Neuroscience Letters 605 (2015) 24–28

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research paper

Preventive effects of ginsenoside Rg1 on post-traumatic stress disorder (PTSD)-like behavior in male C57/B6 mice

Zhongli Wang, Kexuan Zhu, Lin Chen, Liufeng Ou Yang, Yufang Huang, Yunan Zhao*

Basic Medical College, Nanjing University of Chinese Medicine, Nanjing 210023, China

HIGHLIGHTS

- We evaluated the preventive effects of ginsenoside Rg1 on the post-traumatic stress disorder (PTSD) animal model.
- Administration ginsenoside Rg1 could prevent the model animal's PTSD-like behavior changes.
- Administration ginsenoside Rg1 could decrease the levels of CORT and CRH in model animals.

ARTICLE INFO

Article history:
Received 12 March 2015
Received in revised form 12 May 2015
Accepted 11 August 2015
Available online 13 August 2015

Keywords:
Ginsenoside Rg1
Post-traumatic stress disorder
Corticosterone
Corticotrophin-releasing hormone
Traditional Chinese medicine

ABSTRACT

We investigated the preventive effects of Rg1 on a model of mouse post-traumatic stress disorder (PTSD) induced by electric shock combined with situation reminder and explored the underlying mechanism. In the experiment, before the PTSD animal model was developed, Rg1 (10, 5, and 2.5 mg/kg) was orally administered for one week. After the animal model was established, PTSD-like behavior was observed using elevated plus maze, black and light box, and open field tests. One hour after the behavior test, all mice were sacrificed, and then serum corticosterone (CORT) and hypothalamus corticotrophin-releasing hormone (CRH) assays were performed. Results showed that Rg1 (5 mg/kg) treatments relieved PTSD-like behavior by altering elevated serum corticosterone and hypothalamus CRH levels. By contrast, fluoxetine (3 mg/kg) treatment reversed the behavior changes and had no effect on increased CORT and CRH levels. These findings confirmed the preventive effect of Rg1 in PTSD model. Decreasing CORT and CRH levels may be one of the underlying mechanisms.

1. Introduction

Post-traumatic stress disorder (PTSD) is a debilitating anxiety disease that befalls individuals after their exposure to life-threatening events like severe accidents, sexual abuse, combat, or natural catastrophes [1,2]. The pathogenesis of PTSD is still unclear. However, related studies have shown that PTSD is associated with the changes in the endocrine levels of the body after stressful events. Change in the excitability of the hypothalamic-pituitary-adrenal axis (HPA) in particular, is considered one of the most important mechanisms [3]. The HPA axis system mainly includes three kinds of hormones, namely, corticosterone (CORT), adrenocorticotrophin (ACTH), and corticotrophin-releasing hormone (CRH). The researcher found a positive correlation between the hypothalamus CRH level and the anxiety behavior of patients with PTSD [4]. The plasma CORT concentration evidently increases after stressful events. CORT could cross the blood–brain barrier into brain regions, like hippocampus, hypothalamus, and prefrontal cortex, which contain the CORT receptors, and these areas are closely related with emotional control [5,6]. When CORT binds with the receptor, multiple biological effects are produced, such as the start of the immediate early gene, influence on neurotransmitter release, and change in the excitability of neurons [7]. These effects lead to changes in the neuronal plasticity, and even to the loss of neurons, and eventually to the onset of PTSD [8].

Selective serotonin reuptake inhibitor (SSRI) antidepressants, like fluoxetine, are currently in the first line of choices in PTSD drug treatment [9,10]. The response rates to SSRI treatment rarely exceed 60%, and less than 20–30% of SSRI-treated PTSD patients achieve full remission [11]. This unsatisfactory situation together with the fact that no drug that specifically tackles PTSD core symptoms is currently available [9,11], stresses the urgent need for the development of novel PTSD-specific drugs.

* Corresponding author. Fax: +86 24 85811922.
E-mail address: zhaoyunan-js@163.com (Y. Zhao).

http://dx.doi.org/10.1016/j.neulet.2015.08.017
0304-3940/

Herb- and plant-derived medicines have been used since the ancient times and are considered as health remedies. Ginseng has been widely used as a drug in East Asia for thousands of years. At present, ginseng remains one of the most famous and precious herbal medicines consumed around the world. It has a wide range of pharmacological actions on the endocrine, immune, and central nervous systems (CNS) [12]. In our preliminary studies, ginseng crude extracts were screened for their preventive effects on hypercortisolism—induced hippocampal impairment [13].

Several reports evidently showed that ginseng saponins, which are composed of various ginsenosides, are the major active ingredients of ginseng [14,15]. Among the saponins, Rg1 has potential neurotrophic and neuroprotective effects [16,17]. In the present study, we observed the effects of Rg1 on the electric shock combined with situation reminder (SR) induced mouse model of PTSD and explored the underlying mechanism.

2. Materials and method

2.1. Experimental animal

A total of 60 male C57BL/6N mice (weighing 18 g to 20 g, from the Laboratory Animal Center of Nanjing Medical University, Nanjing, China) were allowed five days to adapt to the laboratory environment before the actual experiment. Five animals were housed in each cage with a 12 h light/12 h dark cycle (lights on between 7:00 and 19:00) at a constant room temperature of $22 \pm 1\,^{\circ}C$ and free access to food and tap water. The animals were treated according to the Guidelines of Accommodation and Care for Animals Formulated by the Chinese Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. All efforts were exerted to minimize animal suffering and reduce the number of animals used for the experiments.

2.2. Experimental materials

Ginsenoside Rg1 was purchased from Jinlin Hongjiu Co., Ltd. Fluoxetine, as positive drug, was purchased from Suzhou Pharmaceutical Co., Ltd. Rg1 and fluoxetine were dissolved just before use in saline to concentrations of 0.5 mg/mL and 0.3 mg/mL, respectively. ELISA kit was purchased from the R&D Company.

2.3. PTSD animal models

After five days of adapting to the environment, the PTSD animal model was established as in previous studies [18,19]. The trauma procedure consisted of the exposure to inescapable foot shock (days 1 and 2) followed by three short reexposures to the shock chamber without foot shocks (SR) on days 5, 7, and 9 [20]. In the morning of days 1 and 2, the mice received inescapable, continuous 2 mA foot shock for 5 min. The control group received the same treatment, but the shock was not administered. Both control and shocked animals were reexposed to the context of the trauma (SR) for 5 min once every 2 days for 3 days (Table 1).

Table. 1
The protocol of developed PTSD in animal model.

Day of treatment	Stressor used	Duration
1–2	Foot-shock	5 min
3–4	No stressor applied	–
5	SR	5 min
6	No stressor applied	–
7	SR	5 min
8	No stressor applied	–
9	SR	5 min
10–15	No stressor applied	–
16	Behavioral test	According to need

SR stands for situation reminder.

2.4. Experimental design

A total of 60 male C57BL/6N mice were randomly assigned to six experimental groups ($n = 10$/group) as follows: control group (Control), PTSD animal model group (Model), model +3 mg/kg/d fluoxetine (FLU), model +10 mg/kg/d Rg1 (HDS), model +5 mg/kg/d Rg1 (MDS), and model +2.5 mg/kg/d Rg1 (LDS). The administered doses were based on previous reports [21,22]. The control and model groups were administered with saline (10 mL/kg), whereas the other groups were provided with drugs. After the mice received daily oral administrations of Rg1 and fluoxetine for one week, the animals were exposed to the modeling process. In this procedure, Rg1 and fluoxetine were not administered. The PTSD-like behaviors were observed in the elevated plus maze, black and light box, and open field tests at day 16. An hour after the behavioral tests, the mice were decapitated to obtain blood and tissue (Fig. 1).

2.5. Behavioral tests

All behavioral tests were performed in a shielding room to avoid environmental impacts on the animals. The activities of the animals were recorded by the computer.

2.5.1. Elevated plus maze

Mouse behavior was assessed for 5 min in the elevated plus maze test. According to the description in previous research [23], the apparatus consisted of two open arms (30–10 cm) alternating at right angles with two arms enclosed by 25 cm high walls. Four arms delimited a central area of 5 cm^2. The whole apparatus was placed on a frame with 76 cm height. The test began with the placement of the mouse at the center of the maze with its head facing a closed arm. The time spent and the visits to the open arms were recorded and a four-paw criterion was used for the arm entries.

2.5.2. Black and light box

The apparatus consisted of a chamber subdivided into two compartments, namely, a black closed compartment ($30\,cm \times 32\,cm \times 40\,cm$) and a white open one ($30\,cm \times 32\,cm \times 40\,cm$). The compartments were connected by a small divider [24]. The cross number between the black and light compartments and the time spent in the light compartment were assessed for 5 min. The mice location decision criteria are the same as that in the elevated maze experiment.

2.5.3. Open field test

The open field arena consisted of a dark gray box ($35\,cm \times 35\,cm \times 35\,cm \times 35\,cm$, with a black floor). Each animal was placed at the center of the open field before initiating the recording. After 5 min, the mice were returned to their cages. The pathway of each mouse was recorded and analyzed.

2.6. CORT and CRH assay

After the behavioral test, mouse was anesthetized by 10% chloral hydrate. Blood was collected from the eyes and placed in tubes. The plasma samples were stored at $-20\,^{\circ}C$ until assayed. The brain was removed and the hypothalamus was disconnected. All procedures were performed on an ice plate. The plasma CORT and hypothalamus CRH levels were determined using an ELISA kit. The ELISA analysis results were obtained with an enzyme standard instrument manufactured by the Molecular Devices Company.