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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).

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