and other literature (Gupta