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Research article

Gintonin absorption in intestinal model systems

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ABSTRACT

Background: Recently, we identified a novel ginseng-derived lysophosphatidic acid receptor ligand, called gintonin. We showed that gintonin induces $[Ca^{2+}]i$ transient-mediated morphological changes, proliferation, and migration in cells expressing lysophosphatidic acid receptors and that oral administration of gintonin exhibits anti-Alzheimer disease effects in model mice. However, little is known about the intestinal absorption of gintonin. The aim of this study was to investigate gintonin absorption using two model systems.

Methods: Gintonin membrane permeation was examined using a parallel artificial membrane permeation assay, and gintonin absorption was evaluated in a mouse everted intestinal sac model.

Results: The parallel artificial membrane permeation assay showed that gintonin could permeate an artificial membrane in a dose-dependent manner. In the everted sac model, gintonin absorption increased with incubation time (from 0 min to 60 min), followed by a decrease in absorption. Gintonin absorption into everted sacs was also dose dependent, with a nonlinear correlation between gintonin absorption and concentration at 0.1–3 mg/mL and saturation at 3–5 mg/mL. Gintonin absorption was inhibited by the Rho kinase inhibitor Y-27632 and the sodium–glucose transporter inhibitor phloridzin. Moreover, lipid extraction with methanol also attenuated gintonin absorption, suggesting the importance of the lipid portion of gintonin in absorption. This result shows that gintonin might be absorbed through passive diffusion, paracellular, and active transport pathways.

Conclusion: The present study shows that gintonin could be absorbed in the intestine through transcellular and paracellular diffusion, and active transport. In addition, the lipid component of gintonin might play a key role in its intestinal absorption.

1. Introduction

Oral administration is the first choice for systemic treatment with most drugs because of its advantage over other routes of administration. Herbal and traditional medicines are no exception since most are decocted with water to extract the active component(s) and for easy intake. Orally administered medicines are usually absorbed by the intestine. Ginseng, the root of *Panax ginseng* Meyer, is one of the most popular herbal medicines. Ginseng contains several active ingredients, such as saponins and acidic polysaccharides. Ginseng is used as a general tonic for maintaining homeostasis and is usually administered via the oral route either alone or together with other herbal medicines after decoction [1,2].

Recently, we isolated a lysophosphatidic acid (LPA) receptor ligand from ginseng, which we called gintonin [3,4]. Compared with ginseng saponins and acidic polysaccharides, gintonin is a large molecule, with an apparent molecular weight of approximately 67 kDa in native form and approximately 13 kDa in sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and it consists

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of carbohydrates, proteins, and lipids [3]. We showed that LPAs are a functional component of gintonin [4], as gintonin activates LPA receptors in animal cells. LPA receptor activation by gintonin or endogenous LPA has diverse cellular effects, including intracellular calcium mobilization, morphological changes (i.e., stress fiber formation and cell rounding), induction of proliferation and migration, vascular development, and neurite retraction [5–8]. LPA receptor-mediated cellular effects further extend to biological activities such as neurogenesis in the embryonic brain, angiogenesis, embryo implantation, spermatogenesis, and wound healing [9]. Although the molecular weight of gintonin is much larger than endogenous LPAs, we found that short- and long-term oral administration of gintonin significantly decreased the area of amyloid plaque deposition in the hippocampus and cortex of Alzheimer disease model mice [8,10]. In addition, oral administration of gintonin significantly suppressed metastasis and tumor growth induced by subcutaneous grafts of melanoma cells [11]. These results suggest that orally administered gintonin may be absorbed through intestinal absorption. However, whether gintonin could be absorbed by the intestine was not directly demonstrated.

The parallel artificial membrane permeation assay (PAMPA) is a method by which the permeability of a substance through a lipid-infused artificial membrane can be determined [12,13]. Although there is no active transport in the PAMPA membrane, it is a useful model for predicting the transport behavior of highly lipophilic drug candidates by transcellular absorption. This process is driven by passive diffusion via the concentration gradient. In contrast, the everted gut sac system is used to examine the transport of various substances via *ex vivo* intestinal absorption [14–16]. Recently, we produced a gintonin-specific monoclonal and polyclonal antibodies and developed an enzyme-linked immunosorbent assay (ELISA) for gintonin detection using the monoclonal antibody [17]. In this study, the PAMPA and the mouse everted intestinal sac model were used to investigate gintonin absorption in an artificial biological membrane and the small intestine, respectively. The amount of gintonin transported through the artificial membrane or everted gut sacs was quantified by ELISA using a polyclonal antibody against gintonin [17].

We found that gintonin could permeate artificial membranes in a dose-dependent manner. Gintonin absorption in the mouse everted sac model also increased with incubation time and in a dose-dependent manner. Gintonin absorption in the everted sacs was inhibited by the Rho kinase inhibitor Y-27632 and the sodium–glucose transporter inhibitor phloridzin. We also found that the lipid portion of gintonin plays a role in the intestinal absorption of gintonin. In the present study, we further discuss the relationship between the intestinal absorption of gintonin and gintonin-mediated biological effects, and the possible role of the lipid portion in gintonin absorption.

2. Materials and methods

2.1. Materials

Crude gintonin was isolated from *P. ginseng*, as described previously [3]. Gintonin is a glycolipoprotein containing ginseng proteins complexed with LPA [4]. The BD Gentest Pre-coated PAMPA Plate System was purchased from BD Biosciences (Bedford, MA, USA). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise noted.

2.2. Permeability study using PAMPA

A permeability study was carried out using the BD Gentest Pre-coated PAMPA Plate System. This system is composed of a 96-well microtiter plate (lower plate) and a 96-well filter plate (upper plate). Each composite well is separated by a 125-μm microfilter disc. The hydrophobic filter is coated with lecithin. The filter plate was placed on the microtiter plate containing 300 μL of gintonin (concentration: 0.5–5 mg/mL) dissolved in phosphate buffer (50mM KH_2PO_4 and 3.6mM NaOH; pH 5.8) or phosphate-buffered saline (PBS; pH 7.4). This constituted the donor solution. The acceptor wells (the top of the wells) of the system were hydrated with 200 μL of PBS (pH 7.4). The system was incubated for the indicated time periods (up to 3 h) at room temperature. After incubation, solutions in the donor and acceptor wells were lyophilized using a CentriVap centrifugal vacuum concentrator (Labconco, Kansas City, MO, USA). Samples were reconstituted with deionized water before assay.

2.3. Preparation of the everted sac

The everted sac model is a simple method to estimate the intestinal absorption of a compound. The intestinal absorption of gintonin was examined using the mouse everted gut sac model, as described previously [14,15]. Four-week-old male ICR mice (KOATEC, Pyeongtaek-si, Gyeonggi-do, Korea) were obtained, all surgeries were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering. Then, 7-cm segments of the jejuna were quickly isolated, rinsed with ice-cold Ringer solution (140mM NaCl, 5mM KCl, 1mM $MgCl_2$, 2mM $CaCl_2$, 10mM HEPES-Tris, and 10mM glucose; pH 7.4), and gassed with O_2. These segments were everted and tied at one end with a cotton thread, filled with Ringer solution, and tied at the other end to make sacs. Each sac was individually placed in a 15-mL tube containing 2 mL of oxygenated Ringer solution (mucosal donor solution) and kept in a water bath at 37°C. The donor solution contained gintonin (0.1–5.0 mg/mL). The entire volume of solution inside the sac (serosal acceptor solution) was removed at the indicated time points (5 min, 15 min, 30 min, 60 min, and 90 min). Then, the serosal and mucosal solutions were centrifuged at 3,000 rpm for 10 s, and the amount of gintonin in the supernatant was assayed by ELISA. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Konkuk University (Seoul, Korea).

2.4. Measuring gintonin by ELISA

The amount of gintonin in the samples from the PAMPA and everted sac assay was determined by ELISA as previously described, with some modifications [17]. Briefly, each sample solution was diluted with PBS, added to the wells of a 96-well microplate, and incubated overnight at 4°C. After three washes with PBS containing 0.01% (v/v) Tween 80, the plate was blocked with 100 μL of blocking solution [PBS containing 3% (w/v) bovine serum albumin] for 1 h. After the plate was washed with PBS three times, polyclonal antibodies against gintonin, diluted in blocking solution, were added to each well and incubated for 2 h. The plate was washed with PBS four times, and then 100 μL of diluted horse radish peroxidase-conjugated goat antirabbit immunoglobulin G was added to each well and incubated for 2 h at 37°C. The plate was washed again, and 100 μL of 3,3′,5,5′-tetramethylbenzidine substrate solution was added to each well and incubated for 30 min at room temperature in the dark. The reaction was stopped by the addition of 50 μL of 0.75M H_2SO_4. The activity of the enzyme bound to the solid phase was measured at 450 nm using an ELISA plate reader (SpectraMAX; Molecular Devices, Sunnyvale, CA, USA).

2.5. Recovery of gintonin from the mucosal medium of the gut sac and gintonin uptake by the gut sac

Since gintonin is a glycolipoprotein, it could be a substrate for intestinal enzymes. Therefore, the gintonin recovered (at a concentration of 0.1–1 mg/mL) from the mucosal medium of the gut sac was evaluated. After incubation for 0–90 min, the medium from the mucosal side was centrifuged and gintonin was measured by ELISA. Gintonin recovery (%) was calculated as the amount of gintonin in the mucosal medium divided by the actual amount of gintonin added to the mucosal medium × 100.

2.6. Viability of the everted gut sac

To determine the viability of the gut sacs, glucose concentration in the incubation medium and gut sac contents was determined using a ONETOUCH Ultra glucometer (Lifescan Inc., Milpitas, CA, USA).

2.7. Determination of permeability

The apparent permeability coefficient (P_{app}) was calculated using the following equation:

$$P_{app} = V \times dC/(A \times C_0 \times dT)$$

where P is the permeability coefficient (in cm/s), V is the volume of the acceptor compartment, A is the surface area (in cm^2), C_0 is the starting concentration in the donor compartment (in μg/mL), and dC/dT is the rate of change in the drug concentration (in μg/mL/s) [12]. Gintonin permeation was calculated as the amount of gintonin (in μg) per area (in cm^2) in the receiver (acceptor) compartment of the PAMPA or in the serosal fluid inside the everted sac. Gintonin permeation (%) in the PAMPA was calculated as the amount of gintonin in the acceptor compartment divided by the amount of gintonin in the donor compartment × 100.

2.8. Statistical analysis

Data are expressed as means ± standard deviation. Statistical comparisons of controls and treated experimental groups were performed using Student t test. All statistical evaluations were performed using GraphPad prism, version 5.0 (GraphPad Software Inc., La Jolla, CA, USA). A p value of <0.05 was considered statistically significant.

3. Results

3.1. Permeation of gintonin in the PAMPA

The PAMPA system is widely used as a passive transport model of drug absorption, which is easily influenced by the pH of the donor solution. In this study, absorption of gintonin was investigated in a PAMPA system with a donor solution at pH 5.8 (Fig. 1). This pH was chosen based on the results of a preliminary study, in which the effect of donor pH on gintonin permeation was tested, and the highest permeability was observed at pH 5.8 (Supplementary Fig. S1). The permeability coefficient of gintonin at pH 5.8 was $(1.81 \pm 0.49) \times 10^{-8}$ cm/s. Fig. 1A shows that gintonin permeated the lipid layer of the PAMPA system in a dose-dependent manner after 180 min of incubation at room temperature. The correlation between gintonin absorption (Y, μg/cm^2) and gintonin concentration (X, mg/mL) was linear ($Y = 0.1621X + 0.0074$, $R^2 = 0.9987$). The gintonin permeation percentage was constant (approximately 0.01%) at 0.5–5 mg/mL gintonin (in the donor compartment), indicating typical passive diffusion through the lipid layer (Fig. 1B). When 2.5 mg/mL or 5 mg/mL of gintonin was applied to the donor compartment, the gintonin content in the acceptor compartment gradually increased with increasing incubation time, up to 180 min (Figs. 1C and 1D). Very low gintonin permeation was observed at shorter incubation times, up to 60 min; however, permeation increased with longer incubation time.

3.2. Gintonin absorption in the everted gut sac system

Concentration dependence of gintonin transport was examined by incubation of the everted sac in Ringer solution containing different concentrations (0 mg/mL, 0.1 mg/mL, 0.3 mg/mL, 1 mg/mL, 3 mg/mL, and 5 mg/mL) of gintonin for 30 min. As shown in Fig. 2A, there was a nonlinear increase in gintonin absorption into the sac corresponding to an increase in the gintonin concentration in the mucosal Ringer solution. The transport and absorption of gintonin in the gut sac system were saturated at 3 mg/mL. Owing to the nonlinearity of absorption, the apparent permeation coefficient varied depending on the gintonin concentration and duration of incubation. The apparent permeability coefficients of gintonin at 30 min were $(1.93 \pm 0.68) \times 10^{-7}$ cm/s and $(1.22 \pm 0.16) \times 10^{-7}$ cm/s at 1 mg/mL and 3 mg/mL, respectively. These values are much higher than those obtained from the PAMPA. The time dependence of gintonin transport into the gut sac at different incubation periods, from 0 min to 90 min, is shown in Fig. 2B. When the sac was incubated in 1 mg/mL gintonin, transport reached a maximum at 30 min, followed by a reduction after 60 min (Fig. 2B). This result indicates that serosal gintonin might be removed after transport, possibly through degradation or an efflux system.

The change in mucosal gintonin levels after a 30-min incubation period was measured to calculate gintonin recovery (Fig. 2C). While gintonin recovery from the mucosal side was only approximately 13% at an initial concentration of 0.1 mg/mL after 30 min of incubation, recovery was about 80% at an initial concentration of 1 mg/mL after 30 min (Fig. 2C). When 1 mg/mL gintonin was applied to the mucosal side of the sac, mucosal gintonin recovery gradually reduced with increasing incubation time, showing about 40% recovery after a 90-min incubation period (Fig. 2D).

3.3. Gintonin transport might be partially mediated by activation of Rho kinase and the sodium-dependent glucose transporter, but not by the LPA1/3 receptor

To study whether the LPA1/3 receptor is involved in gintonin absorption, we used the LPA1/3 receptor inhibitor Ki16425 (Fig. 3A). Preincubation with Ki16425 did not attenuate gintonin absorption, suggesting that other LPA receptor subtypes are involved in the intestinal absorption of gintonin. Rho kinase is a downstream signaling molecule of LPA receptor activation. In addition, activation of Rho kinase by LPA is one of mechanisms of LPA-enhanced drug transport. The sodium-dependent glucose transporter (Na^+/glucose transporter) is responsible for the transport of several substances, including glucose. To investigate the involvement of Rho kinase signaling and the Na^+/glucose transporter in gintonin absorption, the Rho kinase inhibitor Y-27632 and the Na^+/glucose transporter inhibitor phloridzin were used (Fig. 3B). Preincubation of the sac with Y-27632 (10μM) or phloridzin (100μM) inhibited gintonin permeation by about 70% (1 mg/mL gintonin for 30 min), suggesting the involvement of Rho kinase activation and the Na^+/glucose transporter in intestinal absorption of gintonin.

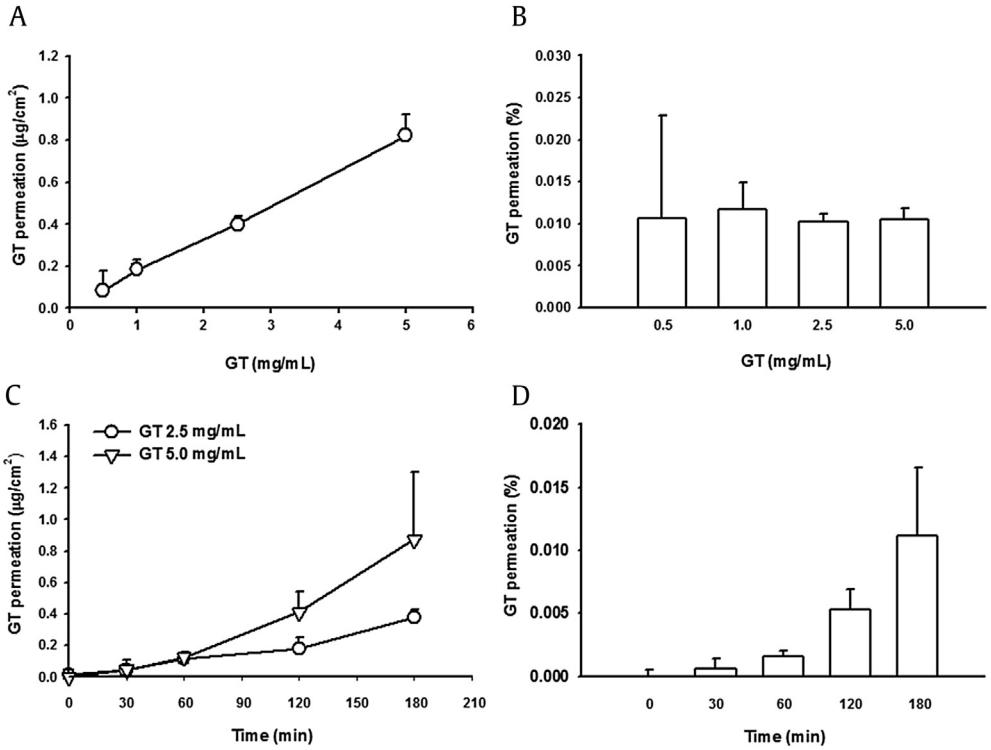

Fig. 1. Permeation of gintonin as measured by the PAMPA. Phosphate buffers (pH 5.8 and 7.4) were added to the donor and receiver compartments, respectively, according to the assay protocol. (A, B) The filter plate was incubated with 0.5–5 mg/mL gintonin in phospate buffer in donor compartment at room temperature for 180 min. (C, D) The filter plate was incubated with 2.5 or 5.0 mg/mL gintonin in phospate buffer in donor compartment at room temperature for 0–180 min. Data represent the means ± SD of four to six independent experiments. GT, gintonin; PAMPA, parallel artificial membrane permeation assay; SD, standard deviation.

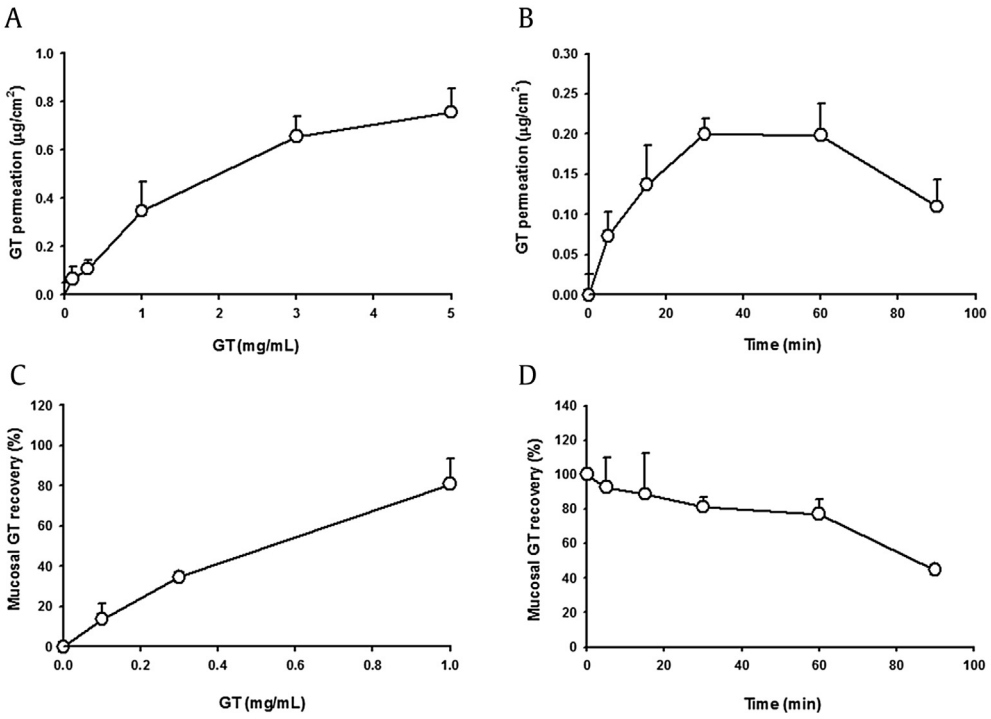

Fig. 2. Permeation and mucosal recovery of gintonin in the mouse everted gut sac model. (A, C) The everted sacs were incubated with 0–5 mg/mL gintonin in Ringer solution at 37°C for 30 min. (B, D) The everted sacs were incubated with 1 mg/mL gintonin in Ringer solution at 37°C for 0–90 min. Permeation and mucosal recovery of gintonin were calculated from the gintonin concentration in serosal and mucosal solutions, respectively. Data represent the means ± SD of four independent experiments. GT, gintonin; SD, standard deviation.

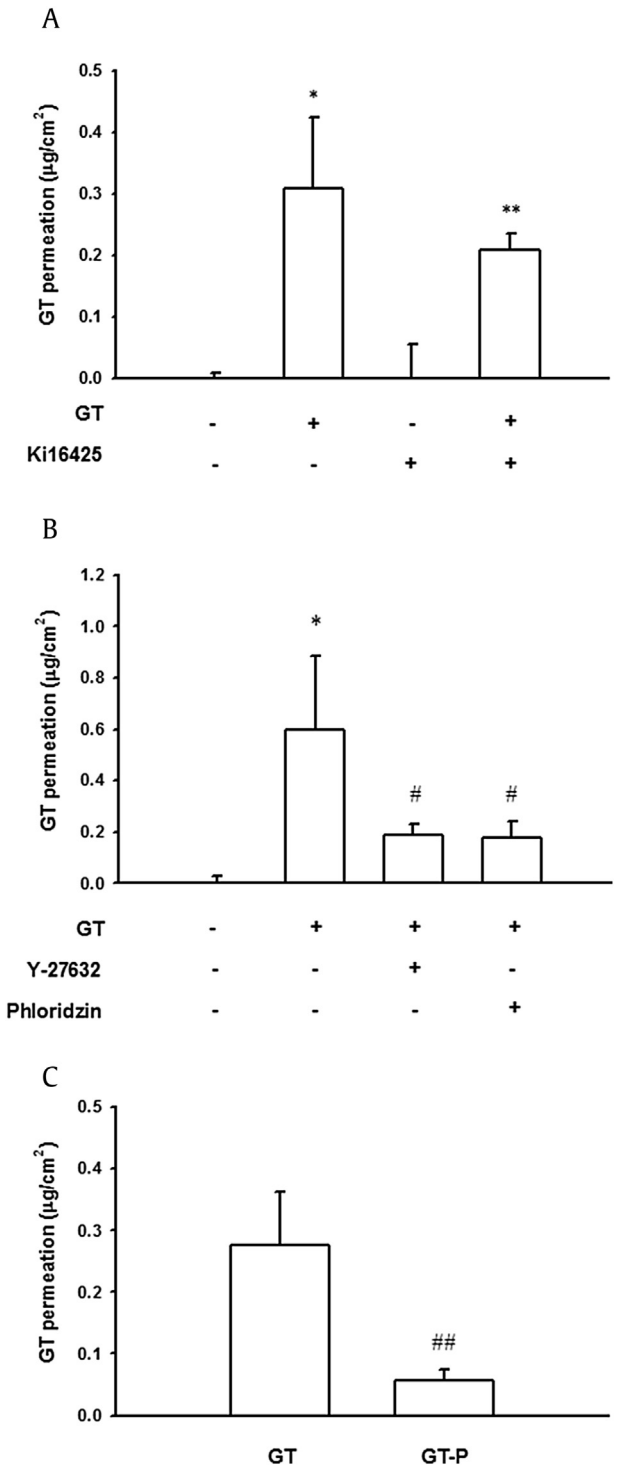

Fig. 3. Intestinal absorption of gintonin. (A, B) Effect of various inhibitors on gintonin permeation and (C) comparison of the permeation of complete gintonin and the protein portion of gintonin in the mouse everted gut sac model. (A) The everted sacs were incubated in the absence or presence of Ki16425 (10μM) at 37°C for 10 min and then incubated with gintonin (GT, 1 mg/mL) for 30 min. (B) The everted sacs were incubated in the absence or presence of Y-27632 (10μM) or phloridzin (100μM) at 37°C for 10 min and then incubated with gintonin (GT, 1 mg/mL) for 30 min. (C) The everted sacs were incubated in the presence of 1 mg/mL complete gintonin or protein portion of gintonin in Ringer solution at 37°C for 30 min. Gintonin permeation was calculated from the gintonin concentration in the serosal solution, as described in the Materials and methods. Data represent the means ± SD of four independent experiments. * $p < 0.05$, versus the negative control. ** $p < 0.01$, versus the negative control. # $p < 0.05$, versus gintonin alone. ## $p < 0.01$, versus gintonin alone. GT, gintonin; GT-P, protein portion of gintonin; SD, standard deviation.

3.4. Role of the lipid portion of gintonin in intestinal absorption and viability of the everted gut sac

In a previous study, we showed that most of the LPAs in gintonin can be extracted by de-lipidation via methanol extraction [4]. To confirm the role of the lipid portion, including the LPAs, of gintonin in absorption, permeation of gintonin that was de-lipidated with methanol was compared with that of untreated gintonin (Fig. 3C). Removal of the lipid portion of gintonin by extraction significantly attenuated gintonin absorption. This result, together with the data from the Rho kinase inhibitor experiment (Fig. 3B), suggested that the lipids including LPAs might contribute to gintonin absorption.

Glucose can be absorbed from the gastrointestinal lumen through the Na^+/glucose transporter. Generally, a continuous increase in serosal glucose levels with increasing incubation time indicates that the gut sac is functional and viable. A 30-min incubation with gintonin (1 mg/mL), Ki16425 (10μM), or Y-27632 (10μM) did not affect glucose absorption levels, while phloridzin (100μM) inhibited glucose transport (Fig. 4). These results indicate that the LPA receptor-related signaling pathway did not affect glucose absorption except the Na^+/glucose transporter inhibitor phloridzin.

4. Discussion

Ginseng, similar to most herbal or traditional medicines, is usually decocted before oral intake. Decocted ginseng extract administered by the oral route can be absorbed by the small intestine [13]. The major intestinal absorption pathways include transcellular, paracellular, and transporter-mediated absorption. In previous reports, we showed that short- or long-term administration of gintonin via the oral route has several biological effects, such as anti-Alzheimer effects, boosting of the cholinergic system, and enhancement of cognitive function [8,10]. Despite the beneficial effects of gintonin, administered via the oral route, on the central nervous system, how gintonin is absorbed in the intestinal system was unknown.

In the present study, we employed the PAMPA and everted gut sac method as *in vitro* model systems to determine the intestinal absorption of gintonin. The major findings of this study are as follows: (1) a dose-dependent linear correlation between gintonin permeation and the initial gintonin concentration in the donor compartment in the PAMPA; (2) a nonlinear correlation between gintonin absorption across the intestinal epithelium into the everted sac (from the mucosal side to the serosal side) and the initial concentration of gintonin; (3) inhibition of Rho kinase or the sodium-dependent glucose transporter leading to reduced gintonin permeation in the everted gut sac; and (4) elimination of the lipid portion of gintonin by de-lipidation resulting in reduced gintonin permeation in the gut sac.

The PAMPA assesses absorption across artificial membranes and is widely used as an initial screening system for drug absorption. Since this assay involves the use of a filter coated with lipid, it can be used to demonstrate the absorption of a drug via the passive diffusion or transcellular pathway [13]. Thus, gintonin may be permeable to the lipid membrane, showing that gintonin can be absorbed through the lipid bilayer via the transcellular pathway according to the concentration gradient. However, when we compared the permeability of gintonin in the PAMPA and everted gut sac model, we observed that more gintonin permeated in the everted sac, suggesting additional routes for gintonin absorption in the gut. These additional routes in the intestine might include the paracellular pathway and energy-dependent pathways such as transporter-mediated transport. Since gintonin-mediated LPA receptor activation is coupled to the activation of Rho kinase [4],

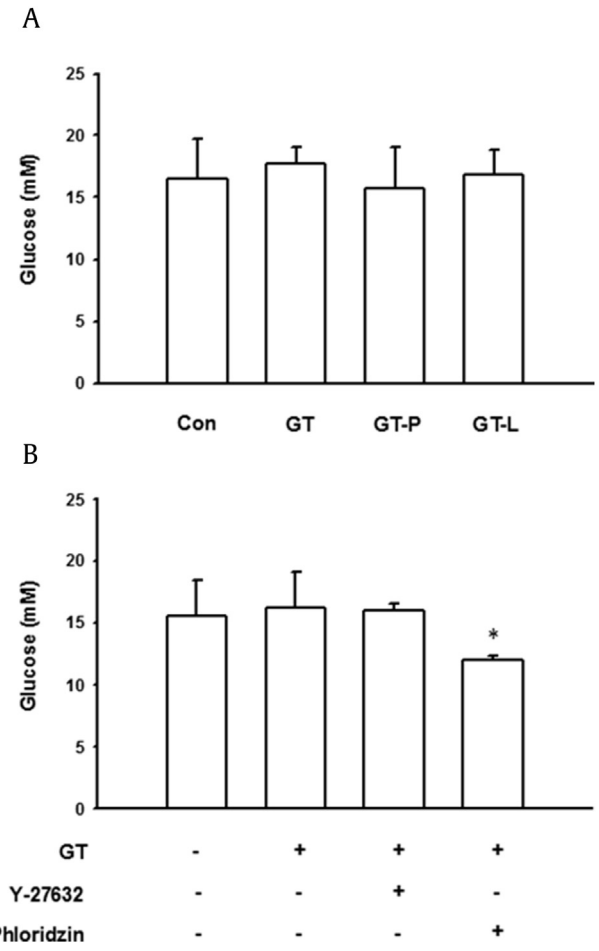

Fig. 4. Influence of gintonin and various inhibitors on the viability of the everted gut sac, as measured by the glucose concentration in the serosal fluid of the sacs. (A) Everted sacs were incubated in the presence or absence of 1 mg/mL complete gintonin, protein portion of gintonin, or lipid portion of gintonin in Ringer solution at 37°C for 30 min. (B) Everted sacs were incubated in the absence or presence of Y-27632 (10μM) or phloridzin (100μM) at 37°C for 10 min and then incubated with gintonin (1 mg/mL) for 30 min. Glucose levels in serosal fluid were determined, as described in the Materials and methods. Data represent the means ± SD of four independent experiments. * $p < 0.05$, versus the negative control. Con, control; GT, gintonin; GT-L, lipid portion of gintonin; GT-P, protein portion of gintonin; SD, standard deviation.

which is known to induce morphological changes in cells via filamentous actin reorganization and opening of intercellular tight junctions in the epithelium (the main barrier of the paracellular pathway) [18–20], we examined the effect of a Rho kinase inhibitor on gintonin intestinal absorption. As shown in Fig. 3B, pretreatment with the Rho kinase inhibitor attenuated gintonin permeation of the everted sac. This result suggests that gintonin might open the tight junctions through activation of LPA receptor-mediated Rho kinase and subsequent regulation of actin reorganization. Thus, the opening of tight junction makes gintonin permeable to the intestinal system, although the molecular weight of gintonin is high. In general, nonlinear saturation in a concentration versus drug absorption plot is believed to indicate carrier-mediated transport [21]. In the everted sac system, permeation increased as the gintonin concentration increased from 0.1 mg/mL to 5 mg/mL, and gintonin permeation into the sac became saturated at approximately 3 mg/mL (Fig. 2A), which suggests that gintonin might be also absorbed through an intestinal transporter. This notion was supported by the observation that incubation with the Na^+/glucose transporter inhibitor phloridzin reduced gintonin permeation (Fig. 3B). Thus, there are at least three ways that gintonin can be absorbed after oral administration—the transcellular pathway, the paracellular pathway, and active transport. In addition, when we examined the integrity of the everted sac in the absence or presence of gintonin by monitoring glucose absorption, we observed that glucose was transported inside the sac and gintonin had no effect on glucose transport. This finding indicates that the gut sacs were functional in the presence of gintonin.

Gintonin is a complex of carbohydrates, lipids, and proteins [4]. Besides LPAs, the main carbohydrate, lipid, and protein in gintonin are glucose, linoleic and palmitic acids, and ginseng major latex-like protein151 and ginseng major storage proteins, respectively [3,4]. Thus, gintonin has an amphiphilic physicochemical property. Recently, we found that ginseng major latex-like protein151 binds to LPA C18:2 and delivers to cognate LPA receptors [22]. Regarding solubility and stability of gintonin in aqueous solutions, gintonin is partially soluble but much more stable than free LPAs in their cellular activity because gintonin LPAs are bound to ginseng proteins and it seems that gintonin LPA is more resistant than free LPA in hydrolysis (Supplementary Fig. S2). Although we did not exactly characterize the physicochemical properties of gintonin, we first examined the role of lipid portion of gintonin in gintonin absorption. Interestingly, when we examined which portion of gintonin contributes to gintonin absorption, we found that a much lower amount of de-lipidated gintonin permeated the everted sac than complete gintonin and the protein portion of gintonin (Fig. 3C). This result suggests that the lipid portion of gintonin might be largely responsible for permeation through the sac, possibly via the transcellular and paracellular pathways. In previous studies, we demonstrated that most of the gintonin-mediated effects in neuronal cells are mediated by LPA1/3 receptors. However, in the present study, the LPA1/3 receptor inhibitor Ki16425 had no effect on gintonin permeation of the gut sac (Fig. 3A). It has been reported that the intestinal system abundantly expresses the LPA2/5 receptor instead of the LPA1/3 receptor [23,24]. Currently, there is no selective LPA receptor antagonist of the LPA2/5 receptors. Therefore, involvement of the LPA2/5 receptors in gintonin absorption in the intestine needs to be evaluated in a future study. In addition, further study will be also required to elucidate molecular mechanisms of how gintonin is absorbed in intestinal systems.

It would be interesting to know how gintonin can reach the target organs to exhibit its biological effects after absorption. It is known that phospholipids can be degraded into fatty acids and lysophospholipids by pancreatic phospholipases. Fatty acids and lysophospholipids, with the help of bile acids, form micelles and can be absorbed into the intestine. Thus, lysophospholipids including LPAs are not further digested by phospholipases [25,26]. The lipid portion of gintonin, especially the LPAs, with some protein components may form micelles with either itself or bile juices, which may then be incorporated into chylomicrons like other lipids, secreted into the lymph by exocytosis, and merge with the bloodstream. When the acyl group of LPA is myristoyl, palmitoyl, stearoyl, and oleoyl, the critical micelle concentrations are 1.850mM, 0.540mM, 0.082mM, and 0.346mM, respectively, in water [27]. Thus, the LPAs of gintonin would reach the cardiovascular system via the intestinal lymph duct without passage through the liver and then circulate to reach the target organs. Previous reports showed that oral administration of LPA attenuated intestinal injury due to radiation and chemotherapeutics in rats [23,28]. In addition, intrarectally administered LPA also attenuated trinitrobenzene-induced colitis in rats [29]. These results suggest that orally or intrarectally administered LPA is not much affected by intestinal digestive enzymes and can exhibit its biological effects. Similarly, we observed that orally administered gintonin attenuated amyloid plaque formation and dysfunction of the cholinergic

system in the hippocampus and cortex of APPswe/PSEN-1 double Tg mice [8,10].

In conclusion, the present study showed that gintonin could permeate into the intestine through passive diffusion, including paracellular and transcellular pathways, and active transport. We also showed that gintonin absorption into the intestine involves Rho kinase activation via Ki16425-insensitive LPA receptors and that the lipid portion of gintonin might play a key role in intestinal absorption.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jgr.2016.12.007.

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