Journal of Ginseng Research 48 (2024) 1–11

Contents lists available at ScienceDirect

Journal of Ginseng Research

journal homepage: www.sciencedirect.com/journal/journal-of-ginseng-research

Review Article

Atypical formations of gintonin lysophosphatidic acids as new materials and their beneficial effects on degenerative diseases

Ji-Hun Kim [a], Ra Mi Lee [a], Hyo-Bin Oh [b], Tae-Young Kim [b], Hyewhon Rhim [c], Yoon Kyung Choi [d], Jong-Hoon Kim [e], Seikwan Oh [f], Do-Geun Kim [g], Ik-Hyun Cho [h], Seung-Yeol Nah [a, *]

[a] Ginsentology Research Laboratory and Department of Physiology, College of Veterinary Medicine, Konkuk University, Seoul, Republic of Korea
[b] Department of Efficacy Study, Institute of Jinan Red Ginseng, Jeollabuk-do, Republic of Korea
[c] Center for Neuroscience, Korea Institute of Science and Technology, Bio/Molecular Informatics Center, Republic of Korea
[d] Department of Bioscience and Biotechnology, Konkuk University, Seoul, Republic of Korea
[e] College of Veterinary Medicine, Biosafety Research Institute, Chonbuk National University, Jeollabuk-do, Republic of Korea
[f] Department of Molecular Medicine, School of Medicine, Ewha Womans University, Seoul, Republic of Korea
[g] Dementia Research Group, Korea Brain Research Institute, Daegu, Republic of Korea
[h] Department of Convergence Medical Science, College of Korean Medicine, Kyung Hee University, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 13 December 2022
Accepted 12 February 2023
Available online 21 February 2023

Keywords:
Degenerative diseases
Ginseng
ginseng-derived neurotrophin
Gintonin lysophosphatidic acids
New material

ABSTRACT

Fresh ginseng is prone to spoilage due to its high moisture content. For long-term storage, most fresh ginsengs are dried to white ginseng (WG) or steamed for hours at high temperature/pressure and dried to form Korean Red ginseng (KRG). They are further processed for ginseng products when subjected to hot water extraction/concentration under pressure. These WG or KRG preparation processes affect ginsenoside compositions and also other ginseng components, probably during treatments like steaming and drying, to form diverse bioactive phospholipids. It is known that ginseng contains high amounts of gintonin lysophosphatidic acids (LPAs). LPAs are simple lipid-derived growth factors in animals and humans and act as exogenous ligands of six GTP-binding-protein coupled LPA receptor subtypes. LPAs play diverse roles ranging from brain development to hair growth in animals and humans. LPA-mediated signaling pathways involve various GTP-binding proteins to regulate downstream pathways like $[Ca^{2+}]_i$ transient induction. Recent studies have shown that gintonin exhibits anti-Alzheimer's disease and anti-arthritis effects *in vitro* and *in vivo* mediated by gintonin LPAs, the active ingredients of gintonin, a ginseng-derived neurotrophin. However, little is known about how gintonin LPAs are formed in high amounts in ginseng compared to other herbs. This review introduces atypical or non-enzymatic pathways under the conversion of ginseng phospholipids into gintonin LPAs during steaming and extraction/concentration processes, which exert beneficial effects against degenerative diseases, including Alzheimer's disease and arthritis in animals and humans via LPA receptors.

1. Introduction

Panax ginseng is a traditional herbal medicine used in Korea, Japan, and China, now used worldwide in various health tonics as an anti-aging ingredient that improves the functioning of nervous and non-nervous systems and also as an immunity booster for body defenses against invasions of pathogenic microorganisms [1]. *Panax ginseng* contains diverse components for its tonic effects [2]. Fresh ginsengs obtained after the harvest of 4–6 years old ginseng are easy to rot at room temperature, as these have a high-moisture content of 70–80% [3]. For example, spontaneous glycolysis reactions in fresh ginseng also occur to increase and release heat, which can easily lead to microbial reproductions and contaminations and subsequently lead to decay [4]. Therefore, in Korea, fresh ginsengs are further processed after harvest for long-term storage traditionally. There are two ways to the preservation of fresh ginseng. The first is to dry fresh ginseng at a specific temperature or

* Corresponding author. Ginsentology Research Laboratory and Department of Physiology, College of Veterinary Medicine, Konkuk University, Seoul, 05029, Republic of Korea.
E-mail address: synah@konkuk.ac.kr (S.-Y. Nah).

https://doi.org/10.1016/j.jgr.2023.02.004

under the sun to reduce the water content to less than 14%, to obtain white ginseng (WG) [5]. Korean Red ginseng (KRG) is also traditionally formed from fresh ginseng using another unique method prevalent in Korea. This method for KRG preparation includes steaming for 2–3 h under high temperatures (95–100 °C), pressure, and dryness [5]. Finally, the water content of KRG is less than 15%. The KRG is sold in the market or subjected to further extraction and concentration using hot water or ethanol at high temperatures to produce KRG products. Thus, most fresh ginsengs after harvest go through two processes before consumer consumption of ginseng.

An accumulating body of evidence shows that steaming fresh ginseng under high pressure to prepare KRG induces changes in ginseng components [5]. For example, KRG has different ginsenoside compositions in content and type of ginsenosides, ginseng polysaccharides, and arginine-fructose-glucose complex formation by Maillard reaction compared to fresh ginseng [5,6]. These changes in ginseng components during the conversion of fresh ginseng to KRG may result from non-specific effects of heat, H_2O/moisture, and pressure during steaming and drying, supported by the finding that new types of minor ginsenoside isomers with low molecular weights are formed from high molecular weight ginsenosides during these processes [5]. Their content changes are dependent on temperature and pressure [5]. KRG hardens, and its color changes to red or dark brown after steaming and drying, whereas the color of fresh ginseng is usually yellowish-white after harvest [6]. It is unlikely that these physical and chemical changes during steaming and extraction affect only ginsenosides, ginseng polysaccharides, and arginine, among other ginseng components [5,7].

Ginseng also contains lipids such as phospholipids, although ginseng phospholipids are 1–2% in content, and the distribution of ginseng phospholipids varies depending on the portion of ginseng [8–11]. However, relatively little is known about the changes in the types and content of ginseng phospholipids during ginseng processing. Recently, Nah et al observed that WG and KRG contain a new material called gintonin, a glycolipoprotein complex, and the active ingredient of gintonin is lysophosphatidic acids (LPAs), one of the bioactive phospholipids in nervous and non-nervous systems [11–13]. Gintonin shows neuroprotective effects *in vitro* and *in vivo* on animal models and clinical trials [14–18]. However, there is no information on how gintonin LPAs are formed from ginseng. This review provides information that ginseng phospholipids might also undergo various changes to turn into gintonin LPAs during ginseng steaming and extraction/concentration with different characteristics compared to the other ginseng components such as ginsenosides and ginseng polysaccharides.

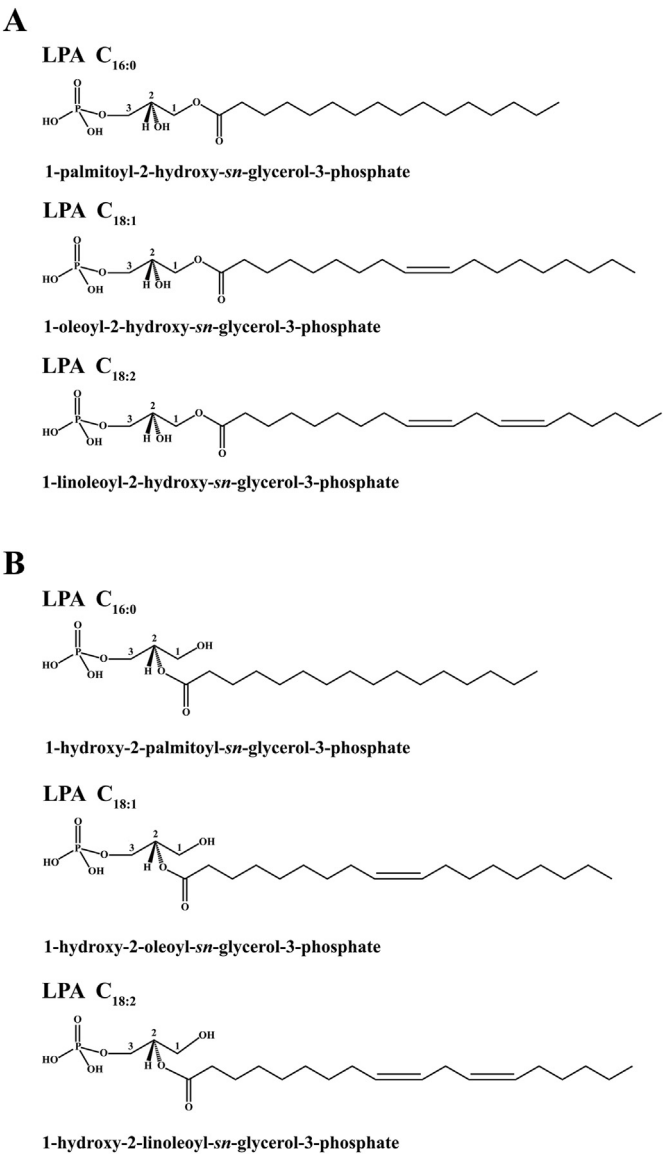

Fig. 1. Structures of lysophosphatidic acids (LPAs) and their isomers that are present in ginseng gintonin. LPA consists of a phosphate head group, glycerol backbone group, and tail fatty acid group. A fatty acid tail acyl group can bind to the glycerol backbone at position 1 or 2, which makes an LPA isomer depending on the position of the acyl group.

2. Chemical structure of LPAs

Phospholipids are major structural components of cell membrane lipid bilayers in animals and plants. However, recent studies have shown that phospholipids play critical roles in information transfer from the extracellular to the intracellular side. LPAs are simple bioactive glycerophospholipids for brain development and functions (Fig. 1) [19]. LPAs are enriched particularly in the animal brain as membrane-derived lipid mediators [20]. LPAs are also precursors for further synthesis of diacyl phospholipids. The chemical structure of LPAs consists of three structural motifs: the phosphate (-PO4$^-$) group of the polar head, the glycerol group of the backbone, and the hydrophobic tail of non-polar fatty acid (Fig. 1). LPAs lack a fatty acid acyl-chain at either *sn*-1-position or *sn*-2-position, containing only a hydroxyl (-OH$^-$) group at 1-position or 2-position instead, which makes them designated "1-acyl-2-lyso-glycerol-3-phosphate or 1-lyso-2-acyl-glycerol-3-phosphate" (Fig. 1A and B). Thus, there are two different LPA isomers, dependent on the position of the acyl chain. LPAs detected in cells have various sizes of acyl chains, i.e., LPA C16:0, LPA C18:1, and LPA C18:2, attached at either the *sn*-1 or *sn*-2 position of the glycerol backbone. LPAs possess better water solubility than other phospholipids because of their -PO4$^-$ head and the free −OH$^-$ group without the fatty acid chain of the glycerol backbone, a different charactersitic from other phospholipids. The -PO4$^-$ group of the polar head acts as a critical functional group for binding to LPA receptors. The hydrolysis of the LPA -PO4$^-$ head group causes a loss of the biological functions of LPA by lipid phosphate phosphatase [21].

3. Ginseng contains LPAs, lysophospholipids, and other phospholipids

A recent study showed that gintonin and a gintonin-enriched fraction (GEF) contain high amounts of LPAs compared to the other herbal medicines [11,22]. The order of LPA amounts was LPA C18:2 >> LPA C16:0 > LPA C18:1 [11]. LPAs can be present as isomers depending on the postition of their acyl groups (Fig. 1). LPA isomers can also have other residues at the 3-phosphate head group, such as choline, ethanolamine, glycerol, and inositol. Further, they can be lysophospholipids. The GEF also contains diverse lysophospholipids incluidng lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), lysophosphatidylglycerol (LPG), and lysophosphatidylinositol (LPI), although their contents are not much high compared to LPAs and PAs, and their order in gintonin is LPC > LPE > LPG > LPI (Fig. 2) [11]. In addition, these lysophospholipids can be classified as 1-acyl-2-lyso-phospholipids or 1-lyso-2-acyl-phospholipids. These LPAs and lysophospholipid isomers act as GTP-binding-protein coupled receptor (GPCR) ligands. Hitherto, 16 such LPA and lysophospholipid GPCRs have been identified, with one, four, and six receptors of LPI, lysophosphatidylserine, and LPA, respectively [23–26]. In addition, previous reports suggest that gintonin LPI could play a role in the GPR55 ligand, known as an atypical cannabinoid receptor in the animal body [25]. We also demonstrated that GEF contains abundant linoleic acid compared to other free fatty acids (FFAs), and GEF linoleic acid could act as a ligand for GPR41, known as one of the fatty acid receptors [27]. Thus, ginseng contains three exogenous ligands for GPR55 and GPR41, the LPA receptor subtypes.

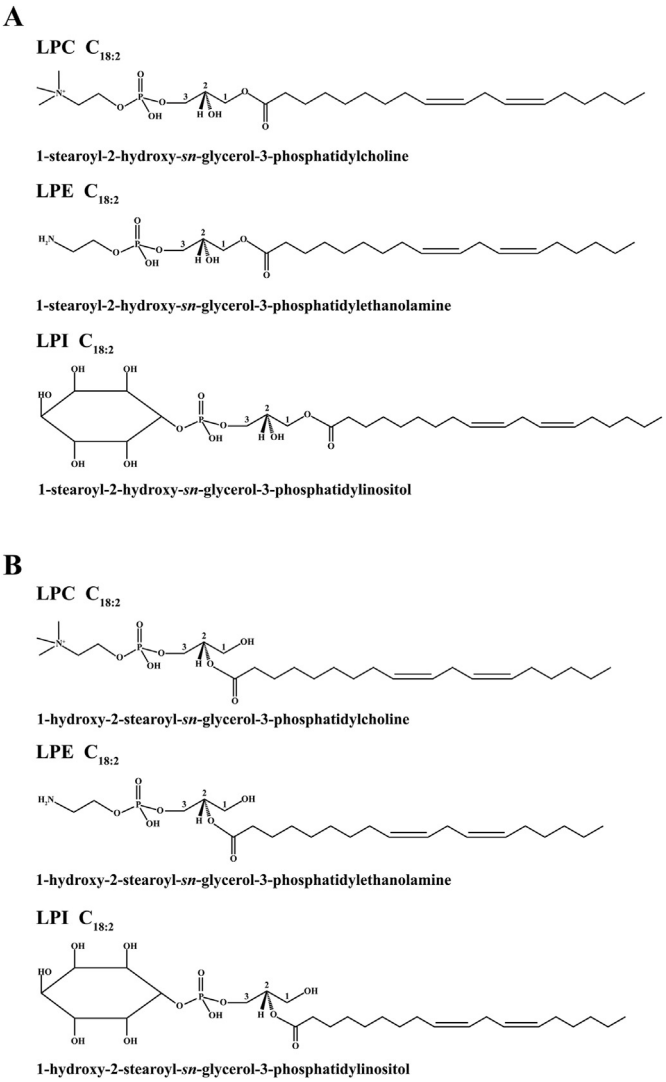

Fig. 2. Lysophospholipids such as lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), and lysophosphatidylinositol (LPI) are present in gintonin-enriched fraction. These can act as a ligand for their respective receptors on the animal cell membrane or can also be converted into LPA C18:2 under steaming and extraction processes. Newly formed lysophosphatidic acids (LPAs) from lysophospholipids can exist as LPA isomers as described in Fig. 1.

The GEF also contains phosphatidic acids (PAs), formed after attachment of one molecule of fatty acid at either 1-acyl-2-lyso-glycerol-3-phosphate or 1-lyso-2-acyl-glycerol-3-phosphate (Fig. 4). Interestingly, GEF contains a high amount of PAs, and their order is PA C16:0-18:2 >> PA C18:2-18:2, that is 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphate >> 1-linoleoyl-2-linoleoyl-*sn*-glycero-3-phosphate (Fig. 4). The GEF also contains phosphatidylcholine (PC) in the order PC C16:0-18:2 >> PC C18:2-18:2 [11], i.e., 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphatidylcholine >> 1-linoleoyl-2-linoleoyl-*sn*-glycero-3-phosphatidylcholine. It also includes other phospholipids, such as phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and phosphatidylinositol (PI). In summary, gintonin LPAs, lysophospholipids, and linoleic acid play crucial roles in biological systems (Fig. 2).

4. A typical processes for the formation of LPAs and other lipids in biological systems

The subset activations of G protein-coupled receptors are closely associated with phospholipid hydrolysis of the plasma membrane through signaling pathways that transfer external stimuli to intracellular sides [28]. Thus, membrane phospholipids can be hydrolyzed by four different phospholipases [28] (Fig. 3). Phospholipase A1 cleaves at position *sn*-1, liberating second messenger FFAs and 1-lyso-2-acyl-lyso-phospholipid (Fig. 3). Phospholipase A2 cleaves at positions *sn*-2, releasing second messenger FFAs such as arachidonic acid and 1-acyl-2-lyso-phospholipid (Fig. 3) [29]. Thus, phospholipase A1 and A2 produce two different isomers in biological systems. Free arachidonic acid is a precursor for the biosynthesis of eicosanoids, i.e., prostaglandins, thromboxanes, and leukotrienes. They have many broad ranging actions, including defenses against exterior damage and pathogens.

Phospholipase C (PLC) removes the phosphoryl headgroup of glycerophospholipids to produce inositol phosphates, second messengers. The generated phosphatidylinositol 3-phosphate diffuses into the cytosol and binds the phosphatidylinositol 3-phosphate receptor at the endoplasmic reticulum to elicit transient intracellular Ca^{2+} mobilization. The remaining diacylglycerols (DAGs) (Fig. 3), the other product after PLC action, induce protein kinase C (PKC) activation. Both calcium and PKC have vital roles in various regulations of intracellular membrane trafficking, cell proliferation, and other cellular processes [30,31].

Phospholipase D (PLD) acts on structural phospholipids in the membane, such as PC and PE, to produce PA [32]. The PA thus synthesized has emerged as a critical modulator of cellular activity in eukaryotes, e.g., for cytoskeletal rearrangements, secretion, endocytosis, and respiratory burst in animals, as well as resistance against freezing, wounding, pathogen elicitation, dehydration, and salts in plants (Fig. 4). On the other hand, serum lyso-PLD, also called autotaxin, acts on animal serum LPCs, abundant in animal serum, to produce plasma LPA [33]. Thus, at least five

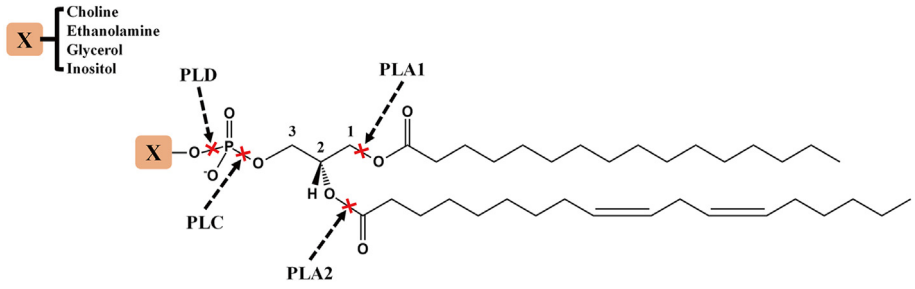

Fig. 3. Phospholipases are enzymes that hydrolyze membrane phospholipids into fatty acids and other hydrophilic or lipophilic substances. There are four major classes, which are distinguished by the type of reaction that these catalyze: Phospholipase A cleaves the *sn*-1 acyl chain (where *sn* refers to stereospecific numbering). Phospholipase A2 cleaves the *sn*-2 acyl chain, usually releasing arachidonic acid. Phospholipase C cleaves before the phosphate, releasing diacylglycerol and a phosphate-containing head group. PLCs play a central role in signaling transduction, releasing 2nd messenger inositol triphosphate. Phospholipase D cleaves after the phosphate, releasing phosphatidic acid and alcohol. Lysophospholipase D (also called autotaxin) cleaves lysophospholipids before the phosphate, releasing lysophosphatidic acids (LPAs) and other hydrophilic components such as choline, ethanolamine, glycerol, or inositol.

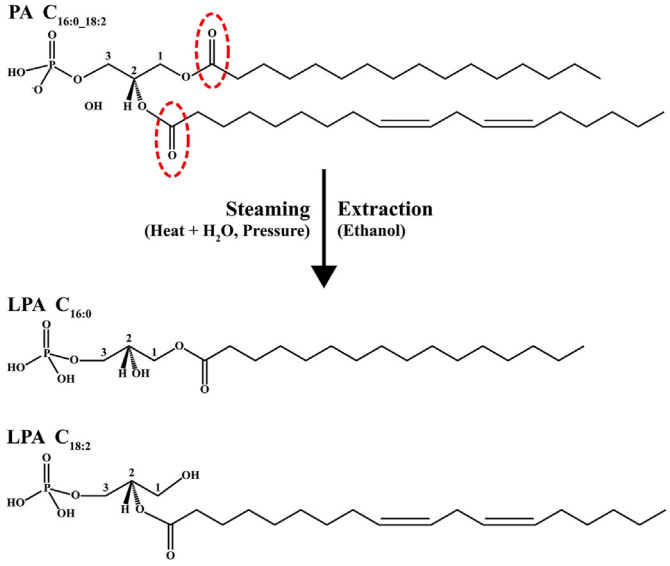

Fig. 4. Phospholipids like phosphatidic acids (PAs) are abundant in gintonin-enriched fraction and they can be converted into LPA C16:0 or LPA C18:2 under steaming and extraction processes. Newly formed lysophosphatidic acids (LPAs) from PAs can exist as LPA isomers as described in Fig. 1.

phospholipases, including lyso-PLD, hydrolyze phospholipids to release FFAs during eicosanoid synthesis to synthesize lysophospholipids as a precursor for LPAs with their water-soluble heads bearing inositol phosphates, choline, ethanolamine, glycerol, and serine. Inositol phosphate and choline act as a second messenger and an acetylcholine precursor, respectively [34].

5. Atypical or non-enzymatic formations of gintonin LPAs and lysophospholipids from ginseng phospholipids

The PA, PC, PE, PG, PI, and other phospholipids and cholesterol constitute lipid bilayers of the cell membrane in animals or plants [8–11]. These membrane phospholipids are relatively unstable and are easily oxidized or hydrolyzed in an atypical manner when exposed to abnormal conditions, e.g., heat, pressure, or oxidative stress, in addition to specific phospholipase actions [34–36]. For example, phospholipids such as PA, abundantly present in animal or plant cell membranes, are hydrolyzed to lysophospholipids (i.e., LPA) and FFAs, if polyunsaturated fatty acids are present at positions 1 or 2 of the glycerol backbone. In addition, the same atypical

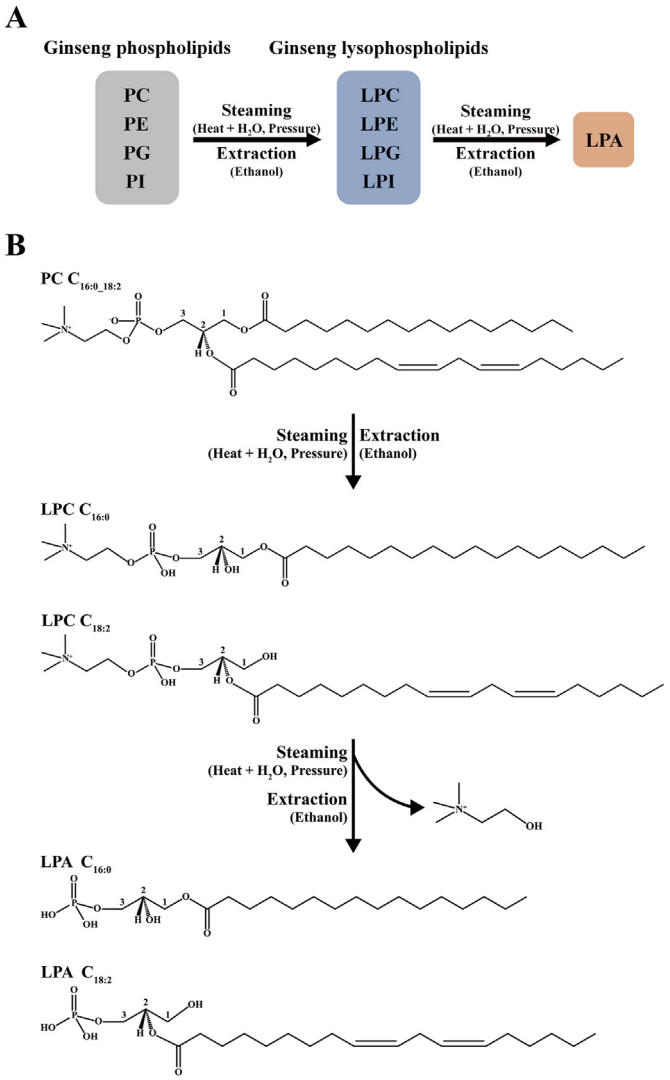

Fig. 5. Summary of a possible atypical process for the formation of lysophosphatidic acids (LPAs) from various phospholipids and lysophospholipids in gintonin and gintonin-enriched fractions. Ginseng contains phospholipids such as phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and phosphatidylinositol (PI). These phospholipids can be converted into the corresponding lysophospholipids and then finally become LPAs under steaming and extraction processes. LPAs can be at once formed from phospholipids or sequentially via lysophospholipids.

principle can be applied to other phospholipids (PC, PE, PG, and PI) to produce lysophospholipids with the production of FFAs. LPAs are formed when choline, ethanolamine, glycerol, or inositol are separated further from the polar head at position 3 of these lysophospholipids [34] (Figs. 4 and 5).

Similarly, ginseng contains phospholipids such as PA, PC, PE, PG, and PI [8–11]. Although not reported until now, ginseng phospholipids might be no exception in changes of phospholipid ingredients during steaming with high pressure and drying processes for KRG. There might be two atypical routes for gintonin LPA formations of ginseng phospholipids by atypical hydrolysis under heat, moisture/H_2O, and pressure. One such way is via the degradation of PAs under heat, moisture/H_2O, and high pressure. As shown in Fig. 4 (red circle), PA has two acyl groups in the glycerol backbone. Unsaturated fatty acids such as linoleic acid and oleic acid are more prone to hydrolysis and oxidation than saturated fatty acids in phospholipids [35–37]. Then, LPAs are formed after the liberation of one of the fatty acids at position 1 or 2. The GEF contains much more PA C16:0-C18:2 than PA C18:2-C18:2 [11]. Therefore, PA C16:0-C18:2 can be converted to produce 1-palmitoyl-2-lyso-glycerol-3-phosphate (LPA C16:0) or 1-lyso-2-linoleoyl-glycerol-3-phosphate (LPA C18:2) under heat, H_2O/moisture, and high pressure with the liberation of FFAs such as linoleic acid or palmitic acid (Fig. 4). In addition, PA C18:2-C18:2 can be converted to produce 1-linoleoyl-2-lyso-glycerol-3-phosphate (LPA C18:2) or 1-lyso-2-linoleoyl-glycerol-3-phosphate (LPA C18:2). Thus, PA C18:2-C18:2 can be converted into two kinds of LPA C18:2, which differ from each other in the position of their acyl groups.

Other phospholipids, such as PC, PE, PG, and PI, can be first converted into lysophospholipids such as LPC, LPE, and LPI by liberating FFA at position 1 or 2 [11] (Fig. 5). These lysophospholipids can be 1-acyl-2-lyso-phospholipids or 1-lyso-2-acyl-phospholipids, dependent on the saturated or unsaturated fatty acid type at position 1 or 2 as shown in Fig. 5. These lysophospholipids can be hydrolyzed further atypically to LPAs by liberating polar head groups such as choline, ethanolamine, glycerol, or inositol (Fig. 5).

6. An acyl-group migration of LPAs and lysophospholipids in gintonin

As shown in Fig. 1, ginseng phospholipids are hydrolyzed under physical actions to produce LPAs from PAs and other phospholipids such as PC, PE, PG, and PI. These phospholipids can be converted into lysophospholipids, which can be converted further into gintonin LPAs. These LPAs will be 1-palmitoyl-2-lyso-*sn*-glycerol-3-phosphate (LPA C16:0), 1-oleoyl-2-lyso-*sn*-glycerol-3-phosphate (LPA C18:1) or 1-linoleoyl-2-lyso-*sn*-glycerol-3-phosphate (LPA C18:2). Interestingly, lysophospholipids including LPAs and LPC have characteristics that acyl and phosphoryl group can migrate other empty position at 1 or 2 [38,39] (Figs. 1 and 5). Thus, it is known that 1-palmitoyl-2-lyso-*sn*-glycerol-3-phosphate, 1-lyso-2-oleoyl-glycerol-3-phosphate, and 1-linoleoyl-2-lyso-*sn*-glycerol-3-phosphate are unstable, and they are quickly converted to the corresponding 1-lyso-2-palmitoyl-glycerol-3-phosphate, 1-oleoyl-2-lyso-glycerol-3-phosphate, and 1-lyso-2-linoleoyl-glycerol-3-phosphate by a spontaneously occurring intra-molecular acyl or phosphoryl migration reactions, respectively [40]. Therefore, it has been challenging to detect and quantify 2-acyl-1-lyso-phospholipids in biological samples as 2-acyl-1-lyso-phospholipids are unstable and yielding a mixture of 1-acyl-2-lyso-phospholipids and 2-lyso-1-acyl-phospholipids [40]. Plückthun and Dennis (1982) demonstrated that 1-palmitoyl-2-lysophosphatidylcholine, an LPC, prepared by PLA2, is the equilibrium mix consisting of approximately 90% of 1-acyl-2-lyso-phospholipids and 10% of 2-acyl-1-lyso-phospholipids [41]. However, it is unlikely that lysophospholipids, including LPAs in gintonin and GEF, also drive spontaneously occurring intra-molecular acyl or phosphoryl migration reactions, as these do not exist in free form and instead bind to ginseng major latex-like protein151 (GLP151) and form complexes with other ginseng-derived carbohydrates and lipids as described below in detail [42].

7. Gintonin LPAs may be more stable than free LPAs

Free LPA spontaneously migrates to 1-acyl-2-lyso-glycerol 3-phosphate or 1-lyso-2-acyl-glycerol 3-phosphate and lysophospholipid as described above (Figs. 1 and 5). Furthermore, if 1-acyl-2-lyso-glycerol 3-phosphate or 1-lyso-2-acyl-glycerol 3-phosphate has a polyunsaturated fatty acid like linoleic acid at acyl position like gintonin LPAs, it will be easily oxidized and lose its biological activity [35] (Fig. 1). Ginseng-derived gintonin LPAs or lysophospholipids have different characteristics from those of free LPAs or other free lysophospholipids, as these are bound with GLP151 proteins and other ginseng components such as ginseng carbohydrates and other lipids [42]. Therefore, gintonin is a kind of glycolipoprotein complex [12] (Fig. 6), although LPAs are a bioactive species in gintonin among lysophospholipids [11,13]. Thus, there are several supporting pieces of evidence that gintonin LPAs are more stable than free LPAs. First, previous reports show that gintonin LPAs are not isolated from ginseng as free LPAs. Instead, gintonin is isolated using anion exchange column chromatography following ethanol or organic solvents extractions, as gintonin is a complex with negatively charged components [11] (Fig. 6). Second, after SDS-PAGE of gintonin, the molecular weight of GEF is approximately 17 kD [11], whereas, the molecular weight of free LPA C18:2 is nearly 433 Da [13]. Gintonin is reported to have carbohydrates, lipids, and proteins, as observed by staining gel on the respective components-Periodic acid-Schiff staining, Sudan black for lipids, and Coomassie staining proteins [11]. The order of carbohydrate, lipid, and protein content is carbohydrate = lipid > proteins [11]. LPAs in gintonin might be tightly bound with the other components of gintonin, as gintonin LPAs are not readily released even with methanol extraction of gintonin several times. Finally, in solution, gintonin LPAs exhibit more prolonged effects on Ca^{2+}-activated chloride channel activation in *Xenopus* oocytes than free LPA [13]. Thus, other components of gintonin besides LPAs might contribute to LPA stability in solution in addition to the role of LPA carrier, storage, and transfer to target LPA receptors with a high affinity [41]. Gintonin itself also showed antioxidant effects [43]. Thus, gintonin has an ability for free radical scavenging effects, providing an additional contributing factor for more stability of gintonin LPAs. These characteristics of gintonin LPAs may contribute to the prolonged impact of their biological actions compared to free LPAs [11].

8. Characterizing the formation of gintonin LPAs

This review article details the mechanism of the formation of gintonin LPAs in an atypical way under harsh conditions such as heat, H_2O/moisture, and high pressure, differing from the typical process of formation of LPAs by phospholipases that are present in cells or body fluids. Interestingly, gintonin LPAs exhibit high affinity with LPA receptors as much as free LPAs do, which means that gintonin LPAs are not only stable in aqueous solution but also in heat under harsh conditions [11]. Gintonin shows some characteristics gintonin is glycolipoprotein complexes, which consist of LPAs, lysophospholipids, and fatty acids/phospholipids/lipids with ginseng proteins and carbohydrates. However, the question of how these glycolipoprotein complexes are formed remains unresolved.

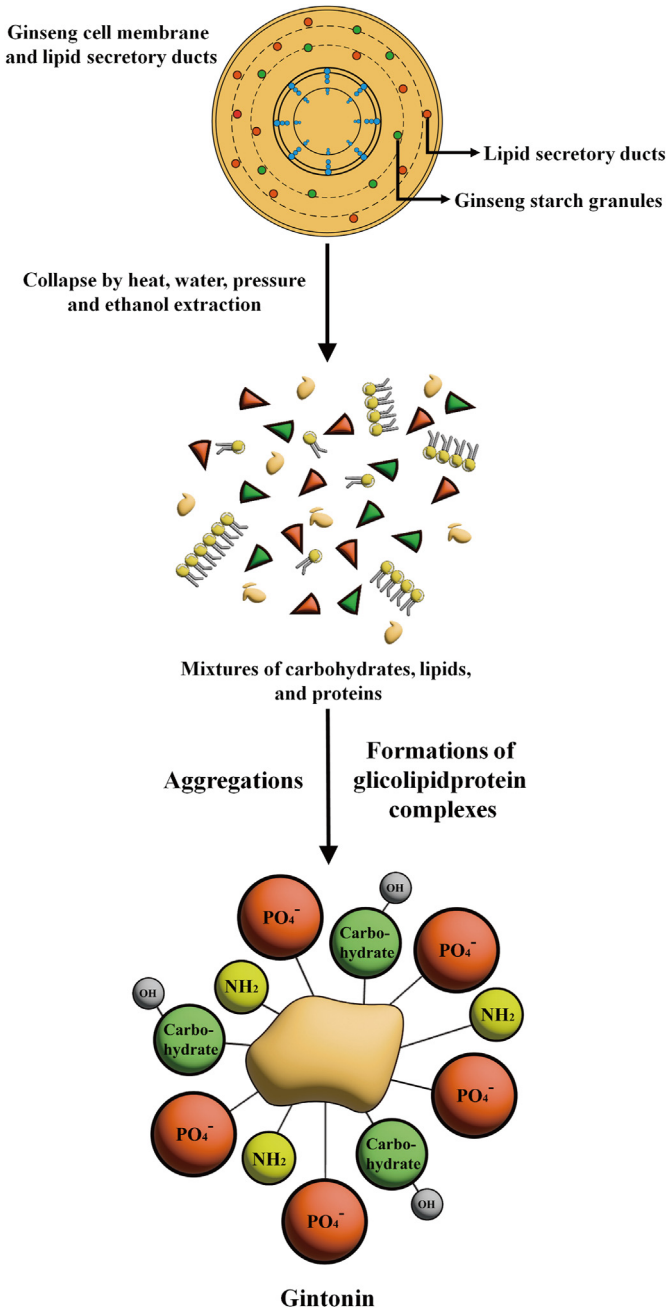

Fig. 6. A hypothetical formation of gintonin glycolipoprotein complex. The cell membranes and lipid secretory ducts of fresh ginseng collapse during steaming processes, accompanying the transformation of ginseng components such as ginsenosides, phospholipids, and other ginseng ingredients under high pressure. There may be a mix of various ginseng components derived from ginseng cell membranes and lipid secretory ducts, which form a complex of glycolipoproteins with hydrophilic components exposed on the outer surface and hydrophobic components sequestered on the inner surface. Most of the outer surface of the gintonin complex has negative charges, as gintonin is isolated using anion exchange chromatography [13.14].

One possibility that follows is that LPAs, lysophospholipids, phospholipids, fatty acids, and other lipids are formed from various phospholipids as well as neutral and acidic lipids, which can be derived from the lipid components of the ginseng cell membrane and can also originate from products secreted by lipid secretory ducts that are abundantly present in ginseng [44,45]. Ginseng lipid secretory ducts are well developed for lipid secretions, and their numbers also increase with ginseng age [45]. These ducts also secrete other ginseng components for ginseng metabolism maintenance [45]. These ginseng cell membranes and ginseng lipid secretory ducts collapse during fresh ginseng processing for WG or KRG preparation and KRG extraction/concentration under heat, H_2O/moisture, and high pressure, which aggregate with each other for the formation of the gintonin complex (Fig. 6). The hydrophobic components of gintonin aggregates come together on the inner surface of gintonin complexes. On the other hand, the hydrophilic components of gintonin such as the -PO_4^- group of LPA and PA, head groups of minor lysophospholipids such as choline, ethanolamine, glycerol and inositol, amine and carboxyl groups of proteins, and the OH^- group of glucose in carbohydrates are exposed on the outer surface of gintonin complexes (Fig. 6) [11–13]. Thus, gintonin LPAs might provide unique characteristics in their compositions and biological actions compared to the free LPAs [11].

9. Beneficial effects of gintonin on nervous and non-nervous systems

9.1. Gintonin can modulate information transfer in brain neurons through the regulation of neurotransmitter and gliotransmitter release

The primary mode of action of gintonin to exhibit its biological effects is through $[Ca^{2+}]_i$ transient via LPA receptor-phospholipase C-IP_3 signaling transduction pathways [11]. Neurotransmitter- or neurohormone-mediated $[Ca^{2+}]_i$ transient is coupled to neurotransmitter or neurohormone release in neuronal systems resembling the mode of action adopted by gintonin [46–50]. The released neurotransmitters or neurohormones in central nervous systems participate in diverse modulations of neuronal activities from learning and memory to sleep and awakening via Ca^{2+}-dependent ion channel enzymes and receptors. Hippocampus is a critical brain region involved in learning and memory, and hippocampal dysfunctions are closely associated with cognitive impairments [51]. In adult hippocampal slices, gintonin treatment induced the release of glutamate and enhanced synaptic transmission by increasing spontaneous firing frequency [52]. The LPA1/3 receptor antagonist attenuated the gintonin-mediated enhancement of synaptic transmission. In addition, gintonin-mediated synaptic transmission enhancement is coupled to long-term potentiation (LTP) induction [53]. In molecular mechanism studies on synaptic transmission and LTP induction, gintonin stimulates glutamate release, and the released glutamate activates *N*-methyl-d-aspartate (NMDA) and non-NMDA receptors for synaptic transmission enhancement as well as LTP induction in the hippocampus [52,53]. In addition to gintonin actions on hippocampal glutamate release, recent studies showed that gintonin also stimulates acetylcholine, dopamine, norepinephrine, and serotonin release in the brain, adrenal gland, and intestinal systems, respectively [46,50]. These gintonin-mediated neurotransmitter or neurohormone releases might play critical roles in neuromodulations in central and peripheral nervous systems besides hippocampal synaptic transmission and LTP induction.

On the other hand, astrocyte, one of the glia, mainly plays a metabolic supporting role for neighboring neurons. Recent studies revealed that astrocytes also play a modulating role in neurons by forming tripartite synapses with neurons in addition to bipartite synapses between neurons [54]. Thus, astrocytes modulate presynaptic and postsynaptic information transfer by forming synapses with presynaptic and postsynaptic neuron terminals by releasing gliotransmitters [47]. In addition to neurotransmitter release, gintonin treatment to astrocytes elicits $[Ca^{2+}]_i$ transient via LPA receptor-phospholipase C-IP_3 signaling transduction pathways and stimulates gliotransmitters such as glutamate and ATP from

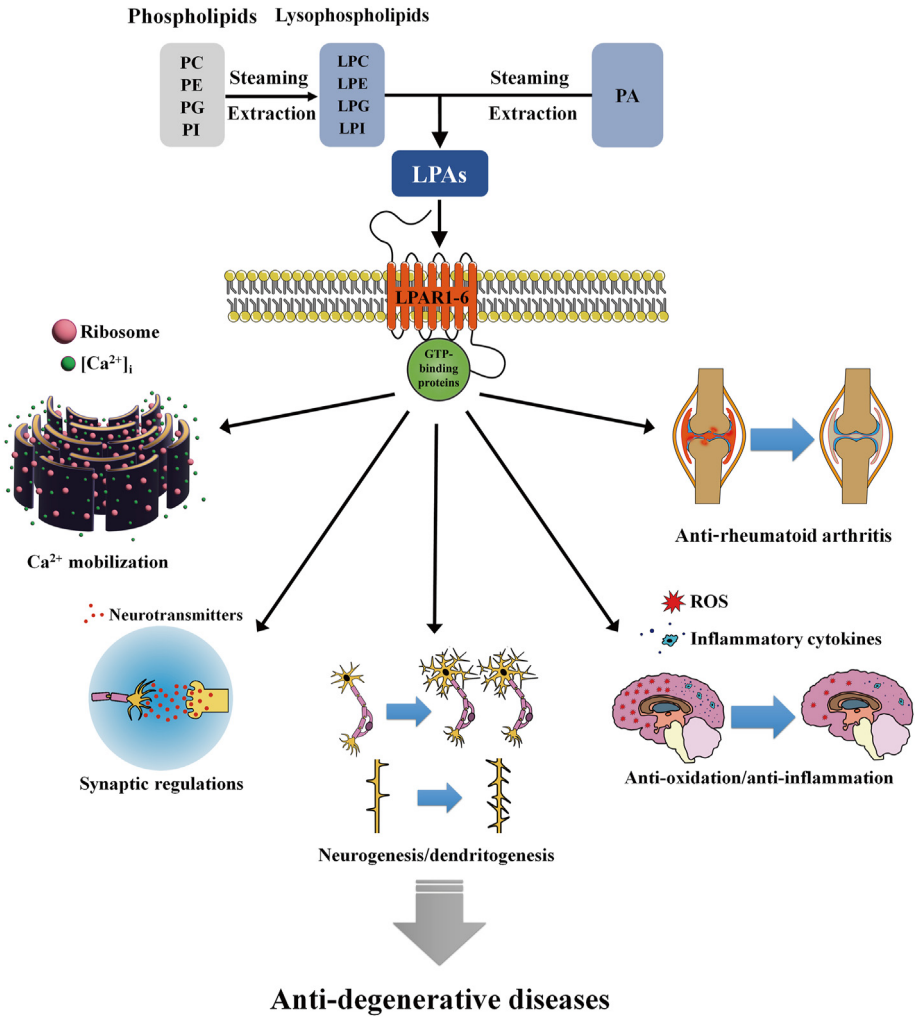

Fig. 7. Summary of a possible linkage of LPA formations in ginseng and their ageing-related degenerative diseases effects via LPA receptor signaling pathway-coupled trophic effects.

astrocytes [47]. Thus, the gintonin-mediated release of gliotransmitter and neurotransmitter supports the notion that gintonin could coordinate synaptic functions or synaptic physiology that transfers information between bipartite neuronal synaptic elements through neurotransmitter release and also impact astrocytic tripartite synapses through the Ca^{2+}-dependent release of gliotransmitters (Fig. 7).

9.2. Gintonin can act as a ginseng-derived neurotrophin with brain-derived neurotrophic factor (BDNF)-like actions

It is known that LPAs are abundant in the brain and saliva. LPA plays as a lipid-derived neurotrophin [55–59]. Neurotrophins are usually peptides or proteins that help to stimulate neurogenesis and dendritogenesis [58]. Interestingly, LPA is a neurolipid acting like a neurotrophin. Brain-derived neurotrophic factor (BDNF) is one of the representative neurotrophins [57]. The roles of BDNF in brain neurons include the survival of existing neurons, encouraging growth, neurogenesis, and differentiation of new neurons with dendritogenesis [58]. Similarly, gintonin stimulates *in vitro* and *in vivo* BDNF production in the brain and spinal cord motor neurons. Gintonin also affects neurons through BDNF-like activity but via LPA receptor activation [48].

Recently, *in vitro* study showed that gintonin stimulates the dendritic growth of striatal neurons in the brain tissue [59]. In addition, gintonin also promotes dendritic spine formation. Dendritic spines originated from dendritic filopodia, and gintonin treatment increased the number of dendritic filopodia [59]. Gintonin-mediated stimulations of dendritic growth, dendritic spine, and filopodia formations were achieved via the LPA1/3 receptor, as gintonin-mediated actions of dendritic growth were blocked by Ki16425, an LPA1/3 receptor antagonist. Another intriguing characteristics of gintonin is its synergistic effects on dendritic growth with BDNF [59]. Sub-concentrations of gintonin or BDNF alone did not elicit any dendritic growth of neurons. However, when a combination of gintonin or BDNF sub-concentrations was co-treated to striatal neurons, there were dramatic increases in dendritic growth. These synergistic actions of gintonin and BDNF on dendritic growth may be due to the convergence of the two growth factors, although their signaling transduction pathways differ. In other words, gintonin is a kind of exogenous neurotrophic factor with different receptors, different from BDNF.

In addition to stimulating effects on gintonin-mediated neuronal dendritic growth, gintonin also induces the *in vitro* proliferation of hippocampal neural progenitor cells (NPCs). Gintonin treatment increased 5-bromo-2′-deoxyuridine (BrdU) incorporation in hippocampal NPCs [48]. Gintonin increased the immunostaining of NeuN, a biomarker protein for mature neurons, confirming that gintonin induced hippocampal neuron

proliferation [48,60]. Gintonin also increased LPA1 receptor expression in hippocampal NPCs [48,60]. However, the gintonin-induced increase in BrdU incorporation and immunostaining of NeuN biomarkers was blocked by an LPA1/3 receptor antagonist and Ca^{2+} chelator, suggesting that gintonin-induced hippocampal neuron proliferation is mediated by LPA receptors in a Ca^{2+}-dependent manner [60]. Oral administration of the GEF also increased hippocampal BrdU incorporation and LPA1/3 receptor expression in adult wild-type and transgenic AD mice [48]. Gintonin increased the number of hippocampal neurons in adult wild-type mice and a transgenic AD mouse model [48,60,61]. Gintonin-mediated *in vitro* and *in vivo* hippocampal neuron proliferation and dendritogenesis support the notion that gintonin could mimic BDNF action in the nervous system as a neurolipid. Thus, gintonin might be a novel candidate for ginseng-derived neurotrophin via LPA receptor regulations (Fig. 7).

9.3. Prevention and improvement of degenerative diseases by gintonin

9.3.1. Alzheimer's disease

It has been recorded in traditional Korean medical books that ginseng consumption for a long time will clear the mind. Based on the information, many studies have investigated the benefits of ginseng against Alzheimer's disease (AD) dementia [62]. AD dementia causes a cognitive dysfunctions with changes in emotions and behaviors of which patients are unaware, resulting in increased levels of stress and economic burdens to family members and caregivers [63]. Gintonin shows anti-AD dementia effects in four ways. First, gintonin inhibits the pathway for Aβ generation, a dementia-causing peptide in the brain, from amyloid precursor protein (APP) in the neuron membrane. Instead, gintonin produces soluble APP α (sAPPα), which is beneficial to the nervous system acting via a non-amyloidogenic pathway [64]. Naturally, less Aβ is produced in the presence of gintonin. Even if Aβ is produced, gintonin prevents the neurotoxic action of Aβ, which kills neurons [64].

Second, gintonin exhibits antioxidant and anti-inflammatory effects against Aβ in the AD animal model brain. Endogenous Aβ formation in the brain in AD animal model mice or direct Aβ injection into mouse brain causes oxidative stress and neuroinflammation through microglia and astrocyte activation [65]. However, oral administration of gintonin reduced the elevated levels of oxidative stress by upregulating the expression of Nrf2/HO-1, thereby reducing the generation of reactive oxygen species (ROS) and lipid peroxidation. In addition, gintonin also suppressed activated microglial cells and inflammatory cytokine mediator formations such as interleukin-6, tumor necrosis factor, and cyclooxygenase-2 in the brains of Aβ-injected mice [65].

It is known that acetylcholine levels in the brain play a key role in AD development, as patients with AD dementia have reduced brain acetylcholine levels in addition to amyloid plaque accumulations. The representative treatment for AD dementia (donepezil; trade name, Aricept) currently used is a drug that increases the concentration of acetylcholine in the brain by inhibiting the enzyme (e.g., acetylcholinesterase) that decomposes acetylcholine. The third role of gintonin is to stimulate acetylcholine release and synthesis in the brain *in vitro* and *in vivo*. Gintonin stimulates acetylcholine release and synthesis in cultured hippocampal neurons [48]. *In vivo* studies have revealed that long-term oral administration of gintonin to both normal mice and AD animal model increases the expression of the enzyme choline acetyltransferase responsible for acetylcholine synthesis in the hippocampus and also neurogenesis [60]. However, it decreases the expression of the enzyme responsible for acetylcholine hydrolysis, resulting in an increase in acetylcholine levels and, thus, the number of neurons in the AD animal model brain [48,60].

Thus, gintonin action in implicated in non-amyloidogenic pathway activation, anti-oxidative stress, and anti-inflammatory activity, and the reinforcement of the cholinergic system in the brain. Administering gintonin to mice with dementia showed improvements in working memory, i.e., an ability to perceive space and retain and remember information received through other sensory organs, and association memory, i.e., the ability to recognize the relationships between unrelated events, compared to mice with dementia that did not ingest ginseng gintonin. In other words, it has been shown that long-term administration of gintonin improves cognitive function-related behaviors among mice with AD dementia compared to mice with dementia that did not take gintonin through hippocampal neurogenesis (Fig. 7) [60].

9.3.2. Parkinson's disease

Another representative neurodegenerative disease prevalent worldwide is Parkinson's disease (PD) [66]. PD is the second most common brain disease in the elderly after AD dementia. PD is caused by the death of dopamine-producing neurons in the basal ganglia, located in the inner central part of the brain below the cerebrum [67]. Most patients with PD have the same mental and cognitive functions as people get old. However, due to a lack of dopamine in the brain, patients with PD themselves show neurological symptoms of motor dysfunctions due to abnormal behaviors they do not want, resulting in changes in their posture, e.g., hunched back and trembling limbs, and the inability to move their body quickly at will [68].

On the other hand, the brain is vulnerable to free radicals as 60–70% of the brain is composed of lipids. Brain lipids lose their original functions when oxidized [69]. Endogenous antioxidants in the brain prevent the oxidation of brain lipids. However, these are insufficient to process all free radicals such as ROS produced by substances that cause PD. In experimental animal PD studies, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration is metabolized into MPP^{+}, a main causative chemical for a lot of ROS production selectively in dopaminergic neurons, which produces dopamine in basal ganglia in the brain, resulting in increased oxidative stress, and only neurons that produce dopamine are targeted selectively. Therefore, animals administered MPTP show neurological symptoms resembling symtpoms of PD in humans [70,71].

Administering gintonin to mice with PD improved the neurological symptoms of PD. Thus, when mice with PD ingested gintonin, their neurological symptoms improved even if these mice had PD. In neurological tests, when gintonin was ingested, the time taken for the mice to descend from a pole was shorter, and exercise time without falling off the treadmill exercise machine was much longer than when ginseng gintonin was not taken [70]. In addition, in nest-building tests conducted among PD mice, the mice who had ingested gintonin built fine nests, whereas those who did not ingest gintonin built poor-quality nests. In addition, the survival period of PD mice who were administered gintonin is prolonged compared to the survival of PD mice who were not administered gintonin [71].

The principle of preventing the occurrence of PD and alleviating the neurological symptoms of gintonin was revealed. Gintonin administration to animal PD model mice reduced the loss of tyrosine hydroxylase-positive neurons, inhibited microglial activation, inhibited inflammatory mediator pathways (interleukin-6, tumor necrosis factor, and cyclooxygenase-2) in regions that correspond to human basal ganglia area, and also protected blood-brain barrier (BBB) damages in the substantia nigra pars compacta or striatum induced by MPTP administration or both [70,71]. In addition, gintonin administration activated the nuclear factor erythroid 2-

related factor 2 and heme oxygenase-1 (Nrf2/HO-1) pathways and the inhibition of phosphorylation of the mitogen-activated protein kinases and nuclear factor-kappa B signaling pathways [70]. In other words, gintonin antioxidant and anti-inflammatory effects are effective in the prevention of PD.

In addition to the preventive and improving effects of gintonin against AD and PD, gintonin administration also alleviated amyotrophic lateral sclerosis, Huntington's disease, and multiple sclerosis in animal models [72–74]. The main mechanisms underlying the alleviation of neurological symptoms and histological lesions in these neurodegenerative diseases due to gintonin administration include the attenuation of oxidative damage and neuroinflammation mediated via inhibition of ROS and proinflammatory cytokines such as TNF-α, IL-6, IL-1, and IL-8 production via LPA receptor regulation [75].

9.3.3. Clinical trials using gintonin

As gintonin administration to AD animal model mice attenuated amyloid plaque accumulation in the cortex and hippocampus with improvements in learning and memory functions [48,60,64], a clinical trial was also performed. In an early clinical trial with a small sample size of 10 participants with mild cognitive impairment or early dementia, gintonin intake (300 mg/day, 12 weeks) markedly improved Korean mini-mental state examination after 4 and 8 weeks compared to basal scores, whereas gintonin intake (300 mg/day, 4 weeks) markedly improved the Korean cognitive subscale of Alzheimer's disease assessment scale (ADAS-Cog-K) and ADAS-non Cog-K scores after 4 weeks compared to the basal scores. In terms of gintonin toxicity to humans, none of the subjects complained of any adverse events throughout the administration of gintonin for 12 weeks. Thus, gintonin administration was safe and well tolerated by cognitively impaired elderly subjects [75].

In preclinical studies, acute gintonin administration opened the BBB transiently [76] and facilitated brain delivery of donepezil, an acetylcholinesterase inhibitor [77], prescribed for current AD treatment as Aricept, into the brain by increasing BBB permeability in addition to improvements in cognitive functions in AD animals [76,77]. Therefore, Lee et al performed another clinical trial to investigate the co-relationship between the enhancement of human BBB permeability and cognitive function improvement after gintonin intake [78]. For this clinical trial, dynamic contrast-enhanced magnetic resonance imaging (MRI) was used for the evaluation of gintonin effects on brain vascular permeability changes in different brain segments in human subjects [78]. In this clinical trial, 10 participants with mild cognitive impairment were randomized for an open-label pilot study. Gntonin intake (300 mg/day) and placebo group and Korean versions of the Alzheimer's disease assessment scale (ADAS-K) and dynamic contrast-enhanced-MRI parameters including K^{trans} and V_p in different brain segments were accessed at baseline at 8 weeks after gintonin intake. Lee et al observed that the increment of the K^{trans} value in the left thalamus from the baseline was significantly correlated with the change of the ADAS-cog scores compared to the placebo group [78]. This clinical trial demonstrated that gintonin intake could enhance the BBB permeability in specific structures involved in cognitive functions in the human brain [78]. In this clinical trial, none of the subjects again also complained of any adverse effects throughout the administration of gintonin, indicating that long-term intake of gintonin does not have side effects. Although gintonin intake for a long-term period provides evidence for improvements of cognitive functions as well as drug delivery through facilitation of BBB permeability, the size of clinical trials of human subjects involved was small. Therefore, further clinical trials might be required with a larger scale of human subjects to secure the effects of gintonin on cognitive functions and the facilitation of BBB permeability.

9.4. Beneficial effects of gintonin in rheumatoid arthritis animal model via LPA2 receptor

Another aging-related disease is arthritis. Arthritis is a disease of joints in the body. Specifically, leg or foot arthritis causes pain when elderly people move to perform a walk or movement. Arthritic pain renders the inability to walk or exercise and long-term lack of exercise in old people is closely associated with an increase in the incidence of Alzheimer's disease. Recent studies also showed that gintonin attenuated arthritis in two kinds of *in vivo* arthritis animal models. In collagen-induced arthritis mouse and carrageenan/kaolin-induced arthritis rat models, the oral administration of gintonin improved the arthritic parameters such as a decrease in body weight, arthritis score, knee joint thickness, squeaking score, and swelling paws [79,80]. The improvements in arthritis by gintonin oral administration were achieved via gintonin-mediated anti-inflammatory effects in *in vitro* and *in vivo* histochemical studies. Thus, treatment of gintonin to fibroblast-like synoviocytes blocked IL-1β-induced pro-inflammatory mediators and inflammatory cytokine productions. Gintonin also exhibited anti-oxidant effects by attenuating ROS formation that was stimulated by IL-1β. Gintonin-mediated anti-arthritic effects were mediated via the LPA2 receptor, as the LPA2 receptor antagonist blocked the gintonin-mediated attenuations of IL-1β-induced pro-inflammatory mediators. Thus, long-term oral administration of gintonin shows anti-arthritic effects via anti-inflammatory and anti-oxidant effects through preclinical studies. Further research is required to verify whether there is an actual effect of improving joint pain in humans through clinical trials.

9.5. Future considerations and conclusion

Many kinds of high molecular weight ginsenosides like ginsenoside Rb1 break down into low molecular weight ginsenosides such as ginsenoside Rg3 or other minor ginsenosides such as ginsenoside Rg5 and Rk1 with fewer sugar components during fresh ginseng processes. Similarly, it is very likely that ginseng phospholipid components can also undergo changes in phospholipid compositions in structures and contents under steaming, drying, and extraction/concentration processes with hot water or ethanol and high pressure as observed in ginsenosides. Thus, gintonin LPAs are newly identified, and gintonin LPAs are a new materials of ginseng obtained from traditional ginseng process methods in Korea [11]. The main sources of gintonin LPAs might be ginseng lysophospholipids and phospholipids, and these phospholipids finally become gintonin LPAs with various beneficial effects (Fig. 7). More importantly, LPAs are found in biological systems such as organs and body fluids but ginsenosides are not. In addition, gintonin LPAs, as exogenous LPA receptor ligands, exhibit diverse physiological and pharmacological functions in nervous and non-nervous systems via LPA receptors (Fig. 7), differing from individual ginsenosides, which do not have their plasma receptors in animal cells.

Although beneficial effects of gintonin in age-related diseases are here reviewed from *in vitro* and *in vivo* studies to human clinical trials, it is exactly unknown, however, in which step(s) ginseng phospholipids are converted into lysophospholipids and newly formed lysophospholipids are further subsequently converted into LPAs during ginseng processes. In addition, since gintonin LPAs are not free forms of LPAs but exist as a glycolipoprotein complex, it will be very interesting to elucidate when gintonin as a glycolipoprotein complex is formed during fresh ginseng processes

including extraction. In future studies, it will be necessary to reveal how and when ginseng phospholipids including lysophospholipids become LPAs and when gintonin glycolipoprotein complexes are synthesized in these ginseng processes for the preparation of KRG and KRG extraction/concentration for ginseng products.

In conclusion, elderly people often suffer from arthritis and dementia at the same time. Thus, 70–80% of the population over 65 years suffer from degenerative arthritis, and one in 10 people in Korea are affected by dementia. Lack of exercise in the elderly due to joint pains is reported to have a close proportional relationship with the onset of dementia such as Alzheimer's disease [81,82]. Finally, gintonin can be a candidate for the prevention of age-related diseases as a new material in an aging society.

Acknowledgments

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (2023R1A2C1003481).

References

[1] Park HJ, Kim DH, Park SJ, Kim JM, Ryu JH. Ginseng in traditional herbal prescriptions. Ginseng Res 2012;36(3):225–41.
[2] Nah SY. Ginseng ginsenoside pharmacology in the nervous system: involvement in the regulation of ion channels and receptors. Front Physiol 2014;5:98.
[3] Gong YJ, Sui Y, Han CH, Ning XF. Drying ginseng slices using a combination of microwave and far-infrared drying techniques. J. of Biosystems Eng 2016 3;41(1):34–42.
[4] Liu YZ, Du EX. Ginseng processing technology. Research on Ginseng 1994;1: 39–40.
[5] Lee SM, Bae BS, Park HW, Ahn NG, Cho BG, Cho YL, Kwak YS. Characterization of Korean red ginseng (panax ginseng meyer): history, preparation method, and chemical composition. J Ginseng Res 2015;39(4):384–91.
[6] Suzuki Y, Choi KJ, Uchida K, Ko SR. Effects of the preheating treatments of raw ginseng in the model system on the synthesis of the maillard type-browning reaction products of red ginseng. Journal of Ginseng Research 2004;28(3): 136–42.
[7] In G, Ahn NG, Bae BS, Lee MW, Park HW, Jang KH, Cho BG, Han CK, Park CK, Kwak YS. In situ analysis of chemical components induced by steaming between fresh ginseng, steamed ginseng, and red ginseng. J Ginseng Res 2017;41(3):361–9.
[8] Choi KJ, Kim DH. Studies on the lipid components of fresh ginseng, red ginseng and white ginseng. Korean J Pharmacog 1985;15(3):141~150.
[9] Kim UG, Lee CS, Jeong BG. Distribution of lipids in panax ginseng root. Journal of Ginseng Research 1988;12(2):93–103.
[10] Kim SH, Kim SY, Choi HK. Lipids in ginseng (panax ginseng) and their analysis. Natural Product Sciences 2018;24(1):1–12.
[11] Cho HJ, Choi SH, Kim HJ, Lee BH, Rhim H, Kim HC, Hwang SH, Nah SY. Bioactive lipids in gintonin-enriched fraction from ginseng. J Ginseng Res 2019;43(2):209–17.
[12] Pyo MK, Choi SH, Hwang SH, Shin TJ, Lee BH, Lee SM, Lim YH, Kim DH, Nah SY. Novel glycolipoproteins from ginseng. J Ginseng Res 2011;35:92–103.
[13] Hwang SH, Shin TJ, Choi SH, Cho HJ, Lee B-H, Pyo MK, et al. Gintonin, newly identified compounds from ginseng, is novel lysophosphatidic acids-protein complexes and activates G protein-coupled lysophosphatidic acid receptors with high affinity. Mol Cells 2012;33:151–62.
[14] Choi SH, Jung SW, Lee BH, Kim HJ, Hwang SH, Kim HK, Nah SY. Ginseng pharmacology: a new paradigm based on gintonin-lysophosphatidic acid receptor interactions. Front Pharmacol 2015;27(6):245.
[15] Kim HJ, Jung SW, Kim SY, Cho IH, Kim HC, Rhim H, Kim M, Nah SY. Panax ginseng as an adjuvant treatment for Alzheimer's disease. J Ginseng Res 2018;42(4):401–11.
[16] Ikram M, Ullah R, Khan A, Kim MO. Ongoing research on the role of gintonin in the management of neurodegenerative disorders. Cells 2020;9(6):1464.
[17] Jakaria M, Azam S, Go EA, Uddin MS, Jo SH, Choi DK. Biological evidence of gintonin efficacy in memory disorders. Pharmacol Res 2021;163:105221.
[18] Choi SH, Lee R, Nam SM, Kim DG, Cho IH, Kim HC, Cho Y, Rhim H, Nah SY. Ginseng gintonin, aging societies, and geriatric brain diseases. Integr Med Res 2021;10(1):100450.
[19] Birgbauer E. Lysophosphatidic acid signalling in nervous system development and function. Neuromolecular Med 2021;23(1):68–85.
[20] Das AK, Hajra AK. Quantification, characterization and fatty acid composition of lysophosphatidic acid in different rat tissues. Lipids 1989;24(4):329–33.
[21] Tigyi G, Parrill AL. Molecular mechanisms of lysophosphatidic acid action. Prog Lipid Res 2003;42(6):498–526.
[22] Lee BH, Choi SH, Kim HJ, Jung SW, Kim HK, Nah SY. Plant lysophosphatidic acids: a rich source for bioactive lysophosphatidic acids and their pharmacological applications. Biol Pharm Bull 2016;39(2):156–62.
[23] Chun J, Hla T, Lynch KR, Spiegel S, Moolenaar WH. International union of basic and clinical pharmacology. LXXVIII. Lysophospholipid receptor nomenclature. Pharmacol 2010;62:579–87.
[24] Inoue A, Ishiguro J, Kitamura H, Arima N, Okutani M, Shuto A, Higashiyama S, Ohwada T, Arai H, Makide K, et al. TGFα shedding assay: an accurate and versatile method for detecting GPCR activation. Nat. Methods 2012;9:1021–9.
[25] Oka S, Nakajima K, Yamashita A, Kishimoto S, Sugiura T. Identification of GPR55 as a lysophosphatidylinositol receptor. Biochem Biophys Res Commun 2007;362:928–34.
[26] Sugo T, Tachimoto H, Chikatsu T, Murakami Y, Kikukawa Y, Sato S, Kikuchi K, Nagi T, Harada M, Ogi K, et al. Identification of a lysophosphatidylserine receptor on mast cells. Biochem Biophys Res Commun 2006;341:1078–87.
[27] Cho YJ, Choi SH, Lee R, Hwang H, Rhim H, Cho IH, Kim HC, Lee JI, Hwang SH, Nah SY. Ginseng gintonin contains ligands for GPR40 and GPR55. Molecules 2020;25(5):1102.
[28] Aloulou A, Rahier R, Arhab Y, Noiriel A, Abousalham A. Phospholipases: an overview. Methods Mol Biol 2018;1835:69–105.
[29] Murakami M, Taketomi Y, Sato H, Yamamoto K. Secreted phospholipase A2 revisited. J Biochem 2011;150(3):233–55.
[30] Nishizuka Y. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science 1992;258(5082):607–14.
[31] Kotzampasi DM, Premeti K, Papafotika A, Syropoulou V, Christoforidis S, Cournia Z, Leondaritis G. The orchestrated signaling by PI3Kα and PTEN at the membrane interface. Comput Struct Biotechnol J 2022;20:5607–21.
[32] Bowling FZ, Frohman MA, Airola MV. Structure and regulation of human phospholipase D. Adv Biol Regul 2021;79:100783.
[33] Jose A, Kienesberger PC. Autotaxin-LPA-LPP3 Axis in energy metabolism and metabolic disease. Int J Mol Sci 2021;22(17):9575.
[34] Blusztajn JK, Slack BE, Mellott TJ. Neuroprotective actions of dietary choline. Nutrients 2017;9(8):815.
[35] Grit M, Crommelin D. Chemical stability of liposomes: implications for their physical stability. Chemistry and Physics of Lipids 1993;64(1–3):3–18.
[36] Grit M, Desmidt J, Struijke A, Crommelin D. Hydrolysis of phosphatidylcholine in aqueous liposome dispersions. International Journal of Pharmaceutics 1989;50(1):1–6.
[37] Grit M, Underberg WJM, Crommelin DJA. Hydrolysis of saturated soybean phosphatidylcholine in aqueous liposome dispersions. J Pharm Sci 1993;82(4):362–6.
[38] Eibl H, Lands WE. Phosphorylation of 1-alkenyl-2-acylglycerol and preparation of 2-acylphosphoglycerides. Biochemistry 1970 Jan 20;9(2):423–8.
[39] Sugasini D, Subbaiah PV. Rate of acyl migration in lysophosphatidylcholine (LPC) is dependent upon the nature of the acyl group. Greater stability of *sn*-2 docosahexaenoyl LPC compared to the more saturated LPC species. PLoS One 2017;12(11):e0187826.
[40] Okudaira M, Inoue A, Shuto A, Nakanaga K, Kano K, Makide K, Saigusa D, Tomioka Y, Aoki J. Separation and quantification of 2-acyl-1-lysophospholipids and 1-acyl-2-lysophospholipids in biological samples by LC-MS/MS. J Lipid Res 2014;55(10):2178–92.
[41] Plückthun A, Dennis EA. Acyl and phosphoryl migration in lysophospholipids: importance in phospholipid synthesis and phospholipase specificity. Biochemistry 1982;21(8):1743–50.
[42] Choi SH, Hong MK, Kim HJ, Ryoo N, Rhim H, Nah SY, Kang LW. Structure of ginseng major latex-like protein 151 and its proposed lysophosphatidic acid-binding mechanism. Acta Crystallogr D Biol Crystallogr 2015;71(Pt 5): 1039–50.
[43] Lee R, Cho HS, Kim JH, Cho HJ, Choi SH, Hwang SH, Rhim H, Cho IH, Rhee MH, Kim DG, et al. A novel protocol for batch-separating gintonin-enriched, polysaccharide-enriched, and crude ginsenoside-containing fractions from ginseng. Journal of Ginseng Research 20 October 2022. Available online.
[44] Kim WK, Kim ES. Structure and function of the secretory ducts in Panax ginseng C. A. Mayer. Kor. Jour. Electron Microscopy 1980;10(2):77–86.
[45] Lee KH, Lee SS, Lee MG, Kim ES. Identification of root age by histochemical staining of secretory duct layers in ginseng. J. Ginseng Res. 2001;25(2):101–5.
[46] Hwang SH, Lee BH, Choi SH, Kim HJ, Jung SW, Kim HS, Shin HC, Park HJ, Park KH, Lee MK, et al. Gintonin, a novel ginseng-derived lysophosphatidic acid receptor ligand, stimulates neurotransmitter release. Neurosci Lett 2015;584:356–61.
[47] Kim H, Lee BH, Choi SH, Kim HJ, Jung SW, Hwang SH, Rhim H, Kim HC, Cho IH, Nah SY. Gintonin stimulates gliotransmitter release in cortical primary astrocytes. Neurosci Lett 2015;603:19–24.
[48] Kim HJ, Shin EJ, Lee BH, Choi SH, Jung SW, Cho IH, Hwang SH, Kim JY, Han JS, Chung C, et al. Oral administration of gintonin attenuates cholinergic impairments by scopolamine, amyloid-β protein, and mouse model of alzheimer's disease. Mol Cells 2015;38(9):796–805.
[49] Lee BH, Kim J, Lee RM, Choi SH, Kim HJ, Hwang SH, Lee MK, Bae CS, Kim HC, Rhim H, et al. Gintonin enhances performance of mice in rotarod test: involvement of lysophosphatidic acid receptors and catecholamine release. Neurosci Lett 2016;612:256–60.
[50] Kim HJ, Park SD, Lee RM, Lee BH, Choi SH, Hwang SH, Rhim H, Kim HC, Nah SY. Gintonin attenuates depressive-like behaviors associated with alcohol withdrawal in mice. J Affect Disord 2017;215:23–9.

[51] Roza C, Campos-Sandoval JA, Gómez-García MC, Peñalver A, Márquez J. Lysophosphatidic acid and glutamatergic transmission. Front Mol Neurosci 2019;12:138.
[52] Park H, Kim S, Rhee J, Kim HJ, Han JS, Nah SY, Chung C. Synaptic enhancement induced by gintonin via lysophosphatidic acid receptor activation in central synapses. J Neurophysiol 2015;113(5):1493–500.
[53] Shin TJ, Kim HJ, Kwon BJ, Choi SH, Kim HB, Hwang SH, Lee BH, Lee SM, Zukin RS, Park JH, et al. Gintonin, a ginseng-derived novel ingredient, evokes long-term potentiation through N-methyl-D-aspartic acid receptor activation: involvement of LPA receptors. Mol Cells 2012;34(6):563–72.
[54] Hulshof LA, van Nuijs D, Hol EM, Middeldorp J. The role of astrocytes in synapse loss in alzheimer's disease: a systematic review. Front Cell Neurosci 2022;16:899251.
[55] Das AK, Hajra AK. Quantification, characterization and fatty acid composition of lysophosphatidic acid in different rat tissues. Lipids 1989;24(4):329–33.
[56] Sugiura T, Nakane S, Kishimoto S, Waku K, Yoshioka Y, Tokumura A. Lysophosphatidic acid, a growth factor-like lipid, in the saliva. J Lipid Res 2002;43(12):2049–55.
[57] Choi SH, Lee RM, Cho HS, Hwang SH, Hwang HI, Rhim H, Kim HC, Kim DG, Cho IH, Nah SY. Visualization of the binding between gintonin, a Panax ginseng-derived LPA receptor ligand, and the LPA receptor subtypes and transactivation of the EGF receptor. J Ginseng Res 2022;46(3):348–56.
[58] Masi G, Brovedani P. The hippocampus, neurotrophic factors and depression: possible implications for the pharmacotherapy of depression. CNS Drugs 2011;25(11):913–31.
[59] Lim HK, Kim K, Son YK, Nah SY, Ahn SM, Song M. Gintonin stimulates dendritic growth in striatal neurons by activating Akt and CREB. Front. Mol. Neurosci. 2022 Oct 26.
[60] Kim HJ, Kim DJ, Shin EJ, Lee BH, Choi SH, Hwang SH, Rhim H, Cho IH, Kim HC, Nah SY. Effects of gintonin-enriched fraction on hippocampal cell proliferation in wild-type mice and an APPswe/PSEN-1 double Tg mouse model of Alzheimer's disease. Neurochem Int 2016;101:56–65.
[61] Kim S, Kim MS, Park K, Kim HJ, Jung SW, Nah SY, Han JS, Chung C. Hippocampus-dependent cognitive enhancement induced by systemic gintonin administration. J Ginseng Res 2016;40(1):55–61.
[62] Choi SH, Lee R, Nam SM, Kim DG, Cho IH, Kim HC, Cho Y, Rhim H, Nah SY. Ginseng gintonin, aging societies, and geriatric brain diseases. Integr Med Res 2021;10(1):100450.
[63] Shoukr EMM, Mohamed AAE, El-Ashry AM, Mohsen HA. Effect of psychological first aid program on stress level and psychological well-being among caregivers of older adults with alzheimer's disease. BMC Nurs 2022;21(1):275.
[64] Hwang SH, Shin EJ, Shin TJ, Lee BH, Choi SH, Kang J, Kim HJ, Kwon SH, Jang CG, Lee JH, et al. Gintonin, a ginseng-derived lysophosphatidic acid receptor ligand, attenuates Alzheimer's disease-related neuropathies: involvement of non-amyloidogenic processing. J Alzheimers Dis 2012;31(1):207–23.
[65] Ikram M, Jo MG, Park TJ, Kim MW, Khan I, Jo MH, Kim MO. Oral administration of gintonin protects the brains of mice against aβ-induced alzheimer disease pathology: antioxidant and anti-inflammatory effects. Oxid Med Cell Longev 2021;2021:6635552.
[66] Iwarsson S, Andersson N, Slaug B, Nilsson MH. People with Parkinson's disease and housing issues: a scoping review. Health Sci Rep 2022;5(2):e511.
[67] Rahman MM, Wang X, Islam MR, Akash S, Supti FA, Mitu MI, Harun-Or-Rashid M, Aktar MN, Khatun Kali MS, Jahan FI, et al. Multifunctional role of natural products for the treatment of Parkinson's disease: at a glance. Front Pharmacol 2022;13:976385.
[68] Rissardo Jamir Pitton, Durante Ícaro, Sharon Idan, Fornari Caprara Ana Letícia. Pimavanserin and Parkinson's disease psychosis: a narrative review. Brain Sci 2022;12(10):1286.
[69] Mijan MA, Kim JY, Moon SY, Choi SH, Nah SY, Yang HJ. Gintonin enhances proliferation, late stage differentiation, and cell survival from endoplasmic reticulum stress of oligodendrocyte lineage cells. Front Pharmacol 2019;10:1211.
[70] Jo MG, Ikram M, Jo MH, Yoo L, Chung KC, Nah SY, Hwang H, Rhim H, Kim MO. Gintonin mitigates MPTP-induced loss of nigrostriatal dopaminergic neurons and accumulation of α-synuclein via the Nrf2/HO-1 pathway. Mol Neurobiol 2019;56(1):39–55.
[71] Choi JH, Jang M, Oh S, Nah SY, Cho IH. Multi-target protective effects of gintonin in 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-Mediated model of Parkinson's disease via lysophosphatidic acid receptors. Front Pharmacol 2018;9:515.
[72] Jang M, Choi JH, Chang Y, Lee SJ, Nah SY, Cho IH. Gintonin, a ginseng-derived ingredient, as a novel therapeutic strategy for Huntington's disease: activation of the Nrf2 pathway through lysophosphatidic acid receptors. Brain Behav Immun 2019;80:146–62.
[73] Choi JH, Oh J, Lee MJ, Ko SG, Nah SY, Cho IH. Gintonin mitigates experimental autoimmune encephalomyelitis by stabilization of Nrf2 signaling via stimulation of lysophosphatidic acid receptors. Brain Behav Immun 2021;93:384–98.
[74] Nam SM, Choi JH, Choi SH, Cho HJ, Cho YJ, Rhim H, Kim HC, Cho IH, Kim DG, Nah SY. Ginseng gintonin alleviates neurological symptoms in the G93A-SOD1 transgenic mouse model of amyotrophic lateral sclerosis through lysophosphatidic acid 1 receptor. J Ginseng Res 2021;45(3):390–400.
[75] Moon J, Choi SH, Shim JY, Park HJ, Oh MJ, Kim M, Nah SY. Gintonin administration is safe and potentially beneficial in cognitively impaired elderly. Alzheimer Dis Assoc Disord 2018;32(1):85–7.
[76] Kim DG, Jang M, Choi SH, Kim HJ, Jhun H, Kim HC, Rhim H, Cho IH, Nah SY. Gintonin, a ginseng-derived exogenous lysophosphatidic acid receptor ligand, enhances blood-brain barrier permeability and brain delivery. Int J Biol Macromol 2018;114:1325–37.
[77] Choi SH, Lee NE, Cho HJ, Lee RM, Rhim H, Kim HC, Han M, Lee EH, Park J, Nah SY. Gintonin facilitates brain delivery of donepezil, a therapeutic drug for Alzheimer disease, through lysophosphatidic acid 1/3 and vascular endothelial growth factor receptors. J Ginseng Res 2021;45(2):264–72.
[78] Lee WJ, Shin YW, Chang H, Shin HR, Kim WW, Jung SW, Choi SH, Kim M, Nah SY. Cognitive improvement effect of gintonin might be associated with blood-brain barrier permeability enhancement: dynamic contrast-enhanced MRI pilot study. Transl Clin Pharmacol 2021;29(1):21–32.
[79] Kim M, Sur B, Villa T, Nah SY, Oh S. Inhibitory activity of gintonin on inflammation in human IL-1β-stimulated fibroblast-like synoviocytes and collagen-induced arthritis in mice. J Ginseng Res 2021 Jul;45(4):510–8.
[80] Kim M, Sur B, Villa T, Yun J, Nah SY, Oh S. Gintonin regulates inflammation in human IL-1β-stimulated fibroblast-like synoviocytes and carrageenan/kaolin-induced arthritis in rats through LPAR2. J Ginseng Res 2021 Sep;45(5):575–82.
[81] Weber A, Mak SH, Berenbaum F, Sellam J, Zheng YP, Han Y, Wen C. Association between osteoarthritis and increased risk of dementia: a systemic review and meta-analysis. Medicine (Baltimore) 2019 Mar;98(10):e14355.
[82] Sangha PS, Thakur M, Akhtar Z, Ramani S, Gyamfi RS. The link between rheumatoid arthritis and dementia: a review. Cureus 2020 Apr 27;12(4):e7855.