Ginsenosides Decrease β-Amyloid Production via Potentiating Capacitative Calcium Entry

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Abstract

Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disorder characterized by extracellular amyloid plaques composed of amyloid β-peptide (Aβ). Studies have indicated that Ca^{2+} dysregulation is involved in AD pathology. It is reported that decreased capacitative Ca^{2+} entry (CCE), a refilling mechanism of intracellular Ca^{2+}, resulting in increased Aβ production. In contrast, constitutive activation of CCE could decrease Aβ production. Panax ginseng Meyer is known to enhance memory and cognitive functions in healthy human subjects. We have previously reported that some ginsenosides decrease Aβ levels in cultured primary neurons and AD mouse model brains. However, mechanisms involved in the Aβ-lowering effect of ginsenosides remain unclear. In this study, we investigated the relationship between CCE and Aβ production by examining the effects of various ginsenosides on CCE levels. Aβ-lowering ginsenosides such as Rk1, Rg5, and Rg3 potentiated CCE. In contrast, ginsenosides without Aβ-lowering effects (Re and Rb2) failed to potentiate CCE. The potentiating effect of ginsenosides on CCE was inhibited by the presence of 2-aminoethoxydiphenyl borate (2APB), an inhibitor of CCE. 2APB alone increased Aβ42 production. Furthermore, the presence of 2APB prevented the effects of ginsenosides on Aβ42 production. Our results indicate that ginsenosides decrease Aβ production via potentiating CCE levels, confirming a close relationship between CCE levels and Aβ production. Since CCE levels are closely related to Aβ production, modulating CCE could be a novel target for AD therapeutics.

Key Words: Alzheimer's disease, Capacitative Ca^{2+} entry, Ginsenoside, Amyloid β-peptide, Panax ginseng

INTRODUCTION

Alzheimer's disease (AD), the most common form of neurodegenerative disorder, is characterized by a comprehensive dementia with memory loss, cognitive decline, personality changes, and behavioral impairments (Selkoe and Hardy, 2016; Breijyeh and Karaman, 2020; Monteiro *et al*., 2023). Etiological features of AD include cerebral accumulation of senile plaques composed of amyloid β-peptide (Aβ) and intraneuronal fibrillary tangles (NFTs) consisting of hyperphosphorylated microtubule-associated protein, tau, leading to severe neuronal atrophy and ultimately death (Selkoe and Hardy, 2016; Breijyeh and Karaman, 2020; Monteiro *et al*., 2023). Although the pathological cause of progressive AD has not been completely understood yet, there are many hypotheses to explain the cause of AD. The "amyloid cascade hypothesis" has long been used to explain the etiology of AD (Selkoe and Hardy, 2016; Breijyeh and Karaman, 2020; Monteiro *et al*., 2023). It states that increased production of Aβ from its precursor (APP) and defective clearance of Aβ are primary events in AD pathogenesis, triggering tau pathology and leading to neurotoxicity and neurodegeneration. Recently, aducanumab was approved by FDA as an anti-Aβ drug. It could alleviate cognitive decline and reduce Aβ plaques in subjects with mild AD during phase III clinical trials (Sevigny *et al*., 2016; van Dyck, 2018). Anti-Aβ drugs have proven that Aβ accumulation is the primary event in AD pathogenesis and that reducing Aβ is a promising therapeutic strategy. However, several alternative hypotheses have been proposed (Monteiro *et al*., 2023). According to the "tau hypothesis", a highly soluble microtubule-associated protein (MAP) known as tau undergoes abnormal and excessive phosphorylation, allowing it to aggregate and transform into paired helical filaments (PHFs). PHFs then aggregate together to form insoluble NFTs as the primary caus-

ative factor of AD pathogenesis (Frost *et al*., 2009; Gulisano *et al*., 2018; Monteiro *et al*., 2023).

Intracellular Ca^{2+} is a critical second messenger involved in various neuronal functions such as synaptic plasticity, neuronal transmission, action potentials, bursting activity, gene transcription, and neurogenesis (Brini *et al*., 2014; Ureshino *et al*., 2019). Dysregulation of intracellular Ca^{2+} signals can participate in pathological mechanisms, including necrosis, apoptosis, and neurodegeneration (Brini *et al*., 2014; Ureshino *et al*., 2019). Intracellular Ca^{2+} is mostly stored in the endoplasmic reticulum (ER) via sarcoendoplasmic reticulum Ca^{2+}-ATPase (Clapham, 2007). Upon stimulation, ER can release stored Ca^{2+} via inositol 1,4,5-trisphosphate receptors (IP3Rs) and ryanodine receptors. In response to ER Ca^{2+} depletion, stromal interaction molecule (STIM) in ER membrane can sense low Ca^{2+} levels and interact with Ca^{2+} release-activated channels in the plasma membrane such as Orai channels to activate capacitative Ca^{2+} entry (CCE), a refilling mechanism of intracellular Ca^{2+}, also known as store-operated Ca^{2+} entry (Clapham 2007; Calvo-Rodriguez *et al*., 2020).

Multiple lines of evidence have suggested Ca^{2+} dyshomeostasis in AD (Calvo-Rodriguez *et al*., 2020, Popugaeva *et al*., 2020). Several studies have reported altered intracellular Ca^{2+} signaling both in neurons and astrocytes using animal models of AD (Calvo-Rodriguez *et al*., 2020), including changes in levels of Ca^{2+} channels and Na+-Ca^{2+} exchangers in AD brain tissues (Colvin *et al*., 1991, Coon *et al*., 1999) and abnormal hypersynchrony in neurons from AD patients (Calvo-Rodriguez *et al*., 2020; Popugaeva *et al*., 2020). In fact, there are reciprocal relationships between Ca^{2+} signaling and APP processing (Popugaeva *et al*., 2020). Perturbation of Ca^{2+} signaling can enhance Aβ production and vice versa (Green and LaFerla, 2008). Aβ42 can elevate intracellular Ca^{2+} levels via metabotropic glutamate receptors 5 (Renner *et al*., 2010) and formation of Ca^{2+} channels in the plasma membrane, which allows the influx of extracellular Ca^{2+} (Arispe *et al*., 1993; Lin *et al*., 2001). Nevertheless, the under-mechanism for the CCE deficits and AD has not been clearly revealed.

P. ginseng is the most common herbal medicine as a source for longevity and a remedy for numerous diseases for over thousands of years, especially in East Asian countries (Lü *et al*., 2009; Kim and Park, 2011). It has been reported that ginseng can enhance memory and cognitive functions in healthy human subjects (Wesnes *et al*., 2000; Reay *et al*., 2005) and AD patients (Lee *et al*., 2008; Heo *et al*., 2011). Ginsenosides are the major active components derived from ginseng. They are triterpene glycosides with numerous structural variations depending on hydroxylation and sugar moiety (Baek *et al*., 2012). Various ginsenosides have been observed to possess multiple pharmacological activities for metabolic diseases such as diabetes (Choi and Song, 2019), cardiovascular disease (Lee and Kim, 2014), immune systems (Choi, 2008), cancer therapy (Choi and Song, 2019), and neurodegenerative diseases such as AD (Razgonova *et al*., 2019; Liang *et al*., 2021). Some ginsenosides can act as inhibitors of β- and γ-secretases and promote non-amyloidogenic pathway via various mechanisms (Cao *et al*., 2016; Wu *et al*., 2022), resulting in the reduction of Aβ levels. In agreement, we have previously found that some ginsenosides derived from heat-processed ginseng can decrease Aβ levels in cultured primary neurons and brains of AD mouse model (Kang *et al*., 2013). However, mechanisms involved in the Aβ-lowering effect of ginsenosides remain unclear. In the present study, we showed that all of Aβ-lowering ginsenosides potentiated CCE. Ginsenosides without Aβ-lowering effects showed no potentiating effects on CCE. Inhibiting CCE not only increased Aβ but prevented the effects of ginsenosides on Aβ. Our results suggest that inhibiting CCE induce the elevation of Aβ, and that ginsenosides decrease Aβ production via potentiating CCE.

MATERIALS AND METHODS

Chemicals

Individual ginsenosides were purified from white ginseng or heat-processed ginseng as described previously (Kim *et al*., 2000, Park *et al*., 2002). Stock ginsenoside solutions were dissolved in dimethyl sulfoxide (DMSO) and kept at -20ºC until they were used at the indicated concentrations by diluting in extracellular solution. The final DMSO concentrations were less than 0.01%. All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Bio-Rad (Hercules, CA, USA) unless indicated otherwise.

Cell culture

Human embryonic kidney (HEK) 293 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37ºC in an atmosphere containing 5% CO_2 (all supplements from GIBCO; purchased from Thermo Fisher Scientific Inc., Waltham, MA, USA). Human neuroblastoma SH-SY5Y cells stably expressing wild-type human APP and wild-type BACE1 (SH-SY5Y-APP/BACE1) were used. Cells were maintained in DMEM supplemented with 10% (v/v) heat inactivated FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 250 μg/mL Zeocin at 37ºC in an atmosphere containing 5% CO_2. SH-SY5Y-APP/BACE cells were treated with 50 μM 2-aminoethyl diphenylborinate (2APB; #D9754, Sigma-Aldrich) and 50 μg Rk1/Rg3/Rg5 mixture (RGK135) for 4 h before measuring Aβ42 levels.

Cytoplasmic Ca^{2+} measurements

Cytoplasmic Ca^{2+} levels ($[Ca^{2+}]i$) were measured using fura2 from HEK cells or SH-SY5Y-APP/BACE1 cells as previously described (Yoo *et al*., 2000). Approximately 10^6 cells were incubated with DMEM containing 4 μM fura2-AM at 22-25°C for 40 min and washed twice with extracellular solution containing 145 mM NaCl, 3.6 mM KCl, 10 mM HEPES, 1.3 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM D-glucose (pH 7.4 with NaOH). To measure CCE, extracellular solution was changed to Ca^{2+}-free extracellular solution containing an ER Ca^{2+}-depleting reagent, cyclopiazonic acid (CPA). The fluorescence was monitored in a stirred quartz-microcuvette (1 mL) in a cell holder of a model CAF-110 fluorescence spectrophotometer (Jasco, Tokyo, Japan) at a wavelength of 340 and 380 nm for excitation and 510 nm for emission. Results were calibrated by adding 10 μM ionomycin with 10 mM $CaCl_2$, which produced the maximum value of fluorescence ratio (340 nm/380 nm, Rmax), and 35 μM EGTA, which produced the minimum value of fluorescence ratio (Rmin).

Aβ42 peptide ELISA assay

SH-SY5Y-APP/BACE cells were treated with 50 μM 2APB or 50 μg/mL RGK135 for 4 h. Following incubation, 1 mL of
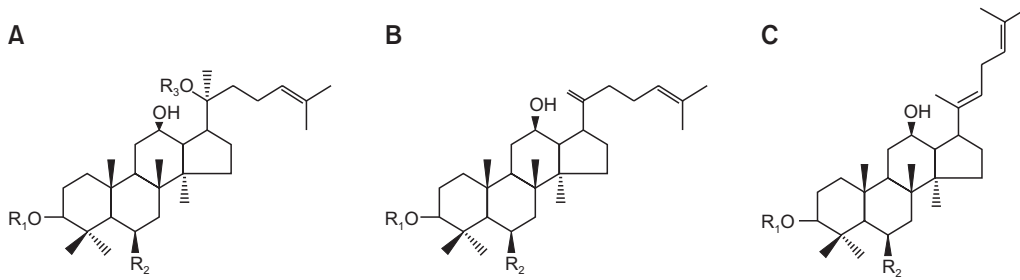
Fig. 1. Chemical structure of ginsenosides. (A) **Rb2**, R1(-Glc-Glc), R2(-H), R3(-Glc-Ara(pyr); **Rg3(20R, S)**, R1(-Glc-Glc), R2(-H), R3(-H); **Re**, R1(-H), R2(-OGlc-Rha), R3(-Glc); **Rg2**, R1(-H), R2(-OGlc-Rha), R3(-H) (B) **Rk1**: R1(-Glc-Glc), R2(-H) (C) **Rg5**: R1(-Glc-Glc), R2(-H). Glc, D-glucopyranosyl; Ara (pyr), L-arabinopyranosyl; Rha, L-rhamnopyranosyl.
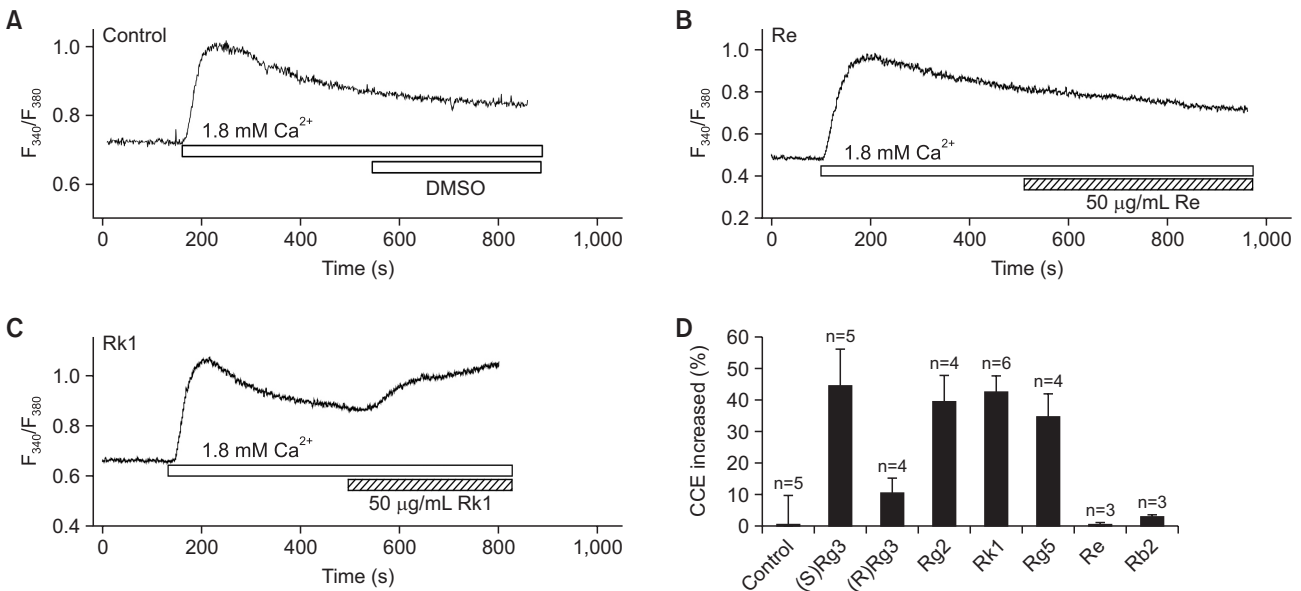

Fig. 2. CCE was potentiated by specific ginsenosides. CCE was induced by incubating HEK 293 cells in Ca^{2+}-free media containing 2 μM CPA for 10 min. After washing cells with Ca^{2+}-free HBSS (0 mM $[Ca^{2+}]_O$), Ca^{2+}-free buffer was replaced with Ca^{2+}-containing media (1.8 mM $[Ca^{2+}]_O$). After CCE level became stable, (A) 0.01% DMSO as control, (B) 50 μg/mL Re, or (C) 50 μg/mL Rk1 was added into the media. Typical results from three different experiments are shown. (D) CCE-potentiating effects of various ginsenosides were compared at the same concentration of 50 μg/mL.

culture medium was collected and centrifuged at 12,000 rpm for 5 min to spin down cell debris. Levels of secreted Aβ42 were measured using an Ultrasensitive Amyloid beta 42 Human ELISA Kit (#KHB3544, Invitrogen, Waltham, USA).

Statistical analysis

Data are expressed as mean ± SEM. We conducted statistical analysis using one way ANOVA between controls and treated experimental groups. Statistical significance was considered at $p<0.05$.

RESULTS

CCE is potentiated by some ginsenosides

We have previously found that some ginsenosides can decrease Aβ levels in cultured primary neurons and in brains of AD mouse model (Kang *et al.*, 2013). Chemical structure

of ginsenosides used in our experiment are shown in Fig. 1. Since levels of CCE and Aβ production are known to be closely related to each other, we investigated the possibility that Aβ-lowering ginsenosides might potentiate CCE. CCE was monitored from HEK293 cells by radiometric imaging as shown in Fig. 2 with typical results. After CCE levels were stabilized, we added 50 μg/mL Re or Rk1 into the media. We also added 0.01% DMSO as control (Fig. 2A). Re failed to increase CCE (Fig. 2B). However, Rk1 induced a significant increase of CCE (Fig. 2C), showing a potentiating effect of Rk1 on CCE.

In a previous report, unprocessed ginseng-derived ginsenosides such as Re, Rg1, and Rb2 showed no effect on Aβ40 or Aβ42 production. In contrast, ginsenosides from heat-processing of ginseng, including Rk1, Rg5, and enantiomers at C20 position of Rg3, (S)Rg3, and (R)Rg3, reduced Aβ production (Kang *et al.*, 2013). Therefore, we compared potentiating effects of different ginsenosides at a concentration of 50 μg/mL on CCE as shown in Fig. 2D. Among tested gin-

senosides, all Aβ-lowering ginsenosides (Rk1, Rg2, Rg3, and Rg5) showed potentiating effects on CCE (Kang *et al*., 2013). In contrast, ginsenosides without Aβ-lowering effects (Re and Rb2) showed no potentiating effects on CCE. These results might suggest that CCE modulates Aβ production.

Among CCE-potentiating ginsenosides, dose-dependency of Rk1, Rg5, and Rg2 were compared. As shown in Fig. 3A, Rk1 at 1, 10, 50, and 100 μg/mL was sequentially added after the induction of CCE. As Rk1 concentration was increased, levels of fluorescence ratio were elevated. Rk1 showed potentiating effect on CCE (22.6%), even at 10 μg/mL (Fig. 3B). Dose-dependency of Rg5, and Rg2 were also shown. Rg5 and Rg2 at 10 μg/mL showed minimal effects on CCE. However, both Rg5 and Rg2 at 50 μg/mL showed significant potentiating effects on CCE.

(S)Rg3 shows stronger potentiating effect on CCE than (R)Rg3

Among ginsenosides, Rk1 and Rg3 showed the most potent inhibiting effects on Aβ production (Kang *et al*., 2013). Interestingly, we also found that (S)Rg3, an enantiomer at C20 position of Rg3, was more effective in inhibiting Aβ production than

(R)Rg3. Thus, we compared the effects of these enantiomers on CCE. When (S)Rg3 and (R)Rg3 at 5, 10, 25, and 50 μg/mL were separately added, CCE was potentiated in a dose-dependent manner. Interestingly, the potentiating effect of (S)Rg3 was significantly higher than that of (R)Rg3 (Fig. 4A). The increased level of fluorescence ratio was calculated, and results are shown in Fig. 4B. At a concentration of 25 μg/mL, (S)Rg3 increased CCE by 37.3%, whereas (R)Rg3 increased CCE only by 3.6%. This result clearly showed differential effects of ginsenoside enantiomers on CCE. Considering that (S)Rg3 had a much stronger Aβ-lowering activity than (R)Rg3 (Kang *et al*., 2013), these findings strongly indicate that levels of CCE might modulate Aβ production.

Ginsenosides reduce Aβ levels by potentiating CCE

It is well known that 2-aminoethoxydipherryl borate (2APB), an inhibitor of IP3R, inhibits CCE (Iwasaki *et al*., 2001, Zhou *et al*., 2007). We tested the effect of 2APB on CCE under our experimental conditions from SH-SY5Y-APP/BACE1 cells. It was revealed that 2APB inhibited CCE in a concentration-dependent manner (Supplementary Fig. 1). To investigate whether 2APB could block the potentiating effect of ginsenosides on CCE, we used RGK135, a mixture of Rk1, Rg3, and Rg5. Separately, these ginsenosides were capable of potentiating CCE (Fig. 2D). As expected, 50 μg/mL RGK135 potentiated CCE (Fig. 5A). Subsequent addition of 50 μM 2APB significantly inhibited CCE. Levels of fluorescence ratio were calculated and compared in Fig. 5B. When 2APB was added prior to RGK135, 2APB prevented the potentiating effect of 50 μg/mL RGK135 on CCE as shown in Fig. 5B. This result confirmed that 2APB blocks the potentiating effect of ginsenosides on CCE.

We showed that all ginsenosides with Aβ-lowering effects potentiated CCE, while ginsenosides without Aβ-lowering effects failed to potentiate CCE. Thus, Aβ production might be closely related to CCE levels, consistent with decreased Aβ production by constitutive activation of CCE (Zeiger *et al*., 2013). To directly examine whether CCE levels affected Aβ production, SH-SY5Y-APP/BACE1 cells were treated with 50 μM 2APB or 50 μg/mL RGK135 for 4 h. Levels of secreted
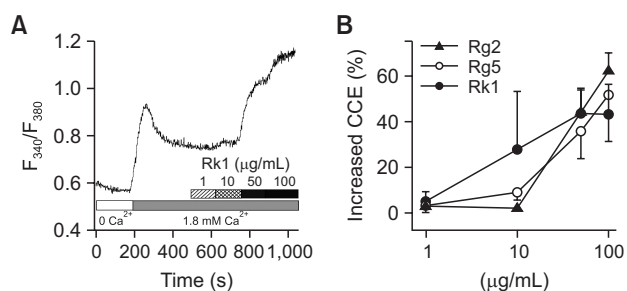

Fig. 3. Dose-dependent CCE-potentiating effects of Rk1, Rg5, and Rg2. (A) CCE was induced as described in Fig. 2. Rk1 at indicated concentration was sequentially added. Levels of fluorescence ratio were calculated. (B) Dose-dependent CCE-potentiating effects of Rk1 (n=6), Rg5 (n=4), and Rg2 (n=4) were compared.
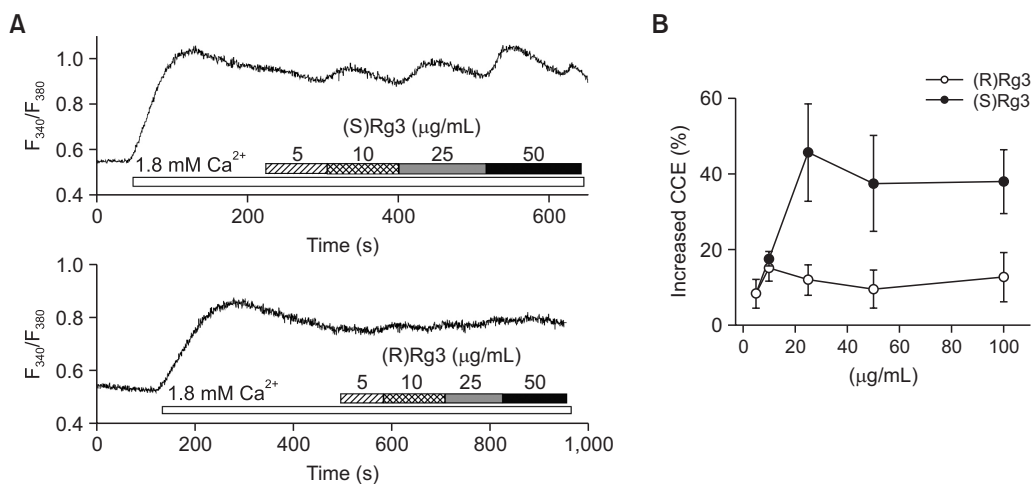

Fig. 4. (S)Rg3 showed higher CCE-potentiating effects than (R)Rg3. (A) CCE was induced as described in Fig. 2. (S)Rg3 and (R)Rg3 were sequentially added at indicated concentrations. (B) CCE-potentiating effects of (S)Rg3 (n=5) and (R)Rg3 (n=4) were compared.
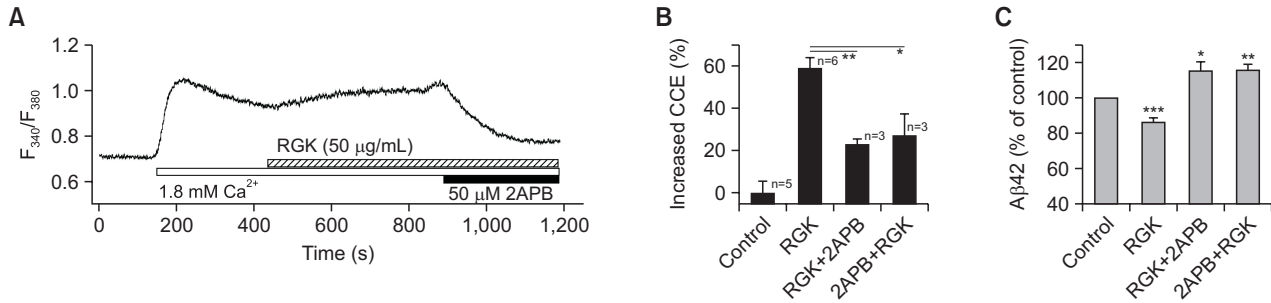
Fig. 5. RGK135 reduces Aβ production through activation of CCE. (A) CCE was induced as described in Fig. 2 from SY5Y-APP/BACE1 cells. Potentiation of CCE was observed by the addition of 50 μg/mL RGK135, which was followed by adding 50 μM 2APB subsequently. (B) Effect of CCE was calculated and compared in different conditions. (C) SH-SY5Y-APP/BACE cells were treated with 50 μM 2APB or 50 μg/mL RGK135 for 4 h. Levels of secreted Aβ42 were measured with an ELISA kit. Relative levels of Aβ42 were normalized to values from non-treat cells (control) shown as percent of control (n=5) (one-way ANOVA, *$p<0.05$; **$p<0.01$; ***$p<0.001$). All values represent mean ± SEM.
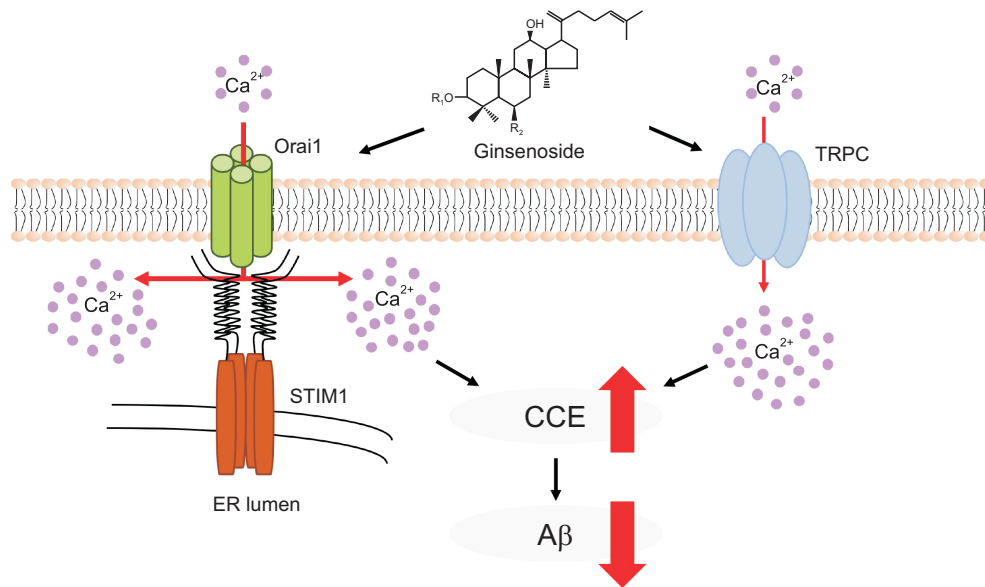

Fig. 6. Our current model for the action of Aβ-reducing ginsenosides on CCE.

Aβ42 were then measured using ELISA kit. Results are shown in Fig. 5C. As expected, RGK135 significantly decreased Aβ42 levels by 13.7 ± 24% (n=5). Treating cells with 2APB itself increased Aβ42 levels by 15.3 ± 5.4% (n=5), consistent with increased Aβ production by decreasing CCE from PS1/PS2 mutant cells (Yoo *et al.*, 2000). When 2APB and RGK135 were added together, Aβ42 levels were increased by 15.9 ± 3.2% (n=5), which is similar to the Aβ42 level observed with 2APB alone. Thus, these results indicated that the Aβ42-lowering effect of RGK135 was prevented by the presence of 2APB. We also treated SH-SY5Y-APP/BACE1 cells with Rk1 and Rg5 in the absence and the presence of 2APB. The levels of Aβ42 were significantly decreased by treating cells with Rk1 or Rg5 alone. In contrast, when cells were incubated with Rk1 or Rg5 in the presence of 2APB, the Aβ42 lowering effects of those ginsenoside was prevented (Supplementary Fig. 2). Taken together, these results show that decreased Aβ42 production by ginsenosides is via potentiating CCE.

DISCUSSION

In this study, we showed that specific ginsenosides (Rk1, Rg5, Rg2, and Rg3) could increase intracellular Ca²⁺ levels by potentiating CCE. The potentiating effect of ginsenosides on CCE was inhibited by 2APB. 2APB alone increased Aβ42 production. In addition, the Aβ42-lowering effect of ginsenoside was prevented by 2APB, suggesting a strong correlation between CCE levels and alternation of APP processing and Aβ production.

More than 30 million people are suffering from AD, the seventh leading cause of death worldwide. However, the pathological cause of AD remains ambiguous. Although traditional AD drugs (donepezil, memantine, galantamine, and rivastigmine) could modestly alleviate AD symptoms, they could not cure AD. They also fail to prevent neuronal degeneration, brain atrophy, and deterioration of cognitive functions. Therefore, the development of new drugs with novel targets is urgently needed. Our results indicate that CCE could be a

novel therapeutic target for AD, and that CCE-activating ginsenosides are potential Aβ-lowering AD drug. Our findings are summarized as a model in Fig. 6.

It has been well documented that the close correlation between intracellular Ca^{2+} signal and the synaptic dysfunction, subsequently neuronal death in AD. Growing evidence indicated that dysregulation of Ca^{2+} homeostasis influenced in amyloidogenic processing of APP and Aβ production (Calvo-Rodriguez *et al.*, 2020; Popugaeva *et al.*, 2020), and the impairment of Ca^{2+} homeostasis has been observed in aging and AD brains (Zeiger *et al.*, 2013; Zhou and Wu, 2020). Furthermore, a series of evidence have proposed a role of familiar AD (FAD)-linked presenilins (PS1/PS2) in Ca^{2+} homeostasis (Popugaeva *et al.*, 2020). Etcheberrigaray *et al.* (1998) have reported that Ca^{2+} responses are altered before senile plaques or NFTs as clinical manifestations of AD in PS mutant AD brain tissues. Subsequent studies have shown that presenilins can form ER Ca^{2+} leak channels (Tu *et al.*, 2006; Popugaeva *et al.*, 2020) and regulate sarcoendoplasmic reticulum calcium ATPase activity (Green *et al.*, 2008) as well as other Ca^{2+} permeable receptors (Popugaeva *et al.*, 2020). Consistent with these results, we have previously reported that PS1/PS2 can function as a negative regulator of CCE (Yoo *et al.*, 2000). By either abrogating PS1 function or eliminating of PS1, CCE was considerably potentiated. In contrast, the significant attenuation of CCE was observed from FAD-linked PS1/PS2 mutant, resulting in increased Aβ42 production. Several studies have also reported that CCE-mediated Ca^{2+} influx is decreased in FAD-associated presenilin mutants (Greotti *et al.*, 2019; Popugaeva *et al.*, 2020). Moreover, it has been revealed that AD stresses such as inhibition of the ubiquitin-proteasome system, ER stress, and impaired proteasome function can induce CCE inhibition, consequently leading to neuronal cell death in sporadic AD (Kuang *et al.*, 2016; Zhou *et al.*, 2019). Additionally, impairment of CCE can enhance AD pathogenesis, synapse loss, and ultimately memory loss (Popugaeva *et al.*, 2020), whereas constitutive activation of CCE decreases Aβ production (Zeiger *et al.*, 2013). Taken together, deficiency of CCE has been regarded as a possible pathogenic factor of AD, suggesting the importance of modulating CCE to decrease Aβ production. In this study, we confirm that there exists a close relationship between CCE levels and Aβ production.

P. ginseng has been extensively studied for its potential to strengthen cognitive functions, including memory, in both healthy individuals (Wesnes *et al.*, 2000; Reay *et al.*, 2005) and patients with AD (Lee *et al.*, 2008; Heo *et al.*, 2011). The numerous ginsenosides, the primary active components extracted from ginseng, have been reported its diverse pharmacological effects, particularly in terms of neurodegenerative diseases such as AD (Razgonova *et al.*, 2019; Liang *et al.*, 2021). Previously, we have demonstrated that several triterpenoid ginsenosides can reduce Aβ42 levels in cultured cell lines, neurons, and AD mouse model brains (Kang *et al.*, 2013). However, the target of ginsenosides with Aβ42-lowering effects has not been fully elucidated. Our results show that CCE could be a target of Aβ-lowering ginsenosides. In addition, small molecule activator for CCE, such as ginsenosides, has never been reported yet.

Even though we elucidated correlation between CCE levels and Aβ production using ginsenosides in this study, further studies are needed to elucidate the underlying molecular mechanism for the modulation of Aβ42 production by CCE lev-els. Several ion channels underlying CCE have been suggested to regulate Aβ production. Increased Ca^{2+} influx through enhanced STIM1-ORAI interaction can alter APP processing and decrease Aβ secretion (Zeiger *et al.*, 2013). Consistent with this result, Tong *et al.* (2016) have reported that mutant PS1 can attenuate STIM1 oligomerization in neuronal cells and human PS1 mutant brain. Therefore, it is possible that increased STIM1 oligomerization and promoted STIM1-ORAI1 recruitment might underlie CCE potentiating effects of ginsenosides. Some transient receptor potential canonical (TRPC) channels have been suggested as Ca^{2+} influx pathways. It has been demonstrated that TRPC1 decreased in AD mouse models can provoke Aβ-mediated memory deficits (Ong *et al.*, 2016; Li *et al.*, 2018). The decreased mRNA expression of TRPC6 in AD patients and patients with mild cognitive impairment has been reported (Lu *et al.*, 2018; Popugaeva *et al.*, 2020). Ginsenosides might also increase TRPC1 and TRPC6 expression levels, leading to enhanced Ca^{2+} signals and reduced Aβ levels.

In summary, we found that some ginsenosides modulate cellular Ca^{2+} levels by potentiating CCE, and that increasing CCE decreases Aβ42 production. Thus, modulating CCE could be a novel therapeutic strategy to treat AD.

ACKNOWLEDGMENTS

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