



Review article

Panax ginseng as an adjuvant treatment for Alzheimer's disease

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ABSTRACT

Longevity in medicine can be defined as a long life without mental or physical deficits. This can be prevented by Alzheimer's disease (AD). Current conventional AD treatments only alleviate the symptoms without reversing AD progression. Recent studies demonstrated that *Panax ginseng* extract improves AD symptoms in patients with AD, and the two main components of ginseng might contribute to AD amelioration. Ginsenosides show various AD-related neuroprotective effects. Gintonin is a newly identified ginseng constituent that contains lysophosphatidic acids and attenuates AD-related brain neuropathies. Ginsenosides decrease amyloid β -protein ($A\beta$) formation by inhibiting β - and γ -secretase activity or by activating the nonamyloidogenic pathway, inhibit acetylcholinesterase activity and $A\beta$ -induced neurotoxicity, and decrease $A\beta$ -induced production of reactive oxygen species and neuro-inflammatory reactions. Oral administration of ginsenosides increases the expression levels of enzymes involved in acetylcholine synthesis in the brain and alleviates $A\beta$ -induced cholinergic deficits in AD models. Similarly, gintonin inhibits $A\beta$ -induced neurotoxicity and activates the nonamyloidogenic pathway to reduce $A\beta$ formation and to increase acetylcholine and choline acetyltransferase expression in the brain through lysophosphatidic acid receptors. Oral administration of gintonin attenuates brain amyloid plaque deposits, boosting hippocampal cholinergic systems and neurogenesis, thereby ameliorating learning and memory impairments. It also improves cognitive functions in patients with AD. Ginsenosides and gintonin attenuate AD-related neuropathology through multiple routes. This review focuses research demonstrating that ginseng constituents could be a candidate as an adjuvant for AD treatment. However, clinical investigations including efficacy and tolerability analyses may be necessary for the clinical acceptance of ginseng components in combination with conventional AD drugs.

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1. Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disease that primarily affects the elderly [1]. It usually occurs after 65 years; its incidence is approximately 10% in people aged ≥ 65 years, but the percentage of individuals with AD drastically increases with age

[2]. AD is one of the most expensive diseases in the world because a therapeutic drug is not currently available. It is clinically characterized by learning and memory impairments, as well as deterioration of other cognitive and noncognitive mental functions [2]. The hallmark of patients with AD is the accumulation of amyloid plaques in the neocortical and limbic areas [3]. The gradual increase in

Abbreviations: $A\beta$, amyloid β -protein; AChE, acetylcholinesterase; AD, Alzheimer's disease; APP, amyloid precursor protein; BDNF, brain-derived neurotrophic factor; EGF, Epidermal growth factor; GLP151, ginseng major latex-like protein 151; LPA, Lysophosphatidic acid; NGF, nerve growth factor; NMDA, n-methyl-d-aspartic acid; PPAR γ , peroxisome proliferator-activated receptor- γ ; PI3K, phosphoinositide-3 kinase; ROS, reactive oxygen species; sAPP α , soluble amyloid precursor protein α .

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amyloid plaque deposits because of brain amyloidogenesis for more than several years induces neuronal cell death, resulting in brain atrophy and deficits. In particular, the loss of cerebral cortical and hippocampal neurons is linked to cognitive impairments and abnormal behaviors [4].

Because amyloid β -protein ($A\beta$) is neurotoxic and accumulates as amyloid plaques and tau tangles in the brains of patients with AD, $A\beta$ and tau hyperphosphorylation is thought to be responsible for the disease [5]. Amyloid precursor protein (APP) is an integral membrane protein present in neuronal bodies and synapses. $A\beta$, containing 37–49 amino acid residues, is produced from APP via amyloidogenesis caused by two enzymes, β - and γ -secretase [6]. In addition to amyloid plaque deposits, another important feature of AD is cholinergic dysfunction, which occurs in several brain areas, such as the basal forebrain, cortical regions, and the hippocampus. The loss of cholinergic neurons in brains with cognitive deficits is closely associated with a decrease in acetylcholine levels due to the reduced activity of enzymes involved in acetylcholine synthesis [7,8]. In addition, several factors are postulated to cause AD, such as oxidative stress, neuroinflammation, mitochondrial dysfunction, glutamatergic excitotoxicity, and reduced levels of neurotrophic factors [9–12]. Deficient hippocampal neurogenesis is also seen in patients with AD [13]. Therefore, it appears that AD is a complex disease accompanied by several deleterious conditions in the brain: amyloid plaque deposits, malfunctions in the brain cholinergic system, oxidative stress, and

brain inflammation accompanying diminished neurogenesis in the adult hippocampus [3,7].

Current Food and Drug Administration–approved treatments of AD are mainly based on two pharmacological modulations of cholinergic [i.e., acetylcholinesterase (AChE) inhibitors, which reduce acetylcholine] and glutamatergic (i.e., NMDA (n-methyl-d-aspartic acid) receptor antagonists, which reduce glutamate excitotoxicity) systems in the brain [14]. Although this can result in symptomatic improvement, it has been reported that the underlying progression of AD is unaffected by these therapies. In addition, long-term treatments can cause various adverse effects [15–17]. Besides the two conventional pharmacologic interventions, there are reports of additional strategies for AD treatment, such as the use of antiinflammatory drugs, lowering of blood pressure, and hormone replacement therapy. In addition, herbal therapy using herbal medicines such as *Panax ginseng* has received attention as an alternative and complementary intervention for neurodegenerative diseases, especially AD [18–21].

Ginseng (*Panax ginseng* Meyer) root has been widely used in the far eastern countries such as China, Japan, and Korea for thousands of years as a traditional tonic for longevity. In traditional medicine, ginseng was simply decocted with water and prepared as a drink (Fig. 1A). The tonic effects of ginseng extract include energizing the body or increasing vital energy, mood elevation, and longevity. Ginseng extract has been previously considered as a nonspecific agent for increasing resistance against various diseases and stresses as an adaptogen, when its mode of action is unknown [22].

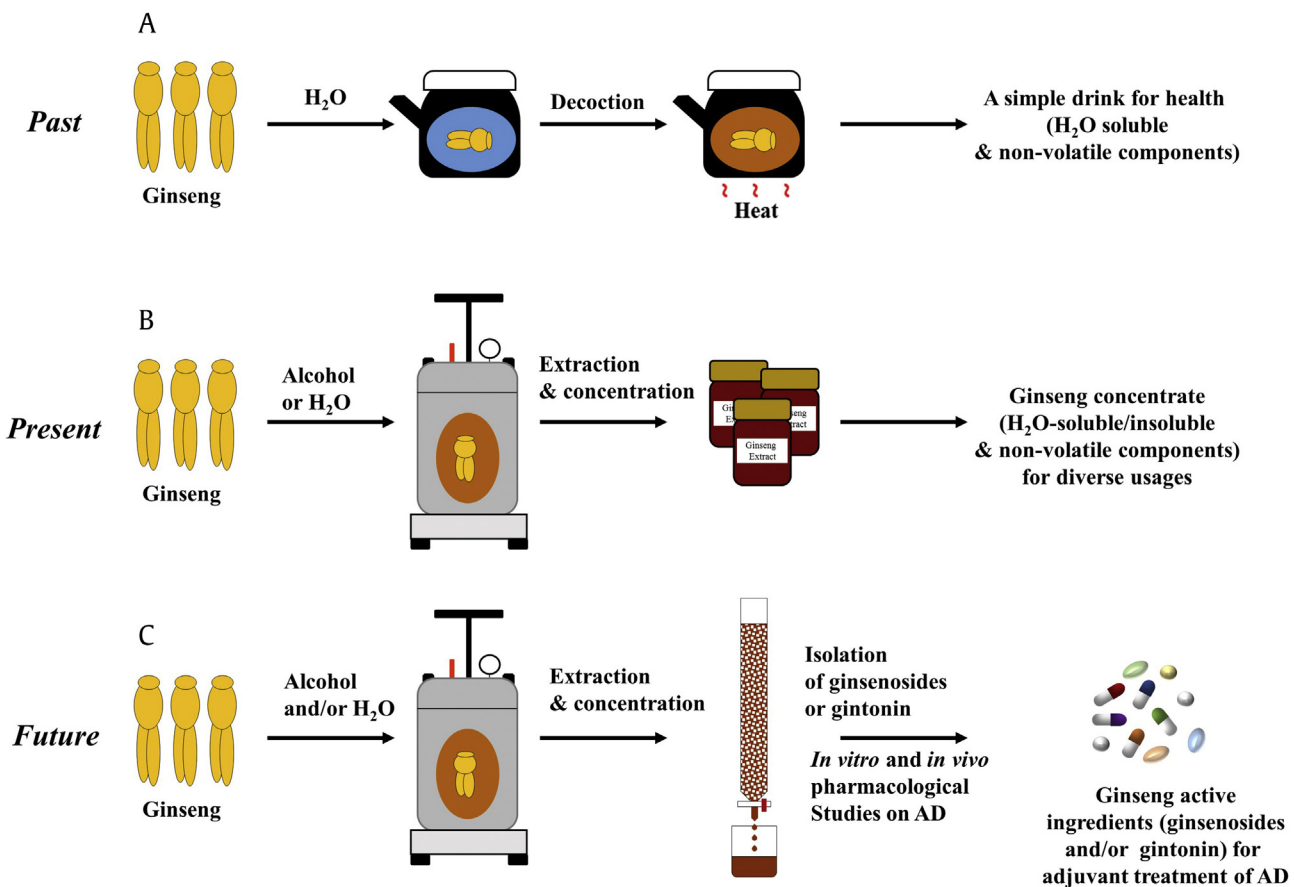


Fig. 1. A brief description of the methods used in ginseng extract preparation from the past to the present. The isolation of the active constituents of ginseng or the isolation or fractionation of the portion containing the active components (C) is required for the production of a nutraceutical or natural medicine instead of using a simple water ginseng extract (A) or a whole ginseng concentrate (B) after water and/or alcohol extraction.

Currently, ginseng is used as a functional food or alternative and/or a complementary medicine worldwide. Ginseng-containing commercial products are prepared by extraction with water and/or alcohol to obtain water-soluble and/or water-insoluble components from ginseng, respectively (Fig. 1B). One of the main merits of ginseng extract is that it exhibits a variety of effects with fewer side effects than those exhibited by other herbal medicines.

Ginsenosides (also called ginseng saponins) are the first active ingredients isolated from ginseng (Fig. 2A). They are a type of triterpenoid dammarane glycosides. The common characteristic of ginsenosides is a hydrophobic, four-ring, steroid-like backbone and carbohydrate moieties at the carbon-3, carbon-6, or carbon-20 positions [23,24]. Approximately 30 ginsenosides have been identified from ginseng, using current ginseng-processing methods. Ginsenosides are classified as protopanaxadiol (e.g., ginsenoside Rb1) or protopanaxatriol (e.g., ginsenoside Rg1). Ginseng and ginsenosides exhibit a variety of beneficial pharmacological effects outside of the nervous systems, such as anticancer, antiinflammation, antioxidant, and vasorelaxative effects [25–28]. In the nervous system, recent studies have demonstrated that ginsenosides exhibit a variety of pharmacologically beneficial effects *in vitro* and *in vivo* in animal models related to neurodegenerative diseases such as AD [29–31].

Recent studies have shown that ginseng also contains a novel ginseng-derived G protein-coupled lysophosphatidic acid (LPA, 1-acyl-2-hydroxy-*sn*-glycero-3-phosphate) receptor ligand, gintonin (Fig. 2B) [32]. Gintonin is distinct from previously identified ginseng saponins and other ginseng components in that it consists of carbohydrates, lipids, and ginseng proteins in a glycolipoprotein complex [32]. The representative functionally active components of gintonin are the LPAs and other lipids [33]. Gintonin LPAs consist of LPA C_{16:0}, LPA C_{18:1}, and LPA C_{18:2} (Fig. 2B). The order of LPA species in gintonin from most to least abundant is LPA C_{18:2} >>> LPA C_{16:0} > LPA C_{18:1}. Interestingly, ginseng contains an extraordinarily high number of LPAs, 10-fold greater than other herbal medicines and foodstuffs [34]. The characteristics of gintonin LPAs enable selective activation of LPA receptors with high affinity [33]. Gintonin LPAs are isolated in a complex with ginseng proteins called ginseng major latex-like protein 151 (GLP151) [35] (Fig. 3). The complex of LPAs and

ginseng proteins may provide at least two advantages to the physiological and pharmacological actions of LPAs. First, free forms of LPAs are susceptible to the action of LPA hydrolysis enzymes, such as lipid phosphate phosphatase [36], whereas gintonin LPAs are more soluble and stable [34]. Second, it appears as if GLP151 acts as a carrier and/or transporter for the delivery of LPA to their respective LPA receptors [35,37] (Fig. 3). Gintonin exerts its physiological and pharmacological actions in the nervous system through the LPA receptor-G $\alpha_{q/11}$ protein-phospholipase C-IP₃ receptor-[Ca²⁺]_i transient pathway [33], which is also associated with *in vitro* and *in vivo* anti-AD activity in the nervous system [35].

This review first introduces that ginseng extract exhibits symptomatically improving effects in patients with AD rather than other neurodegenerative diseases, when it combines with conventional AD drugs, although it is not a disease-modifying drug. This review then shows the extensive studies that ginsenosides and gintonin, refined components derived from *Panax* ginseng extract, exhibit *in vitro* and *in vivo* anti-AD benefits and finally proposes the idea that both components can be used as an adjuvant for the enhancement of conventional AD treatments.

2. Effects of ginseng extract on animal models of and patients with AD

2.1. Effects of ginseng extract on *in vitro* and *in vivo* animal AD model

Recent studies on the efficacy of *Panax* ginseng extract against aging-related brain diseases, such as AD, have shown that ginseng extract inhibits A β -induced neurotoxicity *in vitro* and attenuates A β accumulation in the brain *in vivo* animal studies [38]. Administration of fermented ginseng extract in a mouse model of AD improved memory function and reduced A β formation in the brain [39]. White, red, and black ginseng extract also attenuated hippocampal A β oligomer injection-induced memory dysfunction in mice through AChE inhibition [40]. Oral administration of white ginseng extract to A β oligomer-injected mice restored the reduced synaptophysin and choline acetyltransferase activity [41].

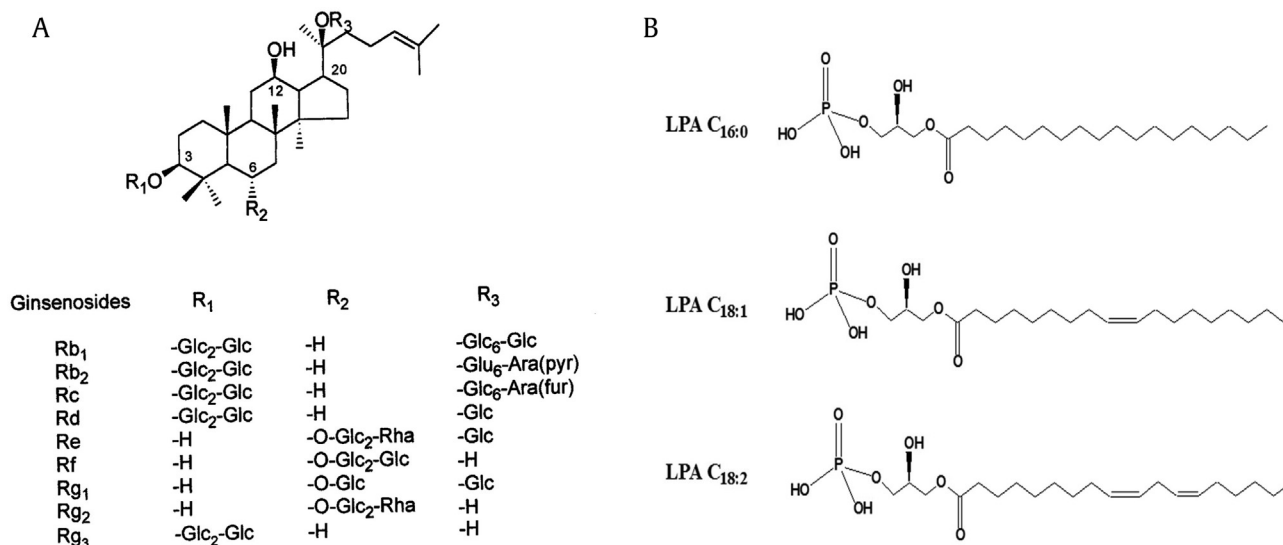


Fig. 2. The two main active ingredients of ginseng that produce anti-Alzheimer's effects. (A) Ginsenosides consist of a terpenoid dammarane backbone with carbohydrates attached at different positions. (B) The chemical structures of lysophosphatidic acids (LPAs) isolated from ginseng gintonin.

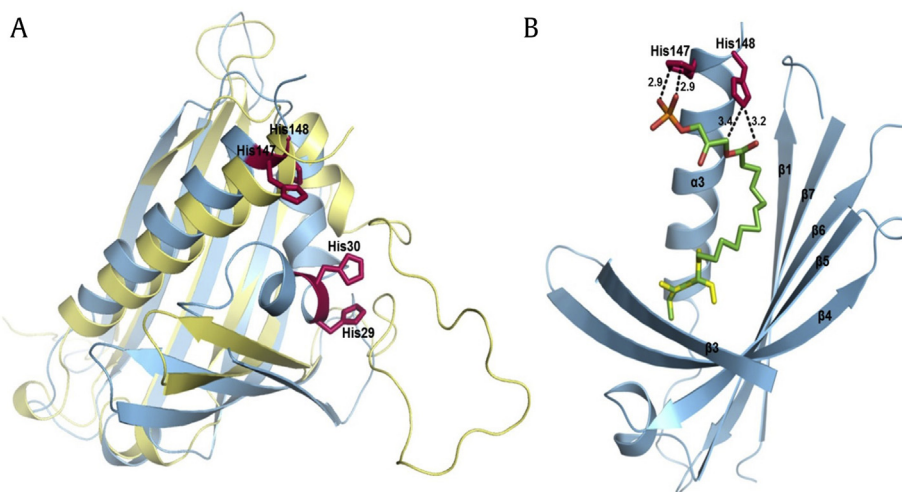


Fig. 3. Ginseng LPAs bind to ginseng major latex-like protein 151 (GLP151) and form a complex. The three-dimensional structure of GLP151 and its binding to LPA C_{18:2}. The electrostatic molecular surface of GLP151 is modeled with LPA C_{18:2} in close conformation. The positions of the residues that recognize LPA C_{18:2} are labeled. Adopted from Choi et al. (2015) for details. LPA, lysophosphatidic acid.

2.2. Effects of ginseng extract on dementia patients with AD

A number of studies have shown that the long-term administration of Korean Red Ginseng extract to patients with AD, combined with conventional AD drugs, gradually improved cognitive function, as assessed using the mini-mental state examination (MMSE) and Alzheimer's Disease Assessment Scale–Cognitive Subscale (ADAS-Cog) tests, with minor adverse effects [42–44]. MMSE test informs us the degree of cognitive impairment including attention, recall, registration, language, ability to follow simple commands, and orientation [36]. ADAS-Cog test gives us on the disturbances of memory, language, praxis, attention, and other cognitive abilities as the core symptoms of AD [45]. Long-term treatment with relatively high amounts of Korean Red Ginseng extract for more than 12 weeks also improved the frontal assessment battery, which is an indication of frontal cortical activity, such as right temporal, parietal, and occipital areas, in elderly patients with AD [45]. These reports show that various ginseng extracts, such as white ginseng, fermented ginseng, and red ginseng, could result in the alleviation of AD symptoms, to some extent, in animal models and patients. This indicates that ginseng extracts contain certain biologically active components that may prevent cognitive dysfunction due to a reduced A β burden and amyloid plaque accumulation.

Furthermore, recent reports have provided information that ginseng components, such as ginsenosides and gintonin, may be responsible for ginseng extract–mediated anti-AD activity via the inhibition of A β -induced neurotoxicity and reactive oxidation stress; stimulation of soluble amyloid precursor protein α (sAPP α) formation (but not A β); antiinflammatory effects; and boosting cholinergic systems, hippocampal neurogenesis, and cognitive functions. The subsequent sections will review the accumulated *in vitro* and *in vivo* evidence indicating that ginsenosides and gintonin are responsible for the anti-AD effects of ginseng extract.

3. Effects of ginseng components on A β formation

3.1. Inhibition of A β formation by ginsenosides

Integral membrane APP processes in the brain involve two pathways, one for the production of neurotoxic A β , and one for the production of beneficial sAPP α . Brain amyloidogenesis is initiated by β - and γ -secretase and results in the production of diverse forms

of A β [6]. These A β s are not soluble and spontaneously aggregate and accumulate in the brain to form amyloid plaques, which damage nearby neurons [6]. Over time, the number of neurons diminishes with an increase in amyloid plaque deposits. As a result, brain function, including cognition, declines. On the contrary, activation of α -secretase cleaves the middle portion of the A β peptide after γ -secretase action to produce sAPP α ; this is the nonamyloidogenic pathway and is beneficial to neurons [46]. sAPP α has been shown to exhibit protective and proliferative properties in a variety of cell types, such as neural progenitor cells in the subventricular zone of adult mice, fibroblasts, thyroid epithelial cells, and embryonic stem cells [46]. Therefore, the inhibition of β - and γ -secretase, which enables suppression of the amyloidogenic pathway, may be a potential target for anti-AD drug development. The stimulation of the nonamyloidogenic pathway through the activation of α -secretase may be an alternative way to reduce the formation of A β in the brain.

Accumulating evidence shows that ginsenosides negatively regulate β -secretase activity. In *in vitro* enzyme assays, ginsenosides Rb1, Rb2, and Rc inhibited β -secretase activity. In molecular docking studies, ginsenosides Rb1 and Rb2 exhibited high binding affinities for β -secretase. Karpagam et al (2013) also demonstrated that ginsenosides CK, F1, Rh1, and Rh2 can act as potential β -secretase inhibitors when evaluating their interactions with β -secretase [47,48]. At the cellular level, ginsenoside Rd increased sAPP α expression level that was reduced by inhibition of the mitogen-activated protein kinase (MAPK) and phosphoinositide-3 kinase (PI3K) pathways in HT22 hippocampal neuronal cells. In addition, one study showed that ginsenoside Rd stimulated sAPP α release via estrogen receptors. In ovariectomized rats, intraperitoneal administration of ginsenoside Rd increased the levels of sAPP α , reduced extracellular A β , and alleviated cognitive and memory impairments. Furthermore, ginsenoside Rd–mediated amelioration of AD-related dysfunction and activation of the MAPK and PI3K pathways was blocked by an estrogen receptor antagonist [49]. These results suggest that ginsenoside Rd likely acts through estrogen receptors to enhance learning and memory function and activation of the nonamyloidogenic pathway for stimulation of sAPP α formation. Ginsenoside Re decreased A β levels in N2a/APP695 cells. A study of the molecular mechanisms showed that ginsenoside Re decreased the levels of both β -secretase mRNA and protein and inhibited β -secretase activity in N2a/APP695 cells. Ginsenoside Re–mediated reductions of β -secretase mRNA and

protein were achieved via an increase of peroxisome proliferator-activated receptor- γ (PPAR γ) expression. Ginsenoside Re also stimulated PPAR γ activity. Therefore, ginsenoside Re-mediated inhibition of β -secretase activity is achieved via activation of PPAR γ , which ultimately reduces the generation of A β_{1-40} and A β_{1-42} [50].

In addition to the effects of ginsenosides on β -secretase activity, ginsenoside Rg1 treatment inhibited the activity of γ -secretase in both B103-APP cells and Tg mAPP mouse brain tissues, indicating that ginsenoside Rg1 is also involved in the APP regulation pathway. The underlying mechanisms of ginsenoside Rg1-induced inhibition of γ -secretase activity involve the enhancement of protein kinase A/CREB (cAMP response element-binding protein) pathway activation in Tg mAPP mice [51]. On the other hand, in *in vitro* studies using HT22 cells and SH-SY5Y cells stably expressing the Swedish mutant APP, ginsenoside Rg1 also increased extracellular secretion of sAPP α , enhanced α -secretase activity, and decreased extracellular release of A β . Ginsenoside Rg1-induced increase of sAPP α was blocked by inhibitors of protein kinase C, extracellular-signal-regulated kinase, and the MAPK and PI3K/Akt pathways [52,53]. Therefore, ginsenoside Rg1 may utilize signaling enzymes for sAPP α instead of its production *per se* through the activation of α -secretase and inhibition of β - and γ -secretase. Ginsenoside Rh2 increased sAPP α levels, increased CTF α/β (C-terminal fragments of APP α/β) ratios, and reduced A β_{1-40} and A β_{1-42} levels in primary hippocampal neurons. Furthermore, ginsenoside Rh2 increased surface APP levels drastically. In addition, ginsenoside Rh2 inhibited APP endocytosis by reducing cholesterol and lipid raft concentrations [54]. In *in vivo* studies, long-term

intraperitoneal administration of ginsenoside Rh2 to a Tg2576 mouse model of AD significantly improved learning and memory performance and reduced the size of senile plaques at 14 months. On the other hand, there are a few reports indicating that ginsenoside affects A β -related tau hyperphosphorylation in the brain, which is also thought to contribute to AD. Treatment of cortical neurons with A β increased tau hyperphosphorylation by increasing GSK-3 β (glycogen synthase kinase-3 β) expression. However, pretreatment with ginsenoside Rb1 attenuated A β_{25-35} -induced tau protein hyperphosphorylation by inhibiting the expression of GSK-3 β [55]. Indirect regulation of tau hyperphosphorylation by ginsenoside Rd has also been reported. Ginsenoside Rd inhibited the tau protein hyperphosphorylation that was increased by okadaic acid, a protein phosphatase inhibitor, in cultured cortical neurons [56] (Fig. 4). Recently, isolated ginseng protein also exhibited anti-AD activity. Ginseng protein improved the memory ability and reduced the amount of A β_{1-42} and p-tau in an AD rat model. The anti-AD effects of ginseng protein appear to be mediated by PI3K/Akt signaling pathway activation [57], raising the possibility that ginseng protein and ginsenosides may also have anti-AD activity.

3.2. Inhibition of A β formation by gintonin

It has recently been reported that gintonin facilitates sAPP α production through the LPA receptor-G $\alpha_{q/11}$ -phospholipase C-IP $_3$ receptor-[Ca $^{2+}$] $_i$ transient pathway [33]. Application of gintonin to SH-SY5Y neuroblastoma cells stimulated sAPP α release in a [Ca $^{2+}$] $_i$ transient-dependent manner via the LPA receptor signaling

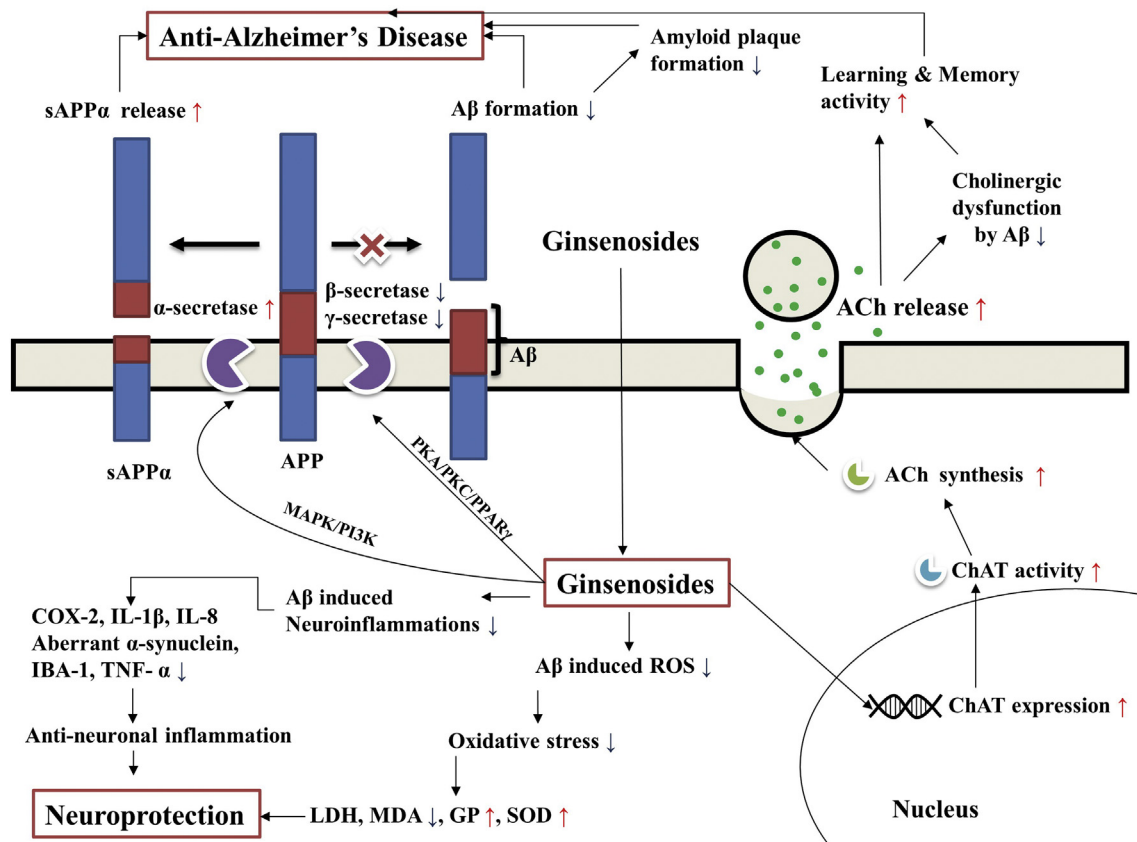


Fig. 4. Schematic diagram showing the ginsenoside-induced anti-AD effects. Ginsenosides act through multiple pathways to exert their effects against AD. A β , β -amyloid; ACh, acetylcholine; AD, Alzheimer's disease; APP, amyloid precursor protein; ChAT, choline acetyltransferase; COX-2, cyclooxygenase-2; GP, glutathione peroxidase; Iba-1, allograft inflammatory factor-1; IL-1 β , interleukin-1 β ; IL-8, interleukin-8; LDH, lactate dehydrogenase; MDA, malondialdehyde; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; PKC, protein kinase C; PPAR γ , peroxisome proliferator-activated receptor γ ; ROS, reactive oxygen species; sAPP α , soluble amyloid precursor protein α ; SOD, superoxide dismutase; TNF- α , tumor necrosis factor- α .

pathway and α -secretase activation. In addition, application of gintonin to SH-SY5Y neuroblastoma cells decreased A β formation and attenuated A β -induced neurotoxicity, indicating that gintonin could affect APP processing in the brain [58]. In another study using a mouse model of AD, long-term oral gintonin treatment decreased amyloid plaque deposits in the cortex and hippocampus [58]. In addition, long-term gintonin treatment in an AD mouse model ameliorated cognitive deficits in various behavioral tests related to spatial and associative memory [58]. Therefore, gintonin-mediated activation of the *in vitro* nonamyloidogenic pathway via LPA receptor signaling pathways is further linked to the *in vivo* attenuation of brain AD-related neuropathies (Fig. 5).

4. Effects of ginseng components on the brain's cholinergic systems

4.1. Effects of ginsenosides on the brain's cholinergic systems

Brain acetylcholine levels are decreased in AD mouse models and in patients with AD due to a decrease in the acetylcholine synthesis enzyme, choline acetyltransferase (ChAT), and an increase in the acetylcholine degradation enzyme, AChE [7,8]. Because acetylcholine plays an important role in cognitive functions, an imbalance in acetylcholine levels may cause cognitive deficits, for example, in learning and memory, in AD animal models and patients with AD. In addition, ginsenosides, such as ginsenosides Rd and Re (but not Rb1, Rg1, and Rg3), are involved in *in vitro* and *in vivo* acetylcholine metabolism in neuronal cells. Ginsenosides Rd and Re induce an increase in acetylcholine synthesis in

neuro-2a cells [59]. Ginsenosides Rd and Re upregulate the expression of ChAT, resulting in an increase in the levels of acetylcholine and vesicular acetylcholine transporter, both of which are required for cholinergic neurotransmission. In addition, these ginsenosides increased the expression of microtubule-associated protein-2, nerve growth factor receptor (p75), p21, and TrkA (tropomyosin receptor kinase A), indicating that ginsenoside Rd and Re affect neuronal differentiation and the nerve growth factor–TrkA signaling pathway, in addition to the cholinergic system. Orally administered ginsenoside Re and Rd increased the expression levels of ChAT and vesicular acetylcholine transporter mRNA in the brain [59]. A ginsenoside Rb1-enriched preparation, Cereboost, attenuated A β _{1–42}-induced cytotoxicity in F3.ChAT stem cells and increased ChAT gene expression. Oral administration of Cereboost reversed A β _{1–42}-induced cognitive dysfunction and increased levels of microtubule-associated protein-2 and synaptophysin in the brain, as well as the acetylcholine concentration [60] (Fig. 4).

4.2. Effects of gintonin on cholinergic systems in the brain

Gintonin-mediated LPA receptor activation has been shown to increase the release of acetylcholine in hippocampal neural progenitor cells and choline acetyltransferase expression levels, indicating that gintonin may induce the differentiation of hippocampal neural progenitor cells into cholinergic neurons [61]. In addition, in an *in vivo* study, oral administration of gintonin for 3 weeks also increased hippocampal ChAT expression levels in wild-type mice. Long-term administration of gintonin in a mouse model of AD

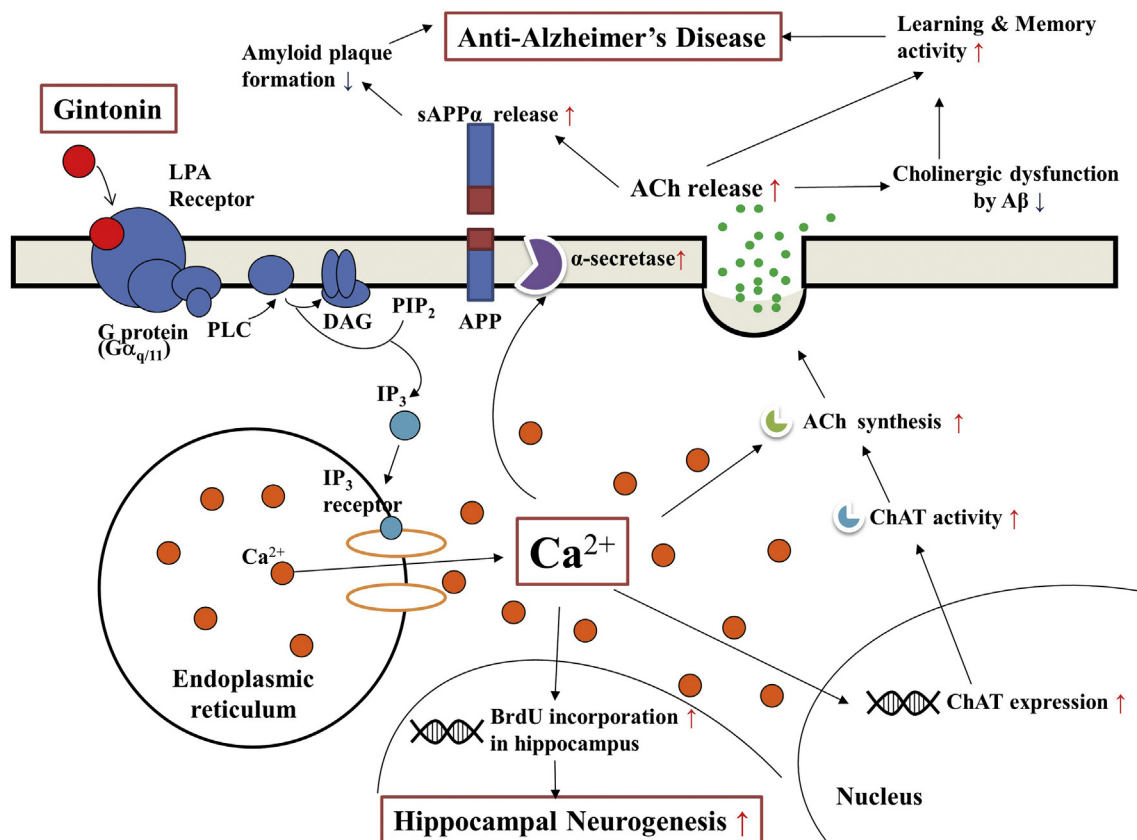


Fig. 5. Schematic diagram showing the gintonin-induced anti-AD effects. Gintonin acts through multiple pathways to exert its effects against AD. A β , β -amyloid; ACh, acetylcholine; AD, Alzheimer's disease; APP, amyloid precursor protein; BrdU, bromodeoxyuridine; ChAT, choline acetyltransferase; DAG, diacylglycerol; IP₃, inositol triphosphate; LPA, lysophosphatidic acid; PIP₂, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; sAPP α , soluble amyloid precursor protein α .

increased acetylcholine levels in the hippocampus. Gintonin also increased ChAT expression levels and reduced AChE expression in the hippocampus [61]. Therefore, gintonin exerts its effects on the cholinergic system in at least two ways: the induction of *in vitro* differentiation of hippocampal neural progenitor cells into cholinergic cells by increasing the level of ChAT expression and by *in vivo* restoration of the hippocampal cholinergic system through the elevation of acetylcholine levels via an increase in ChAT and a decrease in AChE (Fig. 5).

5. Effects of ginseng components on A β -induced reactive oxidative stress

5.1. Effects of ginsenosides on A β -induced reactive oxidative stress

It is well known that A β induces oxidative stress in neuronal cells, resulting in cell death [11]. Accumulating evidence shows that ginsenosides exhibit *in vitro* neuroprotective effects against neuronal cell apoptosis caused by A β s such as A β ₂₅₋₃₅, A β ₁₋₄₀, and A β ₁₋₄₂. The mechanisms of the neuroprotective effects of ginsenosides against A β neurotoxicity are closely related to its attenuation of reactive oxidative stress. For example, exposure of PC12 cells, cortical neurons, or hippocampal cell cultures to A β ₂₅₋₃₅ or A β ₁₋₄₂ led to the accumulation of reactive oxygen species (ROS) and lipid peroxidation, increases of malondialdehyde (MDA) products, and decrease of superoxide dismutase (SOD) activity, respectively, and eventually causing a decrease in cell survival [52,62,63]. Pretreatment with ginsenoside Rb1 or Rg1 not only inhibited A β -induced ROS overproduction and lipid peroxidation but also decreased lactate dehydrogenase release, MDA production, and SOD activity, thereby improving the rate of cell survival. Ginsenosides Rb1 and Rg1 may serve as potent scavengers of ROS produced by A β s [52,62,63]. Another study on ginsenoside Rd antioxidant effects on A β ₂₅₋₃₅-induced neurotoxicity showed that ginsenoside Rd significantly ameliorated A β ₂₅₋₃₅-induced oxidative stress by decreasing ROS production and MDA levels and increasing the levels of SOD and glutathione peroxidase, comparable with other antioxidants, such as probucol and edaravone [64]. Ginsenosides such as ginsenosides Rb1, Rd1, or Rg1 may exert their anti-AD effects through the reduction of oxidative stress and attenuation of reactive oxidative stress-induced neuronal apoptosis. On the other hand, it is known that A β s induce mitochondrial dysfunction by decreasing the mitochondrial membrane potential and ATP (adenosine triphosphate) levels and inhibiting cytochrome c activity in neurons. However, ginsenoside Rg1 treatment reversed A β ₁₋₄₂-induced mitochondrial dysfunction and the death of cortical neurons. The protective effects of ginsenoside Rg1 against the mitochondrial dysfunction induced by A β is achieved through the suppression of intracellular mitochondrial oxidative stress [65]. Therefore, these reports show that the innate antioxidative properties of ginsenosides can be used for the reduction of A β -induced oxidative stress (Fig. 4).

6. Effects of ginseng components on A β -induced neuroinflammation

6.1. Effects of ginsenosides on A β -induced neuroinflammation

Microglia are known as the major inflammatory response cells of the central nervous system [66]. A β , in addition to being a reactive oxidative stress inducer, is an inducer of microglial activation and neuroinflammation. It is known that A β -induced neuroinflammation via microglial activation is an event associated with the onset and progression of the neuropathophysiology of AD [67]. In addition to ginsenoside-induced antioxidative stress, there are

several reports on the attenuation of neuroinflammation by ginsenosides. Ginsenoside Rb1 treatment significantly attenuated neuroinflammation markers, such as cyclooxygenase-2, that were induced by A β ₁₋₄₂ [68]. Li et al (2011) showed that ginsenoside Rg1 exhibited antiinflammatory effects by inhibiting neurotoxic interleukin (IL)-1 β , IL-8, and tumor necrosis factor- α production in THP1 monocytes stimulated by A β ₁₋₄₀ and prevented neuronal cell apoptosis [56]. These results indicate that a direct modulation of immune cells by ginsenoside Rg1 is coupled to its anti-AD activity via attenuation of the A β ₁₋₄₀-induced inflammation-mediated neurotoxic products of immune cells. Ginsenoside Rg3 enhances microglial phagocytosis of A β and promotes A β uptake, internalization, and digestion. Increased maximal A β uptake was observed at 4 h and 8 h after pretreatment with ginsenoside Rg3, and the internalized A β was almost completely digested from the cells within 36 h [69]. In addition, the expression of type A macrophage scavenger receptors was also upregulated by ginsenoside Rg3 treatment in the cytosol. Ginsenoside Rg3 stimulation of the type A macrophage scavenger receptor may contribute to its therapeutic potential for AD via microglial phagocytosis and digestion [69]. Therefore, ginsenosides may achieve their anti-AD effects by attenuating A β -mediated neuroinflammation and facilitating microglial phagocytosis (Fig. 4).

6.2. Effects of gintonin on A β - and inflammatory agent-induced inflammation

There are only a few reports of the inhibitory effects of gintonin on A β - and inflammatory agent-induced inflammation. *In vivo* long-term administration of gintonin in an AD mouse model attenuated the expression level of allograft inflammatory factor-1, a marker for microglia activation, in the cortex and hippocampus, indicating that gintonin exhibits antiinflammatory effects by inhibiting the inhibition of microglial activation and by enhancing microglial phagocytosis [58]. In one *in vitro* study, gintonin applied to RAW 264.7 cells inhibited the lipopolysaccharide-induced production of proinflammatory cytokines such as IL-6, IL-1 β , and tumor necrosis factor- α via the MAPK and nuclear factor-kappa B pathways. Gintonin also restored the levels of miR-34a and miR-93 that were downregulated by lipopolysaccharide treatment [70]. In addition, oral administration of a gintonin-enriched fraction inhibited the increase in expression of inflammatory mediators (e.g., IgE, histamine, IL-4, and interferon - γ) in serum caused by an inflammatory agent, 2,4-dinitrofluorobenzene [71]. Gintonin has also been shown to exhibit antiinflammatory effects in the brain and periphery.

7. Effects of ginseng components on hippocampal neurogenesis

7.1. Effects of gintonin on hippocampal neurogenesis

A recent study showed that a mouse model of AD and patients with AD exhibit increased neurogenesis in the hippocampus [72], indicating the possibility that the brain itself compensates for or protects against A β insults. Neurogenesis in the brain, especially hippocampal neurogenesis in the adult brain, is a target for anti-AD treatments [72]. In fact, several studies have shown that various factors affect hippocampal neurogenesis, such as inflammation inhibition, enriched environments, physical exercise, reduction of stress, and the supply of neurotrophic factors such as brain-derived neurotrophic factor and epidermal growth factor [14]. In addition, several medicinal plants, including ginseng extract, promote hippocampal neurogenesis without a definite mechanism of action [73,74]. Gintonin also stimulates *in vitro* hippocampal neural progenitor proliferation through the LPA receptor signaling pathways

[75]. Previous reports have shown that LPA1 receptor-deficient mice exhibit reduced hippocampal cell proliferation [75]. Interestingly, treating hippocampal neural progenitor cells with gintonin also induced cells to differentiate into neurons and astrocytes [76]. Gintonin also increased the expression of the LPA1 receptor in hippocampal neural progenitor cells, indicating that gintonin-mediated differentiation and proliferation of hippocampal neural progenitor cells is closely related to hippocampal LPA receptors [76]. Oral administration of gintonin to wild-type mice stimulated brain-derived neurotrophic factor expression [77] and cell proliferation in the CA1 (cornu ammonis 1), CA3, and dentate gyrus areas of the hippocampus. Long-term treatment of AD mice with gintonin also increased hippocampal neurogenesis by increasing LPA1 receptor expression [76]. In addition to gintonin-mediated activation of the nonamyloidogenic pathway and restoration of the hippocampal cholinergic system, gintonin-mediated hippocampal neurogenesis may be another target for anti-AD therapy [58,61,75] (Fig. 5).

8. Gintonin improves impaired cognitive functions in elderly AD dementia

Moon et al (2017) further investigated the proof-of-concept effect of gintonin in patients with AD with combination of conventional AD drugs [78]. In parallel to preclinical tests [58,61,75], gintonin administration displayed potential cognition-enhancing effects in cognitively impaired subjects, when compared with those before gintonin administration in two tests, such as MMSE and ADAS-Cog [79]. Administration of gintonin for 4 weeks improved the score of MMSE test significantly on the first and second follow-up visits compared with the baseline. Administration of gintonin for 4 weeks also decreased ADAS-Cog score significantly at the first follow-up visit and continued to decrease at subsequent visits. ADAS-nonCog scores were significantly improved 4 weeks after the daily consumption of gintonin with conventional AD drug treatments [78]. Interestingly, the previous administration of a large amount of whole ginseng extract induced adverse effects such as facial flushing, headache, dizziness, and gastrointestinal disorders [42,44], whereas gintonin administration did not induce any

adverse effects in elderly AD dementia throughout whole investigations [78]. Although this study has a limitation with small sample size and further needs large-scale clinical studies, this study suggests that gintonin is safe as a ginseng component and can be an effective way to improve cognition in dementia patients with combination of conventional AD drugs.

9. Perspective and conclusion

Panax ginseng has been used to promote longevity since the ancient Ch'in dynasty in China. The whole ginseng plant was traditionally decocted, and an extract or concentrate was used as a functional food or an herbal medicine for diverse purposes (Fig. 1A and B). Recent reports have shown that ginseng extracts prepared using diverse processing methods, such as white ginseng, red ginseng, black ginseng, and fermented ginseng, improve AD-related cognitive dysfunctions in patients with AD [42,43,45]. Therefore, accumulating experimental evidence suggests that ginseng extract has anti-AD effects. But treatment with ginseng extract may have certain limitations [42,43,45] because ginseng extract that contains various unidentified ginseng components could not explain exact mode of action on the improvements of AD symptoms.

Two active components of ginseng, ginsenosides and gintonin, may underlie the anti-AD benefits shown by ginseng extract. The molecular basis of the two ginseng components that mediate anti-AD activity in *in vitro* or *in vivo* AD mouse models may include five different mechanisms. These mechanisms include the stimulation of nonamyloidogenic α -secretase activity via the LPA receptor signaling pathway and inhibition of β - and γ -secretase activity, restoration of the hippocampal cholinergic system, attenuation of mitochondrial oxidative stress and neuroinflammation, and enhancement of hippocampal neurotrophic factor expression, and neurogenesis (Table 1). Therefore, ginsenosides and/or gintonin exert their anti-AD activities via multiple target actions in the brain rather than by the single target approach used in conventional AD treatments. Isolated or fractionated ginsenosides or gintonin could be prepared as an adjuvant therapy for the amelioration of AD through multiple targets (Fig. 1C).

Table 1
Summary of anti-Alzheimer's disease effects of *Panax ginseng* extract and its components

Alzheimer's disease-related neuropathies	<i>In vitro</i> or <i>in vivo</i> treatments			References
	Ginseng total extract	Ginsenosides	Gintonin	
β-Amyloids				
Amyloidogenic pathways	β -secretase activity ↓ γ -secretase activity ↓	β -secretase activity ↓ γ -secretase activity ↓	ND	[47,51]
Non-amyloidogenic pathway and sAPP α formation	α -secretase activity inhibition	α -secretase activity and sAPP α ↑ inhibition	α -secretase activity and sAPP α ↑ inhibition	[46,49,58] [52,53,58]
A β formation	Inhibition	Inhibition	Inhibition	[41,58,65]
A β -induced toxicity in neuronal cells	Inhibition	Inhibition	Inhibition	[38,54,58]
<i>In vivo</i> brain amyloid plaque formations	ND	Inhibition	ND	[55]
Tau hyperphosphorylation	ND	Inhibition	ND	
Brain cholinergic system				
Choline acetyltransferase activity and expression	Stimulation	Stimulation	Stimulation	[59,60,61]
Acetylcholinesterase activity and expression	Inhibition	Inhibition	Inhibition	[59,60,61]
<i>In vitro</i> and <i>in vivo</i> brain acetylcholine level	Increase	Increase	Increase	[59,60,61]
Aβ-induced oxidative stresses				
Formations of free radicals	Inhibition	Inhibition	ND	[62,63]
A β -induced neuroinflammation				
Inflammation-related products (Cox-2, IL-1 β , IL-8 and TNF- α , or Iba-1) and microglial activations	Inhibition	Inhibition	Inhibition	[56,58,68]
Hippocampal neurogenesis				
Hippocampal neurogenesis	ND	ND	Increase	[75,77]
Plasma target protein for anti-Alzheimer's disease	ND	ND	LPA receptors	[75]
Applications to AD patients				
Cognitive function in ADAS and MMSE tests	Improvement	ND	Improvement	[42,43,44,45,78]

A β , β -amyloid; AD, Alzheimer's disease; ADAS, Assessment Scale-Cognitive Subscale; COX-2, cyclooxygenase-2; Iba-1, allograft inflammatory factor-1; IL-1 β , interleukin-1 β ; IL-8, interleukin-8; MMSE, mini-mental state examination; ND, not determined; sAPP α , soluble amyloid precursor protein α ; TNF- α , tumor necrosis factor- α .

Some of the anti-AD benefits of ginsenoside overlap with those of gintonin. For example, protection against A β -induced neurotoxicity and inhibition of A β formation have been found for both ginsenosides and gintonin. In addition, both ginsenosides and gintonin also reinforce the brain cholinergic systems. Therefore, indirect ginsenoside- and gintonin-mediated boosting effects on the cholinergic system may prove to be more advantageous than those of direct cholinergic receptor agonists because direct treatment with cholinergic receptor agonists can produce gastrointestinal side effects in humans because of the high level of endogenous muscarinic cholinergic receptors in the gastrointestinal system [80]. However, ginsenoside-mediated attenuation of oxidative stress was not observed with gintonin, whereas gintonin-mediated increases of hippocampal neurogenesis and cognition-enhancing effects in cognitively impaired patients with AD were not observed with ginsenosides [78]. Therefore, ginsenosides and gintonin may complement each other, to some extent. Although some of the ginsenosides have diverse cytosolic kinase signaling pathways [48–50,52,53] (Fig. 4), the concrete molecular mechanisms of the various ginsenosides cannot be easily explained at the membrane level (Fig. 4). This may be because ginsenoside binding proteins at the cell membrane level are unknown or do not exist (Table 1). In contrast, gintonin-mediated anti-AD activity can be clearly explained because gintonin uses LPA receptor signaling pathways (Fig. 5 and Table 1) [37].

Several clinical trials run by large pharmaceutical companies that have targeted the inhibition of only A β formation or antibody therapy against A β for AD treatment have failed. In addition, conventional drugs such as AChE inhibitors and NMDA receptor antagonists currently used by physicians in AD clinics do not demonstrate sufficient therapeutic efficacy against AD with adverse effects. Because AD is a complex multifactorial disease involving multiple aspects of the brain, the multiple actions of ginsenosides and/or gintonin may prove more beneficial if they are combined with conventional single target drugs, such as AChE inhibitors or NMDA receptor antagonists [21]. The combination treatment of ginseng components, which shows multiple modes of actions, with conventional AD drugs which are single target drugs may provide advantages over monotherapy for the effective pharmacological management of AD [81,82]. Combination therapy with ginseng components as an adjuvant may enhance efficacy by inducing additive or synergistic effects. The combination of ginseng components with an AD drug might also improve safety and tolerability potentially with less side effects, making additional neuroprotective effects by prolonging the symptomatic benefits and ultimately delaying disease progression, although the combination therapy with ginseng components were not clinically demonstrated.

In conclusion, although administration of ginseng extract alone did not show enough monotherapeutic effects on AD progression, it can ameliorate cognition deficits in patients when combined with conventional AD drugs. Accumulating evidence has shown that two ginseng components, ginsenosides and gintonin, may be responsible for ginseng extract-mediated AD improvements through diverse molecular mechanisms in mouse models of AD. In the future, clinical investigations might be required for the mass assessment of the efficacy, safety, and tolerability when combining two ginseng components with conventional drugs to enhance AD treatment.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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