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The effect of coniferaldehyde on neurite outgrowth in neuroblastoma Neuro2a cells

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ABSTRACT

Neurite outgrowth is the differentiation process by which neurons establish synapses. In the dentate gyrus of the hippocampus, new neurons are constantly produced and undergo neurite outgrowth to form synapses, and this process is involved in cognitive ability. Therefore, if an agent could modulate neurite outgrowth, it could potentially be developed as a compound for modulating cognitive ability. In this study, we examined whether coniferaldehyde, a natural compound, regulates neurite outgrowth in Neuro2a cells. We ascertained morphological changes and measured the percentage of neurite-bearing cells and neurite lengths. Coniferaldehyde significantly increased the percentage of neurite-bearing cells, and the length of neurites in a concentration-dependent manner, without inducing cell death. We then have identified that, coniferaldehyde activates the extracellular signals-regulated Kinase 1 and 2 (ERK1/2), and further noted that, U0126, an ERK1/2 inhibitor, blocks coniferaldehyde-facilitated neurite outgrowth. Moreover, Subchronic administration of CA enhanced learning and memory, and increased neurite length of newborn neurons in the hippocampus. These results suggest that coniferaldehyde induces neurite outgrowth by a process possibly mediated by ERK1/2 signaling and enhances learning and memory.

1. Introduction

Aging is accompanied by a natural decline in cognitive ability (Park, 2000), which, if ignored, can lead to dementia or certain mental illnesses (Rajan et al., 2012). As the diseases progress, the rate of cognitive decline accelerates further (Deary et al., 2009). In addition, individuals can suffer cognitive decline due to continual stress such as depression, tension, and fatigue (Brunson et al., 2005). If neurons in the brain are destroyed due to dementia or mental illness caused by cognitive decline, normal brain function cannot be maintained and memory defitits can occur (Glees and Griffith, 1952). Unlike the other cells within the body, brain cells are not regenerated once damaged, thus, it is particularly important to maintain healthy brain cells (Glees and Griffith, 1952).

Neurite outgrowth is a phenomenon in which axons and dendrites spread out from the nerve cell body, forming synapses (Wang et al., 2011a). The hippocampal dentate gyrus is responsible for the storage and consolidation of memories in the adult mammalian brain area. This area is known to contain neural stem cells, suggesting that new neurons are constantly produced (Ming and Song, 2011), that must undergo neurite outgrowth to form synapses, to avoid elimination (Kitabatake et al., 2007). Many studies have suggested that controlling processes such as neurite outgrowth can lead to changes in cognitive ability, including memory (Ming and Song, 2011; Kitabatake et al., 2007).

To date, the effects of medicinal plants on neurite outgrowth have been extensively studied. Coniferaldehyde (CA) (4-hydroxy-3-methoxycinnamaldehyde), a component of *Salvia plebeia, Phyllanthus emblica, Diplomorpha canescens, Cinnamomum cebuense*, and *Allophylus longipes*, is a natural phenolic compound found in many foods such as pears, walnuts, kelp, and citrus fruits (Weng and Wang, 2000; Zhang et al., 2016). CA has been reported to have pharmacological effects, exhibiting anti-oxidant, anti-radical and anti-inflammatory activities (Akram et al., 2015; Hossen et al., 2014; Jung et al., 2009), though the effect of CA on neurite outgrowth has not yet been studied. Therefore, in this study, we investigated the effect of CA on neurite outgrowth in Neuro2a cells with the potential mechanism underlying this effect and learning and memory.

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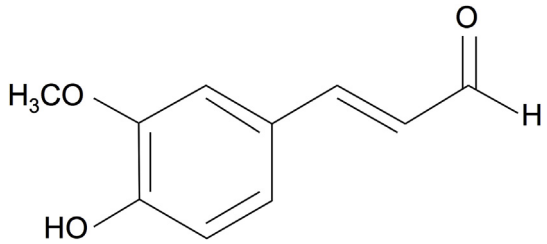

Fig. 1. Structure of CA.

2. Materials and methods

2.1. Material

Coniferadehyde (Fig. 1), retinoic acid (RA), LY294002, U0126, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylatetrazolium bromide (MTT) and Cytotoxicity Detection Kit^{PLUS} (for LDH assay) purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibody against phospho-extracellular signal-regulated kinase (pERK, Thr202/Tyr204) and HRP-conjugated anti-mouse IgG were purchased from Cell Signaling Technology (Beverly, MA, USA). Antibody against ERK1, GAPDH, and HRP-conjugated donkey anti-rabbit IgG, and Akt inhibitor were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). For stock solution, coniferaldahyde dissolved in 100% EtOH (50 mM). RA, LY294002 and U0126 were dissolved in DMSO (RA, 20 mM; LY294002, 50 mM; U0126, 50 mM). All other materials were obtained from normal commercial sources and were of the highest grade available.

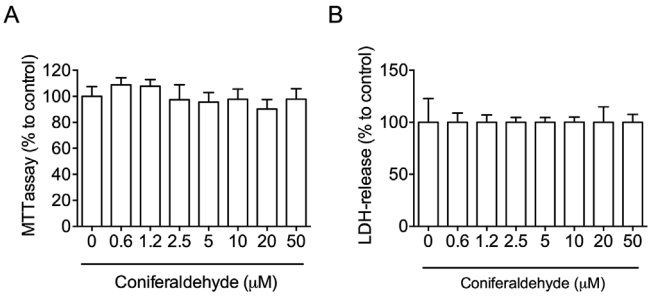

Fig. 3. CA does not affect cell viability. Cytotoxicity assessment of CA against Neuro2a cells. The cells were treated with various concentrations (0.6–50 μM) of CA for 6 h. (A) Cells viability was measured with MTT assay. (B) Cytotoxicity was measured with LDH assay. Data are presented as the mean ± S.D.

2.2. Cell culture

Mouse neuroblastoma cell line, neuero2a, was purchased from

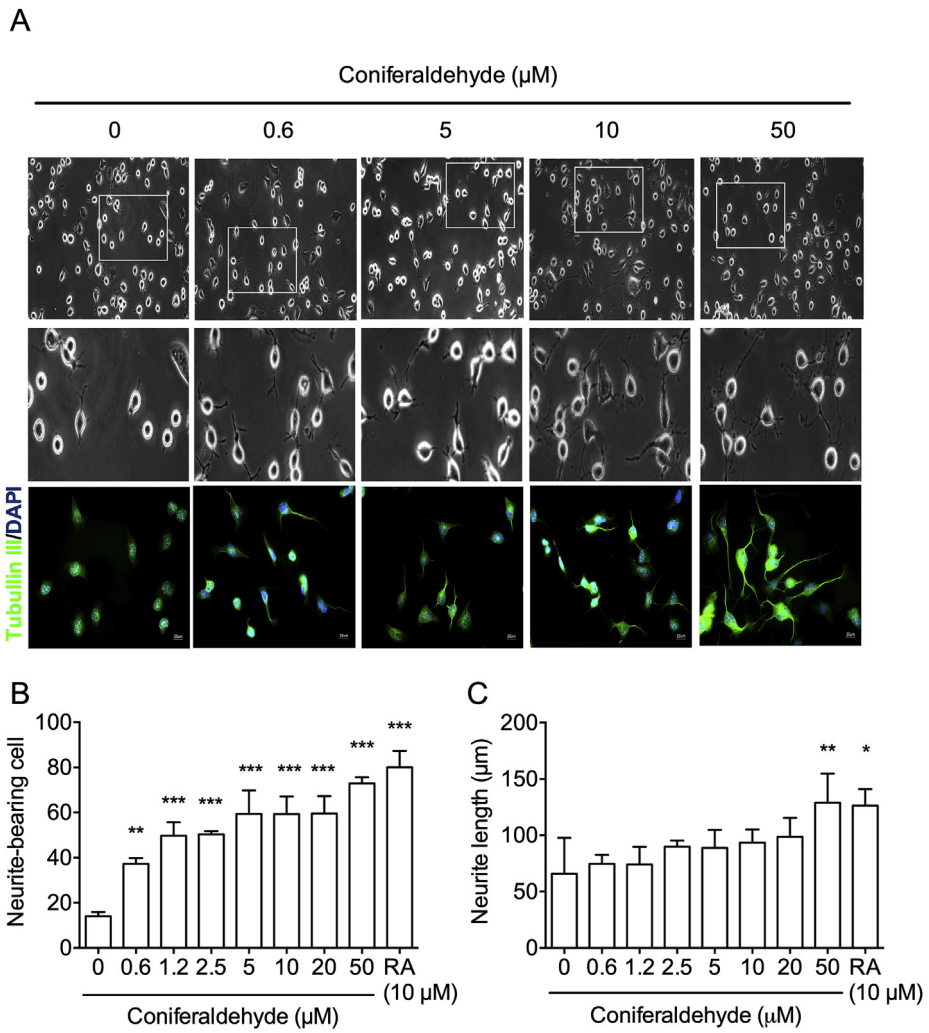

Fig. 2. CA facilitates neurite outgrowth in Neuro2a cells. Neuro2a cells were treated with 10 μM of RA (20 μM) or various concentration of CA (0.6–50 μM) for 6 h. Morphological images of cells were observed by phase contrast microscope (magnification of 200X) (A) and then neurite-bearing cells were counted (B). Length of neurite (C). Data are presented as the mean ± S.D. *p < 0.05, ** < 0.01 and ***p < 0.001 compared to control. CA, coniferaldehyde. RA, retinoic acid.

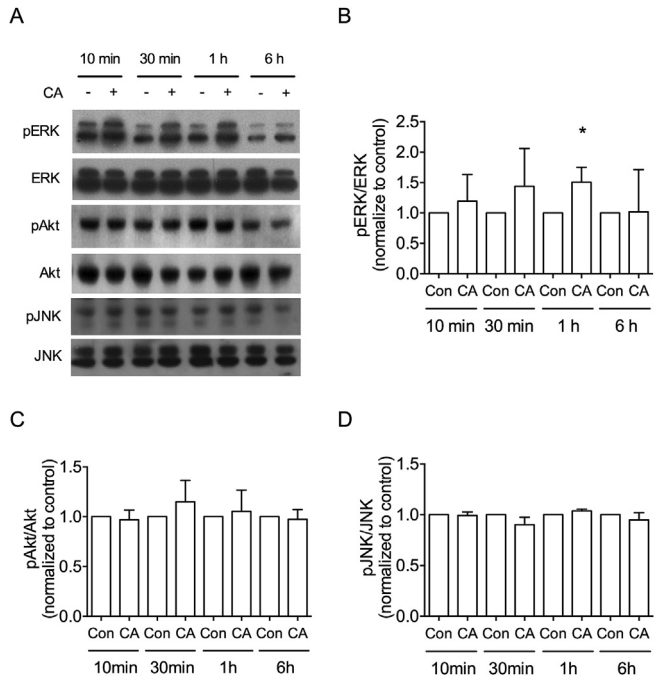

Fig. 4. ERK signaling is required for CA-facilitated neurite outgrowth. CA was treated with 50 μM in Neuro2a cells in a time-dependent and then protein extracts were prepared for western blot analysis. (A) Western blot analysis was performed in time-dependent with phospho-ERK, ERK, phosphor-Akt, Akt, phosphor-JNK and JNK antibodies. (B) Quantitative analysis of western blot of phosphor-ERK and ERK. (B) Quantitative analysis of western blot of phosphor-Akt and Akt. (B) Quantitative analysis of western blot of phosphor-JNK and JNK. Data are presented as the mean ± S.D. *p < 0.05 compared to each control.

American Type Cell Collection (Manassas, VA, 20110, USA). Cells were plated onto 100 mm plastic culture dishes at 37 °C in a humidified atmosphere with 5% CO_2. The culture medium was Minimum Essential Medium with 1% penicillin-streptomycin and 10% fetal bovine serum (WElGENE Co,. Daegu, Korea).

2.3. Evaluation of neurite outgrowth

Cells were seeded in 24-well plate (2×10^4 cells/well) and growth for 24 h. After 24 h, the medium was removed and then the drug was treated to different concentration (CA, 0.6, 1.2, 2.5, 5, 10, 20, 50 μM; RA, 20 μM) for 6 h. For the blocking test, cells were treated with LY294002 (50 μM), U0126 (50 μM) or Akt inhibitor (5 μM) for 30 min, then treated with CA + inhibitor for 6 h further.

Neurite outgrowth of Neuro2a cells was evaluated by measuring the neurite-bearing cell and neurite length. Measurement of the neurite-bearing cells and length of neurites were performed with a phase contrast microscope using application software. Random fields of 100–200 cells were photographed with a Nikon Diaphot phase contrast microscope. At least 3 photographs were taken per experimental point and each experiment was repeated at least three times. The number of cells with at least one neurite longer than its cell body was counted and expressed as a percentage of the total number of cells (neurite-bearing cells). The neurite length of each cell was measured by image J software. Neurites that were longer than a cell nucleus were traced and scaled in micrometers. The average length of neurites was calculated from 200 processes that were measured in each experimental condition.

2.4. Cell viability and cytotoxicity

2.4.1. Cell viability by MTT assay

Cells were seeded in 24-well plate (2×10^4 cells/well) and incubated for 24 h. The drug (0.6–50 μM) was treated to the cells for 6 h. After removing the medium containing CA, MTT mixture (0.5 μg/mL) was treated and incubated for 30 min. And then, MTT reagent was removed and dimethyl sulfoxide was treated. Absorbance was measured at 540 nm with ELISA plate reader (Bio-rad, Hercules, CA, USA).

2.4.2. Cytotoxicity by LDH assay

Cells were seeded in 24-well plate (2×10^4 cells/well) and incubated for 24 h. Drug (0.6–50 μM) was treated to the cells for 6 h. After collecting the drug added medium, centrifuged at 1000 rpm for 1 min to collect the supernatant. The collected supernatant was mixed with Cytotoxicity Detection Kit^{PLUS} A and B (LDH). A and B reagent mixed at a ratio of 45 : 1, and then allowed to react in a shading box for 20 min. Absorbance was measured at 490 nm with ELISA plate reader (Bio-rad, Hercules, CA, USA).

2.5. Protein extraction and western blot assay

Cells were seeded in 12-well plate (5×10^4 cells/well) and incubated for 24 h. The drug (50 μM) was treated to the cells for 6 h. The cells were washed with PBS and scraped off on the ice. Cells were lysed using M-PER buffer (Thermo, Rockford, IL, U.S.A) with protease and phosphatase inhibitor cocktails (Thermo). Proteins from the lysates were quantified using a BCA protein assay kit according to the manufacturer's instructions. Proteins (20 μg) were subjection to SDS-polyacrylamide electrophoresis and transferred to nitrocellulose membranes. The membranes were incubated overnight with the primary antibodies. Reactive bands were visualized by detecting chemiluminescence. Each signal was quantified by image analysis software (ImageJ; National Institute of Mental Healthy, Bethesda, MD, U.S.A.)

2.6. Animals

CD-1 mice (male, 6 weeks old) were obtained from Daehan Biolink (Choongbook, Korea). Mice were deposited in the designated animal care room. Temperature of the room was 23 ± 2 °C. Light cycle was maintained with 12 h interval. Humidity was 50 ± 10%. Food and water were supplied ad libitum. Mice were housed in animal room for 1 week of acclimation. All animal experiments were approved by IACUC of Dong-A University, Korea (DIACUC-approved-17-20).

2.7. Passive avoidance test

Passive avoidance test is an experiment that evaluates learning and memory using the habits of mouse that likes dark place. It was conducted for 2 days and consists of the training trial and test trial. In the training trial, mouse was positioned in acrylic box divided into dark and illuminating room and adapted for 10 s 10 s later, the door connected to the dark room opened. When the mouse enters the dark room, the door was closed and electric shock (0.5 mA, 3 s) was delivered. After 24 h, mouse was re-positioned in the illuminating room and the door opened 10 s later. The latency time was measured by 300 s. To remove the odor cues, box was cleaned with 70% ethanol. Mouse was administrated CPT (1, 3, 10 mg/kg, p.o) dissolved in 10% tween 80 (p.o) 1 h before the training trial. Sensitivity to electrical shock was measured. The criteria for evaluation were screams, jumps and runs, with a maximum of three points each.

2.8. Tissue preparation and immunohistochemistry

Anesthetized mice (Zoletil 50®, 10 mg/kg, i.m.) were perfused transcardially with 100 mM phosphate buffer (PB, pH 7.4). After

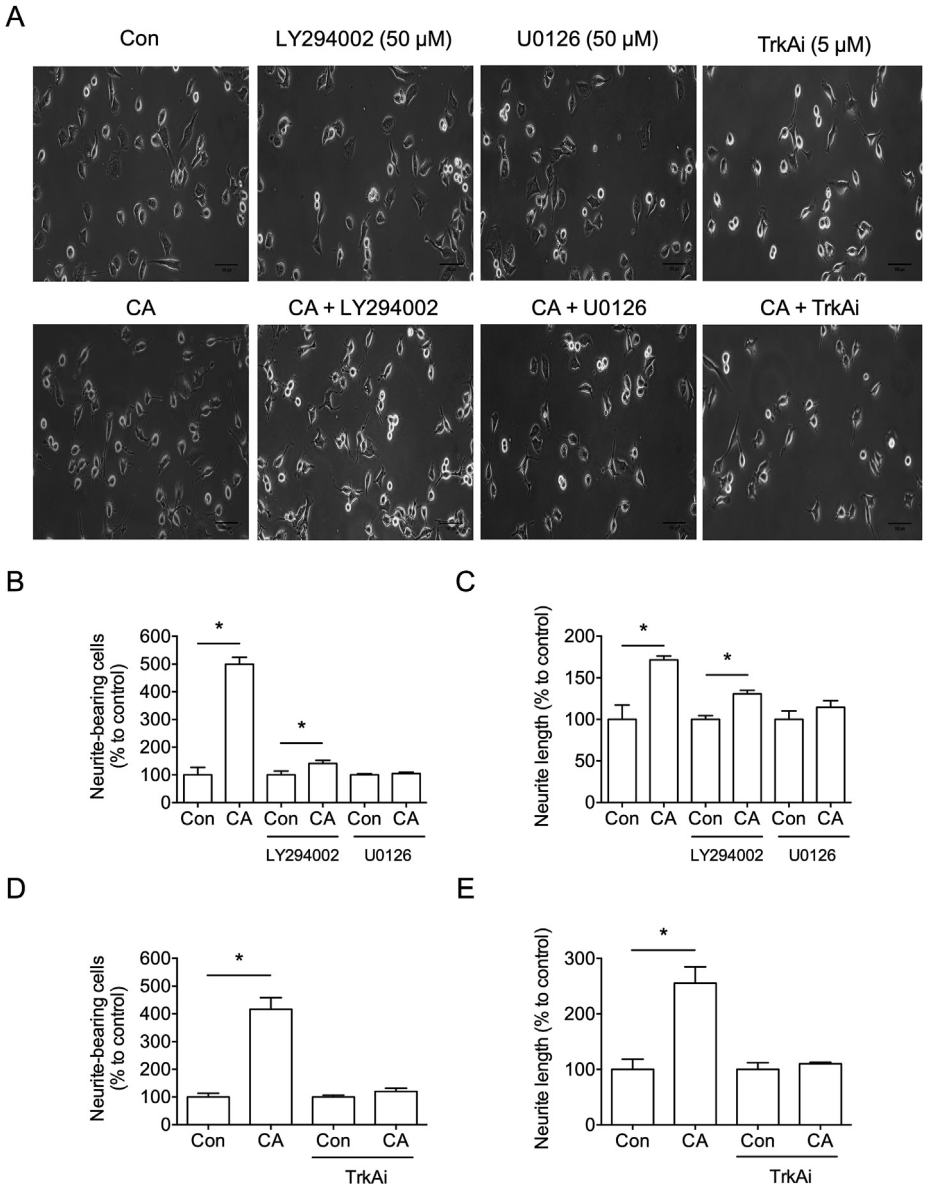

Fig. 5. NGF and ERK signaling is required for CA-facilitated neurite outgrowth. PI3K, ERK1/2 or TrkA inhibitor was used to investigate which signaling pathway is caused by neurite outgrowth. Cells were treated with inhibitor for 6 h, and then cells were treated with CA + inhibitor for further 6 h. Data are presented as the mean ± S.D. *p < 0.05 compared to each control.

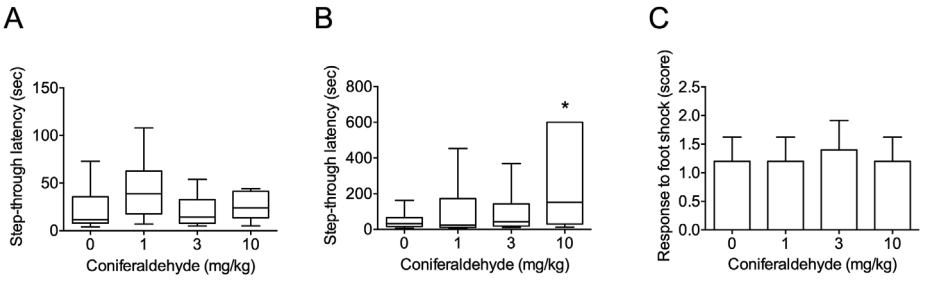

Fig. 6. The effect of CA on learning and memory in passive avoidance test. Mice were administered with CA (1, 3, or 10 mg/kg, p.o.) once a day for 6 days. Acquisition trial (AT) was performed on the sixth day and retention trial (RT) was done on the next da. CA was administered to mice at 1 h before AT. Step through latency in AT (A) and RT (B) were represented. Footshock sensitivity was measured in acquisition trial (C). Data represent as mean ± S.D. *$P < 0.05$ vs. control.

perfusion, the mouse was fixed with ice-cold 4% paraformaldehyde through transcardial perfusion. Brains were isolated and incubated in 4% PFA (in 50 mM, pH 7.4, PB) overnight for postfixation. The brains were incubated in a 30% sucrose (in 50 mM phosphate-buffered saline, PBS) solution at 4 °C before sectioning. Coronal brain slices (30 μm) were made on a cryostat and these were stored in a solution (30% glycerin, 30% ethyleneglycol and 40% DW) at 4 °C. Primary antibodies including goat anti-DCX antibody (1:500, Abcam) was mixed with 0.3% Triton X-100 and 1.5% normal serum. Free-floating sections were incubated for 24 h with the primary antibody solution. After washing, the sections were treated with biotinylated secondary antibody (1:1000) for 90 min, then avidin–biotin–peroxidase complex (1:100) for 1 h. After washing, the sections were then treated with 0.02% 3, 3′-diaminobenzidine and 0.01% H_2O_2 for approximately 3 min. The sections were

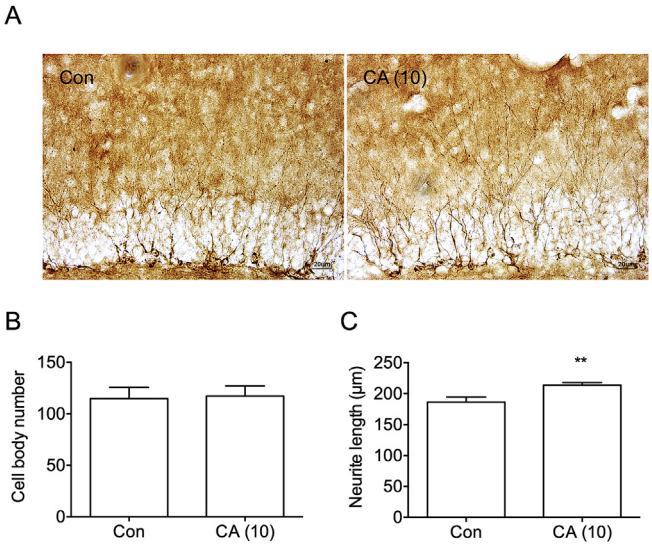

Fig. 7. CA increased neurite length of newborn neurons in the hippocampus. Mouse hippocampus was immediately isolated after retention trial. The DCX-positive cells in the hippocampus was identified (A). (B) The number of DCX-positive cells in the hippocampus. (C) Length of neurite of DCX-positive cells in the hippocampus. Data represent as mean ± S.D. *$P < 0.05$ vs. control.

mounted on gelatin-coated slides, dehydrated with ascending alcohol series, and cleared with xylene. The number of DCX-immunoreactive cells and the neurite length in the dentate gyrus (DG) of hippocampus were estimated by measuring at 200 × magnification using an AnalySIS LS Research (Soft Imaging System Ltd., Münster, Germany). Cells (hilus) were quantified in 5 sections (every tenth section from −1.50 mm posterior to the bregma). The average number of immunopositive cells per section was normalized for the entire hilus by multiplying this average by the number of 30 μm sections used (50 sections) corresponding to the entire hilus.

2.9. Statistics

All statistical analyses and graphs were performed using GraphPad Prism version 5.0 (GraphPad, San Diego, CA). For the multiple comparisons, one-way analysis of variance (ANOVA), followed by *Tukey's* test statistical differences between groups ($P < 0.05$) was used. *T*-test was used for single comparison.

3. Results

3.1. Coniferaldehyde facilitates neurite outgrowth in Neuro2a cells

To investigate whether CA (Fig. 1) induces neurite outgrowth in Neuro2a cells, morphological changes were analyzed after CA (0.6–50 μM) treatment. Cells in the control group showed few neurites, but elongated neurite formations were observed in all groups treated with CA (Fig. 2A). CA increased the percentage of neurite-bearing cells in a concentration-dependent manner to a level similar to that of RA-treated cells ($F_{8,18} = 30.47$, $P < 0.05$, n = 3/group, Fig. 2B). Neurite length was also significantly increased by CA (50 μM), again to a level similar to that of RA-treated cells ($F_{8,18} = 4.71$, $P < 0.05$, n = 3/group, Fig. 2C). Thus, this concentration (50 μM) of CA was used in subsequent experiments. These results suggest that CA induces neurite outgrowth in Neuro2a cells.

3.2. Coniferaldehyde does not affect cell viability

Neuro2a cells were treated with CA (0.6–50 μM) to identify any cytotoxic effects. Cell viability was investigated using MTT and LDH assays. CA did not induce cell death in the MTT assay ($F_{7,40} = 1.85$, $P > 0.05$, n = 6/group, Fig. 3A) or LDH assay ($F_{7,16} = 0.32$, $P > 0.05$, n = 3/group, Fig. 3B). These results suggest that CA does not exhibit cytotoxicity to Neuro2a cells.

3.3. ERK signaling is required for coniferaldehyde-facilitated neurite outgrowth

To investigate its mechanism of action, the effects of CA on ERK, Akt and JNK were examined. Phospho-ERK expression was found to be increased at 1 h (10 min, $t_4 = 0.77$, $P > 0.05$; 30 min, $t_4 = 1.22$, $P > 0.05$; 1 h, $t_4 = 3.56$, $P < 0.05$; 6 h, $t_4 = 0.05$, $P > 0.05$, n = 3/group, Fig. 4A, B). However, CA did not alter the phosphorylation of Akt or JNK (Fig. 4C and D).

To confirm the role of ERK in CA-facilitated neurite outgrowth, an off-target PI3K inhibitor (LY294002, an off target), ERK1/2 inhibitor (U0126) and TrkA inhibitor (Akti) were used. Although LY294002 (50 μM) reduced CA-facilitated neurite outgrowth, redisual outgrowth was observed (vehicle, Fig. 5). U0126 (50 μM) or Akt inhibitor (5 μM), however, completely blocked CA-facilitated neurite outgrowth (neurite-bearing cells: vehicle, $t_4 = 18.91$, $P < 0.05$; LY294001, $t_4 = 4.08$, $P < 0.05$; U0126, $t_4 = 1.52$, $P > 0.05$, n = 3/group, Fig. 5B; TrkAi, $t_4 = 1.37$, $P > 0.05$, n = 3/group, Fig. 5D; neurite length: vehicle, $t_4 = 6.92$, $P < 0.05$; LY294001, $t_4 = 8.40$, $P < 0.05$; U0126, $t_4 = 1.95$, $P > 0.05$, n = 3/group, Fig. 5C; TrkAi, $t_4 = 1.86$, $P > 0.05$, n = 3/group, Fig. 5E). These results suggest that CA facilitates neurite outgrowth through activation of TrkA ERK signaling.

3.4. Coniferaldehyde enhances learning and memory

To test whether CA can cross blood brain barrier and regulate brain function, we first examined the effect of CA on learning and memory using passive avoidance test. In the training trial, CA did not alter step-through latency ($F_{3,36} = 2.205$, $P > 0.05$, n = 10/group, Fig. 6A) and responses to foot shock ($F_{3,36} = 0.500$, $P > 0.05$, n = 10/group, Fig. 6C), suggesting that CA do not affect sensory functions. In the test trial, CA significantly increased step-through latency ($F_{3,36} = 3.553$, $P < 0.05$, n = 10/group, Fig. 6B).

3.5. Coniferaldehyde increases neurite length of newborn neurons in the hippocampus

To test whether CA can cross blood brain barrier and regulate neurite outgrowth, we analyzed the effect of CA on neurite length of newborn neurons (Fig. 7). DCX-positive newborn neurons were observed in hilus of dentate gyrus region of the hippocampus (Fig. 7A). Although CA treatment for 7 days did not alter the number of DCX-positive cells, CA significantly increased the length of neurite of DCX-positive cells (number, $t_8 = 0.371$, $P > 0.05$, n = 5/group, Fig. 7B; length, $t_8 = 6.729$, $P < 0.05$, n = 5/group, Fig. 7C).

4. Discussion

In this study, we found that CA promoted neurite outgrowth in a concentration-dependent manner and did not exhibit cytotoxicity at its highest concentration. CA-facilitated neurite outgrowth was blocked by an ERK inhibitor, and ERK activation was significantly increased by CA. Subchronic administration of CA enhanced learning and memory, and increased neurite length of newborn neurons in the hippocampus. These results suggest that CA promotes neurite outgrowth through the ERK signaling and enhances learning and memory.

Neurite outgrowth is a phenomenon in which axons and dendrites spread out from the cell body, and is essential for the development of the nervous system (Read and Gorman, 2009; Min et al., 2006). Neurite outgrowth plays an important role in determining the connectivity of neural networks (Read and Gorman, 2009) and promoting axonal

regeneration after nerve injury or neurodegenerative diseases (Read and Gorman, 2009; Min et al., 2006; Tang, 2001; Papadopoulos et al., 2009; Schiwy et al., 2009). In general, neurological diseases are caused by the death or damage of nerve cells (Martin, 2001), and thus can be treated by promoting nerve cell production or regeneration (Martin, 2001). It is known that proteins such as neurotrophic factors, cytokines, and transcription factors promote the production of nerve cells (Goldshmit et al., 2004). Nerve growth factor (NGF) has been shown to induce neurite outgrowth in various neurons, namely Neuro2a and PC12 cells (Levi-Montalcini and Angeletti, 1968; Drubin et al., 1985; Wang et al., 2011b). Natural compounds have also been shown to activate neurite outgrowth by promoting the production of nerve cells (Tohda et al., 2005; More et al., 2012). This neurite outgrowth is regulated by a variety of signaling pathways such as Akt, PI3K, ERK and JNK (Chijiwa et al., 1990; Park et al., 2017; Kaplan and Miller, 2000). In the present study, we found that CA activated ERK and not Akt or JNK signaling. This was also confirmed when an ERK inhibitor, U0126, completely blocked CA-induced neurite outgrowth. Interestingly, although CA failed to regulate Akt, the Akt inhibitor, LY294002, significantly suppressed CA-induced neurite outgrowth. This could be due to the presence of an unidentified target of LY294001, other than Akt. Alternatively, downstream elements of the Akt and ERK signaling pathways may overlap, and both may involve the transcription factor cAMP response elements-binding protein (CREB), a transcript factor (Liu et al., 2015; Ditlevsen et al., 2008). ERK signaling is a well-known neuronal survival pathway, and its activation mediates neural differentiation, and protects neurons from drug-induced damage (Boulton et al., 1991). Neurite outgrowth is also known to be mediated by the activation of the ERK signaling by NGF stimulation (Boulton et al., 1991). ERK activates CREB, which then stimulates the transcription of various proteins required for neurite outgrowth (Liu et al., 2015; Won et al., 2015). CA, a natural phenolic compound, thus activates the ERK signaling similar to NGF and induces neurite outgrowth.

In the present study, CA enhanced passive avoidance memory and neurite outgrowth of newborn neurons in the hippocampus. These results are compatible with the data from *in vitro* study. However, we are not sure that CA, itself, can penetrate blood brain barrier (BBB). Because many previous reports suggested CNS effects of penylpropanoids (Yoon et al., 2007; Kwon et al., 2014), we may guess that CA could cross BBB. However, we cannot rule out the possibility that metabolite of CA, which can be generated by liver metabolism, penetrates BBB and exert neurite outgrowth effect.

In summary, treatment with CA significantly induced the neurite outgrowth in Neuro2a cells through the ERK signaling pathway. This suggests that CA could potentially be developed as a new natural drug to promote neuronal differentiation.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuint.2019.104579.

References

Akram, M., Syed, A.S., Kim, K.A., Soo, L.J., Chang, S.Y., Kim, C.Y., Bae, O.N., 2015. Heme oxygenase 1 mediated novel anti-inflammatory activities of Salvia plebeian and its active components. J. Ethnopharmacol. 174, 322–330.

Boulton, T.G., Nye, S.H., Robbins, D.J., Ip, N.Y., Radziejewska, E., Morgenbesser, S.D., DePinho, R.A., Panayotatos, N., Cobb, M.H., Yancopoulos, G.D., 1991. ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. Cell 65 (4), 663–675.

Brunson, K.L., Kramár, E., Lin, B., Chen, Y., Colgin, L.L., Yanagihara, T.K., Baram, T.Z., 2005. Mechanisms of late-onset cognitive decline after early-life stress. J. Neurol. 25 (41), 9328–9338.

Chijiwa, T., Mishima, A., Hagiwara, M., Sano, M., Hayashi, K., Inoue, T., Hidaka, H., 1990. Inhibition of forskolin-induced neurite outgrowth and protein phosphorylation by a newly synthesized selective inhibitor of cyclic AMP-dependent protein kinase, N-[2-(p-bromocinnamylamino) ethyl]-5-isoquinolinesulfonamide (H-89), of PC12D pheochromocytoma cells. J. Biol. Chem. 265 (9), 5267–5272.

Deary, I.J., Corley, J., Gow, A.J., Harris, S.E., Houlihan, L.M., Marioni, R.E., Penke, L., Rafnsson, S.B., Starr, J.M., 2009. Age-associated cognitive decline. Br. Med. Bull. 92 (1), 135–152.

Ditlevsen, D.K., Owczarek, S., Berezin, V., Bock, E., 2008. Relative role of upstream regulators of Akt, ERK and CREB in NCAM- and FGF2-mediated signaling. Neurochem. Int. 53 (5), 137–147.

Drubin, D.G., Feinstein, S.C., Shooter, E.M., Kirschner, M.W., 1985. Nerve growth factor-induced neurite outgrowth in PC12 cells involves the coordinate induction of microtubule assembly and assembly-promoting factors. J. Cell Biol. 101 (5), 1799–1807.

Glees, P., Griffith, H.B., 1952. Bilateral destruction of the hippocampus (cornu ammonis) in a case of dementia. Monatsschr. Psychiatr. Neurol. 123 (4–5), 193–204.

Goldshmit, Y., Galea, M.P., Wise, G., Bartlett, P.F., Turnley, A.M., 2004. Axonal regeneration and lack of astrocytic gliosis in EphA4-deficient mice. J. Neurosci. 24, 10064–10073.

Hossen, S.M., Sarkar, R., Mahmud, S., Aziz, N.A., 2014. Medicinal potential of Phyllanthus emblica (Linn.) fruits extracts: biological and pharmacological activities. Br. J. Pharmaceut. Res. 4, 1486–1499.

Jung, H.J., Song, Y.S., Lim, C.J., Park, E.H., 2009. Anti-inflammatory, anti-angiogenic and anti-nociceptive activities of an ethanol extract of *Salvia plebeia* R. Brown. J Ethnopharmacol. 126, 355–360.

Kaplan, D.R., Miller, F.D., 2000. Neurotrophin signal transduction in the nervous system. Curr. Opin. Neurobiol. 10, 381–391.

Kitabatake, Y., Sailor, K.A., Ming, G.L., Song, H., 2007. Adult neurogenesis and hippocampal memory function: new cells, more plasticity, new memories? Neurosurg. Clin. N. Am. 18 (1), 105–113.

Kwon, G., Kim, H.J., Park, S.J., Lee, H.E., Woo, H., Ahn, Y.J., Gao, Q., Cheong, J.H., Jang, D.S., Ryu, J.H., 2014. Anxiolytic-like effect of danshensu [(3-(3,4-dihydroxyphenyl)-lactic acid)] in mice. Life Sci. 101 (1–2), 73–78.

Levi-Montalcini, R., Angeletti, P.U., 1968. Nerve growth factor. Physiol. Rev. 48 (3), 534–569.

Liu, X., Wang, X., Lu, J., 2015. Tenuifoliside A promotes neurite outgrowth in PC12 cells via the PI3K/AKT and MEK/ERK/CREB signaling pathways. Mol. Med. Rep. 12 (5), 7637–7642.

Martin, L.J., 2001. Neuronal cell death in nervous system development, disease, and injury. Int. J. Mol. Med. 7 (5), 455–478.

Min, J.Y., Park, M.H., Park, M.K., Park, K.W., Lee, N.W., Kim, T., Kim, H.J., Lee, D.H., 2006. Staurosporin induces neurite outgrowth through ROS generation in HN33 hippocampal cell lines. J. Neural Transm. 113, 1821–1826.

Ming, G.L., Song, H., 2011. Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron 70 (4), 687–702.

More, S.V., Koppula, S., Kim, I.S., Kumar, H., Kim, B.W., Choi, D.K., 2012. The role of bioactive compounds on the promotion of neurite outgrowth. Molecules 17, 6728–6753.

Papadopoulos, C.M., Tsai, S.Y., Guillen, V., Ortega, J., Kartje, G.L., Wolf, W.A., 2009. Motor recovery and axonal plasticity with short-term amphetamine after stroke. Stroke 40, 294–302.

Park, D.C., 2000. The basic mechanisms accounting for age-related decline in cognitive function. Cogn. Aging: A primer 11, 3–19.

Park, H.J., Kwon, H., Lee, S., Jung, J.W., Ryu, J.H., Jang, D.S., Kim, D.H., 2017. Echinocystic acid facilitates neurite outgrowth in neuroblastoma Neuro2a cells and enhances spatial memory in aged mice. Biol. Pharm. Bull. 40 (10), 1724–1729.

Rajan, K.B., Hebert, L.E., Scherr, P.A., Mendes de Leon, C.F., Evans, D.A., 2012. Disability in basic and instrumental activities of daily living is associated with faster rate of decline in cognitive function of older adults. J. Gerontol. Ser. A Biol. Med. Sci. 68 (5), 624–630.

Read, D.E., Gorman, A.M., 2009. Involvement of Akt in neurite outgrowth. Cell. Mol. Life Sci. 66 (18), 2975–2984.

Schiwy, N., Brazda, N., Müller, H.W., 2009. Enhanced regenerative axon growth of multiple fibre populations in traumatic spinal cord injury following scar-suppressing treatment. Eur. J. Neurosci. 30, 1544–1553.

Tang, B.L., 2001. Protein trafficking mechanisms associated with neurite outgrowth and polarized sorting in neurons. J. Neurochem. 79, 923–930.

Tohda, C., Kuboyama, T., Komatsu, K., 2005. Search for natural products related to regeneration of the neuronal network. Neurosignals 14, 34–45.

Wang, X., Wang, Z., Yao, Y., Li, J., Zhang, X., Li, C., Ding, Z., 2011a. Essential role of ERK activation in neurite outgrowth induced by α-lipoic acid. Biochim. Biophys. Acta 1813 (5), 827–838.

Wang, Z., Wang, J., Li, J., Wang, X., Yao, Y., Zhang, X., Ding, Z., 2011b. MEK/ERKs signaling is essential for lithium-induced neurite outgrowth in N2a cells. Int. J. Dev. Neurosci. 29 (4), 415–422.

Weng, X., Wang, W., 2000. Antioxidant activity of compounds isolated from Salvia plebeia. Food Chem. 71 489e493.

Won, J.H., Ahn, K.H., Back, M.J., Ha, H.C., Jang, J.M., Kim, H.H., Choi, S.Z., Son, M., Kim, D.K., 2015. DA-9801 promotes neurite outgrowth via ERK1/2-CREB pathway in PC12 cells. Biol. Pharm. Bull. 38 (2), 169–178.

Yoon, B.H., Jung, J.W., Lee, J.J., Cho, Y.W., Jang, C.G., Jin, C., Oh, T.H., Ryu, J.H., 2007. Anxiolytic-like effects of sinapic acid in mice. Life Sci. 81 (3), 234–240.

Zhang, Y., Zhao, L., Guo, X., Li, C., Li, H., Lou, H., Ren, D., 2016. Chemical constituents from *Phyllanthus emblica* and the cytoprotective effects on H_2O_2-induced PC12 cell injuries. Arch Pharm. Res. (Seoul) 39 (9), 1202–1211.