Biosci. Biotechnol. Biochem., **72** (2), 577–581, 2008

Note

Zeatin Supplement Improves Scopolamine-Induced Memory Impairment in Mice

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Received July 30, 2007; Accepted November 14, 2007; Online Publication, February 7, 2008
[doi:10.1271/bbb.70480]

In this study, our aim was to clarify the ameliorative effects of zeatin, a development hormone in plants. Zeatin mitigated cognitive deficits and showed AChE inhibition in scopolamine (Scop)-induced mice following 21 d of zeatin treatment. After administration of Scop for 30 min, each mouse performed Y-maze and step-down latency tasks as a check on immediate against cognitive function. The results showed that zeatin administration attenuated Scop-induced memory damage and decreased AChE activity in the mice. This suggests that zeatin might be useful for protecting cognitive dysfunction, as well as for reducing the activation of AChE in dementia.

Key words: acetylcholinesterase; cognitive function; zeatin [6-(4-hydroxy-3-methyl-trans-2-butenylamino) purine]; scopolamine; Alzheimer's disease

Alzheimer's disease (AD) is characterized by a loss of cholinergic neurons and their cortical projections from the basal forebrain area. The resulting reduction in cholinergic activity is correlated with the degree of cognitive impairment, and is associated with decreased levels of the neurotransmitter acetylcholine (ACh), choline acetyltransferase (the rate-limiting enzyme for ACh synthesis), and increased ACh-hydrolyzing enzyme acetylcholinesterase (AChE). The cholinergic hypothesis for AD has been the basis for the development of presynaptic, synaptic, and postsynaptic treatment approaches designed to maintain and facilitate the activity of the surviving cholinergic system.[1)] The abnormal activities of ChAT and AChE are correlated with the degree of dementia and the severity of the neuropathological hallmarks of AD.[2)]

The principal role of AChE is termination of nerve impulse transmissions at the cholinergic synapses by rapid hydrolysis of ACh. Inhibition of AChE serves as a strategy in the treatment of AD, senile dementia, ataxia, myasthenia gravis, and Parkinson's disease.[3,4)] There are a few synthetic medicines, *e.g.*, tacrine, donepezil, and the natural product-based rivastigmine, for treatment of the cognitive dysfunction and memory loss associated with AD.

These compounds, however, are reported to have adverse effects, including gastrointestinal disturbances and problems associated with bioavailability, which stimulates interest in finding better AChE inhibitors from natural sources. A variety of plants are reported to show AChE inhibitory activity, and hence, may be relevant in the treatment of neurodegenerative disorders such as AD.[5)] In a previous study, Heo *et al.*[2)] proposed that *Fiatoua villosa* showed excellent effects of AChE inhibition among several traditional Korean plants, and zeatin, purified and characterized from *Fiatoua villosa*, was suggested to be an AChE inhibitor. In this study, the ameliorative effects of zeatin against Scop-induced learning and memory impairments were investigated *via* Y-maze tasks and step-through latency tasks in mice. In addition, AChE inhibition was examined by detection of AChE activity in mouse brains.

Male ICR mice (Samtaco BioKorea, Seoul, Korea) were used to measure cognitive function after a one-week adaptation period (20–22 °C, 12 h light/dark cycle). The mice were divided into five groups, and each group consisted of eight mice. Water and feed in the form of dry pellets were available *ad libitum*. All experiments were conducted according to Korea University's Committee on the Care and Use of Laboratory Animals Guidelines. Zeatin (Sigma Chemi-

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cal, St. Louis, MO) was fed every day in water at concentrations of 0.002%, 0.004%, and 0.008% (w/v). The mice were allowed free access to either normal drinking water (control and scop-control groups) or the zeatin solution (zeatin-treated groups) for 21 d. Amnesia was induced in the mice with Scop (1 mg/kg body weight), given subcutaneously. Behavioral tests were started 30 min after injection. Learning and memory were assessed by two separate tests: passive avoidance and Y-maze tests.[6)]

A step-through passive avoidance test was used to evaluate the effects of zeatin on learning and memory. A shuttle box was divided into two chambers, one illuminated and the other dark, separated by a guillotine door. During the training trial, each mouse was placed in the lighted compartment, and when the mouse entered the dark compartment the door closed and the mouse received an inescapable electric shock (0.5 mA, 1 s). The test trial was given 1 d after the training trial, and again the mouse was placed in the lighted compartment and the latency time to enter the dark compartment was measured. If the mouse did not enter the dark chamber within the cut-off time (300 s), it was assigned a latency value of 300 s. A Y-maze test was used to assess the immediate working memory performance of the mice by recording their spontaneous alternation behaviors in a single session.[7)] Each mouse, unfamiliar with the maze, was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The sequence of arm entries was recorded visually, and alternation was defined as successive entries into the three arms in non-overlapping triplet sets. The percent alternation was calculated as the successive entries divided by the total arm entries minus two, multiplied by 100. The animals were sacrificed by decapitation after completion of the Y-maze and passive avoidance tests. The whole brain tissues were immediately removed and homogenized with 12.5 mM sodium phosphate buffer (pH 7.0), and the mixture was centrifuged for 30 min at 34,000 × *g*.[8,9)] The supernatant obtained was used to determine AChE inhibition. The AChE inhibition assay was performed according to the colorimetric method of Ellman *et al.*[10)] using acetylthiocholine iodide as the substrate. AChE inhibition was assessed by modifications of Ellman's method, which is based on the reaction of released thiocholine to give a colored product with a chromogenic reagent. The whole brain tissues were homogenized with 12.5 mM sodium phosphate (pH 7.0) buffer, and the mixture was centrifuged for 30 min at 34,000 × *g*. The supernatant obtained was used to determine AChE inhibition. All the extraction steps were carried out at 4 °C. Protein concentration was measured by the Bradford assay with bovine serum albumin as the protein standard. The rate of hydrolysis by AChE was monitored spectrophotometrically using the 96-well microtiter plate format. The samples were incubated with Ellman's reaction mixture (1 mM 5,5′-dithio-bis (2-nitro benzoic acid), 70 µl: 0.5 mM acetylthiocholine) in 50 mM sodium phosphate buffer (pH 8.0) to the above reaction mixture at 37 °C for 15 min. After incubation, the absorbance was measured at 405 nm.

After evaluation of the experimental values for each group, the value of the control group was converted to 100%. The meaning of 100% AChE inhibition of control was the basis for comparison with the other groups. We converted values of the other groups into percent data using the control value.

All data are presented as the mean ± SE. A one-way ANOVA test was used to determine the effects of the treatment. The differences among the means were inspected by Duncan's multiple range tests, and were considered to be significant at a P value of < 0.05.

Recently, herbal medicines and their compounds have been applied in the treatment of AD patients to improve their memory-related behaviors, but there is little scientific evidence on the effectiveness of such herbal medicines, and there have been no systemic pharmaceutical screenings of the product compounds.[11)] In this study, mice were treated with zeatin *via* drinking water at various concentrations, of 0.002%, 0.004%, and 0.008% (w/v), for up to 21 d prior to Scop prescription. Scop, which is a competitive antagonist at the muscarinic acetylcholine receptor, causes memory deficits and decreased cholinergic activity during behavioral performance.[12)]

Therefore, this method of Scop exposure is a useful *in vivo* model for AD. Spontaneous alternation behavior was evaluated *via* a Y-maze test, and was regarded as a measure of immediate spatial working memory. The number of entries was similar among the experimental groups (Fig. 1A). In contrast, the mice induced only with Scop showed significantly reduced alternation behavior (approximately 20%) as compared to the normal control group, but pretreatment with 0.002%, 0.004%, and 0.008% zeatin reduced the affect of Scop on alternation behavior by approximately 16%, maximally, as compared to the scopolamine-only group (Fig. 1B). This result indicates that Scop injection did not affect the general locomotor activities of the mice, but gave rise to learning disabilities them.

In step-through latency (STL) using the passive avoidance paradigm, the Scop-treated mice showed significantly shorter latency times during the retention trials, with a 47% decrease in STL as compared to control. The mice administered zeatin for 21 d prior to testing showed attenuated Scop-induced memory impairment. The STL decreased approximately 26% and 36% in the 0.002% and 0.008% (w/v) concentration zeatin pretreatment groups respectively (Fig. 2). This result indicates that 21 d of zeatin pretreatment effectively prevented Scop-induced reductions in the passive performance test. AChE activity fluctuated abnormally in the Scop-induced mice, but providing zeatin at concentrations of 0.002%, 0.004%, and 0.008% (w/v) inhibited abnormal changes in AChE activity (Fig. 3). In particular, AChE inhibition was greatly increased in the

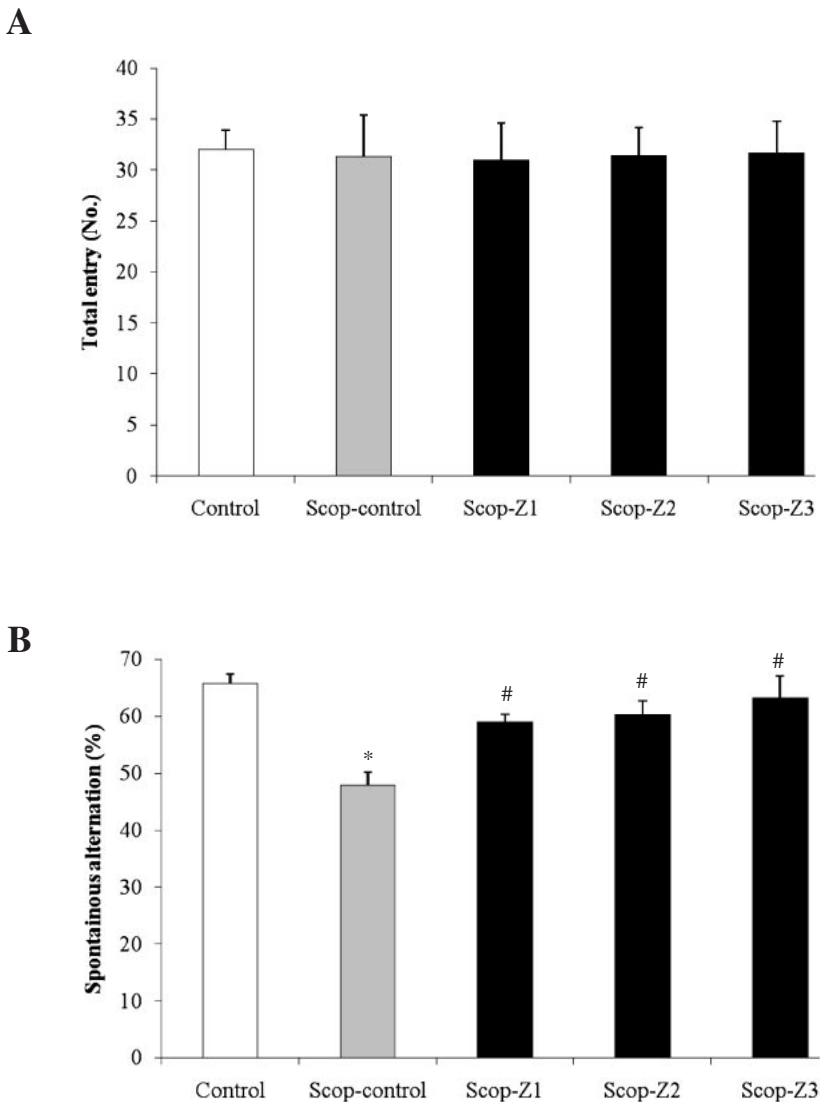

Fig. 1. The Ameliorative Effects of Zeatin against Scop-Induced Learning and Memory Impairments in Mice.

Each behavioral test was conducted 30 min after Scop injection. The numbers of arm entries (A) and spontaneous alternation behavior (B) were measured during an 8-min session. Control mice were injected with only the vehicle (100 µl). The data represent the mean ± SE (n = 8). A is non-significant. $^{*}P < 0.05$ *vs.* control. #$P < 0.05$ *vs.* Scop-treated control. Scop-Z1, Scop-treated mice fed zeatin (0.002%); Scop-Z2, Scop-treated mice fed zeatin (0.004%); Scop-Z3, Scop-treated mice fed zeatin (0.008%).

mice that received the 0.008% zeatin solution. These results indicate that Scop prescription caused cognitive deficits and degeneration of the neurotransmission system, but administration of zeatin improved memory deficits and behavioral disabilities caused by neuronal damage, including those caused by toxic compounds. A consistent finding among AD patients is the loss of cholinergic markers, including reduced levels of ACh and ChAT in the brain. The cholinergic approach to treating AD involves counteracting this loss of cholinergic activity by increasing cholinergic transmission through pharmacological intervention.[13] A relevant animal model of AD is an important tool in the ongoing work of understanding its pathology and in finding better treatments. Although no current model can develop the full pathologic spectrum of the disease, the injection of an excitotoxin has been found to impair memory and elicit a degree of Alzheimer-type neurodegeneration.[14] The key roles of the elements of the acetylcholine system (ChAT, ACh, and AChE) in normal brain function, and in the memory disturbances of AD, have been well documented.[14] In this study, Scop-treated mice showed reductions in memory and behavioral abilities; specifically, they had remarkably shorter step-through latency times and increased AChE activity as compared to control mice.

The Scop model has been used extensively to learn more about the status of the cholinergic system and its role in cognition in aging and AD, as well as in evaluating possible therapeutic agents for treating memory impairment. The muscarinic antagonist Scop, administrated to animals, is capable of producing

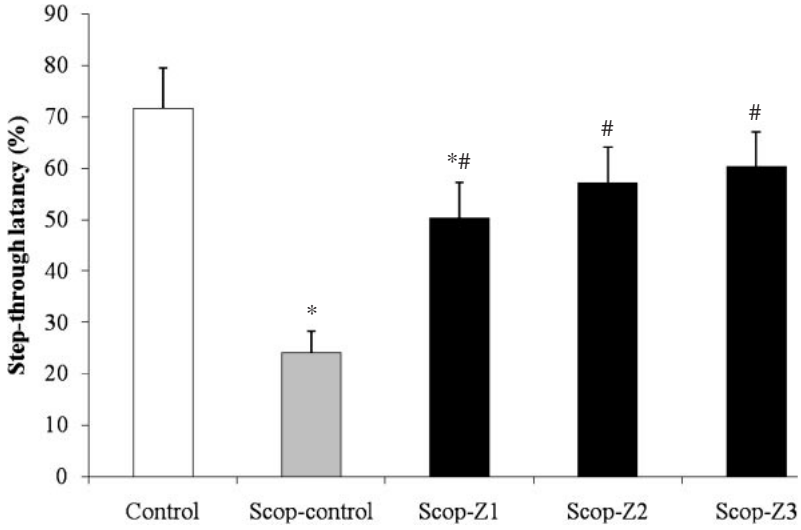

Fig. 2. The Ameliorative Effects of Zeatin against Scop-Induced Step-Through Latency Impairments in Mice.

Effects of zeatin on step-through latency (%) in the retention trial for the passive avoidance task. Each behavioral test was conducted 30 min after Scop injection. Control mice were injected with only the vehicle (100 µl). The data represent the mean ± SE (n = 10). $^{*}P < 0.05$ *vs.* control. $\#P < 0.05$ *vs.* Scop-treated control. Scop-Z1, Scop-treated mice fed zeatin (0.002%); Scop-Z2, Scop-treated mice fed zeatin (0.004%); and Scop-Z3, Scop-treated mice fed zeatin (0.008%).

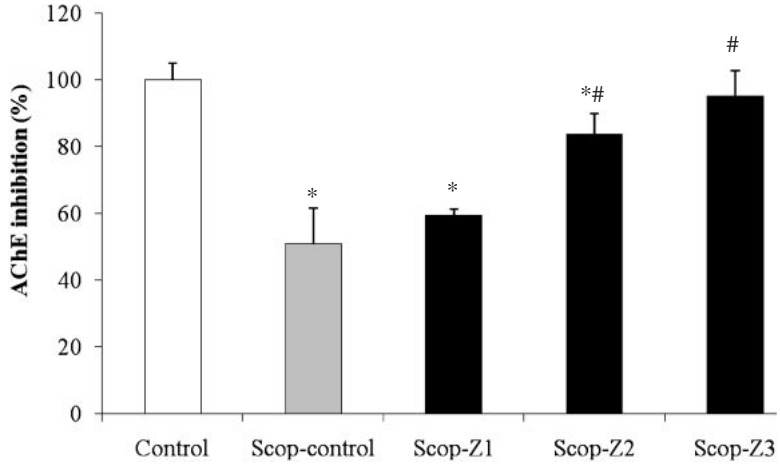

Fig. 3. The Effect of Zeatin on AChE Inhibition in Scop-Treated Mice Brains.

The data represent the means ± SE (n = 10). $^{*}P < 0.05$ *vs.* control. $\#P < 0.05$ *vs.* Scop-treated control. Scop-Z1, Scop-treated mice fed zeatin (0.002%); Scop-Z2, Scop-treated mice fed zeatin (0.004%); Scop-Z3, Scop-treated mice fed zeatin (0.008%).

transiently some of the learning acquisition and short-term memory deficits that are considered to be characteristic of AD.[13] Other studies have reported that Scop administration causes interference with other neurotransmitter systems responsible for alternational processes in learning and memory.[15,16] Zeatin, 6-(4-hydroxy-3-methyl-trans-2-butenylamino) purine, is a division factor isolated from young sweet corn (*Zea mays*) kernels, and it is the most active cytokinin.[17] It is widely used to induce the formation of plants, but the compound has scarcely been studied as a biomaterial against organisms. Cytokinins are hormones believed to be involved in the growth, cell division, and differentiation processes within all plants. When added to cultures of excised auxotropic tissue of higher plants, cytokinins promote rapid cell division and continuous growth of callus.[18] Several studies have suggested that cytokinins affect many types of tumor cell death caused by the inhibition of cyclin-dependent kinase (CDK).[19,20] An increase in endogenous H_2O_2 levels promotes senescence, and H_2O_2 reduction is one effect of cytokinins that leads to delayed senescence. Furthermore, cytokinins might help in the delay of oxidative damage, not only by inducing the activity of some antioxidant enzymes, but also by increasing the content of antioxidants.[21] Although the mechanisms of cytokinins are not well known in animal organisms, these studies suggest that cytokinins affect organisms and plants in many ways. Still, there have been no studies on the aging brain, or disease-attacked brain, and cytokinins.

According to our results, zeatin administration ameliorated Scop-induced memory injury in mice, suggesting that zeatin might be a potential chemo-preventive agent against AD and the memory injuries caused by neurotoxic compounds.

Acknowledgment

This study was supported by a grant (code no. 50401034615) from the BioGreen 21 Program, Rural Development Administration, Republic of Korea.

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