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Enhanced pharmacokinetic behavior and hepatoprotective function of ginger extract-loaded supersaturable self-emulsifying drug delivery systems

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ABSTRACT

The aim of present study was to enhance the nutraceutical properties of ginger extract (GE) by employing supersaturable self-emulsifying drug delivery systems (S-SEDDS). SEDDS of GE (SEDDS/GE), consisting of medium-chain triglyceride, lysolecithin and glycerin, was produced. To prepare S-SEDDS of GE (S-SEDDS/GE), hydroxypropyl methylcellulose was added to the SEDDS/GE as a precipitation inhibitor. Physicochemical, pharmacokinetic, and hepatoprotective properties of GE formulations were characterized. Both formulations improved the dissolution behavior of GE due to the formation of fine micelles with a median diameter of ca. 110 nm. After oral administration of GE samples in rats, the relative bioavailabilities of 6-gingerol and 8-gingerol in the S-SEDDS/GE-treated group were ca. 3-fold higher than those of GE-treated group, respectively. Repeated oral administration of GE/S-SEDDS (100 mg-GE/kg) provided a hepatoprotective effect in a rat model of carbon tetrachloride-induced hepatotoxicity. From these observations, the S-SEDDS approach might be efficacious for enhancing the nutraceutical properties of GE.

1. Introduction

Ginger (*Zingiber officinale*) has been widely used as a dietary condiment and medical plant (Baliga et al., 2011). Phytochemicals in ginger, such as 6-gingerol (6G), 8-gingerol (8G), 10-gingerol, and 6-shogaol (6S), have been identified as the major bioactive components (Semwal, Semwal, Combrinck, & Viljoen, 2015) showing various nutraceutical activities, including a gastro protective effect (Al-Yahya et al., 1989), thermoregulatory effect (Eldershaw, Colquhoun, Dora, Peng, & Clark, 1992), anti-inflammatory effect (Dugasani et al., 2010) and anti-oxidant activity (Dugasani et al., 2010; Lu et al., 2014). In the previous report, gingerols and shogaols could also have potent hepatoprotective effect due to the inhibition of inflammatory and oxidative events (Atta et al., 2010; Yemitan & Izegbu, 2006). The mechanism on these functions of active chemicals are suppression of reactive oxygen species production by scavenging superoxide and inhibiting activity of xanthine oxidase (Dugasani et al., 2010), and reduction of prostaglandin E_2 release by inhibiting activity of cyclooxygenase-2 (COX-2) enzyme and altering COX-2 mRNA levels (Lantz et al., 2007). A number of active compounds have been isolated from ginger (Sekiwa, Kubota, & Kobayashi, 2000; Sekiwa et al., 1999), and gingerols and shogaols likely possess essential properties for hepatoprotective function of lipophilic ginger extract (GE) (Dugasani et al., 2010). There has been growing interest in the therapeutic value of GE supplementation for hepatic injury. In spite of these attractive effects, lipophilic GE components exhibit poor solubility in water and low oral bioavailability, possibly resulting in the limited nutraceutical value.

Recently, considerable attention has focused on lipid-based formulations like self-emulsifying drug delivery systems (SEDDS) as the solubilization technology for the application of poorly water-soluble materials to oral and injectable formulation. SEDDS are defined as isotropic mixtures of lipids, surfactants, co-surfactants, and substances that spontaneously form stable fine emulsions

Abbreviations: ALT, alanine aminotransferase; ANOVA, analysis of variance; CCl_4, carbon tetrachloride; COX-2, cyclooxygenase-2; DLS, dynamic light scattering; ESI, electrospray ionization; GI, gastrointestinal; 6G, 6-gingerol; 8G, 8-gingerol; GE, ginger extract; HPMC, hydroxypropyl methyl cellulose; MCT, medium-chain triglyceride; SEDDS, self-emulsifying drug delivery systems; 6S, 6-shogaol; SCF, supercritical fluid; S-SEDDS, supersaturable self-emulsifying drug delivery systems; TEM, transmission microscopy; UPLC, ultra-performance liquid chromatography.

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even under variable conditions in the gastrointestinal (GI) tract after oral administration (Kollipara & Gandhi, 2014). When exposed to aqueous media, such as the GI fluids, these systems form oil-in-water emulsions and present fine emulsion droplets. The emulsification can improve dissolution and oral absorption of compounds by providing a large interfacial area for substance release. In addition, supersaturable SEDDS (S-SEDDS) were designed to enhance the oral absorption of poorly water-soluble compounds compared with conventional SEDDS (Gao & Morozowich, 2006). In general, the S-SEDDS formulations contain a water-soluble polymeric precipitation inhibitor, such as polyvinylpyrrolidone, hypromellose acetate succinate or hydroxypropyl methylcellulose (HPMC), to maintain supersaturation state by steric and/or specific interactions with lipophilic chemicals, possibly leading to decreasing the precipitation and recrystallization. Although studies on several drugs demonstrated that the S-SEDDS formulations could result in higher oral bioavailability compared with that of the conventional SEDDS formulations (Gao et al., 2003, 2004; Lee et al., 2015), far less is known about its feasibility to offer improved biopharmaceutical properties of GE. These findings prompted us to evaluate the applicability of S-SEDDS technology as a viable formulation option for GE.

This study is the first attempt to design and develop a S-SEDDS formulation of GE. A GE-loaded conventional SEDDS formulation was designed for comparison. The physicochemical properties of SEDDS/GE and S-SEDDS/GE were characterized in terms of the particle distribution and dissolution property. Pharmacokinetic studies on GE, SEDDS/GE and S-SEDDS/GE were conducted to clarify the possible improvement in oral absorption of active ingredients. After repeated oral administration of GE and S-SEDDS/GE, hepatoprotective function was evaluated in a rat model of acute hepatotoxicity induced by carbon tetrachloride (CCl_4)-treatment.

2. Materials and methods

2.1. Materials

GE was supplied by Japan Preventive Medical Laboratory Co., Ltd. (Shizuoka, Japan), and coconard MT®, MCT, was supplied by Kao (Tokyo, Japan). SLP-paste lyso, lysolecithin, was purchased from Tsuji Seiyu Co., Ltd. (Mie, Japan) and glycerin was purchased from Wako Pure Chemical Indurstries, Ltd. (Osaka, Japan). HPMC (6 mPa·s) was supplied by Shin-Etsu Chemical (Tokyo, Japan). All other chemicals and solvents were of reagent or HPLC grade.

2.2. UPLC/ESI-MS analysis of active ingredients in GE

The contents of active ingredients in GE were quantified using a Waters Acquity UPLC system (Waters, Milford, MA) equipped with a single quadrupole detector. A KINETEX C18 (particle size: 2.6 μm, column size: 2.1 mm × 50 mm; Phenomenex) was used, and the column temperature was maintained at 60 °C during analysis. The gradient elution system was used to separate the components with a gradient mobile phase consisting of Milli-Q containing 5 mM ammonium acetate (A) and methanol (B) with a flow rate of 0.25 mL/min (0–0.5 min, 40% A; 0.5–3.0 min, 40–5% A; 3.0–3.5 min, 5% A; and 3.5–4.0 min, 5%–40% A). Analysis was carried out using selected ion recording for specific *m*/*z* 317, 345, 294 and 372 for 6-gingerol $[M+Na]^+$, 8-gingerol $[M+Na]^+$, 6-shogaol $[M+Na]^+$ and tamoxifen $[M+H]^+$, respectively.

2.3. Preparation of GE formulations

SEDDS formulations with GE were prepared by dissolving GE into a mixture of MCT, lysolecithin and glycerin at 50 °C and vortexing until all of the materials were completely dissolved. For the S-SEDDS formulation, HPMC was added into the SEDDS formulation, and then, the mixture was vortexed vigorously to obtain a uniform HPMC suspension. The compositions of the tested GE formulations are shown in Table 1.

Table 1
Composition of GE formulations.

	SEDDS/GE (wt%)	S-SEDDS/GE (wt%)
GE	5	5
MCT	47.5	45
Lysolecithin	38	36
Glycerin	9.5	9
HPMC	–	5

2.4. Physicochemical properties of GE formulation

2.4.1. Transmission microscopy (TEM)

An aliquot of SEDDS/GE or S-SEDDS/GE suspended in distilled water (1 mg-GE/mL) was placed on a Formvar 200 mesh Cu (Nisshin EM, Tokyo, Japan). The sample was allowed to stand for 15–30 s, and then any excess solution was removed by blotting. The samples were visualized by negative staining with 1% (w/v) molybdenum solution and observed under an H-7600 transmission electron microscope (Hitachi, Tokyo, Japan).

2.4.2. Dynamic light scattering (DLS)

Water suspended SEDDS/GE and S-SEDDS/GE (100 μg-GE/mL) were analyzed by a Zetasizer Nano ZS (MALVERN, Worcestershire, UK) to evaluate the micelle size distributions. For the physicochemical characterization of emulsion, transitions of the median particle size of emulsion and polydispersity index (PDI) value were measured.

2.4.3. Dissolution test

Dissolution tests were conducted for 120 min using distilled water (50 mL, 37 °C) by a magnetic stirrer SST-66 (Shimadzu, Kyoto, Japan). GE, SEDDS/GE, and S-SEDDS/GE were weighed (25 mg-GE) in the dissolution vessel with a constant staring at 50 rpm, and then the samples were collected at the determined periods (0, 5, 10, 20, 30, 45, 60, 90 and 120 min). After centrifugation at 3000*g* for 5 min, the supernatants were collected and diluted with 50-fold volume of methanol. The released amounts of 6G, 8G, and 6S were assayed by Waters UPLC/ESI-MS system as described in Section 2.2. UPLC/ESI-MS analysis of active ingredients in GE.

2.5. Animals

Male Sprague-Dawley rats (ca. 200 ± 50 g, 6–9 weeks of age; Japan SLC, Shizuoka, Japan) were housed by 2 rats in cage at 24 ± 1 °C and 55 ± 5% RH, and the room was maintained on a 12/12 h light/dark cycle. Rats can freely access to food and water. All animal experiments carried out in this study were approved by the Institutional Animal Care and Ethical Committee of the University of Shizuoka.

2.6. Pharmacokinetic behavior of GE samples

Blood samples (300 μL) were collected from the tail veins of unanesthetized rats at the indicated times (0.18, 0.33, 0.5, 1, 2, 4, 8 and 12 h) after oral administration of GE (300 mg/kg) dissolved in 1 mL medium-chain triglyceride and SEDDS/GE or S-SEDDS/GE (100 mg-GE/kg) suspended in 2.5 mL of distilled water. Plasma samples were obtained after centrifugation of each blood sample