Effects of an Aqueous Extract of *Asparagus cochinchinensis* on the Regulation of Nerve Growth Factor in Neuronal Cells

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Asparagus cochinchinensis is a medical plant that has long been used to treat fever, cough, kidney disease, breast cancer, inflammatory disease and brain disease in northeast Asian countries. Although several studies have been conducted on the anti-neuroinflammatory effects of *A. cochinchinensis*, the correlation between these effects and nerve growth factor (NGF) has not yet been examined. In this study, we investigated the effects of an aqueous extract of *A. cochinchinensis* (AEAC) on the secretion and action mechanism of NGF in neuronal cells. The concentration of the NGF protein in the supernatant collected from cultured cells increased significantly in B35 cells treated with AEAC in comparison with the vehicle-treated group without any specific cytotoxicity. Furthermore, the mRNA expression of NGF showed a very similar pattern to its protein concentration. To examine the bioactivity of NGF secreted from B35 cells, undifferentiated PC12 cells were cultured in an AEAC-conditioned medium and neuritic outgrowth was observed. The dendrite length of PC12 cells in the AEAC-treated group was significantly higher than that in the vehicle-treated group. Moreover, the level of the downstream effectors p-TrkA and p-ERK of the high-affinity NGF receptor was significantly higher in the AEAC-treated group, while the expression of the downstream effectors of the low-affinity NGF receptor was significantly lower in the same group. These results suggest that AEAC may contribute to the regulation of NGF expression and secretion in neuronal cells; it is therefore an excellent candidate for further investigation as a therapeutic drug for neurodegenerative diseases.

Key words : *Asparagus cochinchinensis*, B35 cells, NGF, NGF receptor, PC12 cells

Introduction

Asparagus cochinchinensis is a perennial herb belonging to the Liliaceae family that is widely distributed in China, Japan and Korea [38]. The root of *A. cochinchinensis* has been used as a traditional medicine in those countries for thousands of years [37, 38], and it is considered a therapeutic drug owing to its anti-inflammatory, diuretic, antiseptic, antitussive, antibacterial, nervine, sialogoue, antipyretic, and stomachic effects, although there is a lack of scientific evidence of these effects. Moreover, it is commonly used in combination with other herbs to treat the lungs, spleen, immune system and aging [37, 38].

Various substances including 19 amino acids, polysaccharides, and more than 20 multi-functional compounds have been identified in the root of *A. cochinchinensis*. These include β-sitosterol [19], daucosterol [25], n-ethatriacontanoic acid [31], palmitic acid [14], 9-heptacosylene [41], smilagenin [3], diosgenin [6], sarsasapogenin-3-O-β-D-glucoside feeding grapes imidacloprid [39], 5-methoxy methyl furfural, yame sapogenin, diosgenin-3-O-β-D imidacloprid feeding glucose glycosides [29, 39], aspacochioside D [30], iso-agatharesinoside [18] and seven steroidal saponins [15]. Among these, some polysaccharides exhibited therapeutic effects against several diseases such as aging [15, 27, 43], inflammatory diseases [15], tumor [11, 20, 36], diabetes [42] and cough [20, 22].

Although several extracts of *A. cochinchinesis* have been applied as traditional medicines to treat various diseases, sci-

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entific evidence is only available for their anti-inflammatory activities. Specifically, these effects and related mechanisms were observed in several models, including Lipopolysaccharide (LPS)/substance P-stimulated mouse astrocytes [10], LPS-stimulated BV-2 microglial cells [12] and cockroach allergen-induced mice [9]. However, the underlying mechanism by which *A. cochinchinensis* influences NGF secretion ability has not yet been clearly identified, even though the effects of *Liriope platyphylla*, another plant in the Liliaceae family, have been thoroughly investigated in neuronal cells and animal models [2, 3, 24].

In this study, we investigated the effects of AEAC on the NGF secretion ability and NGF receptor signaling pathway in B35 neuroblastoma and PC12 cells to provide scientific evidence of the therapeutic effects of *A. cochinchinensis* on neurodegenerative disorders.

Materials and Methods

Preparation of AEAC

The roots of *A. cochinchinensis* used in this study were collected from plantations in the Go-Chang area of Korea and dried in a drying machine (Ilshinbiobase Co., Dongducheon, Korea) at 60°C. Voucher specimens of *A. cochinchinensis* (WPC-14-003) were deposited in the functional materials bank of the PNU-Wellbeing RIS Center at Pusan National University. Dry roots of *A. cochinchinensis* were reduced to powder using a pulverizer (Hanil Electric Co., Seoul, Korea), after which AEAC was purified from 75 g of *A. cochinchinensis* roots for 45 min at 121°C using circulating extraction equipment (IKA Labortechnik, Staufen, Germany) after adding 500 ml of distilled water (Fig. 1A). In addition, a solution of the extracts was concentrated and
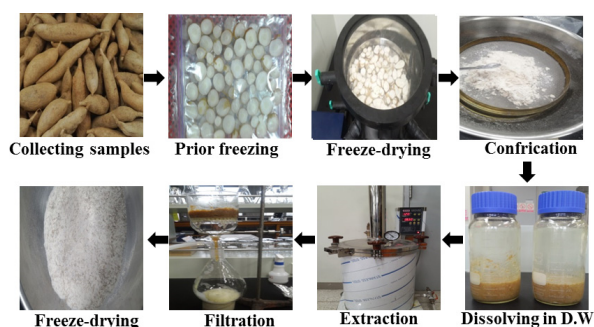

Fig. 1. Preparation scheme of AEAC. AEAC was obtained from the roots of *A. cochinchinensis* using aqueous extraction under the conditions described in the materials and methods.

subsequently passed through a 0.4 µm filter, after which the pellets were dried in a rotary evaporator (EYELA, Tokyo, Japan) and stored at -80°C until needed.

Analysis of total flavonoids and phenolics

Total phenolics in AEAC were measured by the Folin-Ciocalteu method, with slight modification [32]. Briefly, 1 ml of AEAC solution was mixed with 5 ml of Folin-Ciocalteu reagent (Sigma-Aldrich Co., St. Louis, MO, USA), then incubated at room temperature for 5 min. This mixture was subsequently added to 15 ml of 20% Na_2CO_3 and vortexed for 30 sec, after which the absorbance was repeatedly measured at 765 nm using a Versa-max plate reader (Molecular Devices, Sunnyvale, CA, USA). A standard calibration curve was made using different concentrations of gallic acid (Sigma-Aldrich Co., St. Louis, MO, USA), after which the concentration of total phenolic contents in AEAC was presented as mg gallic acid equivalent of extract.

The flavonoid contents in AEAC were also measured as previously described [44]. Briefly, 200 µl of several different concentrations of AEAC were mixed with 60 µl of 5% $NaNO_2$ (Sigma-Aldrich Co.) and 60 µl of 10% $AlCl_3$ (Sigma- Aldrich Co.). Following incubation at 25°C for 5 min, the mixture was added to 400 µl of 1 M NaOH and the absorbance was repeatedly measured at 510 nm using a Versa-max plate reader (Molecular Devices). A standard calibration curve was then made using different concentrations of catechin (Sigma-Aldrich Co.). The flavonoid contents of the AEAC were presented as mg catechin equivalent of extract.

Cell culture

The B35 cell line used in this study is a neuroblastoma that originated from the central nervous system of rats (*Rattus norvegicus*). This cell line was kindly provided by the Korean Cell Line Bank (Seoul, Korea). They were grown with Dulbecco Modified Eagle's Medium (DMEM, Welgene, Gyeongsan-si, Korea) containing 10% fetal bovine serum (FBS, Welgene, Gyeongsan-si, Korea), L-glutamine, penicillin, and streptomycin (Thermo Scientific, Waltham, MA, USA) in a humidified incubator at 37°C under 5% CO_2 and 95% air. The PC12 cell line, which is a pheochromocytoma that originated from the adrenal medulla of rats, was also used in this study and provided by the Korean Cell Line Bank (Seoul, Korea). PC12 cells were grown in Roswell Park Memorial Institute medium (RPMI, Welgene, Gyeongsan-si, Korea) containing 10% FBS (Welgene), L-glutamine, pen-

icillin, and streptomycin (Thermo Scientific, Waltham, MA, USA) in a humidified incubator at 37°C under 5% CO_2 and 95% air.

Cell viability assay

The viability of B35 cells was determined using the tetrazolium compound 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich Co.). To determine the cell viability, B35 cells were seeded at a density of 5×10^4 cells/0.2 ml and grown for 24 hr in a 37°C incubator. When the cells attained 70-80% confluence, they were either treated with vehicle (1x PBS) or pretreated with 100 μg/ml of AEAC dissolved in 1× PBS for 24 hr. After discarding the supernatants, 0.2 ml of fresh MEM media and 50 μl of MTT solution (2 mg/ml in PBS) were added to each well. The cells were then incubated at 37°C for 4 hr. Formazan precipitate was dissolved in DMSO, after which the absorbance at 570 nm was read directly in wells using a Molecular Devices VERSA max plate reader (Sunnyvale, CA, USA). The morphological features of B35 cells in each treated group were also observed using a microscope at 200× magnification (Leica Microsystems, Switzerland).

Western blot

Total protein extracted from PC12 cells using Pro-Prep Protein Extraction Solution (iNtRON Biotechnology, Seongnam, Korea) was collected by the centrifugation at 15,000 rpm for 15 minutes, then quantified using a SMARTTM BCA Protein Assay Kit (Thermo Scientific, Waltham, MA, USA) for Western blot analysis. Briefly, these proteins were separated by 4-20% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for 2 hr, after which resolved proteins were transferred to nitrocellulose membranes for 2 hr at 40 V. Each membrane was then incubated separately at 4°C with the following primary antibodies overnight: anti-TrkA antibody (Cell Signaling Technology, Beverley, MA, USA), anti-p-TrkA antibody (Cell Signaling Technology), anti-Akt antibody (Cell Signaling Technology), anti-p-Akt antibody (Cell Signaling Technology), anti-ERK antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-p-ERK antibody (Santa Cruz Biotechnology), anti-p75[NTR] antibody (Cell Signaling Technology), anti-JNK antibody (Cell Signaling Technology), anti-p-JNK antibody (Cell Signaling Technology) and anti-actin antibody (Sigma-Aldrich Co.). Next, the membranes were washed with washing buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 0.05% Tween 20),

then incubated with 1:1,000 diluted horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (Invitrogen, Carlsbad, CA, USA) at room temperature for 1 hr. Finally, membrane blots were developed using Amersham ECL Select Western Blotting detection reagent (GE Healthcare, Little Chalfont, UK). The chemiluminescence signals that originated from specific bands were detected using FluorChemi®FC2 (Alpha Innotech Co., San Leandro, CA, USA).

Enzyme-linked immunosorbent assay (ELISA) of NGF

The levels of NGF in culture supernatant from B35 cells treated with AEAC were measured using a NGF ELISA kit (Chemicon International Inc., Temecula, CA, USA). Briefly, the samples and standards were incubated overnight on antibody-coated plates in a plate shaker at 100-150 rpm and 2-8°C. The wells were then washed four times with washing buffer, after which 100 μl of anti-mouse NGF monoclonal antibody was added to each well. Plates were subsequently incubated in a shaker for 2 hr at room temperature, after which 100 μl of peroxidase conjugated donkey anti-mouse IgG polyclonal antibody was added to each well and samples were incubated at room temperature for an additional 2 hr. After washing, 100 μl of TMB substrate was added to each well and the plate was incubated at room temperature for 15 min. The reaction was then quenched by the addition of 100 μl of stop solution, after which the plate was analyzed using a SoftMax Pro5 spectrophotometer (Molecular Devices, Sunnyvale, CA, USA).

Dot blot analysis

Total protein prepared from B35 cells treated with AEAC was transferred to a nitrocellulose membrane using a SlotBlot kit (Pharmacia Biotech, CA). The membrane was incubated separately with primary rabbit polyclonal anti-NGF antibody (Cell Signaling Technology, Beverley, MA, USA) at 2 μg in blocking buffer at room temperature for 3 hr, then washed in washing buffer and incubated with secondary antibody, horseradish peroxidase-conjugated goat anti-rabbit IgG (GenTest, MA), at 1:1,000 for 1 hr at room temperature. NGF proteins were detected using Amersham ECL Select Western Blotting detection reagent (GE Healthcare, Little Chalfont, UK). The chemiluminescence signals that originated from specific slot were detected using FluorChemi® FC2 (Alpha Innotech Co).

RT-PCR analysis of NGF transcripts

The relative quantities of NGF transcripts were measured by RT-PCR as previously described [28]. First, total RNA molecules were purified by removing media from each cultured sample and then lysing B35 cells in RNAzol (Tel-Test Inc., Friendswood, USA). The isolated RNA was subsequently measured by UV spectroscopy, after which the expression of NGF genes was assessed using RT-PCR with 5 μg of total RNA from cells of each group. Next, 500 ng of oligo-dT primer (Invitrogen, Carlsbad, CA, USA) were annealed at 70°C for 10 min. The complementary DNA (cDNA), which was used as the template for further amplification, was synthesized by the addition of deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), and deoxythymidine triphosphate (dTTP) with 200 units of reverse transcriptase (Superscript II, Invitrogen, 200 U/μl). Next, 10 pmol of the sense and antisense primers were added, and the reaction mixture was subjected to 28-32 cycles of amplification. Amplification was conducted in a Perkin- Elmer Thermal Cycler using the following cycles: 30 sec at 94°C, 30 sec at 62°C, and 45 sec at 72°C. The primer sequences for target gene expression identification were as follows: NGF, sense primer: 5′-CAT GTT GTT CTA CAC TCT GAT CAC-3′, anti-sense primer: 5′-CTC CTT GCC CTT GAT GTC TGT GG-3′; β-actin, sense primer: 5′-GTG GGG CGC CCC AGG CAC CAG GGC-3′, anti-sense primer: 5′-CTC CTT AAT GCT ACG CAC GAT TTC-3′. The experiment was repeated three times, and all samples were analyzed in triplicate. The final PCR products were separated on 1% agarose gel and then visualized by ethidium bromide staining.

Analysis of neuritic outgrowth

The neuritic outgrowth of PC12 cells was measured as previously described [3, 24]. After incubation of PC12 cells treated with AEAC-conditioned medium for 24 hr, the morphology of PC12 cells was observed under a microscope at 200× magnification (Leica Microsystems, Switzerland). The length of dendrite of PC12 cells was analyzed using the Leica Application Suite (Leica Microsystems, Switzerland).

Cell cycle assay

The cell cycle was measured using a Muse™Cell Cycle Kit (Millipore Co., Billerica, MA, USA) according to the manufacturer's instructions. Briefly, PC12 cells were divided into 100 mm^2 dishes (2.5×10^6 cells/dish), then treated with AEAC-conditioned medium for 24 hr. Total cells from each group were harvested by centrifugation at 3,000 ×g for 5 minutes, then fixed with 70% EtOH at -20°C for 3 hr. The fixed cells were subsequently washed with 1× PBS, then added to 200 μl of Cell Cycle Reagent. Following incubation at 37°C in a CO$_2$ incubator for 30 min, cell cycles were analyzed using FACS (Millipore Co., Billerica, MA, USA).

Statistical analysis

One-way ANOVA was used to identify significant differences between vehicle and AEAC treated groups (SPSS for Windows, Release 10.10, Standard Version, Chicago, IL, USA). All values are reported as the means ± standard deviation (SD) and a p value of <0.05 was considered significant.

Results

Bioactive components of AEAC

To quantify the bioactive components in AEAC, total phenolic contents and total flavonoids were analyzed using the method suggested in previous studies. As shown in Table 1, AEAC contained high concentrations of two important antioxidants, flavonoids (1.32±0.2 mg/g) and phenolics (13.8±0.9 mg/g). These findings suggest that AEAC can have high antioxidative activity.

Toxicity of AEAC

To determine the toxicity of AEAC, cell viability was measured in B35 cells treated with 100 μg/ml using an MTT assay. No significant alterations in cell viability or morphological features were observed in B35 cells treated with AEAC alone for 24 hr (Fig. 2). These findings indicate that AEAC showed no toxicity at less than 100 μg/ml.

Effects of AEAC on NGF secretion and biosynthesis

To investigate the effects of AEAC on the synthesis and secretion of NGF in B35 cells, the levels of NGF protein and mRNA were measured in the culture supernatant and cells after AEAC treatment for 24 hr. The concentration of NGF protein was significantly higher in the supernatant of B35

Table 1. The concentration of total flavonoids and phenol was assayed in a mixture containing different concentrations of AEAC

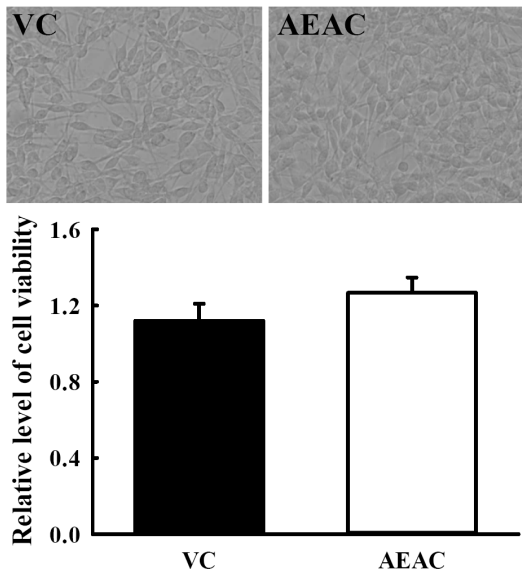| Categories	Concentration (mg/g)
Total flavonoids	1.32±0.2
Total phenol	13.8±0.9

Fig. 2. Toxicity of AEAC. After incubation of B35 cells with 100 μg/ml of AEAC for 24 hr, their morphologies were observed under a microscope at 200× magnification. The viability of B35 cells treated with AEAC was determined by MTT assay in triplicate. The data shown represent the means ± SD of three replicates.

cells treated with AEAC compared with the vehicle treated group (Fig. 3A, Fig. 3B). A similar increase was detected in the mRNA levels of the same group (Fig. 3C). These findings suggest that AEAC treatment can increase NGF secretion and synthesis in B35 neuroblastoma cells.

To verify the activity of secreted NGF, AEAC-conditioned media were inoculated with undifferentiated PC12 cells and their dendritic outgrowth was measured based on microscopic analysis. Dendrites were longer in the AEAC-conditioned medium treated goups than the vehicle-conditioned medium treated group (Fig. 4). These results show that NGF secreted from B35 cells after AEAC treatment can successfully induced the differentiation of PC12 cells.

Effects of AEAC on NGF receptor signaling pathway

Secreted NGF can transduce signals related to survival, proliferation and death into the cytosol through binding of two different types of NGF receptors located on the cell membrane [1]. Therefore, we investigated whether the increase in NGF concentration induced by AEAC treatment could affect the two NGF receptor signaling pathways in PC12 cells. Analysis of the high affinity receptor revealed that the level of p-TrkA was slightly higher in the AEAC-conditioned medium treated group than the vehicle-conditioned medium treated group. A similar enhancement was
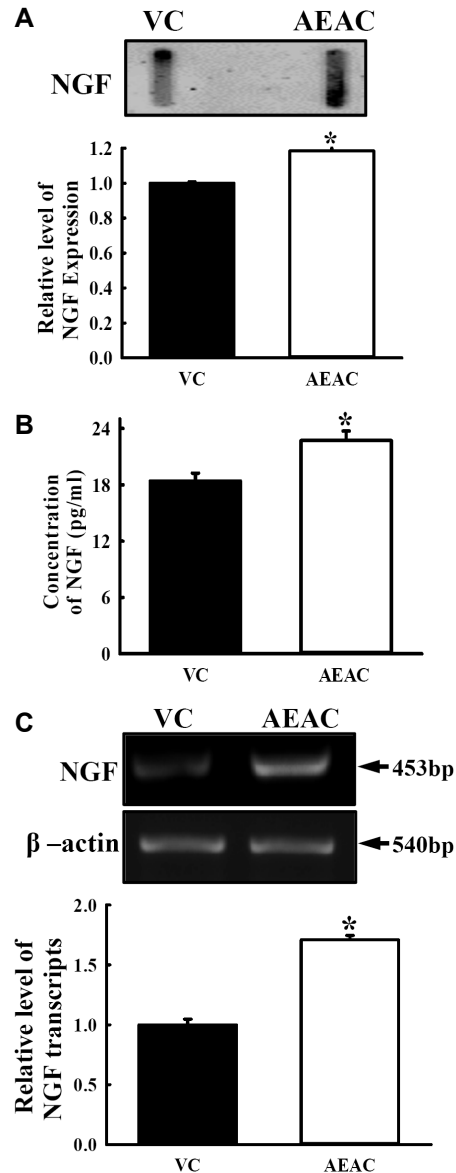

Fig. 3. Secretion and synthesis of NGF. The protein concentration of NGF was detected in the supernatant and cell homogenate of B35 cells treated with AEAC for 24 hr using ELISA (A) and dot blot (B). The level of NGF transcripts was measured in B35 cells using RT-PCR (C). The β-actin signal was used as the endogenous control, and the transcript (540 bp) indicates the RNA loading. The density of the transcript was quantified using a Kodak Electrophoresis Documentation and Analysis System 120. The values of data shown are the means± SD of three experiments. *, *p*<0.05 compared to the vehicle treated group.

observed in the level of p-ERK and p-Akt among downstream members of the high affinity receptor. Following treatment with the AEAC-conditioned medium, their level rapidly increased to 22% and 44%(Fig. 5).
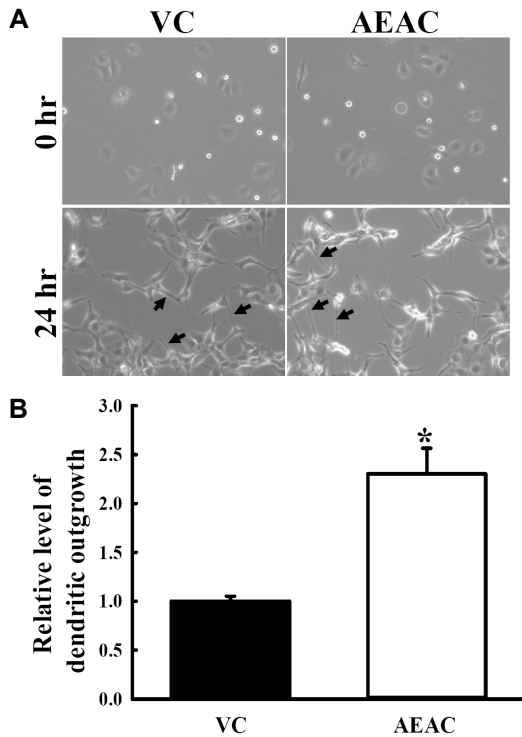
A

B

Fig. 4. Dendritic outgrowth of PC12 treated with AEAC-conditioned medium. After treatment of B35 cells with AEAC for 24 hr, the AEAC-conditioned media were transferred to undifferentiated PC12 cells. (A) The cellular morphology of PC12 was viewed at 200× magnification. (B) The dendritic outgrowth of PC12 cells was measured in specific area (43.9 μm^2). The values shown are the means±SD of three experiments. *, $p<0.05$ compared to the vehicle treated group.

Conversely, in the case of low affinity receptor, the level of p75^{NTR} and p-JNK expression was lower in the AEAC-conditioned medium treated group than the vehicle-conditioned medium treated group (Fig. 6). Taken together, these results showed that NGF secreted by AEAC treatment may stimulate proliferation and inhibit apoptosis of PC12 cells through regulation of the NGF receptor TrkA and p75^{NTR} signaling pathways.

Effects of AEAC on cell cycle regulation

Finally, to examine the effects of AEAC-conditioned medium on the cell cycle of PC12 cells, the number of cells in each stage of the cell cycle was counted in subset groups. After treatment with the AEAC-conditioned medium, the number of cells in the G0/G1 stage was decreased, while those in the S and G2/M stage increased (Fig. 7). These results suggest that AEAC treatment can stimulate progression from the G0/G1 stage to the S and G2/M stage.
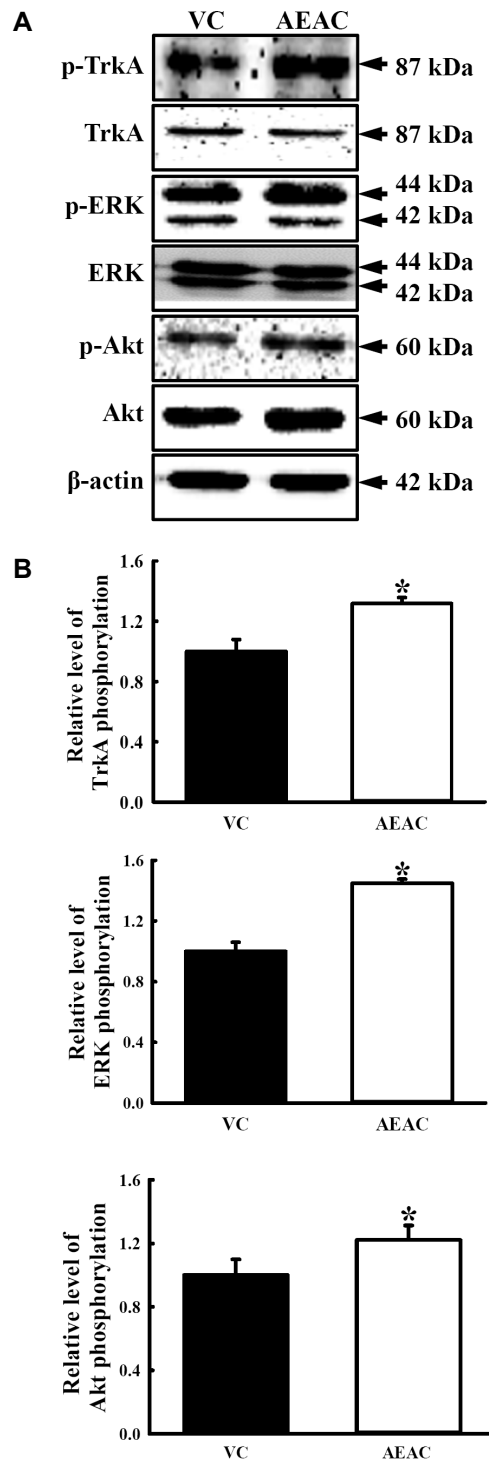
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Fig. 5. Effects of AEAC treatment on the high affinity NGF receptor signaling pathway of PC12 cells. Total tissue lysates were prepared from PC12 cells treated with vehicle or AEAC conditioned medium as described in the Materials and Methods section. Fifty micrograms of protein per sample were immunoblotted with antibodies for each protein. Three samples were assayed in triplicate by Western blotting. Data shown are the means ± SD. *, $p<0.05$ relative to the vehicle treated group.
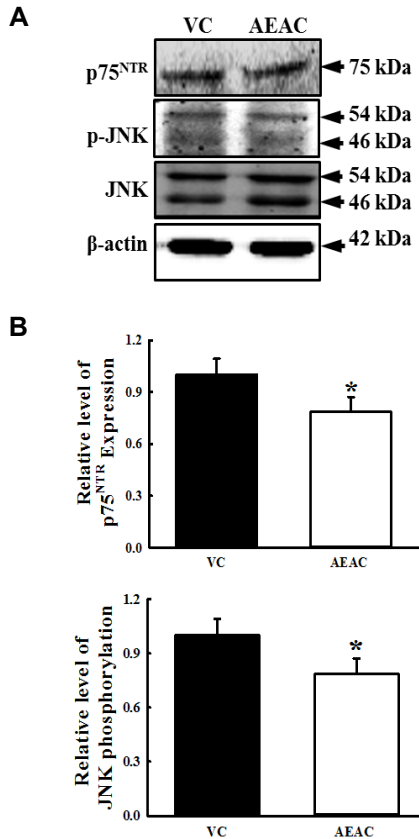
A

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Fig. 6. Effects of AEAC treatment on the low affinity NGF receptor signaling pathway of PC12 cells. Total tissue lysates were prepared from PC12 cells treated with vehicle or AEAC conditioned medium as described in the Materials and Methods section. Fifty micrograms of protein per sample were immunoblotted with antibodies for each protein. Three samples were assayed in triplicate by Western blotting. Data shown are the means ± SD. *, $p<0.0$ relative to vehicle treated group.
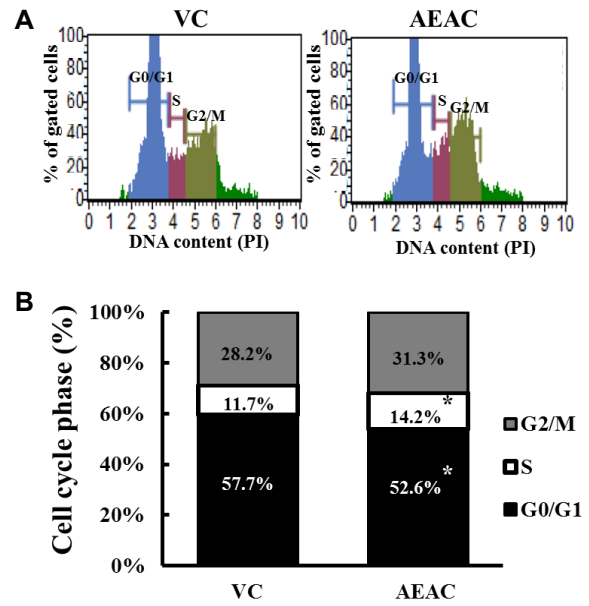
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Fig. 7. Cell cycle analysis. The cell cycle distribution was determined by flow cytometric analysis of the DNA content of nuclei of PC12 cells following staining with propidium iodide. After treatment with AEAC-conditioned medium, the number of cells in the G0/G1, S and G2/M stage was determined. Data shown are the means ± SD. *, $p<0.05$ relative to the vehicle treated group.

Discussion

Nerve growth factor has received recent consideration as a potential treatment regulator for human central and peripheral nervous system related disorders because it has been reported to be an important molecule that regulates neuronal survival and differentiation [1, 34, 35]. Many studies have focused on the identification and development of a novel NGF stimulator to provide therapeutic drugs for the treatment of neurodegenerative disorders. In an effort to identify candidate natural products for the treatment of such diseases and verify their mechanism of action, we investigated the NGF stimulatory effects of AEAC purified from the roots of *A. cochinchinensis* in B35 cells and PC12 cells. Our results showed that AEAC could significantly stimulate the secretion ability and synthesis of NGF in B35 cells, and that secreted NGF induced the differentiation of PC12 cells through regulation of the high affinity and low affinity receptor signaling pathway.

Many natural products have long been considered a source of leads for the development of therapeutic drugs available for the treatment of human diseases because they provide some active principles that can be used as the backbone for synthesis of new drugs [6, 25]. However, a large number of candidates have been discarded because they could not overcome several essential problems. Among these, toxicity has long been considered one of the most important problems during preclinical investigations of natural products [5]. In this study, we investigated the toxicity of AEAC in B35 cells to determine the optimal concentration of AEAC. As shown in Fig. 1, no significant toxicity was detected in B35 cells treated with 100 μg/ml of AEAC. These results were similar to those of previous studies. Three different concentrations (1, 10 and 100 μg/ml) of aqueous extract of *A. cochinchinensis* did not induce significant toxicity in the human hepatoma cell line HepG2 [13]. Furthermore, the viability of A549 cells was maintained at a constant level in 100, 300 and 500 μg/ml of 70% ethanol extract of *A. cochin-*

chinensis [15].

Nerve growth factor was secreted from various cell types including neurons, inflammatory cells (lymphocytes or mast cells), and structural cells (fibroblast, epithelial cells and smooth muscle cells) after stimulation with cytokines and treatment with natural products [7]. Korea white ginseng extract and ginsenoside Rb2 stimulated an increase of NGF mRNA and protein in a steroid-induced polycystic ovary murine model and SD rats [27, 29]. Moreover, NGF-mediated neuritic outgrowth was significantly upregulated by treatment with *Picrohizae Rhizoma* methanol extracts [18]. Furthermore, some extracts (butanol extract, spicatoside A, LP-E, LP-M, LP-M50 and LP2E17PJ) from *L. platyphylla* induced the secretion and synthesis of NGF in B35 cells [9, 10, 24]. In this study, we investigated the effects of *A. cochinchinensis* on NGF secretion and synthesis ability of neuroblastoma cells. The results presented herein provide additional evidence that AEAC purified from *A. cochinchinensis* can be considered a novel NGF stimulator.

Nerve growth factor can transfer the signal for the survival, proliferation and apoptosis of target cells through the binding of high affinity and low affinity NGF receptors [35]. These signal pathways are regulated by various natural products. Trk phosphorylation of the high affinity receptor was rapidly enhanced by spicatoside A and LP-M from *L. platyphylla* in neuronal cells and brain hippocampus cells [10, 24], while the expression of $p75^{NTR}$ and RhoA in low affinity receptor was lower in LP-M treated animal cells [24]. A similar result was observed in AEAC-conditioned medium treated PC12 cells as shown Fig. 5 and 6.

Finally, the results of the present study suggest that AEAC may stimulate NGF synthesis and secretion from neuroblastoma cells without any significant toxicity. Moreover, secreted NGF can successfully induce survival, proliferation and differentiation of undifferentiated PC12 cells through regulation of the NGF receptor signaling pathway.

Acknowledgements

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초록 : 신경세포에서 신경성장인자(nerve growth factor)의 조절에 미치는 천문동(*Asparagus co-chinchinensis*) 열수추출물의 영향

이현아[1]·김지은[1]·송성화[1]·성지은[1]·정민기[1]·김동섭[1]·손홍주[1]·이충열[2]·이희섭[3]·황대연[1]*

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천문동(*Asparagus cochinchinensis*)은 북아시아 지역에서 열병, 감기, 신장질환, 유방암, 염증질환, 뇌질환 등의 치료에 오랫동안 사용되어온 약용식물(medicinal plant)이다. 비록, 천문동의 항염증(ani-inflammatory) 효능에 대한 일부 연구들이 수행되었지만, 신경세포에서 항염증작용과 신경성장인자(nerve growth factor, NGF)의 연관성에 대한 연구는 수행된바 없다. 따라서, 본 연구에서는 신경세포에서 신경성장인자의 분비와 작용기전에 대한 천문동 열수추출물(aqueous extract from *A. cochinchinensis,* AEAC)의 영향을 연구하였다. AEAC로 처리된 B35세포의 배양액에 NGF단백질의 농도는 대조물질(vehicle) 처리군에 비하여 유의적으로 증가하였으며, 특별한 독성은 관찰되지 않았다. 또한, NGF mRNA의 발현도 단백질의 농도변화와 유사한 양상을 나타내었다. 더불어, B35세포로부터 분비된 NGF의 생리활성을 확인하기 위해, AEAC-조정배지(conditioned medium)를 미분화된 PC12세포에 처리한 후 이들 세포의 신경염성 성장(neuritic outgrowth)을 관찰하였다. PC12세포의 수상돌기 길이(dendritic length)는 vehicle처리군에 비하여 AEAC-조정배지처리군에서 유의적으로 증가하였다. 또한, High affinity NGF 수용체의 하위신호전달에 포함된 p-TrkA와 p-ERK의 발현은 AEAC-조정배지처리군에서 높았지만, low affinity NGF 수용체의 하위신호전달에서는 낮은 수준으로 관찰되었다. 이러한 결과는 AEAC가 신경세포에서 NGF발현과 분비의 조절에 기여하기 때문에 신경퇴행성질환(neurodegenerative disease) 치료제로서 우수한 후보물질임을 제시하고 있다.