Journal of Ethnopharmacology 216 (2018) 89–96

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Yokukansan, a traditional Japanese herbal medicine, enhances the anxiolytic effect of fluvoxamine and reduces cortical 5-HT_{2A} receptor expression in mice

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ARTICLE INFO

Chemical compounds:
Fluvoxamine maleate (PubChem CID: 9560989)
Ketanserin tartrate (PubChem CID: 16219944)

Keywords:
Yokukansan
Fluvoxamine
Contextual fear conditioning
Anxiolytic effect
5-HT_{2A} receptor
Mouse

ABSTRACT

Ethnopharmacological relevance: Yokukansan is a traditional Japanese herbal medicine that has been approved in Japan as a remedy for neurosis, insomnia, and irritability in children. It has also been reported to improve behavioral and psychological symptoms in patients with various forms of dementia.

Aim of the study: To evaluate the usefulness of co-treatment with an antidepressant and an herbal medicine in the psychiatric field, the current study examined the effect of yokukansan on the anxiolytic-like effect of fluvoxamine in mice.

Materials and methods: The anxiolytic-like effect in mice was estimated by the contextual fear conditioning paradigm. Contextual fear conditioning consisted of two sessions, i.e., day 1 for the conditioning session and day 2 for the test session. The expression levels of 5-HT_{1A} and 5-HT_{2A} receptor in the mouse brain regions were quantified by western blot analysis.

Results: A single administration of fluvoxamine (5–20 mg/kg, i.p.) before the test session dose-dependently and significantly suppressed freezing behavior in mice. In the combination study, a sub-effective dose of fluvoxamine (5 mg/kg, i.p.) significantly suppressed freezing behavior in mice that had been repeatedly pretreated with yokukansan (0.3 and 1 g/kg, p.o.) once a day for 6 days after the conditioning session. Western blot analysis revealed that the expression level of 5-HT_{2A} receptor was specifically decreased in the prefrontal cortex of mice that had been administered yokukansan and fluvoxamine. Furthermore, microinjection of the 5-HT_{2A} receptor antagonist ketanserin (5 nmol/mouse) into the prefrontal cortex significantly suppressed freezing behavior.

Conclusion: The present findings indicate that repeated treatment with yokukansan synergistically enhances the anxiolytic-like effect of fluvoxamine in the contextual fear conditioning paradigm in mice in conjunction with a decrease in 5-HT_{2A} receptor-mediated signaling in the prefrontal cortex. Therefore, combination therapy with fluvoxamine and yokukansan may be beneficial for the treatment of anxiety disorders.

1. Introduction

A growing body of evidence suggests that the brain serotonin (5-HT) nervous system plays a role in the mechanism of fear memory and the pathology of fear-related mental disorders (Bauer, 2015). Selective 5-HT reuptake inhibitors (SSRIs), antidepressants that preferentially activate brain 5-HT neurotransmission, are clinically used for the treatment of these disorders, including obsessive-compulsive disorder (OCD) and post-traumatic stress disorder (PTSD) (Figgitt and McClellan, 2000). This clinical evidence indicates that SSRIs have not only antidepressant but also anxiolytic properties. It is well known that rodents that are re-exposed to the same environment where they had been previously exposed to aversive stimuli including inescapable foot-shock show freezing behavior, a response characterized by a period of crouching and complete immobility, and this behavior can be used as a model of anxiety caused by fear memory (Fanselow and Helmstetter, 1988). Furthermore, freezing behavior induced by contextual fear conditioning has been reported to typically be reduced by both acute and chronic treatment with SSRIs (Inoue et al., 2011), indicating that this animal model may be useful for evaluating the efficacy of SSRIs for the treatment of anxiety disorders.

Yokukansan is a traditional Japanese herbal medicine, which has

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https://doi.org/10.1016/j.jep.2018.01.032
Received 14 October 2017; Received in revised form 26 January 2018; Accepted 26 January 2018
Available online 31 January 2018
0378-8741/

been approved in Japan as a remedy for neurosis, insomnia, and irritability and night crying in children. Increased recent clinical evidence indicates that yokukansan is also effective and well-tolerated treatment for behavioral and psychological symptoms, such as excitement, aggression, hallucinations, insomnia, anxiety, wandering and depression, in patients with Alzheimer's disease and other forms of dementia when used clinically (Matsuda et al., 2013). Interestingly, an in vitro binding study demonstrated that yokukansan binds to the 5-HT_{1A} receptors and acts as a partial agonist (Terawaki et al., 2010). Moreover, repeated administration of yokukansan increases and decreases 5-HT_{1A} and 5-HT_{2A} receptor function in the prefrontal cortex, respectively (Egashira et al., 2008; Ueki et al., 2015a, 2015b). Previously, both agonism of 5-HT_{1A} receptor and antagonism of 5-HT_{2A} receptor have been shown to have anxiolytic-like effects in animal models of anxiety (Motta et al., 1992; Hashimoto et al., 1996), although paradoxical findings have also been reported regarding the latter (León et al., 2017). These reports led us to speculate that yokukansan may have a beneficial effect on anxiety disorders. Indeed, a few studies reported that yokukansan showed inhibitory effects on anxiety-like behaviors induced by both innate fear and memory-dependent fear in rat (Mizoguchi et al., 2010; Yamaguchi et al., 2012; Shoji and Mizoguchi, 2013).

Clinical evidence has indicated that there are treatment-resistant populations in patients suffering anxiety disorder (Patterson and Van Ameringen, 2016). Recently, complementary and integrative medicine is attracting attention for the purpose of enhancing the efficacy and/or reducing side effects of Western medicine. Thus, we expected that yokukansan might be beneficial for augmentation therapy in treatment-resistant anxiety disorders. As a basic research to prove this hypothesis, the present study investigated the effect of yokukansan on the anxiolytic-like effect of fluvoxamine as estimated by the contextual fear conditioning paradigm in mice. Furthermore, changes in the expression of 5-HT_{1A} and 5-HT_{2A} receptors in the brains of mice that had been subjected to the fear conditioning paradigm were also examined.

2. Material and methods

2.1. Animals

Male ICR mice (Japan SLC Inc., Shizuoka, Japan) were housed at a room temperature of 23 ± 1 ℃ 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 during the light period.

This study was 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.

2.2. Drugs

Yokukansan is composed of seven kinds of dried medicinal herbs: 4.0 g of Atractylodis lanceae Rhizoma (*Atractylodes lancea* De Candolle), 4.0 g of Poria (*Wolfiporia cocos* Ryvarden et Gilbertson (*Poria cocos* Wolf)), 3.0 g of Cnidii Rhizoma (*Cnidium officinale* Makino), 3.0 g of Uncariae Uncis cum Ramulus (*Uncaria rhynchophylla* Miquel), 3.0 g of Angelicae Radix (*Angelica acutiloba* Kitagawa), 2.0 g of Bupleuri Radix (*Bupleurum falcatum* Linné), and l.5 g of Glycyrrhizae Radix (*Glycyrrhiza uralensis* Fischer). These herbs are registered in the Pharmacopeia of Japan ver. 17. The powdered water extract of yokukansan used in the present study was manufactured according to the formulation previously reported (Mizukami et al., 2009; Terawaki et al., 2010; Tsuji et al., 2014; Nakatani et al., 2014) and supplied by Tsumura & Co. (Tokyo, Japan). Raw materials for crude drug of yokukansan that were delivered to Shenzhen Tsumura Medicine Co., Ltd. in China or to the Ishioka Processing & Quality Control Center in Japan were stored in a low-temperature warehouse. The storage condition of these clued drugs was regulated strictly at a warehouse with low temperature (15 ℃) and low humidity (below 60%) before use. The amount of materials that is cut and mixed was based on the unit quantity of yokukansan formulation for production. Once cut, they are used immediately to prevent any deterioration. To manufacture the powdered water extract of yokukansan, approximately 500 kg of cut materials was decocted by purified 6000 L hot water. Next, a continuous centrifugal was used to separate the liquid extract from the residues of crude drugs. To concentrate the liquid extract, low-temperature concentration with a continuous film evaporator was performed. Then, the concentrated liquid that was sprayed from the top of the dryer was dried and cooled to obtain the powdered water extract. 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 (see Supplementary Fig. 1). 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 ℃, 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 ℃, 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 ℃, 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. Fluvoxamine maleate, a selective serotonin reuptake inhibitor, was provided by Meiji Seika Pharma Co., Ltd. (Tokyo, Japan). Ketanserin tartrate, a selective 5-HT_{2A} receptor antagonist, was purchased from Sigma-Aldrich Co., Ltd. (MO, USA). Yokukansan and fluvoxamine were dissolved in purified water and saline, respectively. Ketanserin was prepared in a vehicle of saline to which a few drops of Tween 80 had been added. The dosage and injection route of drugs were decided based on previous reports (Miyamoto et al., 2000, 2004; Nunes-de-Souza et al., 2008; Dobi et al., 2013; Tsuji et al., 2014).

2.3. Apparatus and procedure for the contextual fear conditioning paradigm

For the experiments, we used a plastic box (20 × 18 × 30 cm high) with a stainless steel grid floor. Intermittent inescapable electric foot-shocks were delivered through the grid floor by an isolated shock generator (Muromachi Kikai, Co., Ltd., Japan).

The contextual conditioned fear stress procedure was performed over 2 days in accordance with our previous reports (Miyamoto et al., 2000; Takeda et al., 2002) with a minor modification; i.e., a day for the conditioning session and a day for the test session. In the conditioning session, mice were placed in the box and subjected to 36 inescapable foot-shocks (intensity 1 mA, duration 1 s) at 1–10 s intervals. After the last foot-shock, mice were immediately returned to their home cage. Twenty-four hours or a week later, mice were used in the test session. In the test session, the mice were again placed in the same box without being exposed to foot-shocks, and the duration of freezing behavior was