Anti-Alzheimer and Antioxidant Activities of Coptidis Rhizoma Alkaloids

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Coptidis Rhizoma and its isolated alkaloids are reported to possess a variety of activities, including neuroprotective and antioxidant effects. Thus, the anti-Alzheimer and antioxidant effects of six protoberberine alkaloids (berberine, palmatine, jateorrhizine, epiberberine, coptisine, and groenlandicine) and one aporphine alkaloid (magnoflorine) from Coptidis Rhizoma were evaluated *via* β-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1), acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) assays, along with peroxynitrite ($ONOO^-$) scavenging and total reactive oxygen species (ROS) inhibitory assays. Six protoberberine alkaloids exhibited predominant cholinesterases (ChEs) inhibitory effects with IC_{50} values ranging between 0.44—1.07 μM for AChE and 3.32—6.84 μM for BChE; only epiberberine (K_i=10.0) and groenlandicine (K_i=21.2) exerted good, non-competitive BACE1 inhibitory activities with IC_{50} values of 8.55 and 19.68 μM, respectively. In two antioxidant assays, jateorrhizine and groenlandicine exhibited significant $ONOO^-$ scavenging activities with IC_{50} values of 0.78 and 0.84 μM, respectively; coptisine and groenlandicine exhibited moderate total ROS inhibitory activities with IC_{50} values of 48.93 and 51.78 μM, respectively. These results indicate that Coptidis Rhizoma alkaloids have a strong potential of inhibition and prevention of Alzheimer's disease (AD) mainly through both ChEs and β-amyloids pathways, and additionally through antioxidant capacities. In particular, groenlandicine may be a promising anti-AD agent due to its potent inhibitory activity of both ChEs and β-amyloids formation, as well as marked $ONOO^-$ scavenging and good ROS inhibitory capacities. As a result, Coptidis Rhizoma and the alkaloids contained therein would clearly have beneficial uses in the development of therapeutic and preventive agents for AD and oxidative stress-related disease.

Key words protoberberine; cholinesterase; peroxynitrite; β-site amyloid precursor protein cleaving enzyme 1; Coptidis Rhizoma; total reactive oxygen species

Alzheimer's disease (AD) is an age-related neurodegenerative disease and the most frequent and predominant cause of dementia in the elderly, provoking progressive cognitive decline, psychobehavior disturbances, memory loss, the presence of senile plaques, neurofibrillary tangles, and the decrease in cholinergic transmission.[1,2] Until recently, two major hypotheses have been proposed regarding the molecular mechanism of pathogenesis: the cholinergic hypothesis and the amyloid cascade hypothesis.[2] In order to treat and prevent AD, most pharmacological research has focused on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitors to alleviate cholinergic deficit and improve neurotransmission.[3] Since amyloid β peptide (Aβ) results from the proteolysis of amyloid precursor protein (APP) by β- and γ-secretases, and the formation and accumulation of Aβ is a crucial cause in AD pathogenesis, the β-site APP cleaving enzyme 1 (BACE1; aspartyl protease, Asp2, and memapsin2) has recently emerged as a prevalent therapeutic target for AD.[4,5] However, two major hypotheses are not sufficient to explain all the pathological pathways of AD. Apart from two major approaches, several activities relevant to anti-AD have been proposed: anti-inflammatory, nicotinic receptor-stimulating, and antioxidant effects.[3,6] Recently, numerous studies have been performed supporting the correlation between AD, inflammation, and oxidative stress and/or nitrosative stress.[7—9] In particular, AD has been reported to be highly associated with cellular oxidative stress, including augmentation of protein oxidation, protein nitration, glycoloxidation, and lipid peroxidation as well as accumulation of Aβ.[8—10] Among cellular oxidative stress, reactive oxygen species (ROS) and reactive nitrogen species (RNS), including superoxide anion radicals ($\cdot O_2^-$), hydrogen peroxides (H_2O_2), hydroxyl radicals ($\cdot OH$), singlet oxygen ($^1O_2$), alkoxyl radicals ($RO\cdot$), peroxyl radicals ($ROO\cdot$), and peroxynitrites ($ONOO^-$), stand accused of the etiology of numerous human degenerative diseases. In particular, $ONOO^-$, formed by the *in vivo* reaction of nitric oxide ($NO\cdot$) with $\cdot O_2^-$, has been implicated in Aβ formation and accumulation, with high levels of Aβ also augmenting $ONOO^-$ generation in the brain of AD patients.[11,12] Therefore, the simultaneous studies on both cholinesterases (ChEs) and BACE1 inhibitory effects, as well as antioxidant effects, including $ONOO^-$ scavenging and ROS inhibitory effects of Coptidis Rhizoma alkaloids, is worthy of development of promising anti-AD agents.

Coptidis Rhizoma (the rhizomes of *Coptis chinensis* FRANCH, Ranunculaceae) is known for 'Huang Lian' and used in the treatment of various diseases in traditional Chinese medicine due to their anti-diabetic, relaxant, pyretic, antibacterial, and antiviral effects.[13] Coptidis Rhizoma is also known to hold a diversity of alkaloids, including berberine, palmatine, jateorrhizine, epiberberine, magnoflorine, and coptisine,[14] and known to exert a variety of activities including anti-hypertensive,[15] anti-diabetic,[16] anti-inflammatory,[17] hypolipidemic,[18] anti-diabetic complications,[19] and antioxidant effects.[20—23] In particular, Coptidis Rhizoma and its isolated alkaloids have been reported to exhibit cognitive-en-

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hancing,[24] anti-depressing,[25] and cholinesterase-inhibitory effects.[26,27] Although much research has been conducted considering the anti-AD and neuroprotective effects of Coptidis Rhizoma and its alkaloids,[28,29] the relationship between individual alkaloid structure and anti-AD effects, as well as its relevance to antioxidant effects, remain limited. Furthermore, protoberberine alkaloids with relatively low molecular weight and high lipophilicity are supposed to meet the requirements of promising therapeutic drugs for AD.

Therefore, the objectives of the present work are to evaluate the inhibitory effects of isolated alkaloids from Coptidis Rhizoma in BACE1, AChE, and BChE assays, along with antioxidant effects in the $ONOO^-$ scavenging and total ROS inhibitory assays.

MATERIALS AND METHODS

Chemicals and Reagents Electric-eel AChE (EC 3.1.1.7), horse-serum BChE (EC 3.1.1.8), acetylthiocholine iodide (ACh), butyrylthiocholine chloride (BCh), 5,5′-dithiobis [2-nitrobenzoic acid] (DTNB), eserine, L-penicillamine (L-2-amino-3-mercapto-3-methylbutanoic acid), ethylenediaminetetraacetic acid (EDTA) diethylenetriaminepentaacetic acid (DTPA), and phenylmethylsulfonylfluoride (PMSF) were purchased from Sigma Co. (St. Louis, MO, U.S.A.). BACE1 FRET assay kit (β-Secretase) was purchased from the PanVera Co. (Madison, WI, U.S.A.). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) was purchased from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). Dihydrorhodamine 123 (DHR 123) and 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) were of high quality and were purchased from Molecular Probes (Eugene, OR, U.S.A.), and $ONOO^-$ from Cayman Chemicals Co. (Ann Arbor, MI, U.S.A.). All chemicals and solvents used in the column chromatography and the assays were of reagent grade, and were purchased from commercial sources.

Isolation of Alkaloids Six protoberberine alkaloids (berberine, palmatine, jateorrhizine, epiberberine, coptisine, and groenlandicine) and one aporphine alkaloid (magnoflorine) were isolated from Coptidis Rhizoma, as mentioned previously,[19] and its chemical structures were elucidated on the basis of spectroscopic evidences and by comparison with published data.[19,30] The chemical structures are shown in Fig. 1.

***In Vitro* BACE1 Enzyme Assay** The assay was carried out according to the supplied protocol with select modifications. Briefly, a mixture of 10 μl of the assay buffer (50 mM sodium acetate, pH 4.5), 10 μl of BACE1 (1.0 U/ml), 10 μl of the substrate (750 nM Rh-EVNLDAEFK-Quencher in 50 mM, ammonium bicarbonate), and 10 μl of the tested samples (final concentration (f.c.) 100 μM) dissolved in 10% dimethyl sulfoxide (DMSO) was incubated for 60 min at 25 °C in the dark. The proteolysis of two fluorophores (Rh-EVNL-DAEFK-Quencher) by BACE1 was monitored by the formation of the fluorescent donor (Rh-EVNL), which increases in fluorescence wavelengths at 530—545 nm (excitation) and 570—590 nm (emission), respectively. Fluorescence was measured with a microplate spectrofluorometer (Gemini EM, Molecular Devices, Sunnyvale, CA, U.S.A.). The mixture was irradiated at 545 nm and the emission intensity was recorded at 585 nm. The percent inhibition (%) was obtained by the following equation: % inhibition$=[1-(S_{60}-S_0)/(C_{60}-C_0)]\times 100$, where C_{60} was the fluorescence of the control (enzyme, buffer, and substrate) after 60 min of incubation, C_0 the initial fluorescence of the control, S_{60} the fluorescence of the tested samples (enzyme, sample solution, and substrate) after 60 min of incubation, and S_0 the initial fluorescence of the tested samples. To allow for the quenching effect of the samples, the sample solution was added to reaction mixture, and any reduction in fluorescence by the sample was then investigated. The BACE1 inhibitory activity of each sample was expressed in terms of the IC_{50} value (μM required to inhibit the proteolysis of the BACE1 substrate by 50%), as calculated from the log-dose inhibition curve. Quercetin was used as the positive control.

Kinetic Parameters of Groenlandicine and Epiberberine in BACE1 Inhibition In order to determine the inhibition mechanism, BACE1 inhibition at three different concen-

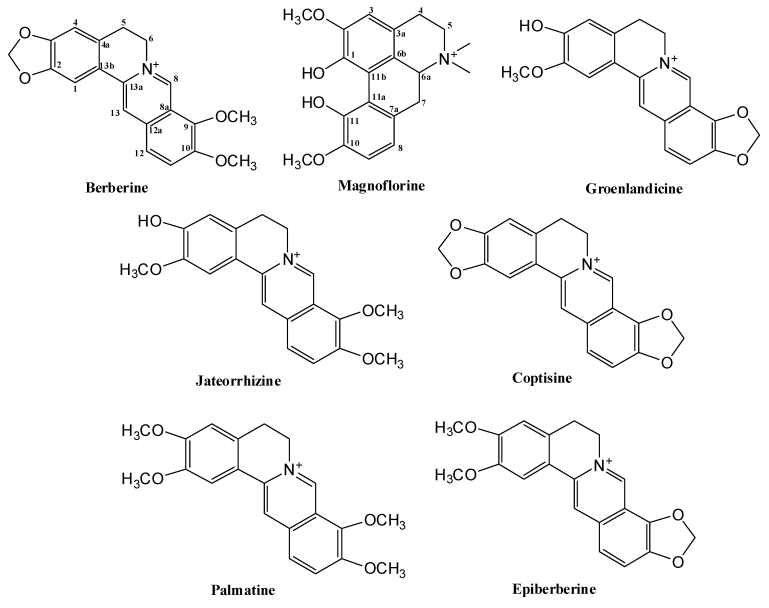

Fig. 1. Structures of Alkaloids from Coptidis Rhizoma

trations of two alkaloids (2, 4, 10 μM) was evaluated by monitoring the effects of different concentrations of the substrates (150, 250, 375 nM), respectively. The reaction mixture consisted of the same, aforementioned BACE1 assay method: a mixture of 10 μl of the assay buffer (50 mM sodium acetate, pH 4.5), 10 μl of BACE1 (1.0 U/ml), 10 μl of the substrate (Rh-EVNLDAEFK-Quencher in 50 mM, ammonium bicarbonate), and 10 μl of the tested samples dissolved in 10% DMSO. The inhibition constants (K_i) were determined by interpretation of the Dixon plot, where the value of the x-axis implies $-K_i$.[31]

***In Vitro* ChEs Enzyme Assay** The inhibitory activities of the ChEs were measured using the spectrophotometric method developed by Ellman *et al.*[32] ACh and BCh were used as the substrates to assay the inhibitions of AChE and BChE, respectively. The reaction mixture contained: 140 μl of sodium phosphate buffer (pH 8.0); 20 μl of test sample solution (f.c. 100 μM); and 20 μl of either AChE or BChE solution, which were mixed and incubated for 15 min at room temperature. All tested samples and the positive control (eserine) were dissolved in 10% DMSO. The reactions were nitrated with the addition of 10 μl of DTNB and 10 μl of either ACh or BCh, respectively. The hydrolysis of ACh or BCh was monitored by following the formation of the yellow 5-thio-2-nitrobenzoate anion at 412 nm for 15 min, which resulted from the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of either ACh or BCh, respectively. All reactions were performed in triplicate and recorded in 96-well microplates, using VERSA max (Molecular Devices, Sunnyvale, CA, U.S.A.). Percent inhibition was calculated from $(1-S/E)\times100$, where E and S were the respective enzyme activities without and with the test sample, respectively. The ChEs inhibitory activity of each sample was expressed in terms of the IC_{50} value (μM required to inhibit the hydrolysis of the substrate, ACh or BCh by 50%), as calculated from the log-dose inhibition curve.

$ONOO^-$ Scavenging Activity $ONOO^-$ scavenging was measured using a modified version of the method of Kooy *et al.*[33] by monitoring DHR 123 oxidation. DHR 123 (5 mM) in EtOH, which was purged with nitrogen, was stored at -80 °C as a stock solution. This solution was then placed in ice and remained unexposed to light prior to the study. The samples were dissolved in 10% DMSO at a final concentration of 40 μM for the compounds. The buffer used consisted of 90 mM sodium chloride, 50 mM sodium phosphate, 5 mM potassium chloride at pH 7.4, and 100 μM DTPA, each of which was prepared with high quality deionized water and purged with nitrogen. The final concentration of DHR 123 was 5 μM. Five minutes after treating with or without the addition of authentic $ONOO^-$, the background and final fluorescent intensities of the samples were measured. DHR 123 was oxidized rapidly by the authentic $ONOO^-$, and its final fluorescent intensity remained unchanged over time. The fluorescence intensity of the oxidized DHR 123 was measured with a microplate fluorescence reader (FL 500, Bio-Tek Instruments, Winooski, VT, U.S.A.) at the excitation and emission wavelengths of 485 nm and 530 nm, respectively. The results were expressed as the percent inhibition of oxidation of DHR 123 and calculated from the final fluorescence intensity minus background fluorescence. L-Penicillamine was used as the positive control.

Inhibition on Total ROS Generation ROS generation was assessed using the ROS-sensitive fluorescence indicator DCFH-DA.[34] Male Wistar rats weighing 150—200 g were sacrificed by decapitation and the kidneys were quickly removed and rinsed in iced cold-buffer [100 mM Tris, 1 mM EDTA, 0.2 mM PMSF, 1 μM pepstatin, 2 μM leupeptin, 80 mg/l trypsin inhibitor, 20 mM β-glycerophosphate, 20 mM NaF, 2 mM sodium orthovanadate (pH 7.4)]. The tissues were immediately frozen in liquid nitrogen and stored at -80 °C. Ten microliters of each test sample (f.c. 100 μM, dissolved in 10% DMSO) was added to 190 μl of kidney postmitochondrial fraction in a 50 mM potassium phosphate buffer. Then, the mixtures were loaded with 50 μl of DCFH-DA (12.5 mM) in a potassium phosphate buffer and shaken for 5 min. Finally, the fluorescence of 2′,7′-dichlorodihydrofluorescein (DCF), the oxidation product of DCFH-DA was measured on a microplate fluorescence spectrophotometer (Bio-Tek Instruments, Inc., Winooski, VT, U.S.A.) for 30 min at an excitation wavelength of 485 nm and an emission wavelength of 530 nm. Trolox was used as the positive control.

Statistics The Kruskal–Wallis test and the Mann–Whitney U test were used to determine the statistical significance of differences between values for various experimental and control groups. Data were expressed as the mean±S.E.M. in triplicate.

RESULTS AND DISCUSSION

To evaluate the potential of the Coptidis Rhizoma alkaloids as anti-AD agents, their ChEs and BACE1 inhibitory activities were measured using the modified method of Ellman *et al.*[32] and the manufacturer protocol, respectively. As shown in Table 1, six protoberberine alkaloids isolated from Coptidis Rhizoma, including berberine, palmatine, groenlandicine, jateorrhizine, coptisine, and epiberberine, exerted potent AChE inhibitory effects without significant differences in their IC_{50} values, 0.44, 0.51, 0.54, 0.57, 0.80, and 1.07 μM, respectively. In spite of slightly higher AChE inhibitory effects, our present results were consistent with previous reports.[27,35,36] Ingkaninan *et al.*[35] reported that the AChE inhibitory activity of protoberberine alkaloids is associated with planarity, substitutions on the molecule, and the

Table 1. Anti-ChEs and BACE1 Inhibitory Effects of Coptidis Rhizoma Alkaloids

	AChE[a]	BChE[b]	BACE1[c]
	IC_{50} (μM) Mean±S.E.M.	IC_{50} (μM) Mean±S.E.M.	IC_{50} (μM) Mean±S.E.M.
Berberine	0.44±0.04	3.44±0.26	>100
Palmatine	0.51±0.00	6.84±0.07	>100
Jateorrhizine	0.57±0.03	6.34±0.60	>100
Coptisine	0.80±0.01	5.81±0.49	>100
Groenlandicine	0.54±0.01	3.32±0.01	19.68±1.42
Epiberberine	1.07±0.00	6.03±0.06	8.55±1.29
Magnoflorine	>100	18.14±3.12	>100
Eserine[d]	0.02±0.00	0.05±0.02	
Quercetin[e]			9.63±1.54

a—*c*) Final concentrations of test samples were 100 μM for test compounds, dissolved in 10% DMSO; 50% inhibition concentrations (IC_{50}, μM) are expressed as the mean±S.E.M. of triple experiments. *d*) Eserine and *e*) queretin were reference compounds in the ChE and BACE1 assays.

positive charge and aromaticity at the nitrogen. Although several studies have been conducted regarding the AChE inhibitory effects of individual Coptidis Rhizoma alkaloids,[27,35—37] this is the first work on AChE inhibition of groenlandicine. Groenlandicine, berberine, coptisine, epiberberine, jateorrhizine, and palmatine also exhibited significant BChE inhibitory effects with IC_{50} values of 3.32, 3.44, 5.81, 6.03, 6.34, and 6.84 μM, respectively (Table 1). In this study, groenlandicine and berberine exerted the most effective BChE inhibition, and two-fold stronger than palmatine, jateorrhizine, coptisine, and epiberberine. Our BChE inhibitory studies of the Coptidis Rhizoma alkaloids were inconsistent with previous reports that berberine, palmatine, and coptisine showed weak BChE inhibitory effects.[38,39] Magnoflorine exhibited marginal to no inhibitory effects within test concentrations in the ChEs assays, corresponding to Adsersen's study where two aporphine alkaloids, bulbocapnine and corydine, showed no inhibitory effects against ChEs.[40]

Among the test alkaloids, only groenlandicine and epiberberine exhibited good BACE1 inhibition in a dose-dependent manner with IC_{50} values at 19.68 and 8.55 μM, respectively, as compared with a positive control, quercetin (IC_{50} 9.63 μM) (Fig. 2). Although several of the alkaloids possessed protective effects against Aβ-induced neurotoxicity,[28,29] the direct BACE1 inhibitory effects of the alkaloids have yet to be evaluated. In order to determine the manner of inhibition, kinetic analyses were investigated at different concentrations of alkaloids and substrate. As shown in Figs. 3 and 4, groenlandicine (K_i=21.2) and epiberberine (K_i=10.0) showed non-competitive inhibition with a substrate in the Dixon plots, clearly indicating that the presence of the methylenedioxy group in the D ring was responsible for key contributors to the BACE1 inhibition of protoberberine alkaloids. Considering present results in the ChEs and BACE1 assays, the Coptidis Rhizoma alkaloids have a strong possibility of inhibiting and preventing AD mainly through ChEs rather than Aβ pathways. Interestingly, groenlandicine and epiberberine exhibited both significant ChEs and BACE1 inhibition due to the methylenedioxy group in the D ring. This additional activity suggests that the two alkaloids may possess therapeutic advantages over other test alkaloids as ChE inhibitors.

As mentioned above, there has been much growing interest in multifactorial mechanisms other than ChEs and Aβ pathways, including inflammation and cellular oxidative stress in AD pathogenesis.[3,6] Thus, protection and inhibition against oxidative stress may play an important role in the development of anti-AD agents. The mechanisms of $ONOO^-$ and ROS have been relatively well-known in both cellular inflammation and oxidative stress-related neurodegenerative disorders.[1,7] In particular, $ONOO^-$, formed from NO· and $\cdot O_2^-$, is a highly reactive oxidizing and nitrating agent, leading to oxidize cellular components, including proteins, lipids, carbohydrates, and DNA, increased aggregated Aβ, and stimulated inflammatory response.[7,8] Since there are no endogenous antioxidant enzymes to scavenge $ONOO^-$ and a variety of ROS and/or RNS is partly involved in the Aβ pathway, it might be important to evaluate alkaloids, harboring $ONOO^-$ scavenging and ROS inhibitory effects, as potential anti-AD candidates. As illustrated in Table 2 and Fig. 5, groenlandicine and jateorrhizine exhibited significant

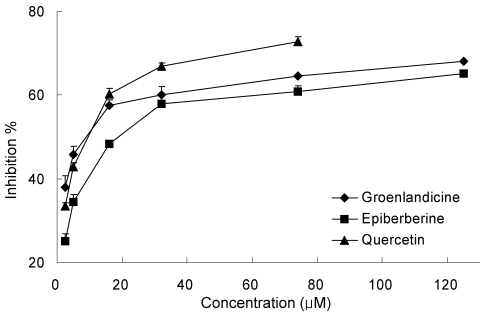

Fig. 2. Concentration-Dependent Inhibitory Effects of Groenlandicine, Epiberberine, and Quercetin on BACE1

Results are expressed as means±S.E.M. in triplicate.

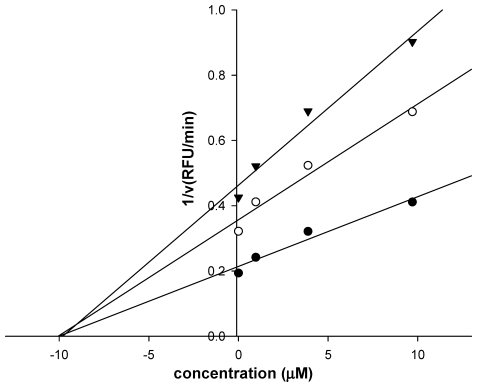

Fig. 3. Dixon Plots for Inhibition of Epiberberine on BACE1 in the Presence of Different Concentrations of Substrate: 375 nM (●); 250 nM (○); and 150 nM (▼)

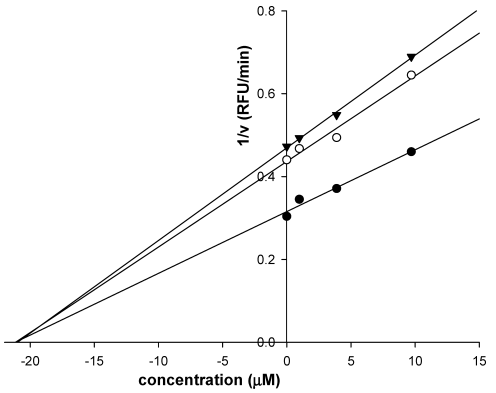

Fig. 4. Dixon Plots for Inhibition of Groenlandicine on BACE1 in the Presence of Different Concentrations of Substrate: 375 nM (●); 250 nM (○); and 150 nM (▼)

$ONOO^-$ scavenging effects in a dose-dependent manner, with IC_{50} values of 0.84 and 0.78 μM, respectively, and exerted activity ten-fold stronger than a well known $ONOO^-$ scavenger, penicillamine, with an IC_{50} value of 7.67 μM. Coptisine and epiberberine came in second with respect to IC_{50} values, 17.73 and 16.83 μM, followed by berberine and palmatine with IC_{50} values of 23.06 and 28.70 μM, respectively. In the total ROS system, only groenlandicine and coptisine exhibited moderate inhibitory effects with respective IC_{50} values of 51.78 and 48.93 μM (Table 2). Although there are several works concerning antioxidant capacities of magnoflorine,[20,22,23] its $ONOO^-$ scavenging and total ROS in-

Table 2. Scavenging/Inhibitory Effects of Coptidis Rhizoma Alkaloids against Authentic $ONOO^-$ and Total ROS Generation

	$ONOO^{-}$ [a]	Total ROS[b]
	IC_{50} (μM) Mean±S.E.M.	IC_{50} (μM) Mean±S.E.M.
Berberine	23.06±0.63	>100
Palmatine	28.70±1.20	>100
Jateorrhizine	0.78±0.09	>100
Coptisine	17.73±1.10	48.93±0.71
Groenlandicine	0.84±0.01	51.78±0.69
Epiberberine	16.83±0.78	>100
Magnoflorine	>40	>100
Penicillamine[c]	7.67±0.67	
Trolox[d]		15.40±0.16

a) Final concentrations of test samples were 40 μM for test compounds, with *b*) final concentrations at 100 μM. All were dissolved in 10% DMSO. *a*, *b*) The 50% inhibition concentrations (IC_{50}, μM) are expressed as the mean±S.E.M. of triple experiments. *c*) Penicillamine and *d*) trolox were reference compounds in the $ONOO^-$ and the total ROS assays.

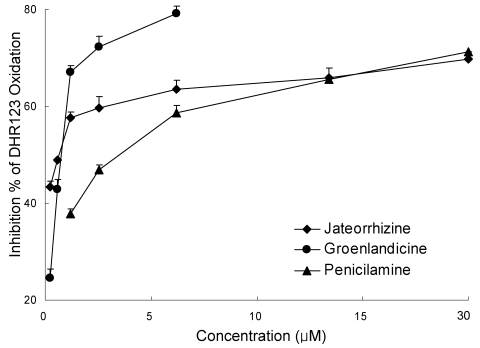

Fig. 5. Concentration-Dependent Scavenging Effects of Jateorrhizine, Groenlandicine, and Penicillamine on Authentic $ONOO^-$

Results are expressed as means±S.E.M. in triplicate.

hibitory effects did not show within test concentrations. According to a previous study on Coptidis Rhizoma alkaloids, coptisine, palmatine, epiberberine, jateorrhizine, and magnoflorine were protective against $ONOO^-$- and 3-morpholinosydnonimine (SIN)-1-induced cellular damage, except for berberine.[20] In particular, berberine has been focused upon for its antioxidant activities, including strong radical scavenging, inhibition of lipid peroxidation, LDL oxidation, $NO\cdot$ generation, and chelation of metal ions.[21,41] In addition, berberine, jateorrhizine, magnoflorine, and palmatine also have strong anti-photooxidative activities due to their basic structures and singlet oxygen quenching activities.[42] In spite of many antioxidant activities of protoberberines, studies on other minor components from Coptidis Rhizoma and its bioactivities leave much to be explained. To the best of our knowledge, this detailed study herein on the anti-AD and antioxidant effects of groenlandicine and Coptidis Rhizoma alkaloids is the first. Moreover, groenlandicine exerted BACE1 inhibitory and $ONOO^-$ scavenging/total ROS inhibitory activities prior to the various antioxidant effects of berberine that have been researched extensively.

CONCLUSIONS

Since berberine and palmatine are major alkaloids in Coptidis Rhizoma and they exhibited potent ChEs inhibitory activities, there is no denying that these two alkaloids might be attributed to over-all anti-AD effects. However, it is interesting that other minor alkaloids, including groenlandicine, epiberberine, coptisine, and jateorrhizine participate in the additional AD-related pathways, such as the BACE1, ROS, and RNS systems.

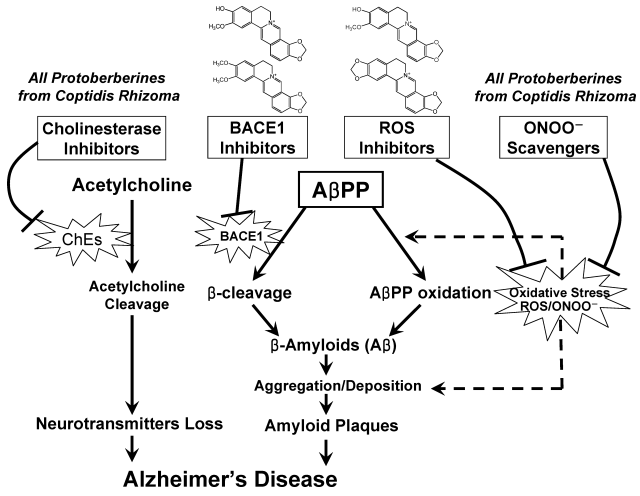

Fig. 6. Plausible Anti-AD Mechanism of Coptidis Rhizoma Alkaloids

Considering these results regarding Coptidis Rhizoma alkaloids, groenlandicine could potentially exhibit anti-AD effects through both ChEs and Aβ pathways and antioxidant capacities to scavenge/inhibit ROS and RNS. This multi-effective alkaloid also possess the methylenedioxy group in their D ring as the hydrophobic ring system and have a low molecular weight, which is sufficient for the important structural requirements of an anti-AD agents (Fig. 6). However, the precise and detailed mechanism of groenlandicine and other alkaloids from Coptidis Rhizoma remains to be scrutinized. Consequently, groenlandicine and Coptidis Rhizoma alkaloids would clearly have beneficial uses in the development as therapeutic and/or preventive agents for AD.

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