Sedative Effects of Combined Administration of 4-Hydroxy-3-methoxybenzaldehyde, a Component of *Gastrodia elata*, and 2,3-Dihydroxybenzaldehyde in Rats

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The present study was performed to investigate the sedative effects of the combined administration of phenolic compounds. 4-hydroxy-3-methoxybenzaldehyde, a component of *Gastrodia elata*, showing positive GABAergic neuromodulation was administered intraperitoneally together with an identical dose of 2,3-dihydroxybenzaldehyde, a potent antioxidant, to the rats and then evaluated for its effects on the convulsion, the hypnosis, the anxiety and the muscle relaxation. Combined administration of both compounds significantly reduced the pentyleneterazole-induced lethality. In addition, this mixture significantly enhanced the pentobarbital-induced sleeping time. Contrary to the anticonvulsive and sedative effects, the combined administration did not exhibit anxiolytic or muscle relaxant activities. These results indicated that the combined treatment of 2,3-dihydroxybenzaldehyde and 4-hydroxy-3-methoxybenzaldehyde with different effects leads to the anticonvulsion and/or sedation

Key words – *Gastrodia elata*, sedatives, phenolic compound, combined administration

Introduction

Gastrodiae Rhizoma is the dry tuber of *Gastrodia elata* Blume (Orchidaceae) and has been used in traditional medicine in Korea, China and Japan as a sedative, an anticonvulsant and an analgesic against epilepsy, general paralysis, vertigo and tetanus [10]. Many phenolic constituents for *Gastrodia elata* Bl. have been known: besides a major phenolic glucoside - gastrodin, more than 15 phenolics have been isolated [9,12]. In our previous report, 4-hydroxybenzaldehyde, among them, showed an significant inhibitory effect on brain lipid peroxidation in pentylenetetrazole-treated rats and on the GABA transaminase activity [3]. We also demonstrated that 4-hydroxy-3-methoxybenzaldehyde and 4-hydroxybenzaldehyde significantly inhibited the GABA transaminase activity and the enhancement of [^{3}H]flunitrazepam binding to the benzodiazepine receptor on the $GABA_A$ receptor complex [4]. Recently, we compared the antioxidant activities of above components with those of some analogues *in vitro* and found that 2,3-dihydroxybenzaldehyde, one of the analogues, exhibited much better activity than other phenolic compounds.

Anticonvulsive or sedative actions of *G. elata* Bl. have been reported to be closely related with anti-lipid peroxidation [7,8] and positive GABAergic neuromodulation [3,4] in the mammalian central nervous system. So, we are interested in the sedative effects of the mixture of a potent antioxidant compound and a positive GABAergic neuromodulator having similar structure.

In this study, 4-hydroxy-3-methoxybenzaldehyde showing positive GABAergic neuromodulation was administered intraperitoneally together with an identical dose of 2,3-dihydroxybenzaldehyde, a potent antioxidant, to the rats and evaluated its effects on the convulsion, the hypnosis, the anxiety and the muscle relaxation.

Materials and Methods

General

All assay reagents and chemicals including 2,3-dihydroxybenzaldehyde and 4-hydroxy-3-methoxybenzaldehye

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were purchased from Sigma (St. Louis, MO, USA) except sodium pentobarbital (Han-Rim Pharmaceuticals, Korea). Male Sprague-Dawley rats weighing 250-350 g for the lipid peroxidation and 120-150 g for *in vivo* test were used. Animals were housed in a regulated environment (temperature: 21±1℃ humidity: 50±5%) with a 12 h light-dark cycle.

Lipid peroxidation assay

In accordance with the US guidelines (NIH publication # 85-23, revised in 1985), rats (Sprague-Dawley, 250-350 g, male) were anesthetized with diethyl ether, and perfused with normal saline. Brains were isolated, and the homogenized with glass Teflon homogenizer in 4 volumes of 0.1 M potassium phosphate buffer (pH 7.4). Tissue homogenates, 8.1% sodium dodesyl sulfate, 20% acetate buffer (pH 3.5) and 0.8% 2-thiobarbituric acid were incubated for 1 h at 95℃, then cooled in room temperature. Thiobarbituric acid reactive substance in the reactant was transferred into the mixture of *n*-butanol: pyridine (15:1) and measured by spectrophotometer (Spectronic Genisis 5, Mitron Roy, USA) at 532 nm.

Pentylenetetrazole-induced lethality assay

The effects of the compounds on pentylenetetrazole (PTZ)-induced lethality were evaluated according to Viola et al. [11] with slight modifications. For this purpose, mice were divided into groups (n=10, each). The first group served as control and was injected with the vehicle, while the 2nd group received compounds (100 mg/kg) 15 minutes before the administration of a convulsive dose of PTZ (50 mg/kg, i.p.). Percent(%) lethalities of animals were calculated.

Pentobarbital-induced sleeping time assay

In this test, the time(min) elapsing from the loss to the regaining of the righting reflex after drug administration is recorded and referred to as sleeping time. The hypnotic effect of compounds (100 mg/kg) in combination with sodium pentobarbital was evaluated. For this purpose, mice were divided into groups (n=10, each). The first group served as control and was injected with the vehicle, while the 2nd group received compounds 15 minutes before the administration of a sunthreshold dose of sodium pentobarbital (25 mg/kg, i.p.).

Horizontal wire test

Wire (1mm diameter and 15cm long) was horizontally strung 20 cm above the laboratory table. Rats were lifted by the tail, allowed to grasp the wire with their forepaws and released. After two trials, perfomed at 5 min intervals, the rats were intraperitoneally injected as stated previously and subjeced to the test 15 min later. A myorelaxant drug impairs the rats to grasp the wire at least with one hindpaw within

3 s. Mice were divided into groups (n=10, each). The first group served as control and was injected with the vehicle, while the 2nd group received compounds 15 minutes before the test.

Elevated plus maze test

This test has been validated to measure anxiety in rodents [1,6]. The maze is a horizontal cross made by two open platforms of 25 cm x 5 cm crossed by two platforms of the same dimensions, closed by walls 35 cm high. The maze is suspended 50 cm from the room floor. Rats were placed on the central part of the cross facing a walled arm. The number of entries and the timespent into open and closed arms were counted during 5 min using plus maze monitoring program (Elevated Plus maze, Vatican Production, Inc). A selective increase in the parameters corresponding to open arms reveals an anxiolytic effects.

$$\text{Percent time in Open (\%)} = \frac{\text{Time in the open arms}}{\text{Time in the open arms} + \text{Time in the closed arms}} \times 100$$

$$\text{Percent Open Crosses (\%)} = \frac{\text{Number of entries into the open arms}}{\text{Total number of entries}} \times 100$$

Statistical analysis

Data represents the mean±SE. Intergroup comparisions of data were made by Student *t*-test or ANOVA, followed by Neuman-Keuls multiple comparision test (Systat, Intelligent Software, Evanston, IL, USA).

Results and Discussion

Our previous reports [3] revealed that 4-hydroxybenzaldehyde, a phenolic component of G. *elata* possesses an inhibitory effect on lipid peroxidation: malondialdehyde content was significantly diminished

almost to the level of control in the pentylenetetrazole-treated rats by its intraperitoneal administration. This compound and 4-hydroxy-3-methoxybenzaldehyde, another constituent of *G. elata*, inhibited the [^{3}H]Ro15-1788 binding and the [^{3}H]flunitrazepam binding to the benzodiazepine receptor on the $GABA_A$receptor complex without concentration-response relationship. Furthermore, 4-hydroxy-3-methoxybenzaldehyde enhanced the binding of [^{3}H] flunitrazepam in the presence of GABA. In conclusion, we suggested that 4-hydroxy-3- methoxybenzaldehyde would allosterically modulate the GABAergic neurotransmission via enhancement of the binding of endogenous receptor agonist in the presence of GABA, a major inhibitory neurotransmitter in the mammalian brain [4]. During our continuing search for the antioxidant activities of the phenolic constituents and their analogues, we observed that 2,3-dihydroxybenzaldehyde (1 mM) potently inhibited the lipid peroxidation generation in *in vitro* assay, its activity was much better than those of above 4-hydroxy-3-methoxybenzaldehyde and 4-hydroxybenzaldehyde (Fig. 1). These results suggest that 2,3-dihydroxybenzaldehyde may play a more important role for the sedative or anticonvulsant effect *in vivo*. It has been reported that antiepileptic or anticonvulsive effects of *G. elata*is closely associated with the antioxidant activities of their phenolic constituents [7,8]. Moreover, oxidative stress has been suggested to induce the status of excessive excitatory neurotransmission in the mammalian central nervous system [1,2,5].

On the basis of above results and reports, we administered the same dose (100 mg/kg) of 2,3-dihydroxybenzaldehyde and 4-hydroxy-3-methoxybenzaldehyde to young rats for 7 days. Combined administration of these compounds significantly reduced the pentyleneterazole-induced lethality ($p<0.05$, Fig. 2) against control. However, each 2,3-dihydroxybenzaldehyde and 4-hydroxy-3-methoxybenzaldeyde alone did not diminish the pentylenetetrazole-induced lethality (data not shown). In addition to anticonvulsive activity, this mixture showed a sedative effect in young rats. As shown in Fig. 3, pentobarbital-induced sleeping time (126.1±9,90 min) of the

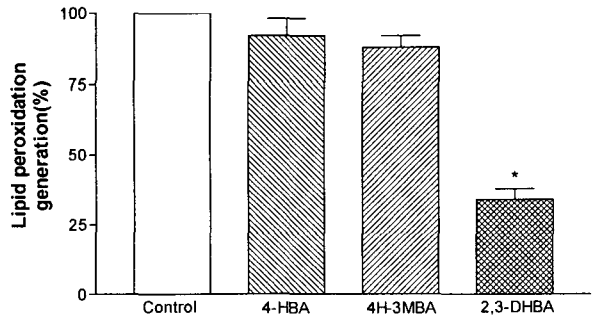

Fig. 1. Effects of phenolic compounds on the lipid peroxidation generation in rats. *$p<0.05$: Significantly different from control. Data represent mean±SE of eight experiments. 1mM concentration of compounds were added. 4-HBA; 4-hydroxybenzaldehyde, 2,3-DHBA; 2,3-dihydroxybenzaldehyde, 4H-3MBA: 4-hydroxy-3-methoxybenzaldehyde.

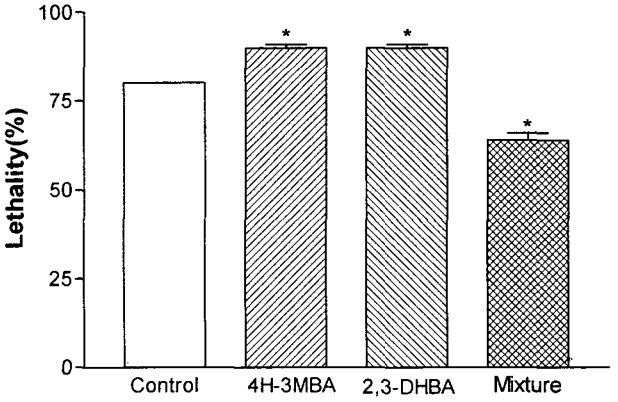

Fig. 2. Effects of phenolic compounds on the pentylenetetrazole (50 mg/kg)-induced lethalities in young rats. *$p<0.05$: Significantly different from control. Data represent mean±SE of 10 animals. 100 mg/kg dose of compounds were administered. 2,3-DHBA; 2,3-dihydroxybenzaldehyde, 4H-3MBA: 4-hydroxy-3-methoxybenzaldehyde.

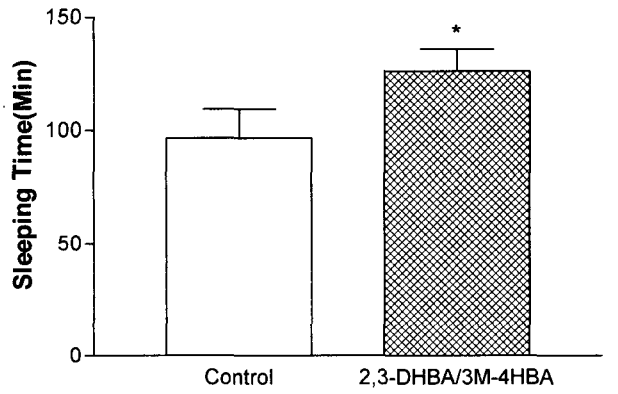

Fig. 3. Effects of phenolic compounds on the pentobarbital (25mg/kg)-induced sleeping time in young rats. *$p<0.05$: Significantly different from control. Data represent mean±SE of 10 animals.100 mg/kg dose of compounds were administered. 2,3-DHBA; 2,3-dihydroxybenzaldehyde, 4H-3MBA: 4-hydroxy-3-methoxybenzaldehyde.

group treated with the combined compounds was significantly enhanced in comparison with that of control (96.6±12.7 min, $p<0.05$). Contrary to the anticonvulsive and sedative effects, the combined administration of both compounds did not exhibit anxiolytic or muscle relaxant activities. Although percent time in open (1.9±0.3 %) of treated groups was significantly lower than that of control (10.9±2.6, $p<0.05$, Fig 4), the percent open crosses (%) did not show any differences between two groups.

As a result, the combined treatment of 2,3-dihydroxybenzaldehyde which is a potent antioxidant and 4-hydroxy-3-methoxybenzaldehyde, a positive GABAergic neuromodulator, leads to the anticonvulsive and/or sedative action. Although further study might be needed for the establishment of detailed therapeutic regimens, phenolic compounds including 2,3-dihydroxybenzaldehyde and 4-hydroxy-3-methoxybenzaldehyde may be expected to be candidates for clinical trials for sedatives.

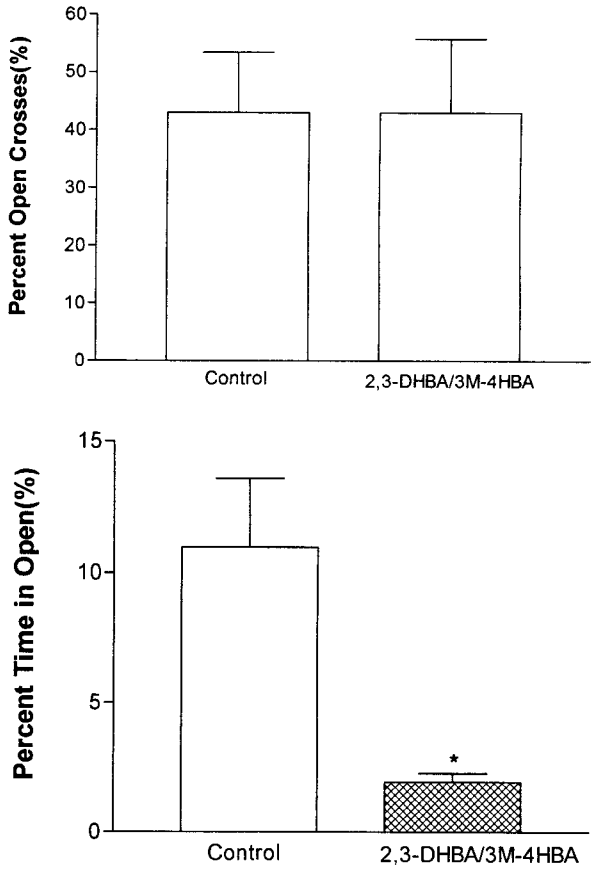

Fig. 4. Effects of phenolic compounds on the plus maze performance of young rats. *$p<0.05$: Significantly different from control. Data represent mean±SE of 10 animals. 100 mg/kg dose of compounds were administered. 2,3-DHBA; 2,3-dihydroxybenzaldehyde, 4H-3MBA: 4-hydroxy-3-methoxybenzaldehyde.

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초록 : 천마성분인 4-hydroxy-3-methoxybenzaldehyde와 2,3-dihydroxy- benzaldehyde의 병용투여에 의한 진정효과

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페놀성 화합물들의 병용투여가 rat의 진정효과에 미치는 영향을 조사하였다. 천마성분으로서 positive GABAergic neuromodulation을 보이는 4-hydroxy-3-methoxybenzaldehyde와 강력한 항산화물질인 2,3-dihydroxybenzaldehyde를 동량 투여한 결과, pentyleneterazole에 의해 유발된 사망률이 유의하게 감소되었으며 pentobarbital에 의해 유도된 수면시간은 유의하게 연장되었으나 항불안효과와 근육이완효과는 나타나지 않았다. 이러한 결과로 보아 항경련효과를 가진 화합물과 항산화 효과를 가진 화합물을 병용투여함으로서 경련이나 불면 등의 신경증상을 효과적으로 억제할 수 있을 것으로 사료된다.