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Sulforaphane alleviates scopolamine-induced memory impairment in mice

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ABSTRACT

Sulforaphane, an organosulfur compound present in cruciferous vegetables, has been shown to exert neuroprotective effects in experimental *in vitro* and *in vivo* models of neurodegeneration. To determine whether sulforaphane can preserve cognitive function, we examined its effects on scopolamine-induced memory impairment in mice using the Morris water maze test. Sulforaphane (10 or 50 mg/kg) was administered to C57BL/6 mice by oral gavage for 14 days (days 1–14), and memory impairment was induced by intraperitoneal injection of scopolamine (1 mg/kg) for 7 days (days 8–14). Mice that received scopolamine alone showed impaired learning and memory retention and considerably decreased cholinergic system reactivity in the hippocampus and frontal cortex, as indicated by a decreased acetylcholine (ACh) level and an increased acetylcholinesterase (AChE) activity. Sulforaphane significantly attenuated the scopolamine-induced memory impairment and improved cholinergic system reactivity, as indicated by an increased ACh level, decreased AChE activity, and increased choline acetyltransferase (ChAT) expression in the hippocampus and frontal cortex. These effects of sulforaphane on cholinergic system reactivity were confirmed *in vitro*. Sulforaphane (10 or 20 μM) increased the ACh level, decreased the AChE activity, and increased ChAT expression in scopolamine-treated primary cortical neurons. These observations suggest that sulforaphane might exert a significant neuroprotective effect on cholinergic deficit and cognitive impairment.

1. Introduction

Sulforaphane (1-isothiocyanato-4-methylsulfinylbutane; Fig. 1A) is an organosulfur compound found in cruciferous vegetables *e.g.*, broccoli, cabbage, watercress, and Brussels sprouts [1]. Sulforaphane first attracted interest because of its potential anti-cancer activity [2]. However, because of its diverse pharmacological properties including cardiovascular protection, protection against diabetic nephropathy, neuropathy, and *Helicobacter pylori* infection, and restoration of skin integrity [2], this compound is currently being tested in several clinical trials (www.clinicaltrials.gov). Recent *in vitro* and *in vivo* studies have examined the potential neuroprotective actions of sulforaphane. For example, sulforaphane has been shown to protect cortical neurons against 5-*S*-cysteinyl-dopamine-induced toxicity [3] and Neuro2A and N1E 115 cells against amyloid β-induced toxicity [4]. In rodents, sulforaphane reduces infarct volume following focal cerebral ischemia [5] and attenuates brain edema following traumatic brain injury [6]. However, the potential effects of sulforaphane on cholinergic system and cognitive function have not yet been elucidated.

The central cholinergic system is important in the regulation of cognitive function. The neurotransmitter acetylcholine (ACh),

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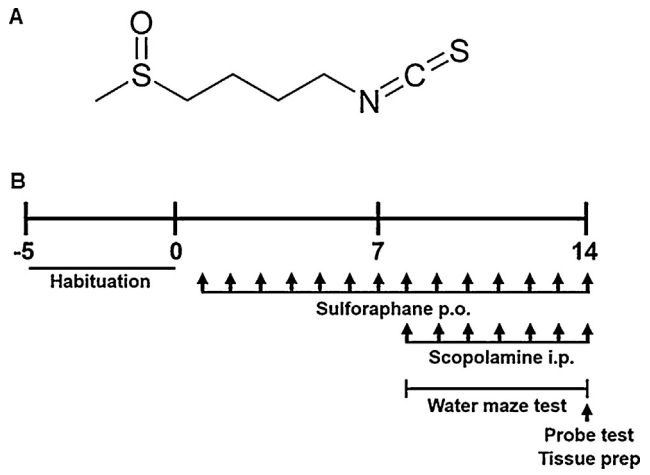

Fig. 1. Chemical structure of sulforaphane and study design. (A) Structure of sulforaphane. (B) After a 5-day adaption period, the mice were given sulforaphane (10 or 50 mg/kg, p.o.) for a total of 14 days. Beginning on day 8, scopolamine (1 mg/kg, i.p.) was also administered for 7 days, and the Morris water maze test was used to assess behavior during this period (days 8–14). On day 14, each mouse was sacrificed, and the hippocampus and frontal cortex were removed for the analysis of acetylcholine level, acetylcholinesterase activity, and choline acetyltransferase expression.

which is synthesized by choline acetyltransferase (ChAT) in cholinergic neurons [7], is required for central and peripheral control of multiple cognitive processes including timing, attention, learning, and memory [8]. Accordingly, lesions of the cholinergic neurons that decrease ACh release into the synaptic cleft result in learning and memory dysfunction [9]. The duration of ACh action is dependent upon the activity of acetylcholinesterase (AChE), which hydrolyzes ACh after its release [10,11]. Inhibition of AChE is the therapeutic approach used for Alzheimer's disease, other types of dementia, traumatic brain injury, and delirium, and it may potentially be useful in the treatment of schizophrenia [12]. ChAT is the most specific marker of cholinergic neuron function in the central and peripheral nervous systems [13]. The activity of ChAT is strongly reduced in aged brains, and the degree of reduction in activity is significantly correlated with the severity of cognitive impairment [14].

Scopolamine is a nonselective muscarinic ACh receptor (mAChR) antagonist that mainly targets M1AChR and M2AChR, thereby impairing learning acquisition and short-term memory in rodents and humans [15–17]. Scopolamine-induced amnesia has been used to generate experimental animal models for the screening of antiamnesic drugs [10]. To tease out the effects that result in cognitive benefits, it is essential to test drugs using a memory impairment model that is nondegenerative and shows cholinergic dysregulation. The hippocampus and frontal cortex are particularly vulnerable to scopolamine-induced neuronal injury [18].

To elucidate the potential effects of sulforaphane on cognitive function and the cholinergic system, we evaluated learning and memory retention in C57BL/6 mice using the Morris water maze test and assessed cholinergic markers (ACh, AChE, and ChAT) in C57BL/6 and primary cortical neurons exposed to scopolamine.

2. Materials and methods

2.1. Materials

Sulforaphane was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Scopolamine hydrochloride, fetal bovine serum (FBS), and antibodies against β-actin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium, Neurobasal medium, Ca^{2+}/Mg^{2+}-free Hank's Balanced Salt Solution (HBSS), L-glutamine, B-27 supplement, and penicillin/streptomycin were purchased from Gibco BRL (Carlsbad, CA, USA). The Amplex Red ACh/AChE assay kit was purchased from Invitrogen (Grand Island, NY, USA). Antibodies against ChAT were purchased from Abcam (Cambridge, United Kingdom).

2.2. Animals

Female C57BL/6 mice (age, 8 weeks; weight, 25–27 g) were purchased from Orient Bio (Seoul, South Korea) and housed in a regulated environment (21 ± 2 °C, 12-h light/dark cycle, light period starting at 8 AM) with free access to food and water. All experiments were conducted in compliance with Konkuk University's Council Directive for the care and use of laboratory animals (KU11025). To test the effect of sulforaphane on cognitive function, the mice were randomly assigned to four treatment groups (n = 10 per group): (1) vehicle (phosphate buffered saline [PBS]), (2) scopolamine (1 mg/kg/day, 100 μl/day), (3) sulforaphane (10 mg/kg/day, 100 μl/day) + scopolamine (1 mg/kg/day, 100 μl/day), and (4) sulforaphane (50 mg/kg/day, 100 μl/day) + scopolamine (1 mg/kg/day, 100 μl/day). Sulforaphane was dissolved in PBS and administered by oral gavage (p.o.) on days 1–7, prior to training for the Morris water maze test, and continued on days 8–14, during which the mice underwent the Morris water maze test. Scopolamine was dissolved in PBS and administered by intraperitoneal (i.p.) injection on days 8–14. On days 8–14, sulforaphane was administered 60 min before the trial, and scopolamine was injected 30 min before the trial. On day 14, the mice were decapitated immediately following the Morris water maze test. The hippocampus and frontal cortex were removed, dissected on ice, and frozen at −80 °C until analysis. The experimental design is summarized in Fig. 1B.

2.3. Cell culture

Primary cortical neurons were prepared from ICR mice at embryonic day 15. Cerebral cortices from the embryos were dissected and placed in ice-cold Ca^{2+}/Mg^{2+}-free HBSS (4 ml/embryo). The tissue was centrifuged (300 × g, 2 min), dissociated with 0.05% trypsin in HBSS for 10 min at 37 °C, resuspended in minimal essential medium containing 10% FBS, 10% heat-inactivated horse serum, 2 mM L-glutamine, and 1% penicillin/streptomycin and then filtered twice through a 70-μm cell strainer. Cells were seeded on 0.2-mg/ml poly-D-lysine-coated plates and incubated in a 37 °C humidified atmosphere. The cells were allowed to adhere to the plates for 45 min before the culture media was changed to Neurobasal medium supplemented with B27, 1% L-glutamine, and 1% penicillin/streptomycin. Neurites were observed sprouting from neuronal cell bodies on day 3 after the initial plating. Neurobasal medium was changed every other day. The cortical neurons were treated with sulforaphane (10 or 20 μM) for 1 h, followed by 20 μM scopolamine for 6 or 12 h; PBS was used as the vehicle.

2.4. Morris water maze test

The water maze test was conducted in a circular tank (diameter 183 cm, height 58 cm) filled with water maintained at 25 ± 2 °C. The tank was divided into four quadrants with a hidden escape platform (diameter, 20 cm; height, 48 cm) submerged 1.5 cm below the water surface in the center of one quadrant. The mice were trained to find the hidden platform by learning and memorizing several visual cues placed outside the maze. The position of the cues remained unchanged throughout the experiments. Four trials were conducted on each day of the training period (days 8–13). Each mouse was given 60 s to find the hidden platform and allowed to remain on it for another 30 s. The mean time used to find the