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Arousal Inhibitory Effect of Phlorotannins on Caffeine in Pentobarbital-Induced Mice

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Abstract

Sleep is vital to maintain health and well-being; however, insomnia is currently a widespread health complaint worldwide. In particular, caffeine, a psychoactive component of coffee, tea, and caffeine beverages may lead to sleep disorders such as insomnia. In this study, our primary objective was to investigate the inhibitory effect of high-purity phlorotannin preparation (HP-PRT) on caffeine-induced wakefulness. The sleep test of pentobarbital-induced mice was used as an *in vivo* animal model. Caffeine (50 and 100 mg/kg) showed significant arousal effects (an increase in sleep latency and a decrease in sleep duration). Co-administration of caffeine (50 mg/kg) and the sedative-hypnotic diazepam (DZP, 1 mg/kg) did not result in similar arousal activity. HP-PRT (500 mg/kg) also inhibited caffeine-induced wakefulness. Our results suggest that HP-PRT would be a useful additive for developing coffee products without the arousal effect.

Key words: Phlorotannins, Somnogenic effect, Caffeine, Arousal effect, Pentobarbital-induced sleep test

Introduction

Sleep is vital to maintain health and well-being because of its primary function of providing rest and restoring the body's energy levels (Krueger et al., 2008). Sleep disorders impair not only cognitive and psychological functioning but also physical health (Brand and Kirov, 2011). In addition, obesity and cardiovascular disease are closely related to sleep disorders (Gangwisch et al., 2005; Wolk et al., 2005; Miller and Cappuccio, 2007).

Coffee is one of the most consumed beverages in the world (Tuomilehto et al., 2004), and has an arousal effect on the central nervous system (Davis et al., 2003). Caffeine, the major psychoactive constituent in coffee (Brown et al., 2001), induces wakefulness by inhibiting adenosine A_{2A} receptors (Huang et al., 2005). According to the "Caffeine Intake by the U.S. Population" report, the average amount of caffeine consumed is approximately 300 mg/person/day (Somogyi, 2010). A large amount of caffeine may lead to sleep disorders, such as insomnia (Sanchez-Ortuno et al., 2005; Strassnig et al., 2006). Therefore, to identify foods, and their constituents, that can inhibit the arousal effect of caffeine would be promising from the perspective of the food industry.

In our previous studies (Cho et al., 2012a; Cho et al., 2012b), we have demonstrated that phlorotannin extracts from the brown alga *Ecklonia cava* induce sleep via modulation of the benzodiazepine site of the $GABA_A$ receptor. In the present study, the effect of phlorotannins on caffeine-induced wakefulness was evaluated using a pentobarbital-induced sleep test in mice.

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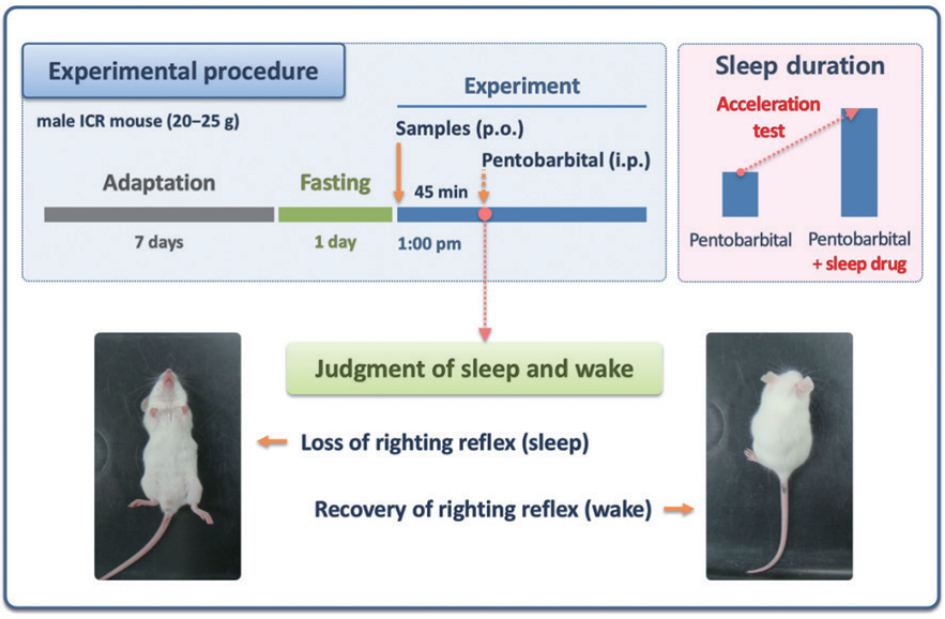

Fig. 1. The experimental procedure of the pentobarbital-induced sleep test in mice.

Materials and Methods

Materials

The high-purity phlorotannin preparation (HP-PRT, lot no. SD-GT-E-004) was obtained from S&D Co., Ltd. (Yeongi-gun, Chungcheongnam-do, Korea). HP-PRT was purified from the ethanol extract of the brown alga *Ecklonia cava*, and was standardized to 67 mg/g dieckol. The total phlorotannin content of HP-PRT is 90% (900 mg phloroglucinol equivalents/g, w/w). Diazepam (DZP; Myungin Pharm. Co. Ltd., Seoul, Korea), a $GABA_A$-benzodiazepine agonist, was used as a reference hypnotic drug. All other chemicals and reagents were of the highest grade available.

Animals

All procedures involving animals were conducted in accordance with the animal care and use guidelines of the Korea Food Research Institutional Animal Care and Use Committee (permission number: KFRI-M-12027). Every effort was made to minimize the number of animals used and any pain and discomfort that they might experience. ICR mice (male; 23–28 g; 4 weeks) were purchased from Koatech Animal Inc. (Pyeongtaek, Korea). The animals were housed in an insulated, soundproof recording room maintained at an ambient temperature of 23 ± 0.5°C, with a relative humidity (55 ± 2%) on an automatically controlled 12-h light/12-h dark cycle (lights on at 5:00). They had free access to food and water.

Pentobarbital-induced sleep test

For better understanding the pentobarbital-induced sleep test in mice, the experimental procedure is shown in Fig. 1. All samples were dissolved in 0.5% (w/v) carboxymethyl cellulose (CMC)-physiological saline before use. Control mice (0.5% CMC-saline, 10 ml/kg) were tested in parallel with the animals receiving test sample treatment. All experiments were performed between 13:00 and 17:00, and mice were fasted for 24 h prior to the experiment. Test solutions were administered (post-oral injection, p.o.) to mice by using a sonde needle 30 min prior to the intraperitoneal injection (i.p.) of pentobarbital (42 mg/kg). Following the pentobarbital injection, the mice were placed in individual cages and observed for measurements of sleep latency and sleep duration. Observers were blinded to the individual treatments. The sleep latency was recorded from the time of pentobarbital injection to the time of sleep onset, and sleeping duration was defined as the difference in time between the loss and recovery of the righting reflex.

Statistical analysis

For multiple comparisons in the pentobarbital-induced sleep test, data were analyzed using one-way ANOVA followed by Dunnett's test. Comparisons between group data were analyzed using an unpaired Student's *t*-test. Differences with $p < 0.05$ were considered statistically significant. The significance analysis was performed using Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA).

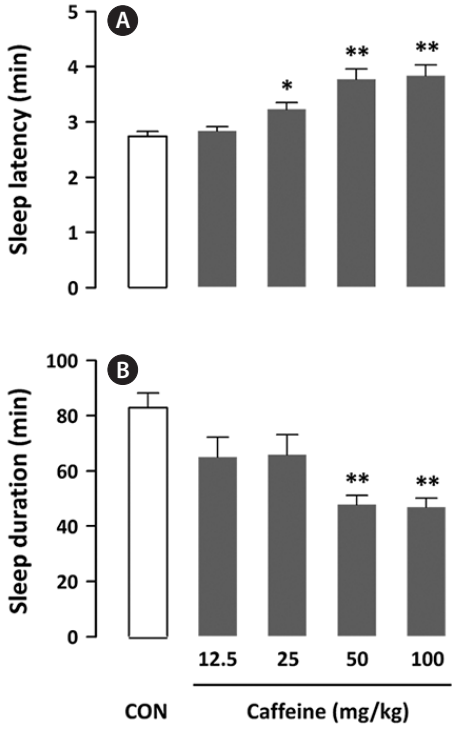

Fig. 2. Effects of caffeine on sleep latency (A) and sleep duration (B) in mice administered a hypnotic dose (42 mg/kg, i.p.) of pentobarbital. Mice received pentobarbital 30 min after oral administration (p.o.) of the control group (CON) and caffeine. Each column represents mean ± SEM (n = 10). * $p < 0.05$, ** $p < 0.01$, significant difference as compared to the control group (Dunnett's test).

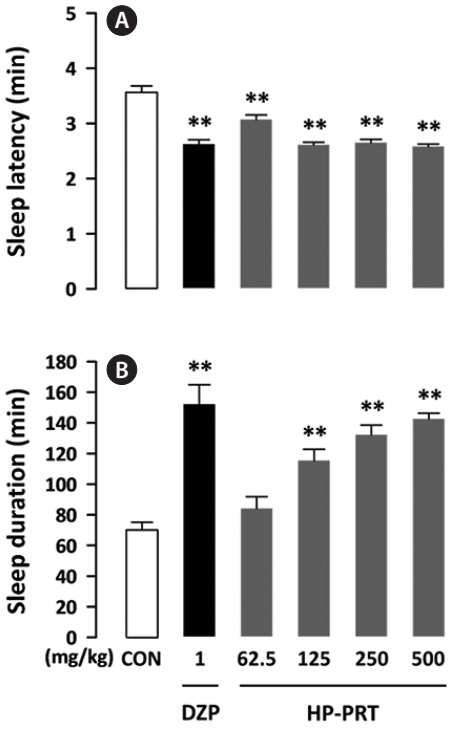

Fig. 3. Effects of high-purity phlorotannin preparation (HP-PRT) and diazepam (DZP) on sleep latency (A) and sleep duration (B) in mice administered a hypnotic dose (42 mg/kg, i.p.) of pentobarbital. Mice received pentobarbital 30 min after oral administration (p.o.) of samples. Each column represents mean ± SEM (n = 10). ** $p < 0.01$, significant difference as compared to the control group (Dunnett's test).

Results and Discussion

Although a large amount of caffeine intake is known to lead to sleep disorders such as insomnia, few studies have been conducted on foods and their constituents with inhibitory effects on caffeine-induced wakefulness. In this study, we evaluated the effect of HP-PRT with sedative-hypnotic activity on caffeine-induced wakefulness by using the pentobarbital-induced sleep test in mice. This method is useful to evaluate *in vivo* hypnotic and arousal activities (Ma et al., 2009). It is important that the active compounds are able to pass the blood–brain barrier to produce hypnotic or arousal activities (Risa et al., 2004). Therefore, it is necessary to confirm activities through *in vivo* animal model assays (Fang et al., 2010).

To determine a statistically significant dosage of caffeine, its effects on sleep latency and sleep duration in mice were evaluated. With a hypnotic dose of pentobarbital (42 mg/kg), caffeine (12.5, 25, 50, and 100 mg/kg, p.o.) produced a dose-dependent increase in sleep latency and a decrease in sleep duration (Fig. 2). It showed a significant ($p < 0.01$) arousal activity from a concentration of 50 mg/kg. According to the results of Huang et al. (2005), caffeine significantly produced arousal activity in analysis of sleep-wake profile based on electroencephalography and electromyography. In this study, 50 mg/kg was selected as a dosage of caffeine for the experiment to test the inhibitory effects of phlorotannins. A dosage of HP-PRT was determined by testing four concentrations of 62.5, 125, 250, and 500 mg/kg. Diazepam (DZP), one of the most well-known sedative-hypnotics, was tested as a positive control in parallel with HP-PRT. One mg/kg of DZP has been tested as the positive control in the pentobarbital-induced sleep test (Amos et al., 2003; de Sousa et al., 2005). As expected, it was found that DZP (1 mg/kg, p.o.) significantly potentiated the pentobarbital-induced sleep in mice ($p < 0.01$) relative to the control group (Fig. 3). HP-PRT also caused a dose-dependent decrease in sleep latency (Fig. 3A) and an increase in sleep duration (Fig. 3B). Effect of HP-PRT on caffeine-induced wakefulness was evaluated at 500 mg/kg with the significant hypnotic activity similar to DZP at 1 mg/kg.

The inhibitory effect of DZP, a positive control on caffeine-induced wakefulness, is shown in Fig. 4. DZP induces sleep via positive allosteric modulation of the benzodiazepine site of the $GABA_A$ receptor (Kopp et al., 2003; Tobler et al., 2001). As expected, administration of caffeine (50 mg/kg) alone

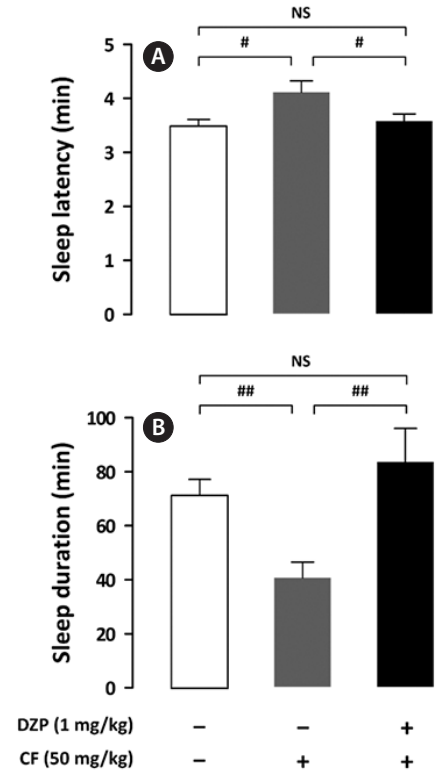

Fig. 4. Effects of co-administration of diazepam (DZP, 1 mg/kg) and caffeine (CF, 50 mg/kg) on sleep latency (A) and sleep duration (B) in mice administered a hypnotic dose (42 mg/kg, i.p.) of pentobarbital. Each column represents mean ± SEM (n = 10). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, significant difference between each group (unpaired Student's *t*-test). NS, not significant.

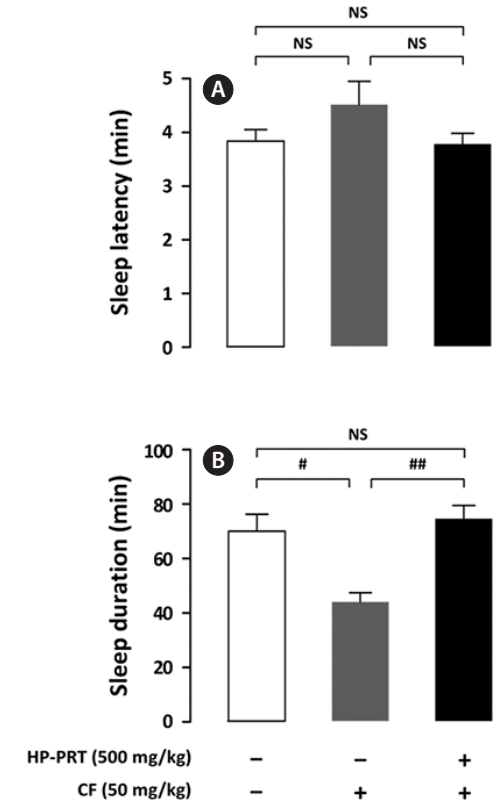

Fig. 5. Effects of co-administration of high-purity phlorotannin preparation (HP-PRT, 500 mg/kg) and caffeine (CF, 50 mg/kg) on sleep latency (A) and sleep duration (B) in mice administered a hypnotic dose (42 mg/kg, i.p.) of pentobarbital. Each column represents mean ± SEM (n = 10). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, significant difference between each group (unpaired Student's *t*-test). NS, not significant.

showed significant arousal effects; however, sleep latency and sleep duration of mice treated with both DZP (1 mg/kg) and caffeine were similar to those of the control group. HP-PRT (500 mg/kg) also reversed the arousal effect of caffeine (Fig. 5). The pentobarbital-induced sleep test using rodents is a useful in vivo assay for evaluating hypnotic or arousal effect. Pentobarbital, an anesthetic, produces hypnosis and anesthesia by modulating the barbiturate site of $GABA_A$ receptor (Gerak et al., 2004). Regardless of molecular mechanisms of sleep-wake regulation, hypnotic or arousal compounds can accelerate or decelerate the pentobarbital-induced sleep. In this study, arousal effect of caffeine via the adenosine A_{2A} receptor was offset by hypnotic effect of DZP and HP-PRT via the benzodiazepine site of the $GABA_A$ receptor. Therefore, this offset did not alter duration and latency of the pentobarbital-induced sleep. In addition, according to the report by El Yacoubi et al. (2003), caffeine reduced the hypnotic effect of alcohol by modulating the $GABA_A$ receptor (Koob, 2004; Lobo and Harris, 2008; Kumar et al., 2009).

In this experiment, no adverse effects were observed following HP-PRT administration. It is known that benzodiazepine agents, such as DZP increase non-rapid eye movement sleep without altering rapid eye movement sleep (Qiu et al., 2009) but decrease delta activity, an indicator of sleep quality (Bastien et al., 2003). HP-PRT does not alter delta power (data not shown) and would thus be a better agent for inhibiting caffeine-induced wakefulness. Phlorotannins, which are oligomers and polymers of phloroglucinol (1,3,5-tri-hydroxybenzene), have a brown color and a bitter taste (Shibata et al., 2002). Their sensory characteristics would be suitable as an additive to coffee. In addition, phlorotannins have various biological activities, such as antioxidative (Zou et al., 2008), anti-inflammatory (Kim et al., 2009), antibacterial (Nagayama et al., 2002), and antiallergic (Sugiura et al., 2006) effects. Therefore, HP-PRT may be a good additive for the preparation of coffee products without an arousal effect. Further studies are needed to evaluate the effects of co-administration of HP-PRT and caffeine on sleep-wake architecture and profile.

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