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Anxiolytic-like effect of danshensu [(3-(3,4-dihydroxyphenyl)-lactic acid)] in mice

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ABSTRACT

Aims: Danshensu [3-(3,4-dihydroxyphenyl)-lactic acid], a phenylpropanoid compound isolated from *Prunella vulgaris* var. *lilacina*, is a well-known antioxidant. Although its antioxidant activity and cardioprotective effect have been reported, the pharmacological properties of danshensu in the central nervous system remain unclear. We investigated whether danshensu exerts anxiolytic-like activity in mice.

Main methods: We conducted monoamine oxidase A (MAO-A) inhibition assay on danshensu in vitro, and behavioral tests including the elevated plus-maze test (EPM), the hole-board test, the rotarod test and the open field test were employed.

Key findings: We found that danshensu significantly inhibited the activity of MAO-A in vitro. The administration of danshensu (3 or 10 mg/kg) produced a significant anxiolytic-like effect in the EPM and hole-board test. In addition, no changes in the spontaneous locomotor activity and no myorelaxant effects were observed compared to the control group; these effects were confirmed with the open field test and the rotarod test. Moreover, the anxiolytic-like properties of danshensu were antagonized by a dopamine D_1 receptor antagonist (SCH 23390) but not by a 5-HT_{1A} receptor antagonist (WAY 100635) or an α_1-adrenergic receptor antagonist (prazosin).

Significance: These results indicate that danshensu exerts its anxiolytic-like properties, in part, through dopaminergic neurotransmitter signaling.

Introduction

Anxiety disorders are the most prevalent class of psychiatric disorders throughout a lifetime, and 6–12% of the population suffers from a specific phobia, the highest incidence of all anxiety disorders (Kessler et al., 2010). Benzodiazepines (BZDs), including diazepam, lorazepam and alprazolam, have been the drugs most frequently prescribed for anxiety disorders since the 1960s. BZDs reduce anxiety by enhancing the GABAergic activity by binding with a $GABA_A$ receptor complex (Salzman et al., 1993). However, the agents of this class of drugs inherently have side effects such as cognitive impairments, physical or psychiatric dependence, sedation, myorelaxation and withdrawal seizures (Uzun et al., 2010). In addition, BZDs have little therapeutic effect on over one quarter of patients (Martin et al., 2007). This narrow therapeutic index thereby encourages the advent of new agents with an advanced anxiolytic-like efficacy and fewer adverse effects.

A considerable amount of evidence suggests that monoamine neurotransmitters like serotonin (5-HT), norepinephrine (NE) and dopamine (DA) play an important role in the pathophysiology of anxiety disorders (Martin et al., 2007; Legoabe et al., 2011). It is well established that the dysfunction of the serotonergic neurotransmitter system is a major cause of depression (Meltzer, 1989) and abnormalities in the dopaminergic neurotransmitter system of striatum lead to social anxiety disorder (Stein et al., 2002). Because the monoaminergic neurotransmitter systems are also involved in the modulation of mood and emotion (Bortolato et al., 2008), the regulation of the monoamine neurotransmitter system, including the inhibition of monoamine degradation, provides a foundation for studying the neurobiological and therapeutical bases of anxiolytic drugs (Jindal et al., 2013).

Monoamine oxidase (MAO), a mitochondria-bound flavin enzyme, catalyzes the degradation of biogenic amines, such as 5-HT, NE and DA, of the synaptic cleft in the brain. The therapeutic inhibition of MAO affects neuropsychiatric disorders by elevating the synaptic neurotransmitters to an optimum level (Bortolato et al., 2008). Of the two types of MAO enzyme isoforms, MAO-A mainly contributes to the

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metabolism of 5-HT, NE and DA in rodents, and MAO-A inhibitors exert an anxiolytic-like effect (Bortolato et al., 2008). The patients who failed to respond to BZDs as first-line treatment, can get therapeutic effect from MAO-A inhibitors in some cases (Stahl and Felker, 2008). Recently, we found that the ethanolic extract of *Prunella vulgaris* var. *lilacina* has a MAO-A inhibitory activity. Similar results were also reported in a domestic journal and issued in a form of patent (Hwang, 2006). Based on those findings by us and others, we isolated danshensu [3-(3,4-dihydroxyphenyl)-(2*R*)-lactic acid] as an active compound through activity-guided fractionation (Fig. 1). There are reports that danshensu has a neuroprotective effect on the cerebral ischemic condition (Seetapun et al., 2013) and lipopolysaccharide-induced insults in mice (Li et al., 2012). The cardioprotective effect of danshensu via its antioxidant activity also has been studied by many research groups (Wu et al., 2007). However, any behavioral outcomes associated with its MAO-A inhibitory activity have not been investigated until now.

In the present study, we investigated whether danshensu that was isolated from *P. vulgaris* var. *lilacina* has an inhibitory activity on MAO in vitro. In addition, several behavioral tests were conducted in rodent models to investigate the anxiolytic-like effects of danshensu which might be related to MAO-A inhibitory activity.

Methods

Animals

Male ICR mice (6 weeks old; 25–30 g body weight) were purchased from the Orient Co. Ltd., a branch of the Charles River Laboratories (Gyeonggi-do, Korea), and kept in the University Animal Care Unit for 1 week prior to the experiments. The mice were housed 5 per cage, provided food and water ad libitum and kept in a 12 h light/dark cycle (the light was on from 07:30 to 19:30 h) at a constant temperature (23 ± 1 °C) and humidity (60 ± 10%). The facilities were approved by the Association for the Assessment and Accreditation of Laboratory Animal Care International. The animal treatment and maintenance were carried out in accordance with the Animal Care and Use Guidelines issued by Kyung Hee University in Korea. All of the mouse experiments were performed according to the protocols approved by the Institutional Animal Care and Use Committee of Kyung Hee University (approved protocol number: KHP-2012-03-09).

Drugs

Danshensu was donated by one of the authors (D.S. Jang) and the purity was greater than 99%. SCH 23390, WAY 100635, prazosin, vanillic acid, 4-aminoantipyrine, clorgyline and peroxidase were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). The human recombinant MAO enzymes were obtained from BD-Supersomes™. Diazepam was donated by Daewon Pharmaceutical Co. (Korea). All other materials were of the highest grade commercially available. Danshensu, SCH 23390, WAY 100635 and prazosin were dissolved in 0.9% saline solution. Flumazenil was dissolved in 10% Tween 80 solution.

MAO-A or B inhibition assay

The monoamine oxidase inhibition assays were performed using a tyramine substrate and human recombinant MAO enzymes using a spectrophotometric method, described elsewhere with slight modifications (Novaroli et al., 2005). The enzyme concentration was set as 0.0025 mg protein/mL for MAO-A and 0.0075 mg protein/mL for MAO-B. After the enzymes were treated with the danshensu stocks (0.01, 0.1, 1, 10 and 100 μM) at 37 °C for 30 min, the chromogenic solution composed of vanillic acid, 4-aminoantipyrine and peroxidase was added. The absorbance was measured at 490 nm for 30 min in kinetic mode immediately after adding the tyramine solution (100 μM) to the reaction mixtures (OPTIZEN 2120UV, Mecasys Co. Ltd., Korea). Clorgyline was used as a positive control. The IC_{50} values were calculated using the Prism software package (GraphPad) by plotting the initial rate of tyramine oxidation on a logarithmic scale against the inhibitor concentration.

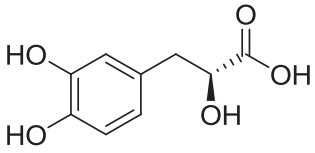

Fig. 1. Structure of danshensu [(3-(3,4-dihydroxyphenyl)-(2*R*)-lactic acid)].

Elevated plus-maze test (EPM)

The apparatus consisted of a black Plexiglas maze with two enclosed arms and two open arms. The maze was raised to a height of 50 cm from the floor in a dimly lit room (20 lx) equipped with a video-based Ethovision System (Noldus, Wageningen, The Netherlands) above the maze to analyze the movements as follows: the number of entries into the open and closed arms, the time spent in each arm, and the total distance moved in the EPM. The procedure for the EPM test was similar to those previously reported (Yoon et al., 2007; Jung et al., 2006; Hong et al., 2012). In brief, the mouse was placed in the center of the maze platform with its head facing towards an open arm. Danshensu (1, 3 or 10 mg/kg) was dissolved in 0.9% saline solution and orally administered 1 h before the experiments. Diazepam as a positive control was administered 30 min before the experiments (Rago et al., 1988). The mice were tested once, individually, for 5 min, and the maze was cleaned with 70% ethanol to remove any residue or odors after each trial. In separate experiments, the mice were co-administered with danshensu and SCH 23390, a dopamine D_1 receptor antagonist; WAY 100635, a 5-HT_{1A} receptor antagonist; or prazosin, an α_1-adrenergic receptor antagonist, for antagonism studies. Danshensu (3 mg/kg) was orally administered 1 h before testing, and SCH 23390 (0.023 mg/kg) (Rodgers et al., 1994), WAY 100635 (0.2 mg/kg) (Jung et al., 2006) and prazosin (0.065 mg/kg) (Ishola et al., 2012) were injected intraperitoneally 30 min before testing. All of the behavioral experiments using animals were carried out between 10:00 and 16:00 h.

Hole-board test

The hole-board apparatus (Ugo Basile, Italy) consisted of gray Perspex panels (40 cm × 40 cm, 2.2 cm thick) with 16 equidistant holes, 3 cm in diameter. The center of each hole was 10 cm from the nearest wall (File and Wardill, 1975). The board was positioned 15 cm above a table. Photocells below the holes measured the number of head-dips. The mice were placed individually in the center of the board facing away from the observer and animal behavior and the number of head-dips were recorded over a 5 min period (Jung et al., 2006). A head-dip was scored if both eyes disappeared into the hole. Danshensu (1, 3 or 10 mg/kg) was orally administered 1 h before the experiments. Diazepam (1 mg/kg, i.p.) as a positive control was administered 30 min before the experiments. The results are expressed as the mean total number of head-dips (Ishola et al., 2012).

Rotarod test

The rotarod apparatus consisted of a rod (3 cm in diameter) with a rough surface, as well as accessories to control its rate of rotation (Daejong, Seoul, Korea). Each mouse was first trained to remain on the rod for 2 min while the rod rotated at 30 rpm. Only those mice which demonstrated this ability were used for the test on the following day. Pre-selected animals were administered with danshensu (1, 3 or 10 mg/kg, p.o.) 1 h before or diazepam (1 mg/kg, i.p.) 30 min before the experiments, and each mouse was placed onto the bar. The latency