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Novel Cognitive Improving and Neuroprotective Activities of *Polygala Tenuifolia* Willdenow Extract, BT-11

Cheol Hyoung Park,[1] Se Hoon Choi,[1] Ja-Wook Koo,[2] Ji-Heui Seo,[1] Hye-Sun Kim,[1] Sung-Jin Jeong,[1] and Yoo-Hun Suh[1]*

[1]Department of Pharmacology, College of Medicine, National Creative Research Initiative Center for Alzheimer's Dementia and Neuroscience Research Institute, MRC, Seoul National University, Seoul, South Korea

[2]School of Biological Sciences, Seoul National University, Seoul, South Korea

We carried out this study to search a new active constituent that had cognitive enhancing activity and low side effects from natural source. We found that the extract of dried root of *Polygala tenuifolia* Willdenow (BT-11, 10 mg/kg, i.p.) could significantly reverse scopolamine-induced cognitive impairments in rat, using a passive avoidance and a water maze test. We also investigated the effects of BT-11 on neurotoxicity induced by glutamate (Glu) and toxic metabolites of amyloid precursor protein (APP) such as amyloid β protein (Aβ) and C-terminal fragment of APP (CT) in primary cultured neurons of rat. The pretreatment of BT-11 (0.5, 3, and 5 μg/ml) significantly reduced cell death induced by Glu (1 mM), Aβ (10 μM) and CT105 (10 μM) in a dose-dependent manner. In addition, BT-11 inhibited acetylcholinesterase (AChE) activity in a dose-dependent and non-competitive manner (IC_{50} value; 263.7 μg/ml). Our novel findings suggest the possibility that this extract may have some protective effects against neuronal death and cognitive impairments in Alzheimer's disease (AD), or other neurodegenerative diseases related to excitotoxicity and central cholinergic dysfunction.

Key words: *Polygala tenuifolia* Willdenow; cognitive function; excitotoxicity; amyloid β protein; anticholinesterase

Clinical and neuropathological characteristics of Alzheimer's disease (AD) are progressive deterioration in cognitive function and widespread cerebral deposition of a 40–43-amino acid peptide called amyloid β protein (Aβ) in the form of amyloid fibrils (Glenner and Wong, 1984; Selkoe 1994). Numerous studies have demonstrated that Aβ plays a crucial role in the pathogenesis of AD by inducing dysregulation of ionic homeostasis (Mattson et al., 1993), generation of reactive oxygen species (Behl et al., 1994), neuroinflammation (Eikelenboom et al., 1994), interaction with acetylcholinesterase (AChE) for aggregation (Inestrosa et al., 1996), and inhibition of choline uptake (Kar et al., 1998). Increasing evidence has suggested recently that other cleaved products of amyloid precursor protein (APP) such as amyloidogenic C-terminal fragments (CT) might also be an important factor in inducing neuronal death associated with AD (Yankner et al., 1989; Neve et al., 1992; Kim and Suh, 1996, Lee et al., 2000; Cao and Sudhof, 2001; Choi et al., 2001; Rah et al., 2001). Thus, reducing neurotoxicity induced by abnormally metabolized APP products, such as Aβ and CT, may be one beneficial approach to block AD pathogenesis.

It has been reported that excitatory amino acid, glutamate (Glu), may be one of the factors that could contribute to neuronal damages in neurodegenerative diseases such as stroke, brain trauma, Parkinson's disease (PD), and AD (Hagberg et al., 1987; Koh et al., 1990; Iwasaki et al., 1992; Vespa et al., 1998). With these accumulated data suggesting a role of Glu in the pathophysiological mechanisms of neurodegenerative diseases (Beal, 1992; Mattson et al., 1992), an approach to antagonize excitotoxicity has been suggested as a therapeutic method (Turski et al., 1991; Smith et al., 2000).

Profound losses in the cholinergic system of brain are associated closely with cognitive deficits observed in AD (Bartus et al., 1982; Gaykema et al., 1992; Coyle et al., 1993; Cummings and Kaufer, 1996). It is well documented that the selective inhibition of central AChE might increase acetylcholine (ACh) neurotransmission in the synaptic cleft of brain, resulting in improvements in cognitive function (Doležˇ et al., 1991; Tsukada et al., 1997; Wang and Tang, 1998). Although AChE inhibitors

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C.H. Park and S.H. Choi contributed equally to this study.

*Correspondence to: Yoo-Hun Suh, Department of Pharmacology, College of Medicine, National Creative Research Initiative Center for Alzheimer's Dementia, Seoul National University, Seoul 110-799, South Korea. E-mail: yhsuh@plaza.snu.ac.kr

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such as donepezil (Rogers et al., 1998), galantamine (Tariot et al., 2000), and rivastigmine (Rosler et al., 1999) are currently available for the symptomatic treatment of patients with mild to moderate AD, some deficiencies in safety and efficacy limit them for use in the treatment of AD. Therefore, a great need exists for a new cholinergic mechanism-based cognitive enhancer for AD that is safe and effective. An agent that can ameliorate cognitive dysfunction and neurotoxicity induced by toxic metabolites of APP and Glu, or cholinergic dysfunction, should be effective in the treatment of various neurodegenerative diseases, including AD.

Extensive studies to search for new active extracts or components derived from various natural plants that can be used in the treatment of brain diseases have been carried out recently, in attempts to obtain advanced therapeutic drugs that possess both high efficacy and safety (Mantle et al., 2000). Although the action mechanisms of plant extracts that have been used medicinally and traditionally should be investigated further, it is thought that they might have various active components responsible for the prevention of diverse brain diseases.

Based on these ideas we have investigated whether an Oriental natural plant, *Polygala tenuifolia* Willdenow, has protective effects against learning and memory impairment, using scopolamine-induced amnesia model of rats. In addition, we examined the effects of the plant on AChE activity and neurotoxicity induced by Glu, Aβ, and CT.

We report that BT-11, the extract of *Polygala tenuifolia* Willdenow, enhances memory and cognitive function in the scopolamine-induced amnesia model of rat, possibly by inhibiting AChE, and protects neurons against Glu, Aβ and CT, suggesting that BT-11 might be beneficial for AD.

MATERIALS AND METHODS

Animals

Male and pregnant female Sprague-Dawley (SD) rats weighing 200–250 g were housed in a specific pathogen-free room, automatically maintained on a 12-hr light-dark cycle at 25°C and proper humidity, and were given food and water ad lib. All experiments were carried out in accordance with the Guidelines for Animal Experiments of Ethics Committee of Seoul National University.

Drugs

Aβ1–42 was purchased from US Peptide (Rancho Cucamonga, CA) and aged by incubation in 0.1 M phosphate-buffered saline (PBS, pH 7.4) at 37°C for 7 days. CT105 was made using a method described previously (Chong et al., 1994). Scopolamine hydrobromide was obtained from Sigma (St. Louis, MO) and dissolved in PBS solution. All other chemicals and drugs were purchased from Sigma. BT-11 was made by 80% ethanol extraction and dissolved initially in PBS solution containing 5% dimethylsulfoxide (DMSO). All drugs were prepared just before use.

Preparation of Extracts From Natural Plants (BT-11)

The dried roots of *Polygala tenuifolia* Willdenow (500 g) were refluxed with 80% ethanol for 3 hr in a boiling water bath. This procedure was repeated twice and the ethanol solution was concentrated under vacuum. Concentrated ethanol fraction (125 g) of the plant, obtained as described above, was used for this study.

Treatment

For in vitro measurement of protective effects of BT-11 against Glu, Aβ , and CT, the rat primary cortical neurons were pretreated with BT-11 (0.5, 3, and 5 μg/ml) for 12 hr before the treatment with Glu or Aβ. Cell viability assays were carried out 12 hr after the treatment with Glu, Aβ, and CT.

Primary Neuron Culture

For primary neuron culture, cerebral cortex was dissected out from embryonic Day 17 SD rat embryos and dissociated by gentle trituration. Cells were cultured in 96-well plates coated with polyethylenimine (0.2 mg/ml in sodium borate buffer, pH 8.3). After overnight incubation in DMEM supplemented with 10% FBS (Gibco BRL, NY), the media was replaced by serum-free defined medium for neurons (Gibco BRL; DMEM supplemented with 2 mM glutamine, 1 mM pyruvate, penicillin-streptomycin-amphotericin B mixture, 5 mM HEPES, 0.5% glucose, 10 μg/ml insulin, 30 nM sodium selenite, 20 nM progesterone, 100 μM putrescine, and 20 ng/ml transferrin). The cultured cells were incubated at 37°C in 5% CO_2 and the media was replaced every other day. For neuronal pure culture, 20 μM of cytosine arabinoside (Sigma) was added to the cells. After 14–15 days, cultured cells were treated with the peptides or drugs for the indicated times.

Passive Avoidance Test

A step-through type passive avoidance test apparatus (Model PACS-30, Columbus Instruments Int., USA) was used to evaluate the effects of BT-11 on learning and memory, essentially as described by Shen et al. (1990). The shuttle box is divided into two chambers of equal size (23.5 × 15.5 × 15.5 cm) separated by a guillotine door (6.5 × 4.5 cm). The light chamber is equipped with an illuminator and the rat can enter the dark chamber through the guillotine door. Rats were placed initially in the light chamber with the door open. Rats displayed an explorative behavior, then entered the dark compartment. Upon entering the dark compartment, the door closed automatically. Training was repeated until the rats entered the dark compartment within 20 sec (training trial).

The rats were given scopolamine or saline (both 1 mg/kg) intraperitoneally, 24 hr after the training trial. A single intraperitoneal administration of BT-11 (10 mg/kg) was given to rats 30 min after treatment with scopolamine and after another 30 min, the rats were placed in the illuminated chamber. When the rat entered the dark chamber, electrical foot shock (1 mA) was delivered for 3 sec through the grid floor and the door was closed automatically (acquisition trial). The rats were again placed in the dark chamber, 24 hr after the acquisition trial, and the latency time to enter the dark chamber was measured for 300 sec (retention trial). If a rat did not enter the dark chamber

within the cut-off time (300 sec), it was assigned a latency value of 300 sec.

Morris Water Maze Task

The testing procedure was basically the same as that described previously by Morris (1984). The experimental apparatus consisted of a circular water tank (140 cm in diameter, 45 cm high). An invisible platform (15 cm in diameter, 35 cm high) was placed 1.5 cm below the surface of the water. Water temperature was 21–23°C. The pool was located in a large test room and many clues external to the maze were visible from the pool (e.g., pictures, lamps, etc.), which could be used by the rats for spatial orientation. The position of the cues remained unchanged throughout the task. Data collection was automated by a video image motion analyzer (Ethovision, Noldus Information Technology h.v., Netherlands). For descriptive data collection, the pool was subdivided into four equal quadrants formed by imaging lines.

Training trial. The training trials were carried out before administration of scopolamine, BT-11, or both. Each rat received 2 trials per day for 5 consecutive days. At the start of a trial rats were placed randomly at one of four fixed starting points facing the wall (designated North, South, East and West) and allowed to swim for 90 sec, or until they escaped the task by finding the platform. The platform was located in a constant position throughout the test period in the middle of one quadrant, equidistant from the center and edge of the pool. In each training session, the latency to escape to the hidden platform was recorded. If the rat found the platform, it was allowed to remain there for 15 sec and then returned to its home cage. The rats that could not reach the platform in 20 sec on the 5th day were abandoned.

Test trial. Immediately after the tenth training trial on the 5th day, the trained rats were injected with scopolamine (1 mg/kg, i.p.); after 30 min, the rats were injected with BT-11 (10 mg/kg, i.p.). At 1, 4, and 18 hr after administration of scopolamine, the rats were allowed to swim for 90 sec and the time spent to reach the platform was measured. The rats were given the second treatment with BT-11 at 17 hr after administration of scopolamine; the third test trial was carried out 1 hr after the second BT-11treatment.

Cytotoxicity Assay (MTT assay)

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazodium bromide (MTT) reduction was carried out according to the manufacturer's instructions. Briefly, after incubating cells for 24 hr with various reagents, MTT (Sigma) was added to a final concentration of 0.5 mg/ml and incubation was continued for 4 hr. The resulting insoluble formazan precipitates were solubilized with DMSO, and the absorbance of converted dye was measured at 570 nm. The assay values obtained on addition of PBS solution were taken as 100%, and complete inhibition of MTT reduction (0%) was defined as the value obtained after addition of 0.9% Triton X-100.

Acetylcholinesterase Assay

Acetylcholinesterase (AChE) assay was carried out by the colorimetric method of Ellman et al. (1961) using acetylthiocholine iodide as a substrate. As an enzyme source, brains of male SD rats (8–10 weeks) were homogenized in a Potter-Elvehjem homogenizer with 10 volumes of homogenization buffer (12.5 mM sodium phosphate pH 7.0, 400 mM NaCl) and centrifuged at 1,000 × *g* for 10 min. After adding homogenization buffer containing 0.5% Triton X-100 to the supernatant, the mixtures were stirred for 30 min and centrifuged again at 10,000 × *g* for 10 min. The resulting supernatant was used as an enzyme source. All extraction steps were carried out at 4°C.

BT-11 was dissolved initially in 5% DMSO and the solution was diluted at a concentration of 200 μg/ml in Buffer 1 (100 mM sodium phosphate, pH 8.0) immediately before use. An aliquot of diluted BT-11 solution (1.5 ml) was mixed with 1.5 ml of Buffer 1, 20 ml of acetylthiocholine iodide solution (75 mM) and 100 μl of buffered Ellman's reagent (10 mM 5,5′-dithio-bis [2-nitrobenzoic acid] and 17.85 mM sodium bicarbonate] and incubated at 25°C for 30 min. Absorbance was measured at 410 nm immediately after adding an enzyme to the reaction mixtures as described above. Reading was repeated at 30 sec intervals for 5 min. A reaction blank, prepared by substituting saline for the enzyme, was measured. AChE activity was calculated using absorption coefficient 1.36 L/mmol/min. All experiments were repeated five times. The concentration of compound required for 50% enzyme inhibition (IC_{50}) was calculated from a linear estimate of the enzyme inhibition dose-response curve.

Statistical Analysis

Data are presented as means ± SE. Passive avoidance and cytotoxicity results were analyzed using one-way analysis of variance (ANOVA). The number of errors and latency times in the water maze test were analyzed using two-way ANOVA.

RESULTS

Effects of BT-11 on Scopolamine-Induced Learning and Memory Impairment

To assess whether the impairment of learning and memory could be improved by the treatment of BT-11, the passive avoidance and water maze tests were carried out using scopolamine-induced amnesia model of rats. As shown in Figure 1, the latency time in the passive avoidance test, which was shortened by scopolamine (1 mg/kg, i.p), was recovered almost to that of the vehicle control group by a single intraperitoneal administration of BT-11 (10 mg/kg).

The water maze test was used to investigate the effects of BT-11 on the disturbance of spatial working memory in the scopolamine-induced amnesia model of rats. Before an injection of scopolamine, all trained rats could reach at the platform in 20 sec (Fig. 2). The latency time to find the platform was increased significantly 1 hr after an administration of scopolamine (1.0 mg/kg, i.p.) but was not changed in the control group. The difference in latency times between the scopolamine-only treated group and the BT-11 treated group was not observed until 1 hr after the treatments with the drugs. The latency time in the group treated with BT-11 (10 mg/kg, i.p.) decreased to a level similar to that of the vehicle-treated group at 4 hr 30 min after the first administration of BT-11 (Fig. 2). Interestingly, after the second treatment, a

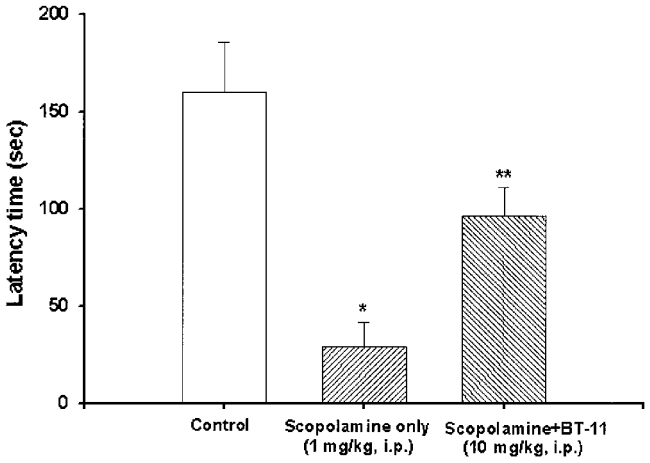

Fig. 1. Effect of a single administration of BT-11 on scopolamine-induced memory deficits in the passive avoidance test. At 30 min after training trials, scopolamine (1 mg/kg, i.p.) or the same volume of saline was administered to rats. At 30 min after scopolamine injection, the rats were injected with BT-11 (10 mg/kg, i.p.). Acquisition trials were carried out 30 min after a single BT-11 treatment. At 24 hr after acquisition trials, the test trials were carried out. Data represents mean ± SE ($n = 10$). *Significantly different from the vehicle control group ($P < 0.05$, one-way ANOVA). **Significantly different from the scopolamine only-treated group ($P < 0.05$, one-way ANOVA).

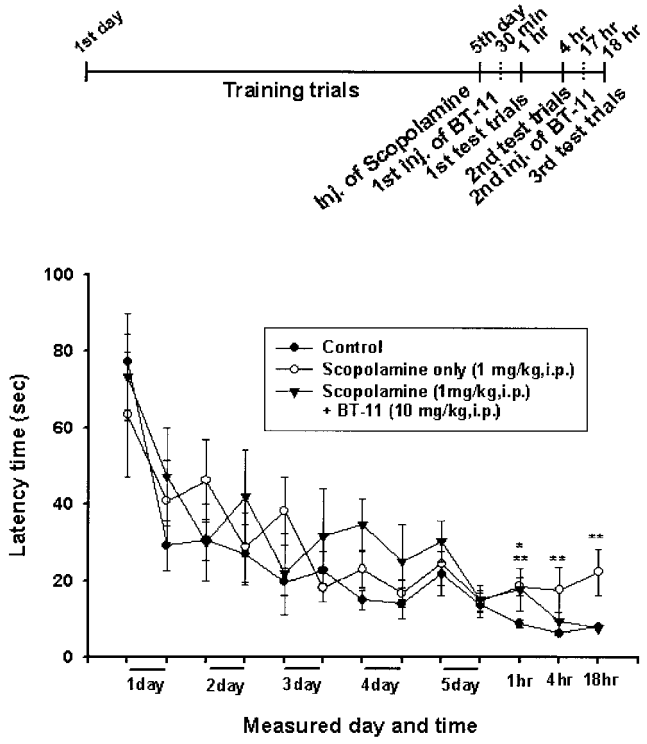

Fig. 2. Effect of repeated administrations of BT-11 on spatial working memory deficits induced by scopolamine in the water maze test. The training trials were carried out before an administration of scopolamine alone or scopolamine and BT-11 twice a day for 5 consecutive days. Scopolamine (1 mg/kg, i.p.) was given to rats immediately after the training trials were completed. BT-11 (10 mg/kg, i.p.) was given to rat 30 min and 17 hr after an scopolamine was administered. The trial tests were carried out at 1 hr (1st), 4 hr (2nd), and 18 hr (3rd) after an scopolamine treatment. Latency time is the time until rat arrived at the platform. Data represents mean ± SE ($n = 10$). *Significantly different from the vehicle control group and the scopolamine only-treated group or the BT-11-treated group. **Significantly different from the vehicle control group and the scopolamine only-treated group ($P < 0.05$ by two-way ANOVA).

greater difference in mean latency time was observed between the scopolamine-only treated group and the BT-11 treated group.

Effects of BT-11 on Cytotoxicity Induced by Glu

BT-11 was introduced into the media of the primary neuron culture 12 hr before treatment with 1 mM Glu. Cell viability was decreased significantly, as measured by MTT reduction in primary cortical neurons at 12 hr after treatment with Glu; however, pretreatment with BT-11 (0.5, 3, and 5 μg/ml) significantly increased cell viability in a dose-dependent manner, compared to the group treated with Glu only (Fig. 3).

Effects of BT-11 on Cytotoxicity Induced by Aβ or CT

Figure 4 shows that exposure of primary neurons to Aβ (10 μM) during 12 hr induced significant neurotoxicity; however, cell viability was increased significantly by pretreatment with BT-11 (3 and 5 μg/ml) for 12 hr before Aβ treatment in a dose-dependent manner, compared to the Aβ-only treated group (Fig. 4). We also investigated the effect of BT-11 on CT105-induced neurotoxicity; as shown in Figure 5, the 12-hr treatment with 10 μM CT105 induced significant inhibition of MTT. The cytotoxicity induced by CT105 (10 μM) was also decreased by a 12-hr pretreatment with BT-11, in a dose-dependent manner.

Effect of BT-11 on AChE Activity

BT-11 inhibited AChE activity in a dose-dependent manner (Fig. 6) confirming the presence of an active anti-cholinesterase component. The concentration required for 50% enzyme inhibition (IC_{50}) was 263.7 μg/ml. The K_m and V_{max} values were calculated from a Lineweaver-Burk plot (Fig. 7). The K_m value of AChE against ACh was not changed significantly upon addition of 270 μg/ml of BT-11 (0.006–0.008 μM); in contrast, BT-11 significantly decreased the V_{max} value (10.74–6.33 dA/min).

DISCUSSION

We have screened for natural compounds in plants that might have protective activities against learning and memory impairment, one of the clinical symptoms observed in all types of dementia. We found that the 80% ethanol extract of root of *Polygala tenuifolia* Willdenow (BT-11) could improve learning and memory in a scopolamine-induced amnesia model of rats. To elucidate the cognitive-enhancing and neuroprotective mechanisms

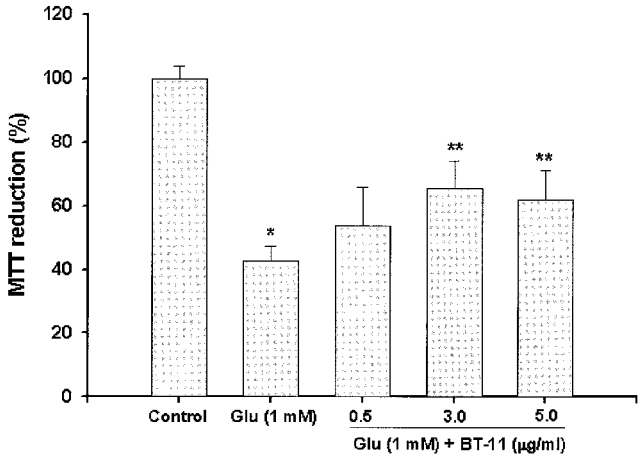

Fig. 3. Protective effects of BT-11 on excitotoxicity induced by Glu. MTT reduction level was decreased significantly over 12 hr by treatment with Glu (1 mM). Pretreatment with BT-11 (0.5, 3, 5 μg/ml) increased cell viability significantly in a dose-dependent manner, however, compared to the group treated with Glu only. Pretreatment with Bt-11 began 12 hr before the treatment with Glu. Data was expressed as percent of control value ± SE. At least two experiments were carried out in triplicate. *Significantly different from the vehicle control group ($P < 0.05$, one-way ANOVA). **Significantly different from the Glu only-treated group ($P < 0.05$, one-way ANOVA).

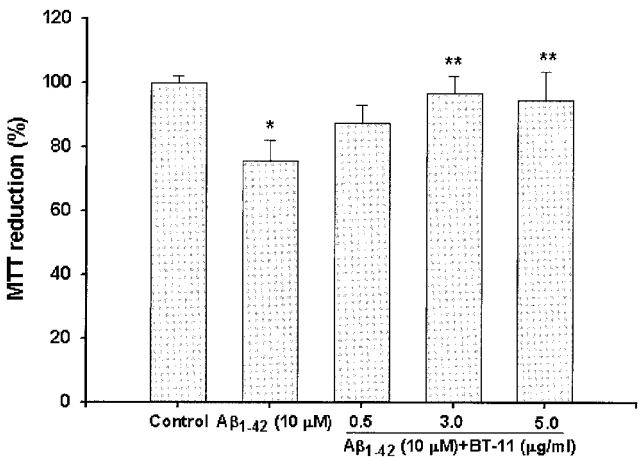

Fig. 4. Protective effects of BT-11 on Aβ-induced neurotoxicity. Exposure of Aβ (10 μM) to primary neurons of rats for 12 hr induced significant cytotoxicity. Note that cell viability was increased significantly by BT-11 pretreatment (0.5, 3, 5 μg/ml) in a dose-dependent manner compared to the Aβ only-treated group. BT-11 pretreatment began 12 hr before treatment with Aβ. Data was expressed as percent of control value ± SE. At least two experiments were carried out in triplicate. *Significantly different from the vehicle control group ($P < 0.05$, one-way ANOVA). **Significantly different from the Aβ only-treated group ($P < 0.05$, one-way ANOVA).

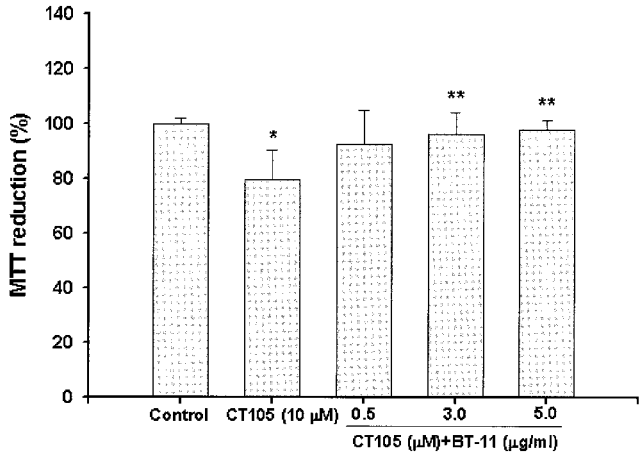

Fig. 5. Protective effects of BT-11 on neurotoxicity induced by CT. CT105 (10 μM) was administered to primary cultured rat neurons for 12 hr. Note that the significantly increased CT105-induced cell death was reduced significantly by pretreatment with BT-11 (0.5, 3, 5 μg/ml), in a dose-dependent manner. BT-11 pretreatment was initiated 12 hr before CT105-treatment. Data was expressed as percent of control value ± SE. At least two experiments were carried out in triplicate. *Significantly different from the vehicle control group ($P < 0.05$, one-way ANOVA). **Significantly different from the CT105 only-treated group ($P < 0.05$, one-way ANOVA).

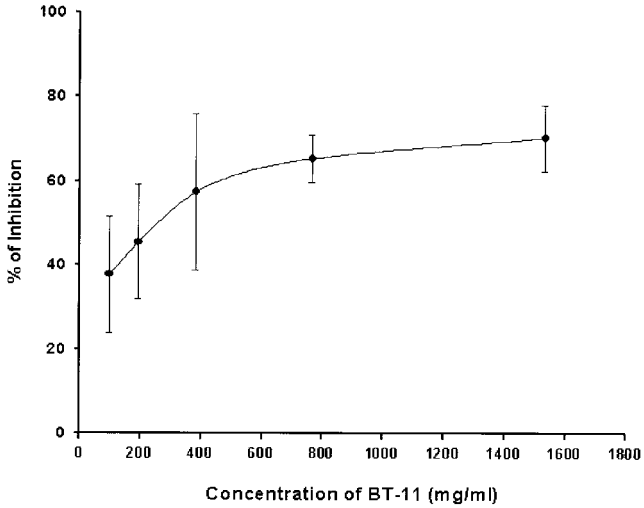

Fig. 6. AChE activity was inhibited by BT-11 in a dose-dependent manner. The concentration required for 50% enzyme inhibition (IC_{50}) was 263.7 μg/ml. Inhibition efficacy was expressed as percent inhibition of enzyme activity compared to the control value (100%). Each value represents the mean ± SE ($n = 5$).

of BT-11 we investigated the effect of BT-11 on cytotoxicity induced by Glu, Aβ, or CT in rat primary cortical neurons, and its inhibitory effect on AChE. Pharmacologic and clinical studies have suggested that Glu-induced excitotoxicity plays a critical role in the pathogenesis of neurodegenerative disease, such as AD and brain ischemia (Meldrum et al., 1990). Glu is well known as an excitatory neurotransmitter and is known to have biological functions including learning and memory (Greenamyre, 1986; Cotman et al., 1987); however, Glu also act as an excito-

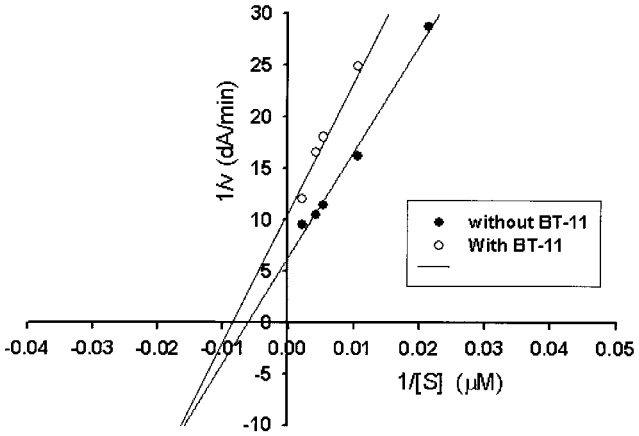

Fig. 7. Lineweaver-Burk plots of AChE activity over a range of substrate concentrations (2–20 μM) in the absence (●) or presence (○) of BT-11 (270 μg/ml).

toxin that leads to neuronal death in the brain (Choi, 1987; Choi et al., 1987, Whetsell, 1996). Overstimulation of excitatory amino acid receptors, *N*-methyl-D-aspartate (NMDA) receptors, which could lead to an increase in intracellular Ca^{2+} level, is involved in excitotoxicity-induced neuronal death (MacDermott, 1986; Nicotera and Orrenius, 1992). In vivo studies on excitotoxicity have also revealed that overstimulation of excitatory amino acid receptors is closely related to neuronal damage and behavioral changes (Obrenovitch et al., 2000; Rossi et al., 2000). Based on these findings, several studies have suggested that a strategy to modulate the excitotoxicity through NMDA receptor antagonists might be beneficial for several brain diseases (Simon et al., 1984; Keyser et al., 1999). We demonstrated that BT-11 could reduce Glu-induced toxicity in rat primary cortical neurons. Although the precise mechanisms of BT-11 against Glu have not been clearly elucidated in this study, this extract might contain one or more identifiable components that are able to affect Glu receptor or intracellular ion homeostasis.

Aβ has been reported to exert neurotoxicity by various mechanisms (Yankner et al., 1989; Rush et al., 1992, Mattson et al., 1993; Behl et al., 1994; Eikelenboom et al., 1994; Brown et al., 2000). Searching for agents effective against Aβ-related neurotoxicity may be one promising strategy for AD therapy. We assessed the protective effect of BT-11 on Aβ-induced neurotoxicity in primary cultured neurons of rats and found that extracellular administration of Aβ inhibited MTT reduction by about 30%. The Aβ-induced decreased viability of neurons by was reversed by pretreatment with BT-11, which is thought to have one or more unidentified components that reduce neurotoxicity induced by Aβ.

With extensive evidence that Aβ may not be the sole active fragment contributing to AD pathogenesis, other potentially amyloidogenic products of APP such as CT have been focused on in relation to the pathological mechanism of AD (Maruyama et al., 1990; Haass et al., 1992b; Zhao et al., 1995). Data from a number of in vitro and in vivo studies have shown that CT can induce neurotoxicity on its own, as well as by Aβ generation (Yankner et al., 1989; Matsumoto and Fujiwara, 1991; Kim and Suh, 1996; Nalbantoglu et al., 1997; Suh, 1997; McKeon-O'Malley et al., 1999; Lee et al., 2000). It has been reported that CT induces strong membrane inward currents, disruption of intracellular Ca^{2+} homeostasis, inflammatory reaction and it renders neuronal cells more vulnerable to Glu-induced excitotoxicity (Fraser et al., 1996, 1997; Kim et al., 1998, 1999, 2000, 2002; Hartell and Suh, 2000; Bach et al., 2001; Chong et al., 2001; Rah et al., 2001). Centrally injected CT induces learning and memory impairments and neuropathological changes in mice (Song et al., 1998; Choi et al., 2001). Thus, pharmacologic methods of reducing toxicity, the production of CT, or both would be invaluable in the treatment of AD. We have shown that BT-11 diminished CT-induced neurotoxicity in a dose-dependent manner. Our results suggest that BT-11 has activities that protect against neuronal loss in the brain induced by CT and by Aβ.

We also found that BT-11 inhibited AChE activity in a dose-dependent manner, indicating that this plant might contain an unidentified anticholinesterase component. Because BT-11 is a crude extract, inhibitory activity (IC_{50} value is 263.7 μg/ml) could not be compared to that of known single compounds, such as donepezil (Snape et al., 1999), galantamine (Bores et al., 1996), rivastigmine (Enz et al., 1991), and DHED (Park et al., 1996). Among the possible strategies aimed at increasing cholinergic neurotransmission, the AChE inhibitor would be a valuable and easily accessible therapeutic agent for maintaining acetylcholine levels in the brain and improving cognitive ability (Giacobini, 1997). In addition, two of the characteristic roles of Aβ on cholinergic function reported recently that AChE activity was increased around amyloid plaque (Sberna et al., 1997) and vulnerable cholinergic neuronal loss in AD was closely related to inhibition of high-affinity choline uptake and ACh release by Aβ (Kar et al., 1998). After the addition of BT-11 (270 μg/ml), the K_m value of AChE against ACh was relatively unchanged, whereas V_{max} value was significantly decreased. These results indicate that BT-11 inhibits AChE in a non-competitive manner. We found that this extract had effects that enhanced cognitive ability for the in vivo scopolamine-induced amnesia rat model. Scopolamine, which is a muscarinic receptor antagonist, can cause amnesia in animals by blocking cholinergic neurotransmission. Therefore, it is thought that the inhibition of AChE by BT-11 might increase ACh neurotransmission in the synaptic cleft of the rat brain, resulting in the reversal of scopolamine-induced cognitive deficits. The enhancing effect on cognitive ability and the inhibitory activity on excitotoxicity and neurotoxicity caused by Aβ and CT provide the possibility that BT-11 might improve cognitive deficits and reduce neurotoxicity induced by Aβ and CT in AD patients as well as in vivo models of dementia including transgenic mouse models.

Polygala tenuifolia Willdenow has been known to have therapeutic effects such as expectorant, sedative, antipsychotic and anti-inflammatory activity. (Chung et al., 1992, 2002; Kim et al., 1998). The polygalasaponins in the dried root of this plant can inhibit cAMP activity (Nikaido et al., 1982). Recently, it was observed also that a crude aqueous extract of this plant might protect ethanol-induced cytotoxicity through inhibition of the apoptosis of Hep G2 cells (Koo et al., 2000); however, its neuroprotective and cognitive enhancing activities have never been reported before.

We first reported that one or more components of BT-11 could penetrate the blood–brain barrier and improve cognitive impairment through elevating cholinergic neurotransmission by blocking ACh hydrolysis and could protect neurons against Glu, Aβ and CT, indicating that BT-11 and its components might be effective in treatment of AD. Further purification processes to isolate one or more active components from BT-11 and further analysis on its structure and effects should be carried out, however, to develop an effective neuroprotective and cognitive deficit-improving drug and to clarify its pharmacologic mechanisms.

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