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Review

Yin and Yang of ginseng pharmacology: ginsenosides *vs* gintonin

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Ginseng, the root of *Panax ginseng*, has been used in traditional Chinese medicine as a tonic herb that provides many beneficial effects. Pharmacologic studies in the last decades have shown that ginsenosides (ginseng saponins) are primarily responsible for the actions of ginseng. However, the effects of ginseng are not fully explained by ginsenosides. Recently, another class of active ingredients called gintonin was identified. Gintonin is a complex of glycosylated ginseng proteins containing lysophosphatidic acids (LPAs) that are the intracellular lipid mitogenic mediator. Gintonin specifically and potently activates the G protein-coupled receptors (GPCRs) for LPA. Thus, the actions of ginseng are now also linked to LPA and its GPCRs. This linkage opens new dimensions for ginseng pharmacology and LPA therapeutics. In the present review, we evaluate the pharmacology of ginseng with the traditional viewpoint of Yin and Yang components. Furthermore, we will compare ginsenoside and gintonin based on the modern view of molecular pharmacology in terms of ion channels and GPCRs.

Keywords: ginseng; ginsenoside; gintonin; lysophosphatidic acid; ion channels; G protein-coupled receptors; Ca^{2+}; traditional Chinese medicine

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Introduction

Since ginsenosides (ginseng saponins) were first identified as active ingredients in ginseng, many studies have shown that ginsenosides negatively regulate ion channels[1]. For example, ginsenoside Rg_3 inhibits not only voltage-gated Ca^{2+} and Na^+ channels but also ligand-gated ion channels such as 5-HT_3 receptors[2–4]. Furthermore, ginsenosides stimulate anion-gated $GABA_A$ and glycine receptors[5, 6]. Therefore, ginsenosides decrease the excitability of excitable cells by inhibition of cation influx and stimulation of anion influx across the plasma membrane.

Crude ginseng saponin fractions elevate the intracellular Ca^{2+} levels in mammalian cells and activate Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes[7]. However, this effect was not explained by ginsenosides. Recently, the active ingredients responsible for the Ca^{2+} rise were elucidated as novel glycolipoproteins and named gintonins[8]. Gintonin is a complex of glycosylated ginseng proteins containing lysophosphatidic acids (LPAs). LPA is a representative mitogenic lipid mediator[9]. Gintonin activates G protein-coupled receptors (GPCRs) for LPA[8]. Unexpectedly, the action of ginseng is now linked to LPA and its GPCRs and *vice versa*. This linkage opens new dimensions for ginseng pharmacology and LPA therapeutics.

Ginsenosides tend to attenuate cell excitations by blocking cation Ca^{2+} and Na^+ influxes or by enhancing anion Cl^- influx. However, gintonin induces transient Ca^{2+} elevation and activation of MAPK, PI3K, PKC, and Rho kinase via LPA receptors to evoke stimulatory cellular responses[4, 8, 10, 11]. Thus, ginsenoside acts as a negative regulator (Yin), and gintonin acts as a positive regulator (Yang) for ginseng pharmacology. The pharmacological actions of ginseng are now explainable and complementary with the opposing actions of gintonin and ginsenoside. Therapeutic application of LPA might be expanded by traditional usages of ginseng and *vice versa*. In this article, both traditional and modern ginseng pharmacology will be discussed from the viewpoint of Yin and Yang.

Ginseng and ginsenosides

Ginseng, the root of *Panax ginseng* CA Meyer, has been used for thousands of years in Asian countries such as Korea, China, and Japan. *Panax* means 'all heal' in Greek, and the

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Chinese characters of ginseng originated from the human-like shape of the ginseng root[12]. The traditional beneficial effects of ginseng are replenishment of vital energy, mood elevation, and longevity. Therefore, in ancient times, ginseng was considered as a panacea that provided eternal youth[1, 12]. In addition, modern pharmacological studies have revealed ginseng's adaptogenic activities against stress, fatigue, cardiovascular dysfunction, and various diseases, including cancer and neurodegenerative disorders. Its active components also have been intensively studied over the past decades.

The representative active ingredients of ginseng are ginsenosides (ginseng saponin), which are derivatives of the triterpenoid dammarane[1]. More than 100 different types of ginsenosides have been isolated and identified from the roots of Korean and American ginseng[13]. Each ginsenoside is composed of three parts, including a hydrophobic four-ring backbone structure, an attached carbohydrate portion, and an aliphatic side chain (Figure 1).

Ginsenosides and the modulation of ion channels

Because ginsenosides were first identified as the active ingredients in ginseng, many studies have demonstrated negative regulation of ion channels by ginsenosides[1]. Their actions are stereoselective, but they lack the specificity and selectivity of other channel inhibitors. The EC_{50} values of ginsenosides are in the μmol/L range, and many ion channels are affected. For example, the EC_{50} values for the ginsenoside Rg_3 are approximately 0.41–97.3 μmol/L[4, 14–20]. The ginsenoside Rg_3 not only inhibits voltage-gated Ca^{2+}, K^+, and Na^+ channels, ligand-gated 5-HT_3, α7 nicotinic acetylcholine, and NMDA receptors but also activates K^+ channels such as KCNQ K^+, BK_{Ca}, and hERG K^+ [4, 14–21]. Other ginsenosides enhance the activation of anion $GABA_A$ and glycine receptors[22, 23] (Table 1). Therefore, ginsenosides decrease the cellular excitability of excitable cells by inhibiting cation influx and by stimulating anion influx across plasma membranes.

Site-directed mutagenesis experiments have identified ginsenoside interaction site(s), and homology docking modeling has provided three-dimensional configurations for ginsenosides and channel proteins[15, 16, 18, 24] (Figure 1). For example, the hydroxyl groups on the second carbohydrate in ginsenoside Rg_3 form stable hydrogen bonds with the core amino acids of channel proteins (Figure 1). The 1st or 2nd amino acids after the K^+ channel 'signature sequence' (TXGYGD) at the pore entrances have been shown to interact with ginsenoside Rg_3. That is, K318 in the KCNQ K^+ channel, S631 in the hERG K^+ channel, Y360 in the BK_{Ca} K^+ channel, K531 in the $K^+_V1.4$ channel, and K859 in the neuronal Na^+ channel. Similarly, the amino acids I417, N418, and L421 in the $Na^+_V1.2$ channel, I433, N434, and L437 in the $Na^+_V1.4$ channel, L417, N428, and L431 in the L-type Ca^{2+} channel, V291, F292, and I295 in the 5-HT_3 channel, and L247 in the α7 nicotinic acetylcholine receptor were found to interact with ginsenosides[4, 14–18, 20] (Table 1 and Figure 1). Therefore, ginsenosides have regulatory effects in a broad range of ion channels with low affinities, but their common factor is that they stabilize membrane potentials and attenuate cellular activities.

Table 1. Summary of the EC_{50} and IC_{50} values of ginsenoside-induced inhibitions or stimulations of the activities of various voltage-gated ion or ligand-gated ion channels.

Voltage-gated ion channels	Ginsenoside	EC_{50} or IC_{50} (μmol/L)	Interacting amino acids
Ca^{2+}			
L	Rg_3, Rb_1	39.9±9.5[1, 2]	L417, N428, L431
N	Rg_3	64.4±13.6[1]	
P/Q	Rg_3	29.6±11.3[1]	
R	Rg_3	57.5±12.5[1]	
T	Rg_3	97.3±12.4[1]	
K^+			
Kv1.4	Rg_3	32.6±2.2[1]	K531
BK_{Ca}	Rg_3	15.3±3.1[3]	Y360
hERG	Rg_3	0.41±0.05[3]	S631
KCNQ	Rg_3	15.2±8.7[4]	K318
Na^+			
Nav1.2	Rg_3	32.0±6.0[5]	I417, N418, L421
Nav1.4	Rg_3	58.5±6.3[6]	I433, N434, L437
Nav1.5	Rg_3	16.1±2.8[7]	
Ligand-gated ion channels	**Ginsenoside**	**EC_{50} or IC_{50} (μmol/L)**	
$GABA_A$	Rc	53.0±12.3[8]	
Glycine	Rf	49.8±9.8[9]	
5-HT_3	Rg_3	27.6±4.3[10]	V291, F292, I295
Nicotinic acetylcholine			
α3β4	Rg_2	60±14[11]	
α1β1δε	Rg_2	16±9[11]	
α7 (L247A mutant)	Rg_3	33.1±1.3[12]	L247
NMDA	Protopanaxatriol	48±16[13]	

EC_{50} values are shown for BK_{Ca}, hERG, and KCNQ K^+ channels and $GABA_A$ and glycine receptors and IC_{50} values are shown for the remainder and were determined in oocytes expressing these ion channels or receptors.

Ginsenosides in ginseng pharmacology

Ginsenosides decrease the excitability of neuronal cells by inhibiting cation influx and/or by stimulating anion influx. These pharmacologic actions of ginsenosides are linked to reductions in the excitabilities of neurons, smooth muscle cells, and cardiomyocytes. Furthermore, the ginsenoside-induced modulation of Ca^{2+} and K^+ channels results in the dilation of blood vessels via relaxation of smooth muscles[25–29]. In bradycardia, it induces relaxation of the cardiomyocytes, which explains its anti-hypertensive and cardioprotective effects[30–35]. In addition, ginsenoside-induced inhibition of the cation-gated NMDA receptor and neuronal Na^+ channels and

Figure 1. Virtual docking model of ginsenoside Rg_3 to chick α7 nicotinic acetylcholine receptor (nAChR mutant L247T) and hydrogen bonds. Virtual dockings of ginsenoside Rg_3 to chick α7 nicotinic acetylcholine receptor (AChR, L247T mutant) channel homology models. (A) Top view of the highest-ranked docking model of ginsenoside Rg_3 to chick α7 nicotinic acetylcholine receptor (nAChR mutant, L247T) channel. The channel is shown as a cartoon diagram, and ginsenoside Rg_3 is represented by a ball and chain model. Subunits are shown in different colors. (B) Side view of the docking model of ginsenoside Rg_3 to nAChR receptor. One of the subunits is omitted in side view for clarity. (C) Poseview analysis of protein-ligand interactions. Hydrogen bonds are denoted by dotted lines. Spline sections indicate hydrophobic contacts, highlight the hydrophobic regions of ginsenoside Rg_3, and provide the identities of contacting amino acids. The roman numerals in parenthesis indicate subunits of the pentamer. Adapted from Lee *et al*[18].

its stimulation of anion-gated $GABA_A$ receptors and glycine receptors explain the neuroprotective and anxiolytic effects of ginseng[10, 36]. It is also possible that ginseng attenuation of cisplatin-induced nausea and vomiting is due its inhibition of $5\text{-}HT_3$ ion channels[37].

Discovery of gintonin

The crude ginseng total saponin (cGTS) fraction contains approximately 50% ginsenosides by weight. Furthermore, the cGTS fraction increases intracellular Ca^{2+} in mammalian cells and activates endogenous Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes, whereas ginsenosides do not. These data imply that some unidentified ginseng component(s) is responsible for the Ca^{2+} increase[7]. In addition, the cGTS fraction-induced Ca^{2+} increases are both reversible and transient. Precise and continuing studies over the past twenty years have elucidated a signaling pathway downstream of cGTS in oocytes, namely the unknown membrane target protein-$G_{q/11}$-$PLC_{\beta3}$-IP_3-Ca^{2+} release[38]. Later, homologous desensitization by cGTS was shown to be mediated through GRK2 and β-arrestin I in oocytes, strongly implying GPCR involvement (Figure 2)[39].

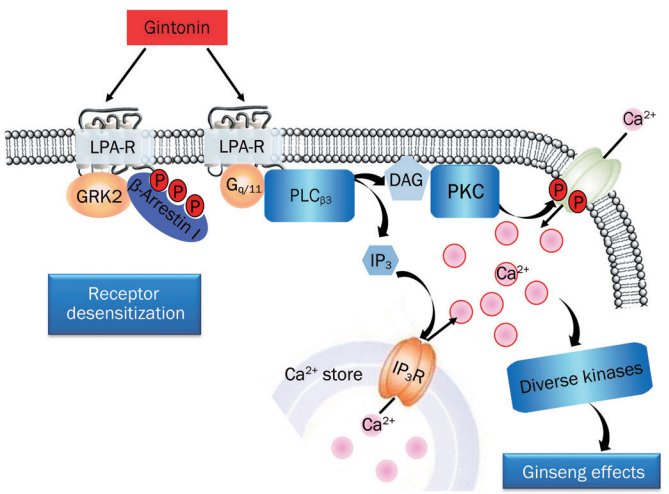

Figure 2. Schematic diagram of gintonin-mediated signal transduction pathways. For the desensitization, gintonin activates LPA GPCRs, which leads to activation of GRK2. The activated GRK2 phosphorylates the LPA GPCRs and then β-arrestin I is recruited. The recruited β-arrestin I inhibits GPCR-G protein coupling. For cellular responses, gintonin activates LPA GPCRs, which leads to activation of phospholipase C (PLC). The activated PLC produces IP_3 and diacylglycerol (DAG). DAG activates protein kinase C (PKC), which phosphorylates Ca^{2+} channels. IP_3 mobilizes Ca^{2+} from internal Ca^{2+} stores through IP_3 receptors. The increased Ca^{2+} levels activate many kinases.

In 2011, the active ingredient in cGTS was finally separated by anion exchange chromatography after ginseng butanol extraction[40]. The novel non-saponin ingredient was designated as gintonin, where *gin* was derived from ginseng, *ton* from the tonic effects of ginseng, and *in* from

protein[41]. Six different gintonin have been identified, and all six induce intracellular Ca^{2+} increases in mammalian cells and *Xenopus* oocytes, confirming that gintonin is responsible for cGTS-induced Ca^{2+} mobilization. Gintonin is composed of carbohydrates, lipids, and proteins, such as, ginseng major latex-like protein and ginseng ribonuclease-like storage proteins. Thus, gintonin is part of a novel class of glycolipoproteins in ginseng that induces intracellular Ca^{2+} increases in mammalian cells[8]. Ginseng contains 0.2% gintonin by weight (Table 2).

Table 2. A brief comparison of ginseng components, gintonin, and ginsenosides.

	Gintonin	Ginsenosides
Molecular weight (M_W)	Native M_W: 67 kDa Apparent M_W: 13 kDa	0.6–1.3 kDa
Composition	Glycolipoprotein: carbohydrates (Glucose), lipids (LPA $C_{18:2}$), and ginseng proteins (GLP and GSP)	Dammarane glycosides
Content in ginseng	0.2%	3%–4% (Sum of individual ginsenosides)
Target protein on cell membrane and signal cascades	LPA receptors, transient $[Ca^{2+}]_i$ elevation via PTX-sensitive and -insensitive G proteins coupled PLC pathway	Non-selective interactions with ion channels and receptors, do not have signal transduction pathway
Desensitization after repeated treatment on cells	Induction of rapid desensitization	No desensitization

Gintonin activation of GPCRs for LPA

During additional studies with the gintonin, it was shown that phospholipase A_1 had attenuating effects on gintonin. This finding highlighted the importance of position 1 esterification on the fatty acid component, which suggests that gintonin contains phospholipids[8]. Previously, Tigyi and Miledi demonstrated that LPA bound to serum albumin could sufficiently activate Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes, and digestion with PLA_1 prevented this activation[42]. Furthermore, using methanol extraction, they demonstrated LPA dissociation from serum albumin[42]. Building upon this reference, researchers examined whether gintonin contained LPAs by liquid chromatography-electrospray ionization/multi-stage mass spectrometry (LC-ESI-MS/MS) analysis[43]. LC-ESI-MS/MS analysis showed that 9.5% of gintonin is composed of lysophosphatidic acids (LPAs), such as LPA $C_{18:2}$, LPA $C_{16:0}$, and LPA $C_{18:1}$, indicating that the bioactive component(s) of gintonin (with respect to the Ca^{2+} response) is a LPA[43].

LPA is an intercellular lipid mediator that functions as a mitogen with hormone- and growth-factor-like activities in most cell types[9, 44]. Currently, three EDG family GPCRs (LPA_{1-3}) and three purinergic GPCRs (LPA_{4-6}) have been reported to act as LPA receptors[9]. Hwang *et al* demonstrated that gintonin activates LPA receptors in GPCR-expressing B103 mammalian cells and in *Xenopus* oocytes[45]. Gintonin only activates LPA receptors specifically, not other GPCRs, such as S1P, fatty acids, 5-HT_{1C}, and muscarinic acetylcholine receptor subtypes (m1, m3, and m5)[8]. Furthermore, the LPA GPCRs have different affinities for gintonin, which follow a decreasing order of LPA_2>LPA_5>LPA_1>LPA_3>LPA_4[8]. Furthermore, gintonin has 3- to 130-fold greater affinity for LPA GPCRs than free LPA. This might be due to ginseng proteins in gintonin because the protein components of gintonin may function as efficient LPA carriers to effectively carry LPA to LPA receptors and protect LPA from hydrolyzing enzymes[8]. Based on the average molecular weight of gintonin (20 kDa), four LPA molecules could most likely bind to one molecule of ginseng protein. The LPA content in ginseng is 80 to 240-fold higher than in other plants, which in part explains its unique pharmacologic properties. Somewhat unexpectedly, the action of ginseng is now linked to LPA and its GPCRs and *vice versa*, and this linkage opens new dimensions for ginseng pharmacology and LPA therapeutics (Table 2).

Gintonin in ginseng pharmacology

Gintonin induces transient Ca^{2+} elevation and activates MAPK, PI3K, PKC, and Rho kinase via LPA receptors to evoke stimulatory cellular responses[4, 11] (Figure 2). Gintonin evokes cell proliferation and migration and morphological changes in human umbilical vein endothelial cells and PC12 neuronal cells[4, 5, 41, 45]. These effects of gintonin are consistent with those caused by LPAs via GPCRs and diverse G proteins, such as $G\alpha_{i/o}$, $G\alpha_{12/13}$, and $G\alpha_{q/11}$[1]. Gintonin hinders the amyloidogenic pathway and induces non-amyloidogenic pathways that produce beneficial soluble APPα (sAPPα) in neurons by activating LPA receptors[41]. Gintonin also reduces the release of $A\beta_{1-42}$ and attenuates $A\beta_{1-40}$-induced cytotoxicity[41]. In addition, gintonin has been shown to rescue $A\beta_{1-40}$-induced cognitive dysfunction in mice[41]. Furthermore, in a transgenic murine Alzheimer's disease model, long-term oral administration of gintonin effectively attenuated both amyloid plaque deposition and short- and long-term memory impairment[41]. Thus, gintonin may contribute to the memory-improving effects of ginseng, which have been proven in human trials.

Autotaxin was found as an autocrine factor released by tumors, which stimulates tumor growth and migration[44]. Later, autotaxin was identified as lysophospholipase D, which produces LPA from lysophosphatidylcholine[46, 47]. Autotaxin and LPA function as mitogenic and motility signals in various cancers, including neuroblastoma, hepatoma, lung cancer, ovarian cancer, metastatic breast cancer, and melanoma[44]. A recent report showed that LPA $C_{18:2}$, which is highly abundant in gintonin, inhibits autotaxin activity. Furthermore, Hwang *et al* showed that gintonin inhibits autotaxin activity *in vitro* and metastasis of B16/F10 melanoma cells *in vivo*[45]. Vessel formation in tumors was also reduced in gintonin-treated mice. These effects may contribute to the anti-cancer effects of

ginseng[45].

Yin and Yang of ginseng pharmacology

In Chinese medicine, Yin and Yang are opposite and complementary forces that combine to form a whole. Ginseng pharmacology is interesting because the two active components have opposite effects and compliment the entire effect of ginseng. For example, ginsenosides stabilize membrane potentials via the dual modulation of ion channels, but gintonin and LPA activate many cellular responses via GPCR activation. Ginsenosides inhibit Ca^{2+} influx, but gintonin induces a transient Ca^{2+} rise, and ginsenosides have low affinity for calcium but are relatively abundant in ginseng, whereas gintonin has a higher affinity for calcium but is only present at low levels (Table 2). Thus, it is highly likely that ginsenosides act as a Yin component to gintonin's Yang component to produce the effects of ginseng. Therefore, it appears that the healing effects of *Panax* are the result of a harmonious balance between the positive and negative actions of ginsenosides and gintonins, reminiscent of the Yin and Yang forces in Chinese medicine.

Concluding remark and perspective

Our understanding of ginseng pharmacology has advanced tremendously during the last few decades. Ginsenosides were considered to be the active ingredients of ginseng for 5 decades. However, many researchers used ginseng extract (the butanol fraction) for pharmacologic studies because individual ginsenosides were scarce and difficult to purify. As a result, other components were included in these pharmacology experiments. Gintonin is now considered as a part of the ginseng fraction, and the LPAs in this fraction are considered responsible for a variety of the biological effects of ginseng, which are mediated through GPCRs. Furthermore, it appears that the pharmacological actions of ginseng can now be explained by the complementary opposing actions of gintonin and ginsenoside. One example might be the anticancer effects of the ginseng extract. Many ginsenosides have shown clear anticancer activity in different cancer cell lines by regulation of cell proliferation[13]. Gintonin exhibited autotoxin inhibition *in vitro* and inhibition of metastasis of B16/F10 melanoma cells *in vivo*[45]. Therefore, both components in ginseng may act synergistically and complementarily for the anti-tumor efficacy of ginseng.

How ginsenosides and gintonin work together in the body or whether ginsenosides affect the actions of gintonin or *vice versa* remains to be established. Future studies are required to elucidate the pharmacological effects of the different actions of ginsenosides and gintonin with respect to their individual contributions and the effects of whole ginseng in biological systems. Future research will undoubtedly expand our knowledge of ginseng pharmacology and the applications of LPA. In addition, the therapeutic application of LPA might be facilitated by knowledge of the traditional usages of ginseng. Although new drug developments based on targeting LPA receptors are in the pipeline, gintonin might provide alternative therapies for pathologic conditions related to LPA and LPA receptor-related diseases.

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