Journal of Pharmacological Sciences xxx (2017) 1–4

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Short communication

Serotonergic and dopaminergic systems are implicated in antidepressant-like effects of *chotosan*, a Kampo formula, in mice

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ARTICLE INFO

Article history:
Received 29 September 2016
Received in revised form
19 December 2016
Accepted 20 December 2016
Available online xxx

Keywords:
Chotosan
Antidepressants
Monoamine

ABSTRACT

We previously demonstrated that *chotosan* (CTS), a traditional herbal formula called *Kampo* medicine, improves diabetes-induced cognitive deficits. In the present study, we investigated the antidepressant-like effects of CTS in mice. The administration of CTS (1.0 g/kg, for 3 days) decreased the immobility time in the forced-swim test, and this decrease was prevented by the prior administration of sulpiride (an antagonist of $D_{2/3}$ receptors) and WAY100635 (an antagonist of 5-HT_{1A} receptors). None of the treatments tested altered the locomotor activity of mice. These results suggest that CTS exerts antidepressant-like effects through changes in the serotonergic and dopaminergic systems.

Chotosan (CTS) is a Kampo formula that consists of 11 different medical herbs and *gypsum fibrosum*, which is generally prescribed to middle-aged and elderly patients with a weak physical constitution and symptoms related to hypertension and chronic headaches (1). In a clinical study, Terasawa et al. found that the administration of CTS improved impaired activities of daily living, delirium, hallucinations, delusion, and insomnia in patients with vascular dementia (2).

CTS and the anxiolytic drug diazepam were shown to improve anxiety-like behavior in C57BLKS/J-*db/db* mice, an animal model of type 2 diabetes (3), and in senescence-accelerated mice (SAMP8), an animal model of aging (4). Although the neuronal mechanisms underlying the effects of CTS on anxiety-related behavior in SAMP8 and *db/db* mice are not yet clarified, the potential of CTS to improve cognitive and emotional deficits may be beneficial in the treatment of dementia patients.

In the present study, we investigated whether CTS exerts antidepressant-like effects in mice. Monoamine neurotransmitters such as serotonin, noradrenaline and dopamine in the central nervous system are known to play key roles in the pathophysiology of depression (5). We thus also evaluated the involvement of the serotonergic and dopaminergic systems in the antidepressant-like effects of CTS.

All experimental protocols were approved by the Institutional Animal Care and Use Committee at Tokyo University of Science, and conducted according to the guidelines of the National Institute of Health and the Japanese Pharmacological Society. We used five-week-old male ddY mice (SLC, Shizuoka), and made efforts to minimize the number of animals used, as well as animal pain and distress. All animals were kept in a controlled environment, with a 12:12-h light schedule, temperature of 23 °C, and relative humidity of 55 ± 5% for at least 5 days before experiments were conducted, and were provided *ad libitum* access to food and water.

The CTS extract used in this study was purchased from Tsumura Co. (Japan) in the form of a spray-dried powder extract prepared according to the standardized extraction method of medical plants registered in *Japanese Pharmacopoeia XV*. CTS was extracted from a mixture of 3.0 parts *Uncariae Uncis cum Ramulus* (the hooks and branch of *Uncaria rhynchophylla* MIQUEL), 3.0 parts *Aurantii Nobilis pericarpium* (the skin of *Citrus unshiu* MARKOVICH), 3.0 parts *Pinelliae tuber* (the tuber of *Pinellia ternate* BREITENBACH), 3.0 parts *Ophiopogonis tuber* (the root of *Ophiopogon japonicus* KERGAWLER), 3.0 parts *Hoelen* (the sclerotium of *Poria cocos* WOLF), 2.0 parts *Ginseng radix* (the root of *Panax ginseng* C.A. MEYER), 2.0 parts *Saposhnikoviae radix* (the root and rhizome of *Saposhnikovia divaricata* SCHISCHKIN), 2.0 parts *Chrysanthemi flos* (the flower of

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Peer review under responsibility of Japanese Pharmacological Society.

http://dx.doi.org/10.1016/j.jphs.2017.01.002

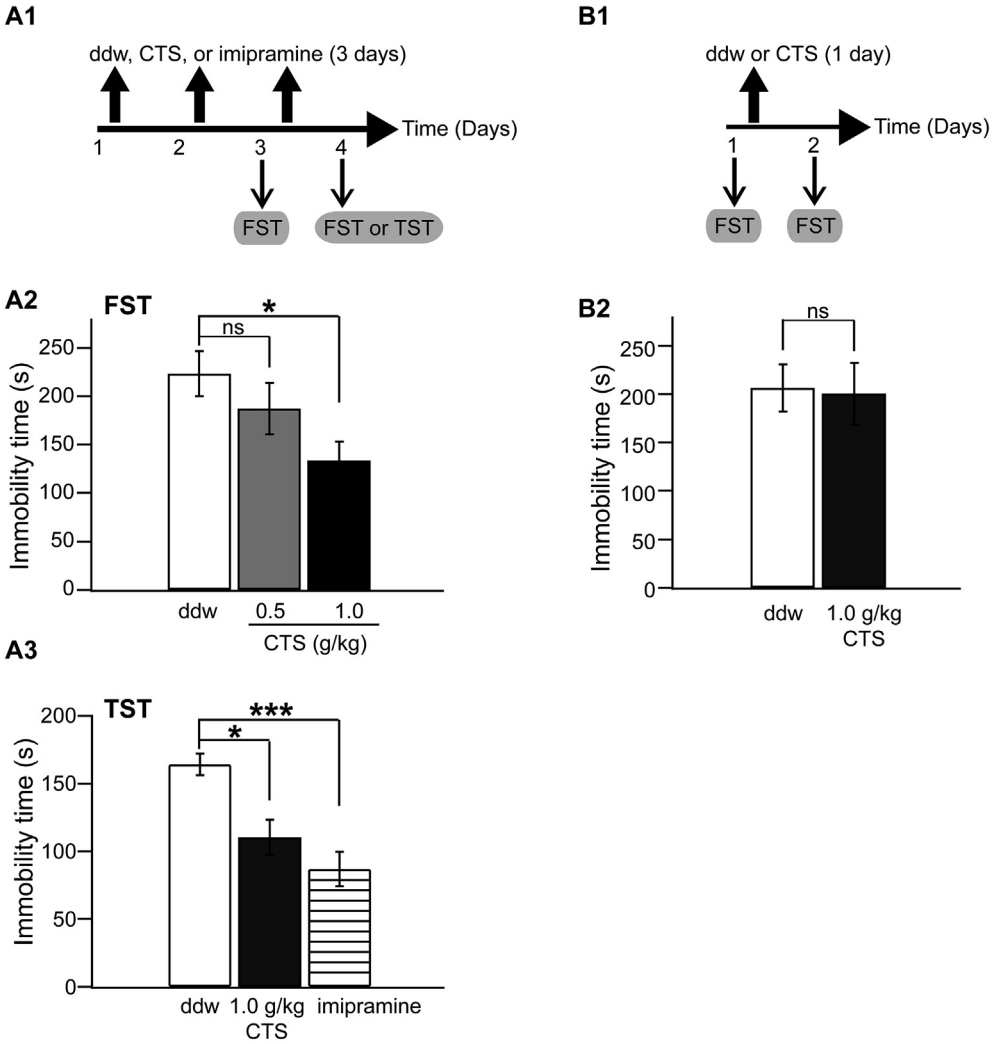

Fig. 1. Effects of CTS on the immobility time in the FST or TST. Experimental schedule (**A1** and **B1**). Effects of CTS (0.5 or 1.0 g/kg/day, p.o., 3 days) or ddw (vehicle) on the immobility time in the FST (**A2**). Effect of CTS (1.0 g/kg/day, p.o., 3 days) on the immobility time in the TST with imipramine as a positive control (**A3**). Effects of CTS (1.0 g/kg/day, p.o., 1 day) or ddw on the immobility time in the FST (**B2**). Results are expressed as means ± SEM. $^{*}P < 0.05$, $^{***}P < 0.001$, ns: non-significant [a one-way ANOVA followed by Bonferroni's multiple comparison test (A2 and A3), or Student's t-test (B2)]. n = 6, respectively.

Chrysanthemum morifolium RAMATULLE), 1.0 part *Glycyrrhizae radix* (root of *Glycyrrhiza uralensis* FISHER), 1.0 part *Zingiberis rhizoma* (the rhizome of *Zingiber officinale* ROSCOE), and 5.0 parts *Gypsum fibrosum* ($CaSO_4 \cdot 2H_2O$). The yield of the CTS extract was 16.1%. Protocols for the extraction and chemical profiling of CTS were the same as those described in a previous study (3) and mass spectrometry data obtained from the extract were stored in the Wakan-Yaku Database system (WakanDB ID: LCMS:Chotosan/11000001, http://wakandb.u-toyama.ac.jp/wiki/LCMS:Chotosan/11000001), Institute of Natural Medicine, University of Toyama. CTS was dissolved in distilled water (ddw). CTS (1 g/kg) was orally (p.o.) administered at 4–5 p.m for 3 days according to our previous study (6). All behavioral studies performed at 10–12 a.m.

The forced-swim test (FST) was performed by placing a mouse in an acrylic cylinder (50-cm in height, 18-cm in diameter) containing a 7-cm water column (25 ± 1 °C). Water was replaced between every trial. Two swimming sessions were conducted: an initial 15-min pretest, followed by a 6-min test 24 h later. Test sessions were recorded through a web-camera system in order to measure the time of immobility, with immobility being defined as floating passively in the water and only making slight movements to keep the head above the water line. The scored immobility time was blindly checked by the co-authors. The following drugs were used: metergoline (Tocris Cookson Ltd., Bristol, U.K.), 4-chloro-DL-phenylalanine (PCPA), imipramine, ketanserin tartrate salt, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridyl)cyclohexanecarboxamide (WAY100635), SCH23390 (Sigma–Aldrich, St. Louis, MO, USA), sulpiride, and yohimbine hydrochloride (Wako Pure Chemical Industries, Osaka). Metergoline (6 mg/kg), SCH23390 (0.03 mg/kg), WAY100635 (0.1 mg/kg), or yohimbine (1 mg/kg) was administered subcutaneously (s.c.), and ketanserin (5 mg/kg), or sulpiride (50 mg/kg) was administered intraperitoneally (i.p.) 45 min before the second swimming session. PCPA (150 mg/kg, i.p.) was pretreated once a day for 4 consecutive days. Six hours after the last PCPA treatment, mice were administered CTS or vehicle. The dosage, time schedules and routes of drug administration were based on previous studies (7,8).

The tail suspension test (TST) was performed. Mice were individually suspended by the tail from a horizontal ring (distance from the floor = 27-cm) in a gray acrylic box (30 × 15 × 15 cm) using

Please cite this article in press as: Sasaki-Hamada S, et al., Serotonergic and dopaminergic systems are implicated in antidepressant-like effects of *chotosan*, a Kampo formula, in mice, Journal of Pharmacological Sciences (2017), http://dx.doi.org/10.1016/j.jphs.2017.01.002