

Korea Red Ginseng Alters Electroencephalogram Spectra of Sleep-Wake Stage in Rats

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(Received July 30, Accepted August 28, 2008)

Abstract : The present investigation was performed to evaluate the homeostatic regulation of sleep architecture by the ethanol extract of Korea red ginseng (KRG), since the available data were often controversial. In addition, it was also interested in whether the sleep-wake stages were differently affected by low and high doses of KRG. Each adult Wistar male rat was implanted with a transmitter for recording EEG and activity via telemetry. After one week of surgery, polygraphic signs of undisturbed sleep-wake activities were recorded for 12 h (between 9:00 am and 9:00 pm) after KRG administration. KRG (10 and 100 mg/kg) increased non-rapid eye movement (NREM) sleep as well as total sleep. The total percentages of wakefulness were decreased comparably. KRG (10 mg/kg) decreased the power density of the δ -wave (0.75-4.5 Hz) and increased α -wave (8.0-13.0 Hz) in the NREM and rapid eye movement (REM) sleep. KRG also decreased δ -wave power density in wake time. However, KRG (100 mg/kg) increased δ -wave and decreased θ -wave (5.0-9.0 Hz) power density in wake time, while showed little effect on the power density in NREM and REM sleep. In conclusion, low and high doses of KRG increase spontaneous sleep and NREM sleep and differently regulate the EEG spectra in REM and NREM sleep.

Keywords : Korea red ginseng (KRG); sleep; electroencephalogram (EEG); non-rapid eye movement (NREM); rapid eye movement (REM); power density.

INTRODUCTION

A wide range of behavioral studies have provided considerable evidence that the regulation of the total amount of sleep is a homeostatic process¹. Homeostatic control mechanisms are activated to compensate for insufficient or excess sleep. In mammals, sleep consists of two major stages: 1) rapid eye movement (REM) sleep and 2) non-REM (NREM) sleep. REM sleep is a distinctive sleep stage that alternates with episodes of NREM sleep. The spontaneous NREM-REM sleep cycle in the rat takes about 12 to 20 min²⁻⁶. Over the last four decades, most of the sleep research has focused on identifying relevant brain structures, neuronal networks, and their transmitters that are

involved in the generation and regulation of NREM and REM sleep⁷⁻¹⁰. Few studies have focused on understanding the possible mechanisms and modulating methods for the ultradian periodic occurrence of NREM-REM sleep.

Panax Ginseng, a well-known herbal medicine, has been used as a traditional medicine for thousands of years. Ginseng is now a popular and worldwide used natural medicine. Ginseng had a complex profile of activity. It is sometimes difficult to reconcile on the available neurochemical reports on the plant. Ginseng has the effects of stabilizing and balancing the whole physiology¹¹. Ginseng has been used for treatment of insomnia clinically¹².

However, some controversial reports indicated that sleep disorders were most commonly experienced adverse events of ginseng¹³. This study was undertaken to assess the role of ginseng in regulation sleep/wake fluctuation and architecture of sleep, and to determine possible mechanisms of ginseng on modulation of NREM/REM sleep.

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We also examined the amount of total sleep and wakefulness, and investigated power density changes in recorded electroencephalogram (EEG) of specific sleep-wake stage in freely moving rats.

MATERIALS AND METHODS

Materials

Six-year Korean red ginseng (KRG) was purchased from a market of Nong-Hyup in Cheongju area. KRG was extracted three times with 1,000 ml of boiling water for 4 hours under reflux at the room temperature. This extract was subjected to filtration and concentration. Finally, ethanol extract of KRG was freeze-dried followed by evaporation of the remaining supernatants and used as the test samples.

Animals

Experiments were performed on 24 adult male Wistar rats (Samtako, Korea) weighing between 250~350 g. Rats were housed individually with food and water provided *ad libitum* under an artificial 12-h light/dark cycle (light on at 7:00) and at a constant temperature ($22 \pm 2^\circ\text{C}$). The rats were housed in the departmental holding room for 1 week before testing. All the rats were maintained 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

The animals were divided into 3 groups (control, 10 and 100 mg/kg of KRG) with 8 rats in each. Each rat was implanted with a transmitter (Data Sciences TA11CTA-F40) for recording EEG and activity via telemetry as described previously¹⁴. The body of the transmitter was implanted subcutaneously off midline and posterior to the scapula, and it was attached to the skin with 3 sutures for stabilization. Leads from the transmitter were led subcutaneously to the skull and the bare ends placed in contact with the dura through holes 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. All surgical procedures were performed using stereotaxic apparatus. Surgical anesthesia was achieved with pentobarbital (50 mg/kg, ip).

Data collection

Following 7 day post-surgical recovery, telemetric recording of cortical EEG and activity were conducted using procedures similar to previous reports¹⁴. For the

EEG signal, the gain of transmitters was set at $-0.5/+0.5$ volts per/unit X 2 and raw signals generated from the transmitter were in the range of 0.5-20.0 Hz. The signals were processed by a Data Sciences analog converter and routed to an AD converter (Eagle PC30) housed in a PC class computer. The AD converter digitized the EEG and activity signals at 128 Hz. The digitized data were transferred to the computer and displayed graphically by the program on the computer monitor. An on-line fast Fourier transformation (FFT) was performed on EEG data in every 2 sec of data (256 samples) after a Hanning window treatment. The FFT analyses generated the power density values from 0.0 to 20.0 Hz at a resolution of 0.5 Hz. The FFT data were further averaged in the range of 0 to 20 Hz for every 10 sec. The sleep data and FFT results were saved to the hard disk every 10 sec for additional off-line analyses. Movement of the animal in relation to the telemetry receiver generated transistor-transistor logic (TTL) pulses that were collected and counted as a measure of activity. KRG was administered orally 10 min before EEG recording. Recording began at 9:00-12:00 AM (Fig. 1).

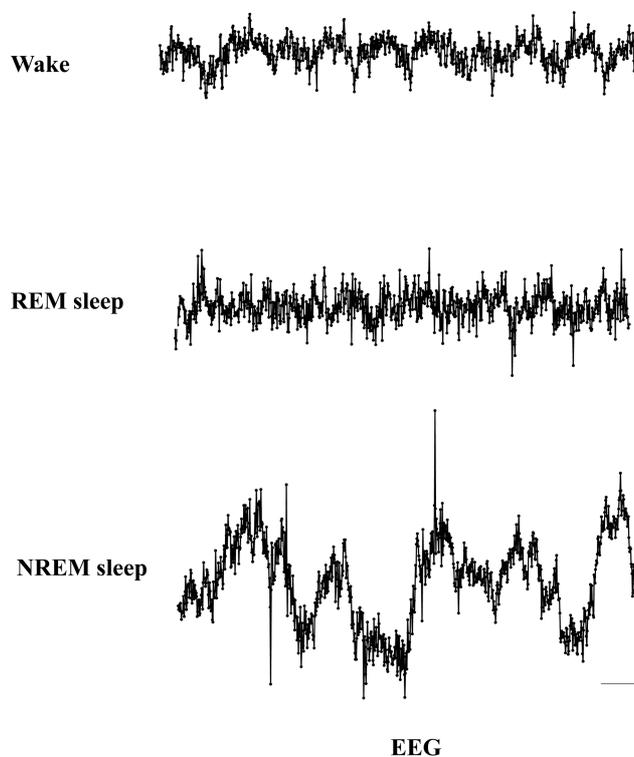


Fig. 1. Typical cortical EEG recordings of each stages of wakefulness, NREM and REM sleep in rats. EEG: electroencephalogram. Vertical bar: 50 μV , horizontal bar: 20 s in the panel of right corner.

Determination of behavioral states and analysis in EEG power

The amount of time in wakefulness, NREM and REM sleep were determined from the digitized data on 10 sec epochs using professional animal sleep analysis software SleepSign 2.1 (KISSEI Comtec Co Ltd. USA). Briefly, the software discriminates wakefulness as high-frequency low-amplitude EEG. NREM was scored based on the presence of spindles interspersed with slow waves in the EEG. EEG power during lower frequency δ -wave (0.75-4.5 Hz) is significantly reduced in REM sleep and the range of θ -wave activity (5.0-9.0 Hz, peak at 7.5 Hz) was increased.

Data analysis

The time spent (min) in NREM, REM, and total sleep time (NREM+REM) were processed to obtain 12 h period totals for each rat. We further calculated the time of each recording spent in each sleep-wake state (wake, NREM, REM). The absolute EEG power during wakefulness, NREM, and REM were calculated in 0.5 Hz bins from 0.5 to 20 Hz for the entire 12 h records of each recording process. Afterwards, in each state, EEG power density in 3 selected frequency bands for wakefulness, NREM, and REM [δ -wave (0.75-4.5 Hz), θ -wave (5.0-9.0 Hz) and α -wave (8.0-13.0 Hz)] were evaluated.

Statistical analysis

All statistical analyses were conducted using SigmaStat software (SPSS, Inc.). One-way repeated measures of analysis of variance (ANOVA) procedures were used in the data analyses across days. After significant ANOVAs, post hoc comparisons of means were conducted with Tukey tests.

RESULTS

Effects of KRG on sleep architecture

No change in REM sleep was observed in all the tested rats during the 12 h recording time. While the amount of wake and NREM after KRG administration changed in the course of the experiment. KRG 10 mg/kg induced a significant decrease in total wake time and increase in NREM and total sleep. Similarly, KRG 100 mg/kg decreased total wake time and increased total sleep time, the NREM and REM sleep increased but not statistically significant (Fig. 2).

Effects of KRG on EEG power density during NREM sleep

During NREM, δ -wave was reduced, α -wave was

increased, and θ -wave power was not changed after 10 mg/kg KRG administration. Differently, KRG 100 mg/kg did not induce any changes in EEG power density of all the 3 selected frequency bands (Fig. 3).

Effects of KRG on EEG power density during REM sleep

During REM sleep, KRG 10 mg/kg significantly decreased δ -wave, increased α -wave power density, without influencing power density of θ -wave; KRG 100 mg/kg did not induce any changes in EEG power density

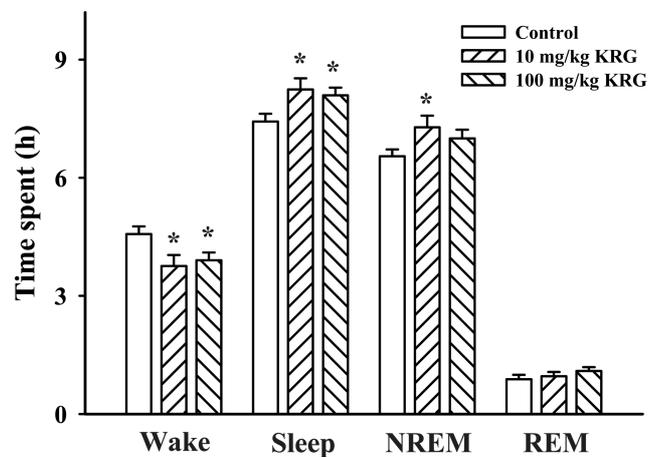


Fig. 2. Effects of KRG on sleep architecture. The data represents the mean \pm SE of time spent in sleep-wake state [Wake, Sleep (total sleep), NREM sleep, REM sleep]. * $P < 0.05$, significantly different from control.

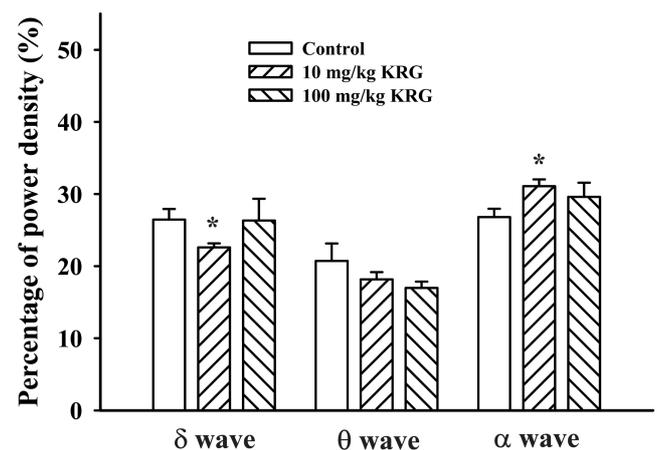


Fig. 3. Effects of KRG on EEG power density during NREM sleep. EEG power density in δ -wave, θ -wave and α -wave for NREM were evaluated. The data represents the mean \pm SE of EEG power density in 3 selected frequency bands in NREM sleep stage. * $P < 0.05$, significantly different from control.

of the 3 selected frequency bands (Fig. 4).

Effects of KRG on EEG power density during wake time

KRG (10 and 100 mg/kg) did not change EEG power density of α -wave. While low and high dose of KRG showed different effects on δ wave, KRG 10 mg/kg significantly decreased power density of δ -wave, but KRG 100 mg/kg increased δ -wave and decreased θ -wave

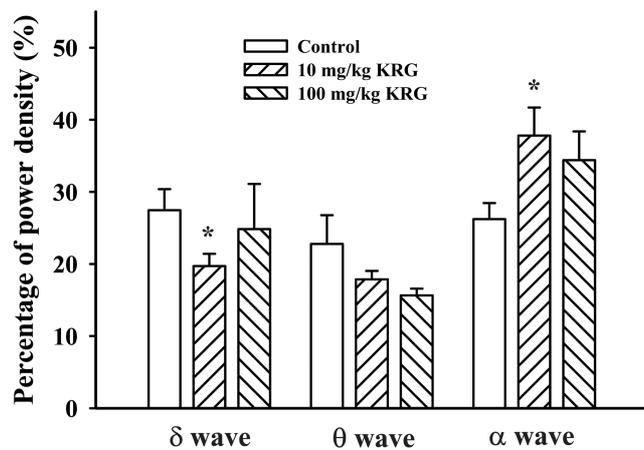


Fig. 4. Effects of KRG on EEG power density during REM sleep. EEG power density in δ -wave, θ -wave and α -wave for REM were evaluated. The data represents the mean \pm SE of EEG power density in 3 selected frequency bands in NREM sleep stage. * $P < 0.05$, significantly different from control.

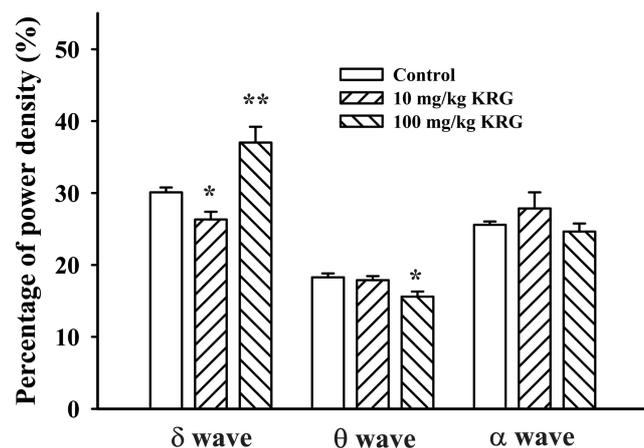


Fig. 5. Effects of KRG on EEG power density during wake time. EEG power density in δ -wave, θ -wave and α -wave for wakefulness were evaluated. The data represents the mean \pm SE of EEG power density in 3 selected frequency bands in wake stage. * $P < 0.05$; ** $P < 0.01$, significantly different from control.

power density during wake time (Fig. 5).

DISCUSSION

Our investigation indicated that KRG increased total and NREM sleep in rats, and decreased wakefulness. *Panax ginseng* is extensively used for a wide variety of clinical ailments and to improve general physical and mental wellbeing¹⁵. People believe that ginseng has the sleep stabilizing and balancing effects. Our results are consistent with the belief that ginseng is effective in improving sleep and prove further that these effects are more specifically focused on NREM¹⁶.

Over many years of research on the homeostatic regulation of sleep, it has been demonstrated that the homeostatic demand for its total amount and frequency depends on the specific activity patterns of the cortical EEG waves¹⁷⁻²³. A number of studies have suggested that the intensity of δ -wave activity (or slow-wave activity; spectral power in the 0.75 to 4.0 Hz range) in the cortical EEG is the single most important process for the homeostatic regulation of NREM sleep^{17,19-25}. In support of this suggestion, studies have shown that the δ -wave activity in NREM sleep typically declines in the course of the daily sleep period and increases in recovery sleep after a period of prolonged waking^{20,21,23,24}. Furthermore, it has also been reported that the δ -wave activity is reduced in the subsequent NREM sleep after a nap and/or excess sleep^{22,25}. However, in our results, KRG increased NREM sleep with a decreased δ -wave activity; therefore, it seems possible that KRG modulate sleep architecture with a similar way as physiological regulation in excess sleep.

Unlike NREM sleep, the EEG correlation for the REM sleep homeostatic process remained poorly understood. Some studies in humans suggested that the EEG activity of the α -wave activity (frequency range of 8-13 Hz) might be a marker of REM sleep homeostasis²⁶. Yet, it is suggested that the α -wave activity may not be involved in REM sleep homeostasis of rats²⁷. Thus, it has been demonstrated clearly that the δ -wave activity is still involved in the homeostatic regulation of REM sleep²⁸. In our study, KRG 10 mg/kg showed similar effects on NREM and REM effects, but significantly increased NREM and total sleep. REM. KRG 100 mg/kg did not change EEG power density of δ -, θ - and α -waves. Only, total sleep was increased. NREM and REM sleep were changed. These differences indicate that ginseng is more effective in lower dose in modulating of sleep, and it is more specifically related to the function of NREM sleep.

Our results also found out that low dose (10 mg/kg) and high dose (100 mg/kg) of KRG showed different effects on δ -wave and θ -wave power density in wake time. It is another possible reason for the difference of sleep modulating effects between low and high doses of KRG. In summary, the results of the current study demonstrated that KRG increases spontaneous sleep and NREM sleep and differently regulate the EEG spectra in REM and NREM sleep.

ACKNOWLEDGEMENT

This work was supported by the research grant of the Chungbuk National University in 2008.

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