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RESEARCH NOTE

Effect of the Licorice Flavonoid Isoliquiritigenin on the Sleep Architecture and Profile in Mice

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Abstract The sleep-promoting effect of isoliquiritigenin (ILTG) was investigated by analyzing the sleep architecture in mice. A hypnotic diazepam (DZP, 2 mg/kg) significantly decreased sleep latency by 39.7% and increased the amount of non-rapid eye movement sleep (NREMS) by 103.8% for the first 3 h after administration. ILTG (50 mg/kg) also produced a significant decrease in sleep latency (30.7%) and an increase in the amount of NREMS (61.1%). DZP significantly decreased delta (0.5-4 Hz) activity as compared with the vehicle; however, ILTG did not alter the delta activity. These results mean that ILTG induces sleep similar to physiological sleep without a decline in sleep quality.

Keywords: isoliquiritigenin, sleep-promoting effect, sleep architecture, electroencephalogram, electromyogram

Introduction

Licorice is one of the most frequently consumed medicinal plants in Korea (1) and has been used as a food ingredient and functional food. The biological activity of licorice has been widely studied for decades and its biologically active constituents remain attractive to many research groups (2). A large number of clinical and experimental studies have reported several useful biological properties of licorice, such as its antioxidant, anticancer, immunomodulatory, cardioprotective, and anti-inflammatory effects (3).

In our previous report (4), licorice ethanol extract was shown to impart *in vivo* sedative-hypnotic effects and *in vitro* binding activity to a γ-aminobutyric acid type A-benzodiazepine ($GABA_A$-BZD) receptor. We also showed that isoliquiritigenin (ILTG), a flavonoid in licorice, induces sleep through positive allosteric modulation of the $GABA_A$-BZD receptor in the central nervous system (5). Sleep-inducing mechanism of ILTG is shown in Fig 1A. ILTG was also characterized as a partial agonist of the $GABA_A$-BZD receptor through an electrophysiological study (5). However, the effects of ILTG on changes in sleep architecture and profile have not been evaluated.

The present study aimed to examine the sleep-promoting effects of ILTG by investigating the effects of an oral administration of ILTG on sleep-wake regulation and profile through electroencephalogram (EEG) and electromyogram (EMG) recordings in C57BL/6N mice.

Materials and Methods

Chemicals Isoliquiritigenin (ILTG) and diazepam (DZP) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Myungin Pharm Co., Ltd. (Seoul, Korea), respectively.

Animals Male C57BL/6N mice (27-30 g) were purchased from Koatech Animal Inc. (Pyeongtaek, Korea). All animals were given food and water *ad libitum* and housed in a room set at 24ºC, controlled humidity at 55%, and a 12 h light/dark cycle (light on at 9:00 AM). All procedures

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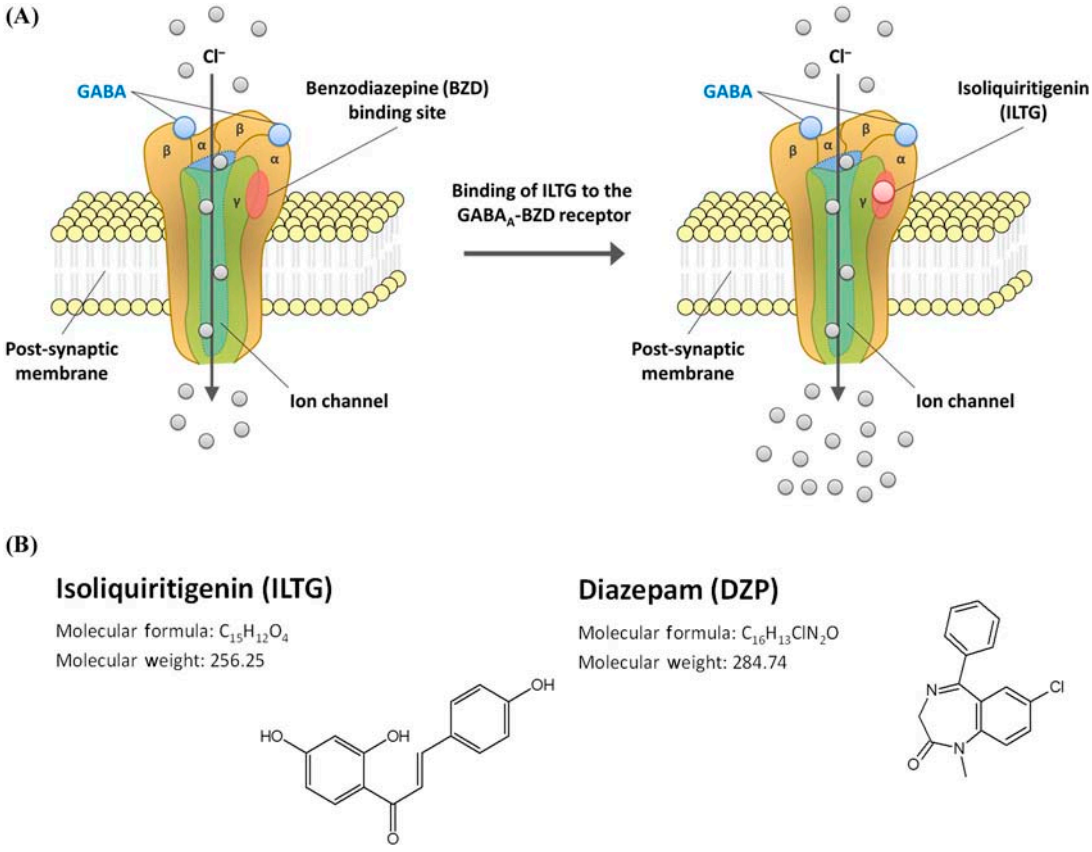

Fig. 1. Sleep-inducing mechanism of isoliquiritigenin (A) and structure and molecular weight of isoliquiritigenin and diazepam (B).

involving animals were conducted in accordance with the guidelines of the Korea Food Research Institutional Animal Care and Use Committee (Permission no.: KFRI-M-09118).

Surgical procedure Under pentobarbital (50 mg/kg, i.p.) anesthesia, C57BL/6N mice were chronically implanted with the head mount (#8201; Pinnacle Technology Inc., Lawrence, KS, USA) for polysomnographic recording. The front edge of the head mount was placed 3.0 mm anterior to the bregma of the mouse skull. Four stainless steel screws for the electroencephalogram (EEG) were passed through the head mount into 4 predrilled holes of the skull, and 2 electromyogram (EMG) wires were sutured onto the nuchal muscles in the back of the neck and then fixed with dental cement. The skin was sutured around the head mount. After surgery, each mouse was allowed 7 days in an individual transparent barrel for recovery.

Recordings of EEG and EMG After recovery, the mice were habituated to the recording conditions for 4 days before the actual testing. The samples were orally administered to the mice, and each mouse was immediately transferred to a recording chamber and connected to a recording cable. The recording was started at 9:00 AM and was continued for 12 h. Time-synchronous digital video was recorded along with the EEG and EMG. Cortical EEG and EMG signals were recorded using the PAL-8200 data acquisition system (Pinnacle Technology Inc.).

Sleep-wake state analysis The sleep-wake states were automatically classified by 10-s epoch as wakefulness (Wake), rapid eye movement sleep (REMS), and non-REM sleep (NREMS) by SleepSign ver. 3 software (Kissei Comtec, Nagano, Japan) according to the standard criteria (6). Sleep latency was defined as the time elapsed between sample administration and the first consecutive NREMS episode lasting at least 2 min. The EEG power spectra were calculated at 0.5 Hz intervals, integrated, and averaged. The EEG power density of NREMS was normalized as a group by calculating the percentage of each interval from the total EEG power (0-20 Hz) of each mouse.

Statistical analysis Comparisons among 2-group data were analyzed by using the unpaired Student's *t*-test. Test for significance was performed using the Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA).

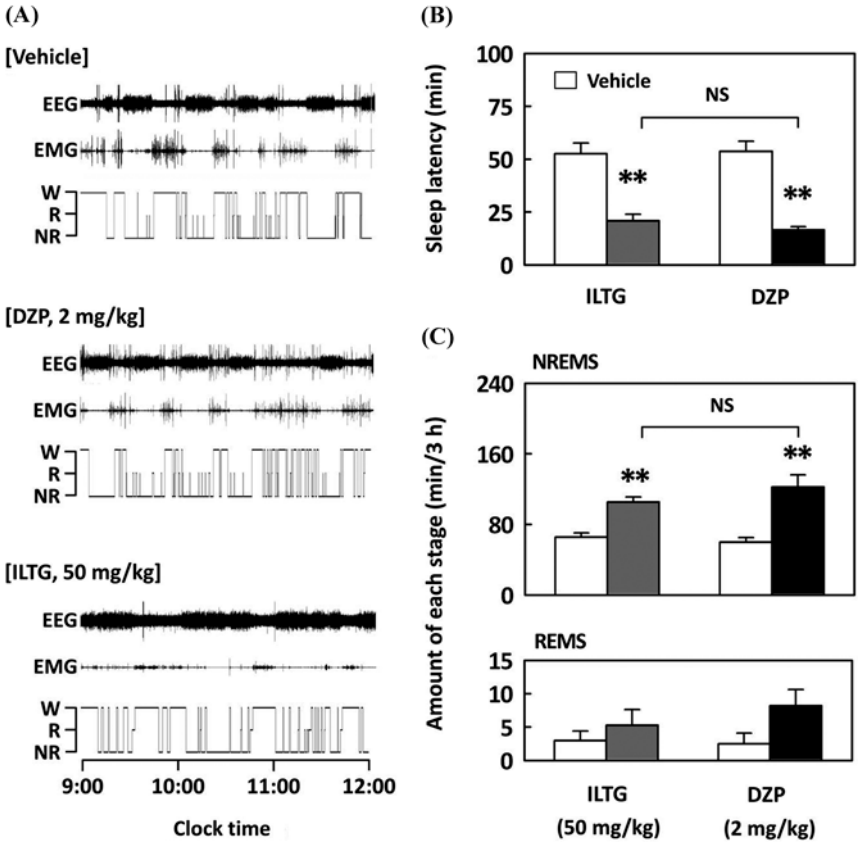

Fig. 2. EEG and EMG recordings and corresponding hypnograms in a mouse treated with vehicle, ILTG, and DZP (A), effects of ILTG and DZP on sleep latency (B), and total time spent in NREMS and REMS (C) for 3 h after the administration. Each column represents the mean±SEM (n=8); **p<0.01, as compared with the vehicle using the unpaired Student's t-test; NS, not significant. DZP, diazepam; EEG, electroencephalogram; EMG, electromyogram; ILTG, isoliquiritigenin; NREMS (or NR), non- rapid eye movement sleep; REMS (or R), rapid eye movement sleep; Wake (or W), wakefulness

Results and Discussion

ILTG is a chalcone that naturally occurs in licorice (7). Chalcones exhibit a basic structure of 2 benzene rings linked through an α, β-unsaturated carbonyl group and belong to a group of flavonoids (8). ILTG has been shown to have valuable pharmacological properties such as antioxidant (9), anti-inflammatory (10), anticancer (11), antiangiogenic (12), and antiallergic (13) activities. In our previous report (5), the administration of 5-50 mg/kg of ILTG increased sleep duration in a dose-dependent manner, and the hypnotic effect of 50 mg/kg ILTG was comparable to the positive control DZP. In the present study, the effects of ILTG on the sleep architecture and profile in mice were evaluated using recordings of EEG and EMG signals.

Effect of ILTG on sleep latency and amounts of NREMS and REMS The signals of EEG and EMG in mice were recorded 12 h after oral administration of ILTG (50 mg/kg) at 9:00 AM, and its effects were compared with the positive control DZP (2 mg/kg). The representative EEG and EMG signals and corresponding hypnograms for the vehicle, ILTG, and DZP are shown in Fig. 2A. The effects of ILTG and DZP on sleep latency and total time spent in NREMS and REMS for 3 h after the administration are shown in Fig. 2B and 2C. As expected, DZP significantly decreased sleep latency by 30.7% (p<0.01) (Fig. 2B) and increased the amount of NREMS by 103.8% (p<0.01) (Fig. 2C), as compared with the vehicle baseline for the first 3 h after the injection. The administration of ILTG also exerted a significant decrease in sleep latency (39.7%, p<0.01) and an increase in the amount of NREMS (61.1%, p<0.01). Both ILTG and DZP did not produce significant change in the amount of REMS (Fig. 2C). It has been known that BZD agents such as DZP increase NREMS without changing REMS (14,15).

Effect of ILTG on hourly time spent in NREMS The time courses of the hourly amounts of NREMS for 12 h after the administration of ILTG and DZP are shown in Fig. 3A. After the injection of ILTG, the amount of NREMS immediately increased. The effect of ILTG was statistically significant (p<0.05), as compared with the vehicle for the first 2 h. No further significant disruption of

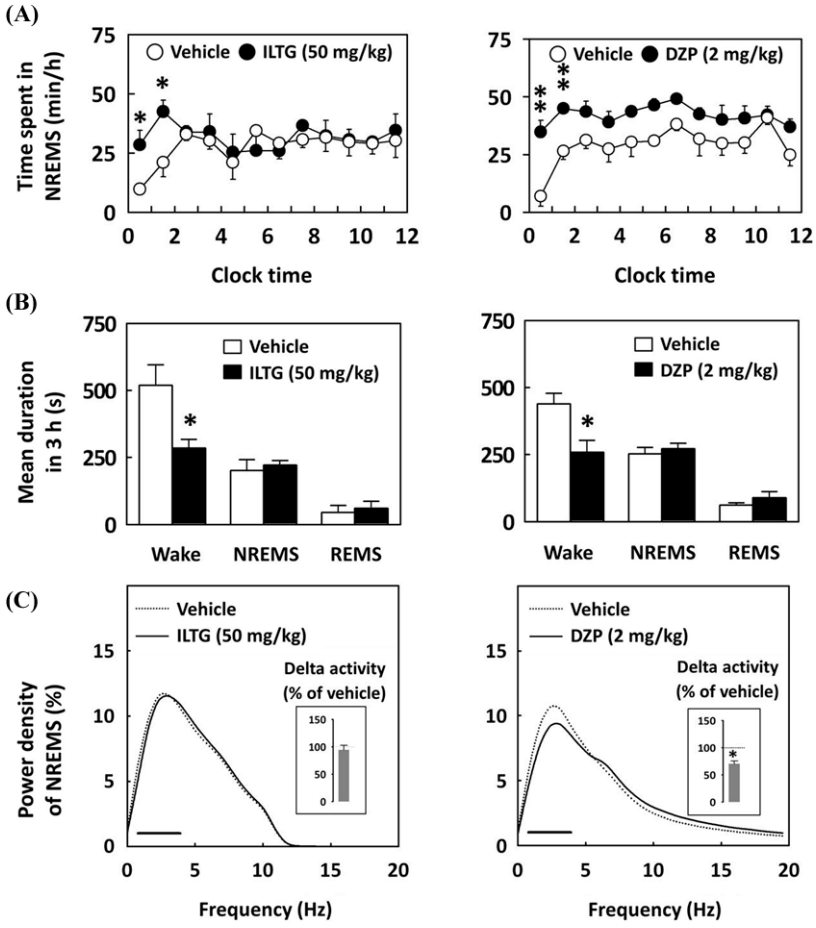

Fig. 3. Effects of ILTG and DZP on time course of NREMS (A), the mean duration of each sleep stage (B), and EEG power density during NREMS (C). Delta activity in NREMS as an index of sleep intensity is shown in the histogram in the inset. Legend: (—) represents the range of delta wave (0.5-4 Hz). *p<0.05, **p<0.01, as compared with the vehicle using the unpaired Student's t-test. DZP, diazepam; EEG, electroencephalogram; ILTG, isoliquiritigenin; NREMS, non- rapid eye movement sleep; REMS, rapid eye movement sleep

the sleep architecture was observed during the subsequent period. These results indicate that ILTG induces NREMS without the occurrence of adverse effects after sleep induction (16). DZP also showed the significant (p<0.01) effect for the first 2 h; however, during the subsequent period, the hourly NREMS readings were higher than those obtained from ILTG.

Effect of ILTG on mean duration of each sleep stage and EEG power density in NREMS To better understand the sleep profile caused by ILTG, the mean duration of each sleep stage and EEG power density in NREMS were calculated. Both ILTG and DZP significantly (p<0.05) decreased the mean duration of Wake by 54.9 and 58.8%, respectively; however, they did not affect the mean duration of NREMS and REMS (Fig. 3B). A decrease in the mean duration of Wake by ILTG without affecting NREMS and REMS indicates that ILTG decreased the maintenance of Wake (16). ILTG did not affect the EEG power density (0-20 Hz) in NREMS as compared with the vehicle, whereas DZP produced a significant (p<0.05) decrease in the delta (0.5-4 Hz) activity, as shown in the inset of Fig. 3C. Delta activity is an indicator of the depth or intensity of NREMS (17,18). The decrease in the delta activity caused by DZP in humans and rodents has been extensively described by previous reports (15,19). BZD agents induce an increase in the quantity of sleep (sleep duration), but decrease the sleep quality (delta activity) (17, 20). DZP produced an increase in beta activity (13-30 Hz), which is the higher EEG frequency generally associated with attention and arousal. Although BZD sleep drugs induce sleep, this increase in beta activity is their own direct effect (19,21). In the present study, DZP showed the typical increase in beta activity; however, the administration of ILTG did not disrupt this feature. In summary, ILTG decreased sleep latency and increased the amount of NREMS, similar to DZP; however, it did not change the EEG power density, which is shown by DZP. These results suggest that ILTG induces NREMS that is similar to physiological sleep.

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