3’-O-Acetyl-24-Epi-7,8-Didehydrocimigenol-3-O-β-D-Xylopyranoside Decreases Amyloid Beta Production in Amyloid Precursor Protein-Transfected HeLa Cells

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Abstract
Extracellular beta amyloid (Aβ) plaques are the neuropathological hallmarks of Alzheimer’s disease (AD). Accordingly, reducing Aβ levels is considered a promising strategy for AD prevention. 3’-O-acetyl-24-epi-7,8-didehydrocimigenol-3-O-β-D-xylopyranoside significantly decreased the Aβ production and this effect was accompanied with reduced sAPPβ production known as a soluble ectodomain APP fragment through β-secretases in HeLa cells overexpressing amyloid precursor proteins (APPs). This compound also increased the level of sAPPα, which is a proteolytic fragment of APP by α-secretases. In addition, 3’-O-acetyl-24-epi-7,8-didehydrocimigenol-3-O-β-D-xylopyranoside decreased the protein level of β-secretases, but the protein levels of A disintegrin and metalloproteinase (ADAM) family, especially ADAM10 and ADAM17, are increased. Thus, 3’-O-acetyl-24-epi-7,8-didehydrocimigenol-3-O-β-D-xylopyranoside could be useful in the development of AD treatment in the aspect of amyloid pathology.

Key Words: Alzheimer’s disease, Anti-amyloidogenic effect, Secretases

INTRODUCTION
AD is a progressive neurodegenerative disorder which is recognized as the most common form of dementia among the elderly characterized by progressive dysfunction of cognition and memory (Scheltens et al., 2016). It affects the millions of the elderly and the number of AD patients has dramatically increased. In the worldwide, approximately 35.6 million people are currently affected from AD, but it is expected that the number of people living with AD will reach 135 million people in 2050 (Prince et al., 2013; Kim et al., 2015a). Although the cause of AD has not yet been fully understood, but several studies investigated that Aβ deposition is one of the major causes of AD pathology in the early onset familial AD (Hardy and Orr, 2006). Senile plaques formed by extracellular Aβ accumulation are one of the AD pathologic hallmarks which believed to not only interfere the synapses neuron communication but also lead to cell death (Karran and De Strooper, 2016). The Aβ is known to be produced from APP (Gu et al., 2018). The shedding of APP generates sAPPβ and CTFβ through β-secretase, and consequential CTFβ cleavage produces Aβ40 as well as Aβ42 by γ-secretase (Park, 2010; Prince et al., 2013). However, α-secretase cleaves APP and then generates sAPPα and CTFα precluding the Aβ formation (Park, 2010). Eventually, the Aβ production will directly be related with the sAPPβ generation and contrary correlated with the proportion of sAPPα (Kim et al., 2015b). Considering such previous findings, reduction of Aβ or delaying the Aβ deposition in the brain would be the possible therapeutic targets in the treatment or prevention of AD (Hardy and Orr, 2006; Kim et al., 2015b; Karran and De Strooper, 2016). Furthermore, various secretases would be the key factors to modulate to deliver such beneficial effects. The ADAM family are known as α-secretases such as ADAM9, ADAM10, and ADAM17.
which catalyze the APP ectodomain shedding. ADAM10 is the major ADAM family member which is responsible for the constitutive activity, while other ADAM family such as ADAM9 and ADAM17 are accountable for the cleavage regulation (Vassar et al., 2009; Kuhn et al., 2015a).

Several drugs are used for slowing down the progression of AD, but they do not significantly delay symptom development or cure the disease. However naturally derived therapeutics showed advantages in slowing down AD development and delaying the onset of symptoms (Kim et al., 2015b; Gu et al., 2018; Chen et al., 2019; Lee et al., 2019). Considering the facts that natural products usually show less side effects compared to the synthetic chemicals and exhibit diversified beneficial effects, natural resources are gaining much attention in the field of neurodegenerative diseases. Cimicifuga dahurica (Turcz.) Maxim. (C. dahurica) is traditionally used as an antipyretic and analgesic in East Asia region such as Korea, China, Japan, and Russia. Several studies have been conducted to determine the activity of specific flavonoid compounds isolated from C. dahurica. Distinguishable compounds such as cycloartane-type triterpenoids, indolone alkaloids, phenolics contained in this medicinal plant are believed to have antioxidative effects, anti-cancer, and anti-inflammatory (Tian et al., 2007; Qin et al., 2016; Zhang et al., 2016; Lv et al., 2017; Nguyen Phuong Thao et al., 2018). However, the Aβ inhibition effects of compounds isolated from C. dahurica have not been studied. In this study, we hypothesized that a compound isolated from C. dahurica roots might exhibit anti-Aβ effects in APP overexpressing HeLa cells. To test this hypothesis, we examined the effect of the compound on Aβ production and its underlying mechanisms by investigating the formation of sAPPα and sAPPβ as well as the activities of both α- and β-secretases.

MATERIALS AND METHODS

Chemicals and reagents

Rabbit anti-APP antibodies to detection the C-terminal of APP were purchased from Sigma-Aldrich Co (St. Louis, MO, USA). FBS was purchased from ATCC Company (Manassas, VA, USA). DMEM, penicillin/streptomycin, G418, and 0.25% trypsin-EDTA were purchased from Gibco–BRL Company (Carlsbad, CA, USA). Zeocin were purchased from Invitrogen Company (Carlsbad, CA, USA). Rabbit anti-GAPDH, anti-rabbit horseradish peroxidase linked IgG, anti-ADAM9 antibodies and lysis buffer were obtained from Cell Signaling Technology (Danvers, MA, USA). Anti-BACE1 antibody, and anti-APP antibody to detection both mAPP and imAPP were obtained from Abbcom Company (Cambridge, UK). Anti-ADAM10 antibody was obtained from Calbiochem Company (San Diego, CA, USA). Anti-TACE and anti-ADAM17 antibodies were obtained from Chemicon Company (Billerica, MA, USA). All other chemicals were of analytical grade obtained from Sigma-Aldrich Co.

Plant materials

The roots of C. dahurica were obtained from Naemome Dah (Ulsan, Korea) which is the herbal resource company on February 2016. Prof. Young Ho Kim from Chungnam National University (Daejeon, Korea) identified and the herbarium of the College of Pharmacy, Chungnam National University de-
minescent Image Analyzer (LAS-4000, Fujifilm, Minato City, Tokyo, Japan) performed for the Imaging and quantitative densitometry analyses. All the protein levels were normalized to that of GAPDH.

**Aβ and sAPPα peptide assay**

APPsw-transfected HeLa cells were cultured with the compound or DMSO in DMEM for 8 h and then the medium was harvested for subsequent analyses. For the secreted Aβ detection, the kits for Aβ42 (KHB3442) and Aβ40 (KHB3482) were obtained from Invitrogen Company and used according to the supplier’s instructions. For sAPPα detection, sAPPα (27734) ELISA kit obtained from IBL Company was used in this study according to the supplier’s instructions as well.

**Statistical analysis**

Data were analyzed with Prism 7.0 software (GraphPad Software Inc., San Diego, CA, USA) using one-way analysis of variance (ANOVA) followed by the Tukey multiple comparison test. Statistical significance was set at p<0.05 and the results are expressed as the mean ± SEM.

**RESULTS**

The structure of comp 27 isolated from *C. dahurica* is shown in Fig. 1A. To test the effect of comp 27 on cell viability, HeLa cells transfected with APPsw were treated with comp 27 (1.25, 2.5, 5, 7.5, and 10 µM) for 8 h. It did not affect cell viability except for 10 µM concentration (Fig. 1B). Therefore, we used these concentrations except 10 µM in this study.

Then, we examined the effect of comp 27 on Aβ secretion. Cells were incubated with 2.5, 5, and 7.5 µM comp 27 for 8 h, and we measured the levels of Aβ42 and Aβ40 used specific ELISA kits from the conditioned media. The production of both were decreased in a dose-dependent manner. The Aβ42 level was reduced by 66.7%, 56.1%, and 46.2% at 2.5, 5, and 7.5 µM of comp 27, respectively (Fig. 1C). The Aβ40 level was also reduced by 75.7%, 64.5%, 52.5% at 2.5, 5, and 7.5 µM of comp 27, respectively (Fig. 1D).

β-Secretase and γ-secretase generated Aβ through sequential cleavage of APP. On the other hand, α-secretase and γ-secretase generated precluding Aβ by cleavage within the Aβ domain. Thus, we further tested the effects of comp 27 on the production of APP proteolytic fragments, sAPPβ and sAPPα, as well as the APP expressions to investigate the two pathways. The secreted level of sAPPβ was increased by 116.2%, 121.1%, and 131.2% at 2.5, 5, and 7.5 µM of comp 27, respectively (Fig. 2A). In addition, treatment of 7.5 µM comp 27 significantly decreased the level of sAPPβ to 50.6% (Fig. 2B, 2C). On the other hand, comp 27 did not change the levels of both mature and immature APP (mAPP and iAPP) (Fig. 2C, 2D).

Comp 27 increased sAPPα secretion and decreased the secretion of Aβ and sAPPβ. However, it did not affect total APP expression. Therefore, we expected that comp 27 may affect either ADAM family or BACE1 which are respectively acting as α- and β-secretases.

Next, we investigated whether comp 27 affect ADAM family expressions and activities. Cells were incubated with 2.5, 5, and 7.5 µM of comp 27 for 8 h. Western blot analysis of sAPPα and sAPPβ was performed for the Imaging and quantitative densitometry analyses. All the protein levels were normalized to that of GAPDH.

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ADAM17 Precursor ADAM10 Precursor and ADAM10 exist as a pro-enzyme state, which are converted to mature form to cleavage of the APP (Lammich et al., 1999). We found that both precursor and active ADAM9 and ADAM10 were increased in a dose-dependent manner (Fig. 3A). Treatment with 7.5 μM of the compound increased ADAM9 levels to 136.5%, and ADAM10 levels to 150.3%, respectively (Fig. 3B, 3C). On the other hand, comp 27 did not change the expression of any forms of ADAM17, precursor and active ADAM17 (Fig. 3D).

We tried to determine whether comp 27 influences BACE1 protein expression. As we expected, compound 27 dose-dependently decreased BACE1 expression. The level of BACE1 was decreased by 77.2%, 68.5%, and 55.5% at 2.5, 5, and 7.5 μM comp 27, respectively (Fig. 4).

**DISCUSSION**

*Cimicifuga dahurica* (Turcz.) Maxim. is commonly called ‘shengma’. It is one of the ancient herbal medicines that has been subject of extensive studies. It is distributed widely in Northeast Asia and Russia, and traditionally used as an anti-pyretic and analgesic agent (Thao et al., 2018). In this study, we first showed that comp 27, one of the isolated compounds from *C. dahurica*, significantly decreased both Aβ42 and Aβ40 secretion in HeLa cells overexpressing mutant APPs within the range of no cytotoxicity. We further investigated the possible underlying mechanism of comp 27. Comp 27 increases sAPPβ secretion which may be attributed to the expression of α-secretases. As expected, comp 27 increased ADAM9 and ADAM10 expression without affecting ADAM 17 activity. This result indicates that the comp 27-induced Aβ reduction occurs through increasing the level of α-secretase, especially ADAM9 and ADAM10. In addition, we found that comp 27 decreases sAPPβ formation concomitantly. Reduction of secreted sAPPβ might be due to either decreasing APP expression itself or inhibiting the expression level of BACE1, which is a β-secretase responsible for the cleavage generating the Aβ peptides in the amyloidogenic pathology. According to our observation, comp 27 did not affect the expressions of both mature and immature APPs. Instead, we detected that comp 27 decreased BACE1 expression which can be the main cause of reduced expression of sAPPβ. BACE1 expression, however, can be regulated at the amount of transcription, translation, or protein degradation. So, further studies need to be performed to investigate the specific mechanisms underlying the comp 27 in such processes.

Aβ oligomers stimulate the kind of biological signaling pathway involving oxidative stress and neuroinflammation (Agostinho et al., 2010). This process leads not only a neuronal synapses and dendrites impairment but also disintegration of the neural circuits and neuronal loss eventually (Vargas et al., 2018). Accordingly, reducing the Aβ generation using any substance is considered to likely a good approach for treatment or prevention of AD. To date, it was not successful to develop an effective drug to stop or modify the progression of AD. Natural products could be an excellent source to reveal a hint for the therapeutic candidates against chronic and complexed disorders including neurodegenerative diseases. Since APP is cleaved within its extracellular domain by α-secretase or β-secretase, the promoting effect of comp 27 on α-secretase expression could decrease amyloidogenic process of APP by β-secretase. It also suggests that inhibitory effect of comp 27 on β-secretase expression could result in the same effect. Thus, our data suggest that comp 27 decreases Aβ production in vitro via modulation of two kinds of enzymes directly involved in APP cleavage. Confirmation of such significant anti-
AjI effects in the future animal studies would promote comp 27 to be a possible therapeutic candidate for the AD pathology.

CONFLICT OF INTEREST

The authors declare no competing financial interest.

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REFERENCES

Agostinho, P., Cunha, R. A. and Oliveira, C. (2010) Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer’s disease. Curr. Pharm. Des. 16, 2766-2778.

Chen, X., Xu, B., Nie, L., He, K., Zhou, L., Huang, X., Spencer, P., Yang, X. and Liu, J. (2019) Flavanol-rich lychee fruit extract substantially reduces progressive cognitive and molecular deficits in a triple-transgenic animal model of Alzheimer disease. Nutr. Neurosci. doi: 10.1080/1028415X.2019.1673527 [Online ahead of print].

Gu, M. Y., Chun, Y. S., Zhao, D., Ryu, S. Y. and Yang, H. O. (2018) Glycyrrhiza uralensis and semilicoisoflavone B reduce Aβ secretion by increasing PPARY expression and inhibiting STAT3 phosphorylation to inhibit BACE1 expression. Mol. Nutr. Food. Res. 62, e1700633.

Hardy, J. and Orr, H. (2006) The genetics of neurodegenerative diseases. J. Neurochem. 97, 1690-1699.

Karran, E. and De Strooper, B. (2016) The amyloid cascade hypothesis: are we poised for success or failure? J. Neurochem. 139 Suppl 2, 237-252.

Kim, J., Park, Y., Chun, Y. S., Cha, J. W., Kwon, H. C., Oh, M. S., Chung, S. and Yang, H. O. (2015a) Effect of licorice cheluesin and its active components on experimental models of Alzheimer’s disease. J. Agric. Food. Chem. 63, 6979-6988.

Kim, J. M., Hwang, K. W., Joo, H. B. and Park, S. Y. (2015b) Antiamyloidogenic properties of dryopteris crassirhizoma roots in Alzheimer’s disease cellular model. J. Food Biochem. 39, 478-484.

Kuhn, P. H., Wang, H., Dlsich, B., Colombo, A., Zeitschel, U., Ellwart, J. W., Kremer, E., Rossner, S. and Lichtenhaler, S. F. (2010) ADAM10 is the physiologically relevant, constitutive alpha-secretase of the amyloid precursor protein in primary neurons. EMBO J. 29, 3020-3032.

Lammich, S., Kojro, E., Postina, R., Gilbert, S., Pfeiffer, R., Jasionowski, M., Haass, C. and Fahrenholz, F. (1999) Constitutive and regulated alpha-secretase cleavage of Alzheimer’s amyloid precursor protein by a disintegrin metalloprotease. Proc. Natl. Acad. Sci. U.S.A. 96, 3922-3927.

Lee, J., Cho, E., Kwon, H., Jeon, J., Jung, C. J., Moon, M., Jun, M., Lee, Y. C., Kim, D. H. and Jung, J. W. (2019) The fruit of Crateagus pinnatifida ameliorates memory deficits in beta-amyloid protein-induced Alzheimer’s disease mouse model. J. Ethnopharmacol. 243, 112107.

Lv, C., Yang, F., Qin, R., Qi, Z., Zhou, W. and Lu, J. (2017) Bioactivity-guided isolation of chemical constituents against H2O2-induced neurotoxicity on PC12 from Cimicifuga dahurica (Turcz.) Maxim. Bioorg. Med. Chem. Lett. 27, 3305-3309.

Park, S. Y. (2010) Potential therapeutic agents against Alzheimer’s disease from natural sources. Arch. Pharm. Res. 33, 1589-1600.

Prince, M., Bryce, R., Albanese, E., Wimo, A., Ribeiro, W. and Ferri, C. P. (2013) The global prevalence of dementia: a systematic review and metaanalysis. Alzheimers Dement. 9, 63-75.e2.

Qin, R., Zhao, Y., Zhao, Y., Zhou, W., Lv C. and Lu, J. (2016) Polyphenolic compounds with antioxidant potential and neuro-protective effect from Cimicifuga dahurica (Turcz.) Maxim. Fitoterapia 115, 52-66.

Scheltens, P., Blennow, K., Breteler, M. M., de Strooper, B., Frisoni, G. B., Salloway, S. and Van der Flier, W. M. (2016) Alzheimer’s disease. Lancet 388, 505-517.

Thao, N. P., Kim, J. H., Thuy Luyen, B. T., Dat, N. T. and Kim, Y. H. (2017a) In silico investigation of cycloartenol triterpene derivatives from Cimicifuga dahurica (Turcz.) Maxim. roots for the development of potent soluble epoxide hydrolase inhibitors. Int. J. Biol. Macromol. 98, 526-534.

Thao, N. P., Lee, Y. S., Luyen, B. T. T., Oanh, H. V., Ali, I., Arooj, M., Koh, Y. S., Yang, S. Y. and Kim, Y. H. (2018) Chemicals from Cimicifuga dahurica and their inhibitory effects on pro-inflammatory cytokine production by LPS-stimulated bone marrow-derived dendritic cells. Nat. Prod. Sci. 24, 194-198.

Thao, N. P., Luyen, B. T., Lee, J. S., Kim, J. H. and Kim, Y. H. (2017b) Soluble epoxide hydrolase inhibitors of indolizine alkaloids and phenolic derivatives from Cimicifuga dahurica (Turcz.) Maxim. Bioorg. Med. Chem. Lett. 27, 1874-1879.

Thao, N. P., Luyen, B. T. T., Lee, J. S., Kim, J. H., Dat, N. T. and Kim, Y. H. (2017c) Inhibition potential of cycloartenol-type glycosides from the roots of Cimicifuga dahurica against soluble epoxide hydrolase. J. Nat. Prod. 80, 1867-1875.

Tian, Z., Si, J., Chang, Q., Zhou, L., Chen, S., Xiao, P. and Wu, E. (2007) Antitumor activity and mechanisms of action of total glycosides from aerial part of Cimicifuga dahurica targeted against hepatitis. BMC Cancer 7, 237.

Vargas, L. M., Cerpa, W., Munoz, F. J., Zanlungo, S. and Alvarez, A. R. (2018) Amyloid-beta oligomers synaptotoxicity: the emerging role of EphA4-c-Ab signaling in Alzheimer’s disease. Biochim. Biophys. Acta Mol. Basis Dis. 1864, 1148-1159.

Vassar, R., Kovacs, D. M., Yan, R. and Wong, P. C. (2009) The beta-secretase enzyme BACE in health and Alzheimer’s disease: regulation, cell biology, function, and therapeutic potential. J. Neurosci. 29, 12787-12794.

Zhang, L. L., Si, J. Y., Zhang, L. J., Xiao-Wei, H., Lin, L., Li, R. Y., Chen, D. and Cao, L. (2016) Synergistic anti-tumor activity and mechanisms of total glycosides from Cimicifuga dahurica in combination with cisplatin. Chin. J. Integr. Med. doi: 10.1007/s11655-015-2108-3 [Online ahead of print].