Phytomedicine 21 (2014) 363–371

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Yokukansan, a traditional Japanese herbal medicine, alleviates the emotional abnormality induced by maladaptation to stress in mice

Minoru Tsuji[a,b,*], Tomoko Takeuchi[a], Kazuya Miyagawa[a], Daisuke Ishii[a], Taro Imai[a], Kotaro Takeda[a], Masaki Kitajima[b], Hiroshi Takeda[a,b]

[a] Department of Pharmacology, School of Pharmacy, International University of Health and Welfare, 2600-1 Kitakanemaru, Ohtawara, Tochigi 324-8501, Japan

[b] Advanced Education and Research Center for Kampo Medicine, International University of Health and Welfare, 2600-1 Kitakanemaru, Ohtawara, Tochigi 324-8501, Japan

ARTICLE INFO

Article history:
Received 5 June 2013
Received in revised form 19 July 2013
Accepted 22 August 2013

Keywords:
Yokukansan
Hole-board
Emotional behavior
Restraint stress
Stress adaptation
Mouse

ABSTRACT

The aim of the present study was to examine the effect of yokukansan, a traditional Japanese herbal medicine that is composed of Atractylodis lanceae Rhizoma, Poria, Cnidii Rhizoma, Uncariae Uncis cum Ramulus, Angelicae Radix, Bupleuri Radix and Glycyrrhizae Radix, on the emotional abnormality induced by maladaptation to stress in mice. Mice were exposed to repeated restraint stress for 60 or 240 min/day for 14 days. From the 3rd day of stress exposure, mice were given yokukansan orally (p.o.) or the 5-HT_{1A} receptor agonist flesinoxan intraperitoneally (i.p.) immediately after the daily exposure to restraint stress. After the final exposure to restraint stress, the emotionality of mice was evaluated using an automatic hole-board apparatus. A single exposure to restraint stress for 60 min induced a decrease in head-dipping behavior in the hole-board test. This emotional stress response disappeared in mice that had been exposed to repeated restraint stress for 60 min/day for 14 days, which confirmed the development of stress adaptation. In contrast, mice that were exposed to restraint stress for 240 min/day for 14 days did not develop this stress adaptation, and still showed a decrease in head-dipping behavior. The decreased emotionality observed in stress-maladaptive mice was significantly recovered by chronic treatment with yokukansan (1000 mg/kg, p.o.) as well as flesinoxan (0.25 and 0.5 mg/kg, i.p.) immediately after daily exposure to stress. These findings suggest that yokukansan may have a beneficial effect on stress adaptation and alleviate the emotional abnormality under conditions of excessive stress.

Introduction

The ability to adapt to stress is an important defensive function of a living body, and impairment of this ability may contribute to some stress-related disorders. Thus, the identification of substances that have beneficial effects on the brain mechanisms that contribute to stress adaptation could help to pave the way for new therapeutic strategies for stress-related mood disorders such as anxiety and depression. A growing body of evidence suggests that the brain serotonin (5-HT) nervous system is an important component related to the etiology, expression and treatment of anxiety and depression (Nordquist and Oreland, 2010). There are now believed to be seven kinds of 5-HT receptor families, 5-HT_{1-7}, that comprise a total of 14 structurally and pharmacologically distinct 5-HT receptor subtypes (Hoyer et al., 2002). The results of our previous studies suggested that brain 5-HT nervous systems, especially 5-HT_{1A} receptors, may be involved, at least in part, in the development of adaptation to stress (Tsuji et al., 2000, 2001, 2003).

Yokukansan is a traditional Japanese herbal medicine that is composed of seven kinds of dried medicinal herbs, i.e., Atractylodis lanceae Rhizoma, Poria, Cnidii Rhizoma, Uncariae Uncis cum Ramulus, Angelicae Radix, Bupleuri Radix and Glycyrrhizae Radix. Yokukansan has been approved in Japan as a remedy for neurosis, insomnia, and irritability in children. Recently, yokukansan has been reported to improve behavioral and psychological symptoms, such as anxiety, hallucinations, agitation, irritability and sleep disturbance in patients with Alzheimer's disease and other forms of dementia when used clinically (Iwasaki et al. 2005a,b; Shinno et al., 2007; Mizukami et al., 2009; Hayashi et al., 2010; Kawanabe et al., 2010; Okahara et al., 2010; Nagata et al., 2012). Interestingly, an *in vitro* binding study demonstrated that yokukansan has a partial agonistic effect toward 5-HT_{1A} receptors (Terawaki et al., 2010). If we consider our previous findings (Tsuji et al., 2000, 2001, 2003), this report led us to speculate that yokukansan may have a beneficial effect on the development of stress adaptation.

* Corresponding author at: Department of Pharmacology, School of Pharmacy, International University of Health and Welfare, 2600-1 Kitakanemaru, Ohtawara, Tochigi 324-8501, Japan. Tel.: +81 287 24 3489; fax: +81 287 24 3489.
E-mail address: mtsuji@iuhw.ac.jp (M. Tsuji).

0944-7113/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.phymed.2013.08.025

A series of behavioral experiments have demonstrated that repeated exposure to the same type of stress stimuli diminishes acute stress responses. For example, Kennett and co-workers reported that male rats exposed to a single restraint stress for 120 min exhibited a reduction in locomotion in an open field, but this change in behavior disappeared after repeated exposure to restraint stress for 120 min/day for 7 days (Kennett et al., 1985a,b, 1986). Similar behavioral adaptive responses to stress stimuli in rats have been confirmed by other researchers (Ohi et al., 1989; Haleem and Parveen, 1994; Haleem, 1996), which suggests that this animal model may be useful for investigating the mechanisms of stress adaptation. Furthermore, to further characterize models of stress adaptation, we examined behavioral responses in rats that were produced by either single or repeated exposure to restraint stress for 60 or 240 min. A single exposure to restraint stress reduces locomotor activity, and this stress response disappears in rats that are exposed to repeated restraint stress for 60 min/day for 7 days, which confirms the development of stress adaptation. However, this adaptive response to stress stimuli was not observed in rats exposed to restraint stress for 240 min/day for 7 days. Thus, we can create stress-adaptive and -maladaptive models by repeatedly exposing rats to different degrees of restraint stress (Takeda et al., 1996; Tsuji et al., 2003).

In the present study, we tried to create a stress-maladaptive model in mice, and examined the effect of yokukansan on the emotional abnormality observed in this animal model.

Materials and methods

The present studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the Committee on the Care and Use of Laboratory Animals of the International University of Health and Welfare, which is accredited by the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

Animals

Male ICR mice (Japan SLC Inc., Shizuoka, Japan) weighing 25–30 g were housed at a room temperature of 23 ± 1 °C with a 12-h light–dark cycle (light on 7:00 a.m. to 7:00 p.m.). Food and water were available *ad libitum*. All experiments were carried out in the light phase of the cycle.

Materials

The powdered water extract of yokukansan used in the present study were manufactured according to the formulation previously reported (Mizukami et al., 2009; Terawaki et al., 2010) and supplied by Tsumura & Co. (Tokyo, Japan). Yokukansan is composed of seven dried medicinal herbs: 4.0 g of Atractylodis lanceae Rhizoma, 4.0 g of Poria, 3.0 g of Cnidii Rhizoma, 3.0 g of Uncariae Uncis cum Ramulus, 3.0 g of Angelicae Radix, 2.0 g of Bupleuri Radix and 1.5 g of Glycyrrhizae Radix. These herbs are registered in the Pharmacopeia of Japan ver. 16. The same active ingredients derived from the herbal medicines in extract powders were also detected in standard solutions for the herbal medicines. The developed plates were either examined by spraying with a 4-dimethylaminobenzaldehyde reagent or dilute sulfuric acid, or irradiated with ultraviolet light. Upon comparison with the standard solutions for the herbal medicines, one spot among the spots from the yokukansan extract showed the same color tone and Rf value. In addition, the amounts of active ingredients such as glycyrrhizin, saikosaponin b_2 and ferulic acid have been determined by high-performance liquid chromatography analysis and stable contents have been secured. The chromatographic conditions for glycyrrhizin were column: a stainless steel column packed with octadecylsilanized silica gel for liquid chromatography, mobile phase: a mixture of H_2O, CH_3CN and CH_3COOH, column temperature: a constant temperature of about 40 °C, flow rate: 1.2 ml/min, detector: an ultraviolet absorption photometer (wavelength: 254 nm). The chromatographic conditions for saikosaponin b_2 were column: a stainless steel column packed with octadecylsilanized silica gel for liquid chromatography, mobile phase: a mixture of H_2O, MeOH and CH_3CN, column temperature: a constant temperature of about 50 °C, flow rate: 1.0 ml/min, detector: an ultraviolet absorption photometer (wavelength: 254 nm). The chromatographic conditions for ferulic acid were column: a stainless steel column packed with octylsilanized silica gel for liquid chromatography, mobile phase: a mixture of H_2O, CH_3CN and $(HCOO)_2$, column temperature: a constant temperature of about 25 °C, flow rate: 1.2 ml/min, detector: an ultraviolet absorption photometer (wavelength: 320 nm). Manufacturing processes and quality are standardized based on the Good Manufacturing Practices defined by the Ministry of Health, Labor and Welfare of Japan. Yokukansan has been approved by the Ministry of Health, Labor and Welfare of Japan as prescriptions covered under the National Health Insurance plan. The three-dimensional high-performance liquid chromatography (3D-HPLC) profile of representative batches of yokukansan is shown in Fig. 1. The compounds shown on the chromatogram were classified on the basis of the constituent herbs of Yokukansan (Table 1) (Mizukami et al., 2009; Nagata et al., 2012). Flesinoxan, a 5-HT_{1A} receptor agonist, was provided by Solvay (Noord-Holland, The Netherlands). Yokukansan was dissolved in purified water and treated orally (p.o.) in a volume of 10 ml/kg. Flesinoxan was dissolved in saline and treated intraperitoneally (i.p.) in a volume of 10 ml/kg. The dosage of drugs were decided based on the previous reports (Tsuji et al., 2000, 2001; Kamei et al., 2009; Kanno et al., 2009; Yamaguchi et al., 2012).

Table 1
Classification of the compounds identified in the three-dimensional chromatogram.

Constituent of TJ-54	Compounds
Atractylodis lanceae Rhizoma	4E,6E,12E-Tetradecatriene-8,10-diyne-1,3,14-triol, 12-isovaleroyl-2E,8E,10E-triene-4,6-diyne-1,14-diol 14-Isovaleroyl-2E,8E,10E-triene-4,6-diyne-1,12-diol, atractylodin
Cnidii Rhizoma	Ferulic acid, ligustilide
Uncariae Uncis cum Ramulus	Geissoschizine methyl erther, hirsuteine, hirsutine
Angelicae Radix	Xanthotoxin, ligustilide
Bupleuri Radix	Saikosaponin b1, saikosaponin b2
Glycyrrhizae Radix	Formononetin, formononetin-7-*O*-glucoside Liquiritigenin, liquiritin, liquiritin apioside, glycyrrhizin, glycyroside, isoliquiritin apioside, isoliquiritin, isoliquiritigenin, glycycoumarin

Apparatus

The automatic hole-board apparatus (model ST-1, Muromachi Kikai Co. Ltd., Tokyo, Japan) consisted of a gray wooden box (50 cm × 50 cm × 50 cm) with four equidistant holes 3 cm in diameter in the floor. An infrared beam sensor was installed on the wall to detect the number and duration of rearing and head-dipping behaviors. The distance that mice moved on the hole-board were recorded by an overhead digital video camera; the heads of the mice were painted yellow and the digital video camera followed their center of gravity. Data from the digital video camera were collected through a custom-designed interface (DVTrack, Muromachi Kikai) as a reflection signal. Head-dipping behaviors were double-checked via an infrared beam sensor and the overhead digital video camera.