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The Effects of Daidzin and Its Aglycon, Daidzein, on the Scopolamine-induced Memory Impairment in Male Mice

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In this study, the effect of daidzin or daidzein isolated from *Pueraria lobata* on the memory impairments induced by scopolamine was assessed in male mice using the passive avoidance and the Morris water maze tasks. Administration of daidzin (5 mg/kg) or daidzein (5 mg/kg) significantly reversed the scopolamine (1 mg/kg)-induced cognitive impairments in male mice as evidenced by the passive avoidance test ($p < 0.05$) and on the Morris water maze test ($p < 0.05$). Moreover, the ameliorating effects of daidzin or daidzein were antagonized by tamoxifen (1 mg/kg), the nonspecific estrogen receptor antagonist. These results indicate that daidzin or daidzein may be useful in cognitive impairment induced by cholinergic dysfunction, and this beneficial effect is mediated, in part, via estrogen receptor.

Key words: Daidzin, Daidzein, Learning and memory, Estrogen receptor

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INTRODUCTION

Isoflavones are estrogen-mimetic compounds isolated from various herbs, such as soy bean and arrowroot. They are known to possess pharmacological properties such as vasodilation (Yamaguchi et al., 2001) and effects on bone metabolism (Tsutsumi, 1995) through its estrogenic activity. Previous studies have shown that isoflavones improve cognitive function in female subjects including rat (Pan et al., 2000; Lund et al., 2001), mouse (Xu et al., 2004) and human (Duffy et al., 2003; Kritz-Silverstein et al., 2003). The isoflavones exert these efforts on the cognitive function primarily by regulating the synthesis of neurotrophic factors (Pan et al., 1999a, 1999b) and choline acetyltransferase expression (Pan et al., 1999b). Isoflavones regulate various brain functions through its effect on estrogenic receptors by regulating gene transcription and second messenger systems (Linford and Dorsa, 2002; Sonee et al., 2004). However, the effects of isoflavones administration on cognitive function in male subjects and its benefits (Heo et al., 2006) or detrimental effects (Lund and Lephart, 2001) still needs to be ascertained. Moreover, acute effects of isoflavones on male young subject are yet to be studied.

Daidzin is a major isoflavone from Puerariae radix and Soy bean. Previously, it has been reported that daidzin suppressed the LPS-induced production of TNF-α in mice (Hasumuma et al., 2007). Its antioxidative activity was reported in the DPPH free radical scavenging study (Cherdshewasart and Sutjit, 2008).

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Daidzein, an aglycon of daidzin, positively regulated cognitive function in woman (Howes et al., 2004; Kreijkamp-Kaspers et al., 2004). Recent studies have indicated that soya isoflavone supplementation for 12 weeks enhances spatial working memory in men (Thorp et al., 2009). Acute effects of isoflavones on learning and memory are yet to be conducted. In view of which, the present study was conducted to identify the effects of acute pre-acquisition trial administration of daidzin and its aglycon, daidzein, isolated from *Pueraria lobata* on scopolamine-induced memory impairment in male mice.

MATERIALS AND METHODS

Animals

Male ICR mice, weighing 25-30 g, were purchased from the Orient Co., Ltd, a branch of Charles River Laboratories. Animals were housed 5 or 6 per cage, allowed access to water and food *ad libitum*, and maintained in controlled temperature (23 ± 1°C) and humidity (60 ± 10%) environment under a 12-h light/dark cycle (light on 07:30-19:30 h). Animal handling, treatment and maintenance were carried out in accordance with the Principle of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and the Animal Care and Use Guidelines of Kyung Hee University, Korea.

Materials

Tacrine (9-amino-1, 2, 3, 4-tetrahydroacridine hydrochloride), (-) scopolamine hydrobromide, and tamoxifen citrate were purchased from the Sigma Chemical Co. Daidzin and daidzein were donated by one of the authors (J.S. Choi). All other materials were obtained from commercial sources and were of the highest grade available.

Passive avoidance task

Passive avoidance performance was carried out in two identical light and dark square boxes (20 × 20 × 20 cm, respectively) separated by a slide-up door (5 × 5 cm), as described in our previous report (Kim et al., 2006). In an acquisition trial, a mouse was initially placed in the light compartment and the door between the two compartments was opened 10 s later. The door automatically closed when the mouse entered the dark compartment and an electrical foot shock (0.5 mA, 3 s) was delivered through the grid floor. One hour prior to the acquisition trial, mice were orally treated with daidzin (1.25, 2.5, 5 or 10 mg/kg), daidzein (1.25, 2.5, 5 or 10 mg/kg), or tacrine (10 mg/kg, a positive control). Thirty min before testing mice were treated with scopolamine (1 mg/kg, i.p.) or vehicle. A retention trial was conducted 24 h after the acquisition trial. The mice were again placed in the light compartment and the latency to enter the dark compartment was recorded. When the mouse did not enter the dark compartment within 300 s, we concluded that the mouse had memorized the passive avoidance training after one acquisition trial. The control group received 10% Tween 80 solution (v/v). In a separate antagonism study, daidzin (5 mg/kg, p.o) or daidzein (5 mg/kg, p.o) treated mice were intraperitoneally co-administered scopolamine (1 mg/kg) with or without tamoxifen (1 mg/kg in 0.5% dimethylsulfoxide solution), 30 and 15 min prior to acquisition trial, respectively.

Morris water maze task

The Morris water maze is a circular pool (90 cm in diameter and 45 cm in height) with a featureless inner surface. The pool was filled to a depth of 30 cm with water containing 500 mL of milk (20 ± 1°C). The tank was placed in a dimly lit, soundproof test room with four visual cues. A white platform (6 cm in diameter and 29 cm high) was then placed in one of the pool quadrants. The first experimental day was dedicated to swimming training for 60 s in the absence of the platform. During the four subsequent days the mice were given two trials per session per day with the platform in place. When a mouse located the platform, it was permitted to remain on it for 10 s. If the mouse did not locate the platform within 60 s, it was placed on the platform for 10 s. The animal was taken to its home cage and was allowed to dry up under an infrared lamp after each trial. The time interval between each trial sessions was 30 min (Kim et al., 2006). During each trial session, the time taken and distance moved to find the hidden platform (latency time) was recorded using a video camera-based Ethovision System (Nodulus). One day after the last training trial sessions, mice were subjected to a probe trial session in which the platform was removed from the pool, allowing the mice to swim for 60 s to search for it. A record was kept of the swimming time in the pool quadrant where the platform had previously been placed. Daidzin (5 mg/kg, p.o), daidzein (5 mg/kg, p.o) or tacrine (10 mg/kg, p.o) was given 1 h before the first trial session at every consecutive day. Memory impairment was induced in mice with scopolamine (1 mg/kg, i.p.) at 30 min after treatment of drugs. Control group received 10% Tween 80 solution only.

Statistics

Values are expressed as mean ± S.E.M. For the

passive avoidance test and for the Morris water maze test, data were analyzed by one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test for multiple comparisons. For the antagonism study, interactions between tamoxifen and daidzin or daidzein were analyzed separately by two-way ANOVA; pairwise comparisons for the assessment of the influence of tamoxifen on the effects of daidzin or daidzein were conducted using Tukey's test. Statistical significance was set at $p < 0.05$.

RESULTS

Effect of daidzin and daidzein on the passive avoidance task

The step-through latency of scopolamine-treated mice in the passive avoidance task was significantly shorter than that of vehicle-treated control mice (Fig. 1, $p < 0.05$). In tacrine (a positive control) with scopolamine-treated mice, step-through latency was significantly higher than that of scopolamine-treated group. Moreover, the shorter step-through latency induced by scopolamine was significantly reversed by daidzin (5 mg/kg, p.o, Fig. 1A) and daidzein (5 or 10 mg/kg, p.o, Fig. 1B) administration, as compared to that of scopolamine-treated group ($p < 0.05$).

Effect of daidzin and daidzein on the Morris water maze task

In the Morris water maze task, the scopolamine-treated group exhibited longer escape latencies throughout the training days than the control group ($p < 0.05$, Fig. 2A and C). Daidzin (5 mg/kg, Fig. 2A) and daidzein (5 mg/kg, Fig. 2C) significantly shortened the escape latencies prolonged by scopolamine treatment ($p < 0.05$). Moreover, tacrine also significantly reduced escape latencies, compared with scopolamine-treated group ($p < 0.05$, Fig. 2A and C). On the day following the final day of training trial sessions, swimming duration within the platform quadrant for the scopolamine-treated group was significantly lower than those of the vehicle-treated control group ($p < 0.05$, Fig. 2B and D). Moreover, the shorter swimming time within the platform quadrant induced by scopolamine was significantly reversed by daidzin, daidzein or tacrine ($p < 0.05$, Fig. 2B and D).

Effects of tamoxifen antagonism on the effect of daidzin or daidzein on the passive avoidance task

The effects of tamoxifen (non-specific estrogen receptor antagonist) on the passive avoidance task were determined, where we observed that tamoxifen (2 or 4 mg/kg, i.p.) effectively impaired the passive avoidance memory (data not shown). In order to study the interaction between the effects of daidzin or daidzein and the estrogen receptors, tamoxifen was administered at 1 mg/kg so as to eliminate the deteriorative activity of tamoxifen on the learning and memory. As shown in Fig. 3, the increased latency time induced by daidzin

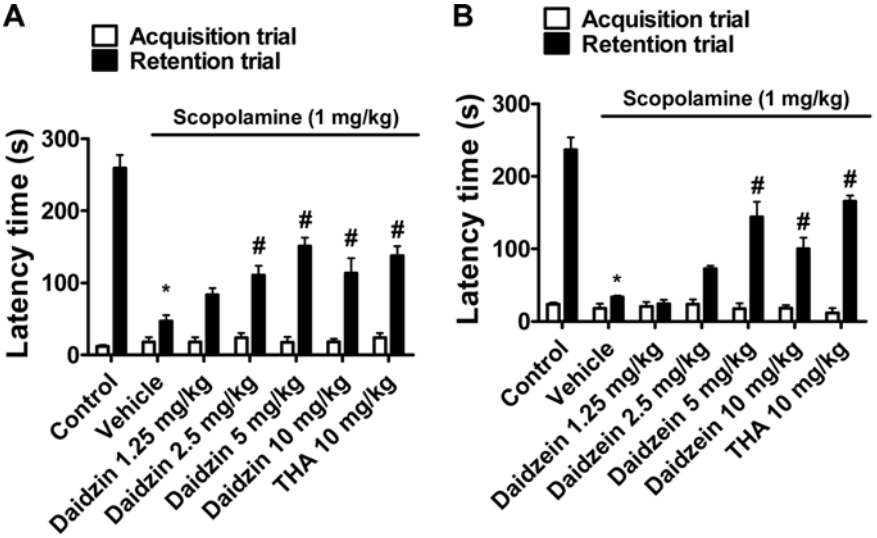

Fig. 1. Effect of a single administration of daidzin or daidzein on scopolamine-induced memory deficits in the passive avoidance task. At 60 min before an acquisition trial, daidzin (1.25-10 mg/kg, p.o, **A**), daidzein (12.5-10 mg/kg, p.o, **B**), tacrine (THA, 10 mg/kg, p.o) or vehicle (same volume of 10% Tween 80) solution were administered to mice. Memory impairment was induced by scopolamine treatment (1 mg/kg, i.p.). Acquisition trial was carried out 30 min after a single scopolamine treatment. At 24 h after the acquisition trial, the retention trial was carried out for 3 min. Data represent mean ± S.E.M. ($n = 10$). *$p < 0.05$ *vs* vehicle control group, $^{\#}p < 0.05$ *vs* scopolamine treated group.

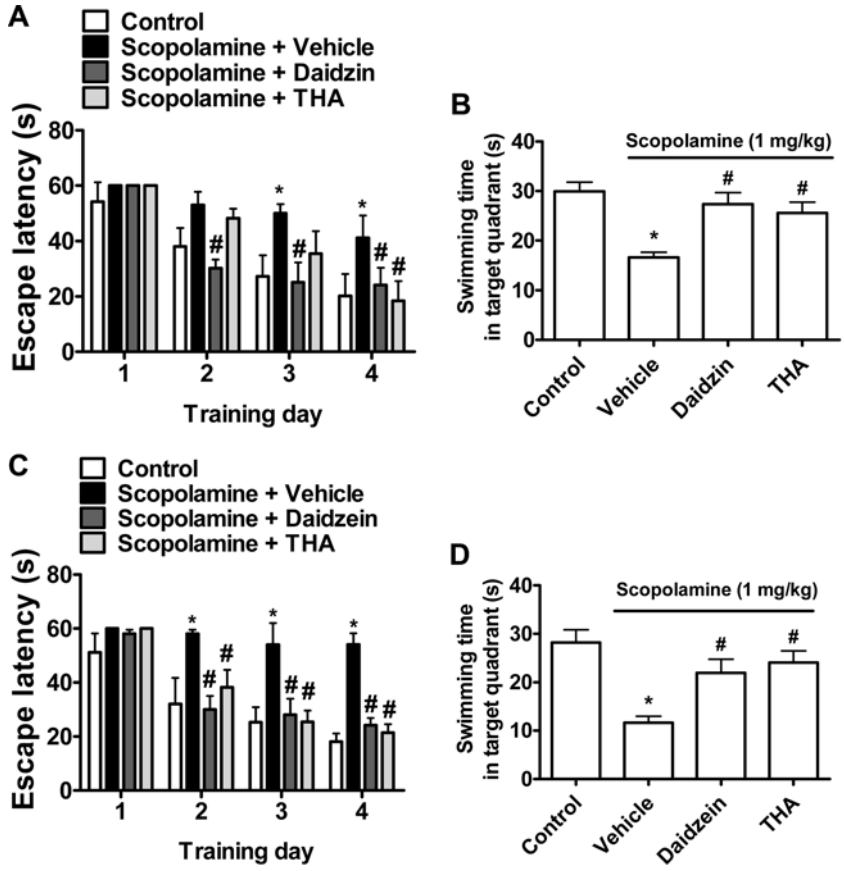

Fig. 2. Effect of a single administration of daidzin (**A**, **B**) or daidzein (**C**, **D**) on the latency time in the training trial sessions (**A** and **C**) and on the swimming time (**B** and **D**) in the probe trial session of the Morris water maze task in scopolamine-induced memory deficits in mice. At 60 min before training trial session, daidzin (5 mg/kg, p.o), daidzein (5 mg/kg, p.o), tacrine (THA, 10 mg/kg, p.o) or vehicle (Con., same volume of 10% Tween 80) solution was administered to mice. Memory impairment was induced by scopolamine treatment (1 mg/kg, i.p.). The training trial and the probe trial sessions were performed as described in the Materials and Methods section. Data represent means ± S.E.M. (n = 10). $^{*}p < 0.05$ *vs* vehicle control group, $^{\#}p < 0.05$ *vs* scopolamine-treated group in graph.

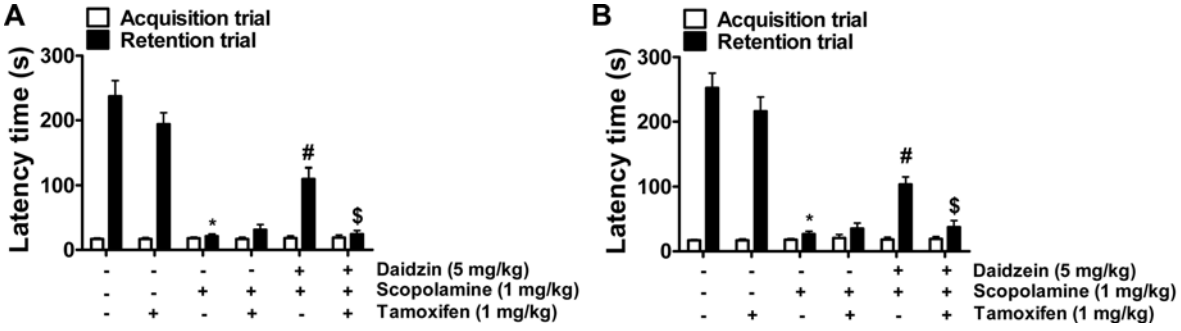

Fig. 3. The effects of a single administration of daidzin (**A**) or daidzein (**B**) on the memory impairment induced by scopolamine via estrogen receptor. At 60 min before an acquisition trial, daidzin (5 mg/kg, p.o), daidzein (5 mg/kg, p.o) or the same volume of 10% Tween 80 solution was administered, and 30 min later, mice were injected with scopolamine (1 mg/kg, i.p.), and 15 min after a single tamoxifen treatment (1 mg/kg, i.p.) was conducted. Acquisition trials were carried out 15 min after a single tamoxifen treatment. Data represent means ± S.E.M. (n = 10). $^{*}p < 0.05$ *vs* vehicle controls, $^{\#}p < 0.05$ *vs* scopolamine treated mice, $^{\$}p < 0.05$ *vs* scopolamine and daidzin or daidzein co-treated mice.

or daidzein with scopolamine treatment was completely eliminated by tamoxifen ($p < 0.05$) specifically evident in the scopolamine-treated group. The interaction between daidzin or daidzein and tamoxifen showed significant group effects [F (1, 36) = 19.113, $p < 0.001$, Fig. 3A; F (1, 36) = 42.328, $p < 0.001$, Fig. 3B].

DISCUSSION

In the present study, we attempted to examine the effects of diadzin and its aglycon, daidzein, on memory impairment induced by scopolamine treatment using the passive avoidance task and the Morris water maze task in male mice. Results of these behavioral studies indicate that daidzin and daidzein definitely have ameliorating effects on the scopolamine-induced memory dysfunction.

It is well-known that the cholinergic system affects memory performance and its antagonist induces amnesia by blocking central cholinergic transmission (Sarter and Bruno, 1997). Therefore, cognition-improving agents may restore cholinergic transmission either by acting directly as agonists on muscarinic receptors or indirectly by overcoming their blockade. In a pilot study, we conducted receptor binding study using daidzein and daidzin on muscarinic receptors. Both of drugs did not exhibit any significant interaction with muscarinic receptors up to 100 μM concentration. Thus, the first hypothesis that drugs directly affected muscarinic receptors has to be excluded (data not shown). Further, we tested the effect of daidzein and daidzin on acetylcholinesterase activity because acetylcholinesterase inhibition is the most powerful concept on indirect effect on scopolamine-induced cholinergic receptor blockade (Giacobini, 1996). In this study, daidzein and daidzin did not show any effect on the acetylcholinesterase activity (data not shown).

Previously, Heo et al suggested that prolonged administration of daidzein might play a role in acetylcholine biosynthesis as a choline acetyltransferase activator, and that it also ameliorates scopolamine-induced amnesia in male mice (Heo et al., 2006). The second dimension to this phenomenon is estradiol, a major estrogen which facilitates hippocampal acetylcholine release (Gibbs et al., 2004; Mitsushima et al., 2009a, 2009b), by an increase in choline acetyltransferase activity and cholinergic neuron in the memory-related brain regions (Ping et al., 2008). Previous study have reported that intra-hippocampal estradiol injections enhance spatial memory in male rat through cholinergic enhancement (Packard et al., 1996). In addition, systemic injection of estradiol also enhanced spatial working memory and this was blocked by sub-effective dose of scopolamine (Packard and Teather, 1997). These reports suggested that acute injection of estradiol enhances cognitive performances, and that estradiol may influence memory through an interaction with muscarinic cholinergic systems. Therefore, there is a possibility that daidzein or daidzin is able to ameliorate memory impairment induced by cholinergic dysfunction through its agonistic property to estrogen receptors. In order to test this hypothesis, we conducted antagonism study using tamoxifen, an estrogen receptor antagonist. The results showed that the effects of daidzin or daidzein on cognitive function were perfectly blocked by tamoxifen. This result suggests that daidzin or daidzein improves the cognitive dysfunction induced by cholinergic blockade through its estrogenic activity in male mice. Lund et al. reported that the performance of a visual spatial memory test was worse in male rats consuming a high-isoflavone diet than in rats consuming an isoflavone-free diet (Lund et al., 2001). On the other hand, it was reported that male rats consuming the soy isoflavone diet significantly outperformed compared to the male rats consuming the isoflavone-free diet in cognitive function (Lee et al., 2004). Several previous reports suggested that the acute and the chronic treatment of estrogens might differently regulate brain function (Osterlund et al., 2000; Gulinello et al., 2006). Previous studies that dealt with phytoestrogens on cognitive function using male mice observed these effects on long-term supplementation of these compounds (Heo et al., 2006). Chronic treatment of estrogen can make estrogen receptor down regulated. Therefore, the period or dose of treatment can seriously affect the regulation of their receptor. In the present study, intrinsic activity of phytoestrogens on brain function can be observed.

In summary, this study suggests that acute administration of daidzin or daidzein acts on estrogen receptor to improve the memory impairment induced by cholinergic dysfunction.

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