Oral Administration of Gintonin Attenuates Cholinergic Impairments by Scopolamine, Amyloid-β Protein, and Mouse Model of Alzheimer's disease: Involvement of Lysophosphatidic Acid (LPA) Receptors

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Introduction

Alzheimer's disease (AD) is the most common age-associated neurodegenerative disease.

The formation of senile plaques and neurofibrillary tangles are well characterized in AD neuropathy. Senile plaques contain amyloid-β protein (Aβ).

Ginseng extracts increased acetylcholine release and enhanced cognitive performance in human and AD patients.

However, little is known about the active ingredient of ginseng and its signaling mechanisms.

We showed that ginseng contains a novel G protein-coupled lysophosphatidic acid (LPA) receptor ligand, gintonin . Gintonin enhanced synaptic transmission in hippocampal slices through LPA receptor signaling pathways.

We showed that gintonin is the active component of ginseng extract and attenuates AD-related neuropathies via activation of non-amyloidogenic pathways; gintonin significantly improved Aβ-induced cognitive dysfunctions in mice.

In addition, long-term oral administration of gintonin attenuated amyloid plaque deposition in the hippocampus as well as short- and long-term memory impairment in a transgenic AD mouse model.

Materials & Methods

Animals
Male ICR or C57BL/6 mice (4- or 8-weeks-old)

Drug Treatment
Gintonin
β-Amyloid (Aβ)40-1 and Aβ1-40

Measurement of intracellular Ca2+ levels
Neural progenitor cells

Measurement of Acetylcholine (ACh) Level
Amplex® Red Acetylcholine/Acetylcholinesterase Assay Kit

Acetylcholinesterase (AChE) and Choline Acetyltransferase (ChAT) Activity

Immunocytochemistry

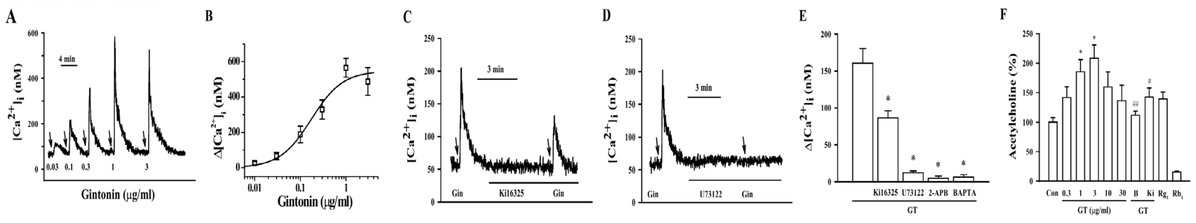
Passive Avoidance Test

Morris Water-Maze Test

Induction of LTP in acute slices in the absence or presence of gintonin

Results

Fig 1. Effects of gintonin (GT) on [Ca2+]i transients in neural progenitor cells (NPCs) and its signal transduction.



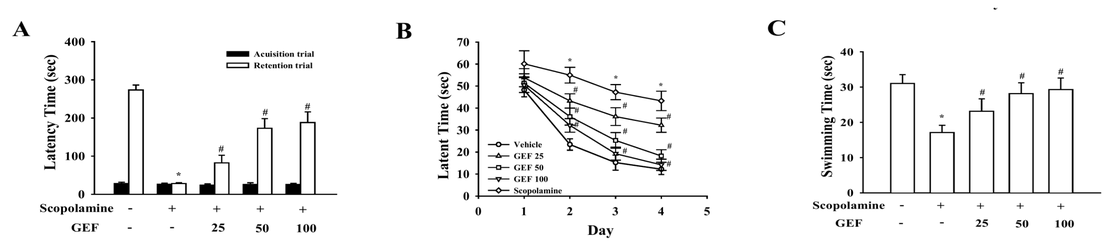
(A) A representative trace obtained after gintonin treatment in NPCs. Gintonin treatment (0.03–3 μg/mL) induces a [Ca2+]i transient.

(B) Concentration-response relationship curve for gintonin-induced [Ca2+]i transients in NPCs.

(C-E) Representative traces of gintonin-mediated [Ca2+]i transients in the absence or presence of various antagonists. An LPA1/3 receptor, antagonist , Ca2+ chelator was added before gintonin application.

(F) Histograms representing net increases of gintonin-mediated [Ca2+]i transients calculated from traces obtained in the absence or presence of various pharmacological agents. * P < 0.05, compared with gintonin only treatment. Data are means ± S.E.M. (n = 3–4). (G) Effect of gintonin (0, 0.3, 1, 3, 10, and 30 μg/mL) and (B) BAPTA-AM, a calcium chelator, (25 μM) on acetylcholine release in the NPCs, determined using an Amplex Red Acetylcholine/Acetylcholinesterase Assay Kit.
Ki16425 (Ki) significantly attenuated gintonin-mediated acetylcholine release. NPCs were pretreated with BAPTA or Ki16425 for 1 h before gintonin treatment and then treated with gintonin or each ginsenoside for 16 h.

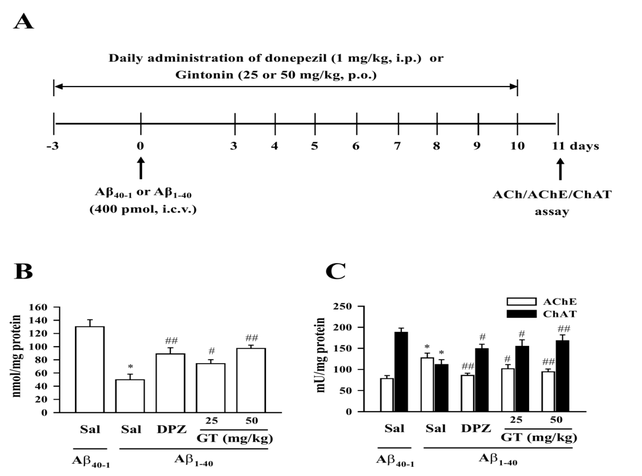
Fig. 2. Effects of gintonin-enriched fraction (GEF) on scopolamine-induced memory deficit in the passive avoidance (A) and Morris water maze tests (B and C).



(A) GEF (25, 50, and 100 mg/kg, p.o.) was administered orally 30 min before treatment with scopolamine. Memory impairment was induced by treatment with scopolamine (0.5 mg/kg, i.p.).

(B and C) The first trial session was performed 30 min after treatment with scopolamine. The training trial and the probe trial sessions were performed for 4 days as described in the Materials and Methods. Values are expressed as means ± SEM (n = 10). Mice were treated with GEF for 3 weeks by oral administration. *P < 0.01 vs. control vehicle group. #P < 0.05 vs. scopolamine-treated group.

Fig. 3. Effect of gintonin (GT; 25 or 50 mg/kg, p.o.) on the Aβ (1-40)-induced changes in cholinergic system.

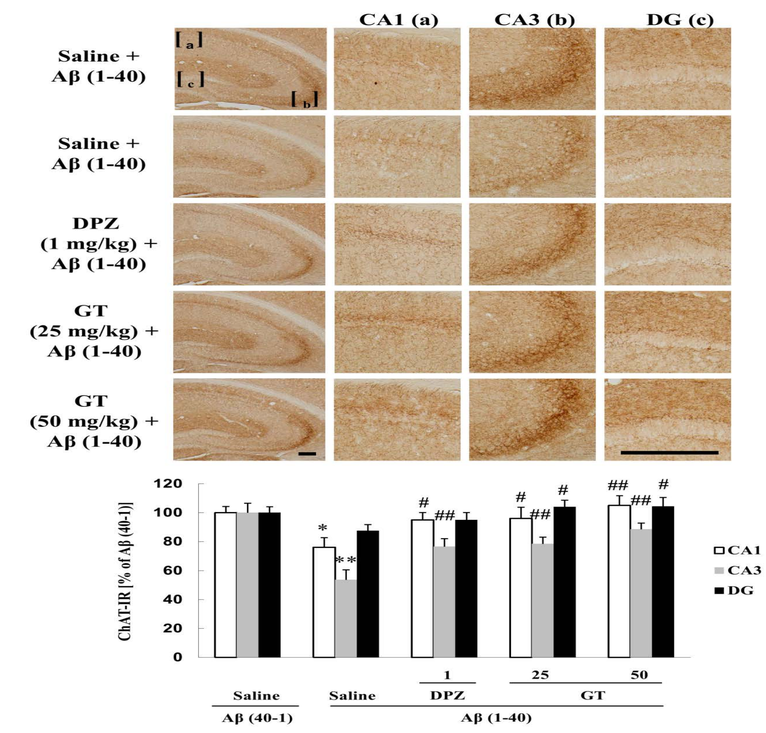


(A) Experimental schedule to evaluate acute gintonin effect in Aβ (1-40)-induced cholinergic system impairment.

(B) Acetylcholine level.

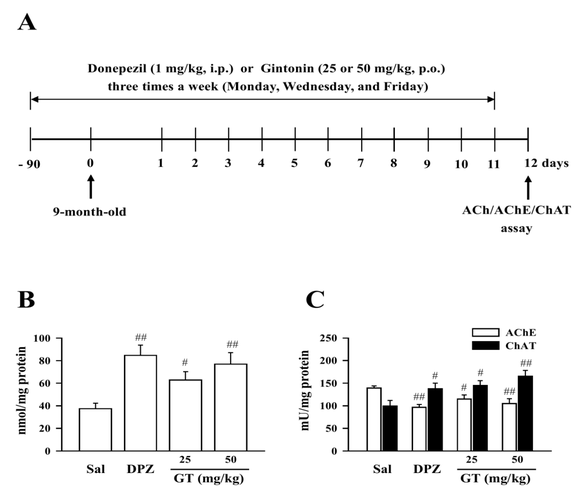
(C)Acetylcholinesterase activity and choline acetyltransferase activity in the hippocampus of the mouse.
Donepezil (DPZ; 1 mg/kg, i.p.) was used as a reference drug. Sal = saline.

Fig. 4. Effect of gintonin (GT; 25 or 50 mg/kg, p.o.) on the Aβ (1-40)-induced decrease in choline acetyltransferase-immunoreactivity (ChAT-IR) in the hippocampus of the mouse.



Donepezil (DPZ; 1 mg/kg, i.p.) was used as a reference drug.

Fig. 5. Effect of gintonin (GT; 25 or 50 mg/kg, p.o.) on cholinergic system changes

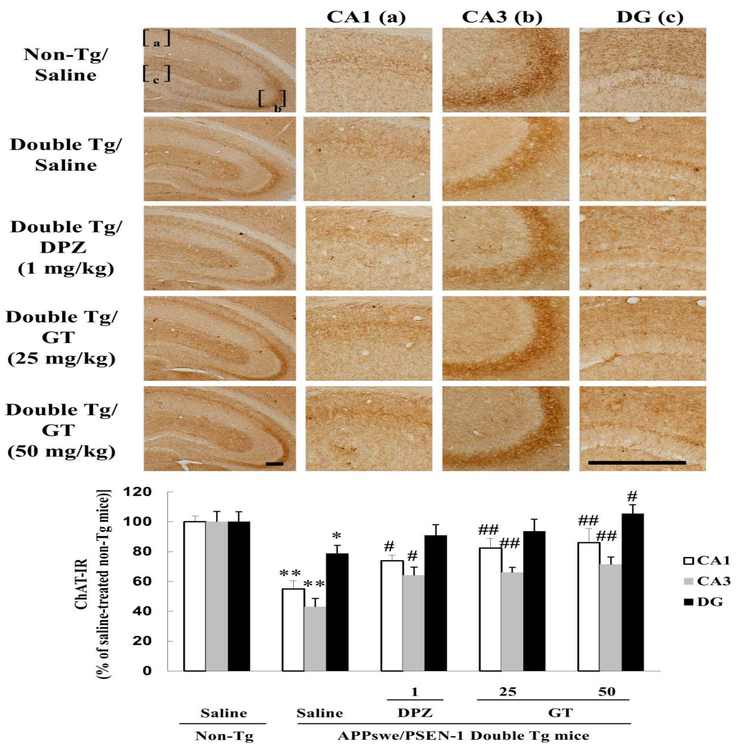


(A) Experimental schedule to evaluate the long-term effect of gintonin on cholinergic system impairment in the APPswe/PSEN-1 double Tg mouse.

(B) Acetylcholine level

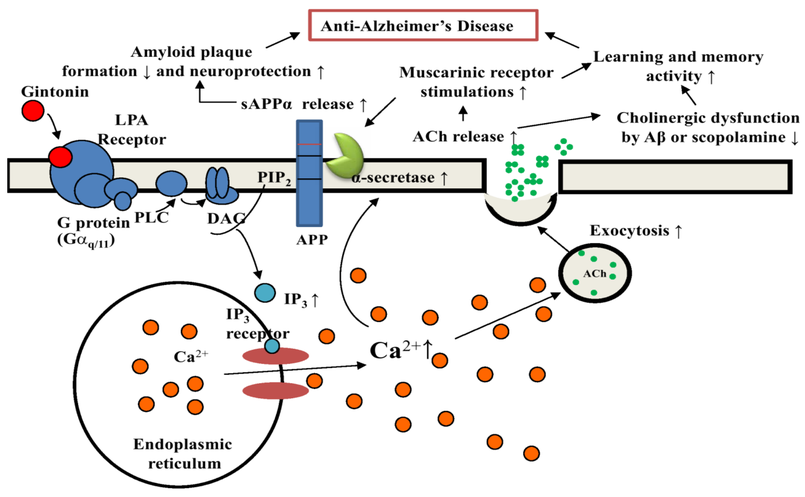
(C) acetylcholinesterase activity, and choline acetyltransferase activity in the hippocampus of the APPswe/PSEN-1 double Tg mouse. Donepezil (DPZ; 1 mg/kg, i.p.) was used as a reference drug.
Sal = saline.

Fig. 6. Effect of gintonin (GT; 25 or 50 mg/kg, p.o.) on the decrease in ChAT-IR in the hippocampus of APPswe/PSEN-1 double Tg mice.



Donepezil (DPZ; 1 mg/kg, i.p.) was used as a reference drug.

Fig. 7. Schematic diagram of the involvement of the cholinergic system in gintonin-mediated anti-Alzheimer's disease (AD) through LPA receptor activation.



We found that gintonin stimulates acetylcholine release, has a protective effect on the cholinergic system, and attenuates acute Aβ-induced and long-term cholinergic dysfunction in the transgenic AD animal model. Gintonin-mediated activation of LPA receptors could be coupled to anti-AD effects via dual actions of the non-amyloidogenic pathway and modulation of the cholinergic system in the brain according to the described pathways.

Discussion & Summary

In a previous report, we showed that gintonin promoted the secreted amyloid precursor protein-α (sAPPα) release in neuronal cells

Supporting this notion is the observation that soluble amyloid precursor protein suppresses acetylcholine esterase. Thus, sAPPα produced by gintonin might contribute to cholinergic recovery.

The last possibility is that LPA receptor activation by gintonin might utilize dual signals for the non-amyloidogenic promotion of sAPPα release and stimulation of the cholinergic system in the nervous system.

Therefore, both reduction of the Aβ accumulation level and maintenance of acetylcholine levels by gintonin might alleviate cognitive deficit due to Aβ.

Finally, we suggest that, in addition to gintonin-mediated non-amyloidogenic pathway activation, the anti-AD effect of gintonin might be achieved via its boosting effects on the cholinergic system. These actions of gintonin might be an additional molecular basis for the neuroprotective effects of ginseng on AD-related neuropathies.