Ginseng Extract Regulates the Alterations of Sleep Architecture and EEG Power Spectra in Restraint Stressed Rats

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(Received December 31, 2009; Revised March 5, 2010; Accepted March 8, 2010)

Abstract: The present investigation was conducted to evaluate the regulation of sleep architecture by the red ginseng water extract (RGE) in acutely and chronically restraint stressed rats. Adult rats were fitted with sleep-wake recording electrodes. Following post-surgical recovery, rats were extensively habituated for freely moving polygraphic recording conditions. Polygraphic signs of sleep-wake activities were recorded for 24 h after RGE administration and induction of stress and were analyzed to understand the regulation of sleep architecture. Acute stress decreased wakefulness and increased total sleep, non-rapid eye movement (NREM) sleep, and rapid eye movement (REM) sleep in both the daytime and nighttime recording. RGE shortened the daytime NREM and REM sleep, without changing the wakefulness and total sleep. RGE increased nighttime wakefulness, and decreased total, NREM and REM sleep. Chronic stress increased wakefulness and decreased total sleep in the daytime recording, and increased REM and decreased NREM sleep in both the day and night time recording. RGE ameliorated chronic stress and induced alterations of REM and NREM sleep in the day and night time sleep architecture. Acute and chronic stress could also induce alternations in cortex electroencephalogram (EEG) recording during NREM, REM sleep and wakefulness. These findings suggest that RGE may modulate the sleep behavior in acutely and chronically stressed rats and the ameliorating effect of RGE on the sleep architecture may involve in modulation of α-, θ- and δ- wave activities of the cortical EEG.

Key words: electroencephalogram (EEG), power density, red ginseng, sleep, stress

INTRODUCTION

Ginseng as a traditional medicinal herb has been used to treat psychiatric disorders such as stress and insomnia for thousands of years in Asian countries. However, ginseng extract has been reported to have stimulant effects on the central nervous systems of humans and animals, and some patients experience CNS excitation, arousal and sleeplessness [1, 2]. Moreover, ginseng has been reported to induce insomnia, nervousness and diarrhea after long term use as well as when it is taken at higher dosages. Nevertheless, it has been said to possess neuroprotective effects anti-stress activity and has been promoted as a tonic that is capable of invigorating the physical and mental well-being [3-7]. Ginseng serves as an “adaptogen”, a term used to describe a chemical that increases the resistance to stresses. This adaptogenic quality, however, is thought to be secondary to the normalization of body processes through the regulation of the production of various hormones. Ginseng stabilizes and balances physiology, and may help to maintain normal sleep and wakefulness [8]. In previous research, red ginseng water extract (RGE) decreased the power density of cortical electroencephalogram (EEG) δ waves (0.75-4.5 Hz) and increased α-waves (8.0-13.0 Hz) in non-rapid eye movement (NREM) and rapid eye movement (REM) sleep. It also decreased δ-wave power density during wakefulness. However, RGE increased spontaneous sleep and NREM sleep [9, 10].

In addition, there has been increased interest in whether RGE regulates the alteration of the sleep architecture and the EEG power spectra in acutely and chronically stressed...
Ginseng and Sleep 31

rats. Growing evidence indicates a close relationship between the stress response and the resultant sleep pattern [11]. This notable alternation of sleep after stress suggests the participation of neurotransmitters and hormones [12-14]. Moreover, chronic stress produces sleep disturbances characterized by a chronic immobilization stress decrease in active waking, NREM and REM sleep, a blunting of the sleep–wake cycle, and a decrease in REM sleep [15]. This sleep-disturbance is usually viewed as one of the deleterious effects that stress has on the CNS [16]. The purpose of this study was to evaluate whether RGE ameliorates the sleep disrupting actions of stress using a rat immobilization model.

**MATERIALS AND METHODS**

**Preparation of red ginseng water extract (RGE)**

Red ginseng water extract (RGE) was kindly provided by Nonghyup Korea Ginseng (Jeungpyung, Korea). Ginsenosides, the major components of RGE were standardized by Nonghyup Co. Ltd., and the saponin fraction yield from RGE was 4.5%.

**Experimental animals**

Experiments were performed on 80 adult male Wistar rats (Samtako, Osan, Korea) weighing between 250 and 350 g. The rats were housed individually with food, and water was provided *ad libitum* under an artificial 12 h light/dark cycle (light on at 7:00) and at a constant temperature (22±2°C). The rats were housed in the departmental holding room for 1 week before testing. All the rats were maintained, and all experiments were conducted, according to the guide for the Care and Use of Laboratory Animals (National Academic Press, Washington, DC, 1996), and in accordance with the National Institute of Toxicological Research on the Korea Food and Drug Administration guideline for the care and use of laboratory animals.

**Surgery**

All surgical procedures were performed stereotaxically under aseptic conditions. Surgical anesthesia was achieved with pentobarbital (50 mg/kg, ip) and all efforts were made to minimize the suffering of the animals. Each rat was implanted with a transmitter (Data Sciences International TA11ICTA-F40, MN, USA) for recording EEG and activity via telemetry as described previously [17]. The body of the transmitter was implanted subcutaneously off midline and posterior to the scapula and was attached to the skin with 3 sutures for stabilization. Leads from the transmitter were led subcutaneously to the skull and the bare ends were placed in contact with the dura through holes that were made in the skull (A: 2.0 [Bregma], L: 1.5; P: 7.0 [Bregma], L: 1.5 contra-lateral). The electrodes were anchored to the skull with screws and dental cement.

**Experiment procedure**

Following 7 days post-surgical recovery, rats were divided into control (non-stress), acute stress, and chronic stress groups, with 8 rats in each group; the RGE-treated groups were also divided into an acute stress and chronic stress groups, with 8 rats in each group. RGE were dissolved in distilled water and administered orally 60 min before the application of the restraint stress. Rats in the acute stress RGE group were fed with RGE 25, 50 and 100 mg/kg for 10 days. On the 8th, 9th and 10th day of RGE feeding, the rats were stressed for 3 h once per day. In the chronic stress RGE group, rats were fed with RGE 25, 50 and 100 mg/kg for 10 days. On the 10th day and 1 h after RGE feeding, the rats were once stressed for 22 h. One hour after ending the stress procedure on the 10th day in both of acute and chronic stress groups, sleeping behavior and EEG data were initiated and continuously recorded for 24 h (12 h day time and 12 h night time recording). The total quantity of RGE consumption for each rats in 25, 50 and 100 mg/kg RGE administration group were 62.5, 125 and 250 mg respectively. Briefly, for acute stress group, rats were fed with RGE at 2:00 pm every day for 10 days. On the 8th, 9th and 10th day of RGE feeding, the rats were stressed for 3 h once from 3:00 pm to 6:00 pm per day; for chronic stress group, rats were fed with RGE at 2:00 pm every day for 9 days, on the 10th day RGE were fed at 7:00 pm, 1 h later, chronic restraint were loaded from 8:00 pm to next day 6:00 pm. Sleep behaviors and EEG were recorded 1 h after the finish of the last acute or chronic restraint, from 7:00 pm and lasted for 24 h.

The stress was produced by restraining the animal inside an adjustable acrylic hemi-cylindrical plastic tube (7.5-cm diameter, 15-cm-long) individually. In order to induce a response of combined or mixed stress, which means rats experienced physical, psychological/emotional and other stressors in restraint process, the stressed rats could not eat food or drink water under restraint process [18, 19]. All experiments were conducted according to the guide for the Care and Use of Laboratory Animals (National Academic Press, Washington, DC, 1996), and in accordance with the National Institute of Toxicological