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Original Research Paper

Enhancement of gastrointestinal absorption of isoliquiritigenin by nanostructured lipid carrier

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ABSTRACT

Isoliquiritigenin (ISL) has various pharmacological effects. Our previous studies demonstrated that the oral bioavailability of ISL was low and the concentration-time profiles of ISL exhibited double peaks after oral administration in rat, but the underlying mechanisms remained unknown. The objective of this study was to clarify the gastrointestinal (GI) absorptive characteristics of ISL using *in situ* intestinal perfusion model as well as explain double peaks phenomenon after oral administration and to evaluate the potential of using nanostructured lipid carrier (NLC) as an oral delivery carrier for poorly water soluble drugs. The results showed that the absorption percent in the stomach for 2 h was less than 10%, the absorption process of intestine was first-order process with passive diffusion mechanism, and the main absorptive segment was colon. Isoliquiritigenin-loaded nanostructured lipid carrier (ISL-NLC) could enhance oral absorption of ISL. The reason for the Double Peak Phenomenon following oral administration in ISL plasma concentrations versus time profiles is Variability of Absorption within different regions of the gut, very low absorption from the stomach, jejunum, duodenum and ileum compared with the absorption from the colon. A pharmacokinetic study was conducted in rats after a single dose oral administration of ISL at 20 mg/kg in the form of either ISL-NLC or isoliquiritigenin solution (ISL-Sol). The AUC $_{(0\sim\infty)}$ values were 5.43 ± 0.67 μg h mL^{-1} and 29.60 ± 2.88 μg h mL^{-1} after administration of the ISL-Sol and ISL-NLC, respectively. The relative bioavailability of ISL-NLC to isoliquiritigenin was 545%. Our studies provide evidence that NLC are valuable as an oral delivery carrier to enhance the absorption of a poorly water soluble drug, ISL.

1. Introduction

Isoliquiritigenin (ISL), a flavonoid with chalcone structure compound originated from *Glycyrrhiza* species, has diverse pharmacological effects such as antioxidant [1–3], anti-inflammatory [4,5], anti-tumor [3,6,7], anti-platelet [8], vasorelaxant effect [9], antiallergic effect [10], estrogenic property [11], antipeptic ulcer actions [12] and attenuating brain injury [13]. Also, ISL is known as a SIRT1 activator, which is an upstream regulator of LKB1 [14,15]. In addition, treatment with ISL alters conformation of Kelch-like ECH-associated protein 1 (Keap1) by alkylating specific cysteine residues, and thus results in NF-E2-related factor-2 (Nrf2) activation [16,17]. Essential for ISL to exert its action *in vivo* is the extent of its absorption after oral administration. However, ISL is poorly water-soluble, and its absorption *in vivo* is very poor and its oral bioavailability is less than 50% in rats after oral administration [18–20], which diminishes its therapeutic effects. In addition, double peaks were observed in the plasma concentration versus time profiles after oral administration in rats regardless of the dose [20]. Therefore, an oral formulation of ISL with improved absorption is highly desired. Moreover, the reason for the double peaks phenomenon following oral administration of ISL in rats needs to illustrate.

Lipid nanoparticles represent drug vehicles composed of physiological lipids such as phospholipids, cholesterol, cholesterol esters and triglycerides [21]. The biological origin of the nanocarrier material offers a number of advantages making lipid nanoparticles one of the ideal drug delivery vehicles [22]. The bioacceptable and biodegradable nature of these systems makes them less toxic as

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compared to other nanocarriers (e.g. polymeric nanoparticles) [22,23]. The nanostructured lipid carriers (NLCs) are presented as an improved generation of lipid nanoparticles [19,24,25], which is developed from solid lipid nanoparticle (SLN) system. It consists of solid lipid matrices with spatially incompatible liquid lipids, results in a structure with more imperfections in crystal to accommodate the drug, and thus gets a higher drug loading capacity. NLC system shares advantages of SLN, e.g. controlled drug release, biocompatibility and the possibility of production on large industrial scale. Furthermore, it minimizes or avoids some potential problems associated with SLN, such as drug leakage during storage and limitation in drug loading capacity [26–30]. Therefore, NLC are promising carrier to increase the drug loading efficiency and prolong the half-life of ISL. Our previous study showed that NLC can prolong exposure of tumor cells to ISL, enhance permeability and retention (EPR) effect, and subsequently increase the therapeutic effect of ISL [19,31]. However, no reports have thus far indicated whether the oral bioavailability of ISL could be significantly improved by NLC technology. The aim of our work is to study the absorption characteristics of ISL using *in situ* intestinal perfusion model as well as double peaks phenomenon following oral administration in rats and to evaluate the potential of using NLC as an oral delivery carrier for poorly water soluble drugs. In addition, pharmacokinetics and relative bioavailability of ISL in rats after single dose oral administration of ISL-NLC were investigated in comparison with isoliquiritigenin solution (ISL-Sol).

2. Materials and methods

2.1. Materials

Isoliquiritigenin (ISL, 99.0% purity) was purchased from Shanghai Bangcheng Chemical Co. (Shanghai, China). Acetanilide (ACE) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) and used as an internal standard (IS). Glycerol monostearate (MS) was purchased from Sinopharm Chemical Reagent Co., Ltd., China. Miglyol® 812 (caprylic/capric triacylglycerols, Sasol, Germany) and Lutrol® F 68 (Poloxamer 188, BASF, Germany) were purchased from Beijing Fengli Jingqiu Commerce and Trade Co., Ltd., China. Tween80 (CRODA, Great Britain) was purchased from Shanghai Chemical Reagent Co., Ltd., China. HPLC grade methanol and acetonitrile were obtained from Shandong Yuwang (Shandong, China). HPLC grade water was provided by the first hospital of Lanzhou University. Sodium chloride (analytical grade) was purchased from Beijing Beihua Fine Chemicals Company, Limited (Beijing, China). Na_2HPO_4, KCl, $CaCl_2$, $MgCl_2$, $NaHCO_3$, glucose and phenol red were obtained from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). Other commercial reagents and solvents were of analytical grade.

The perfusion solution was Krebs–Ringer (K–R) buffer solution containing 7.8 g NaCl, 0.35 g KCl, 1.37 g $NaHCO_3$, 0.02 g $MgCl_2$, 0.32 g NaH_2PO_4 and glucose in 1.48 g/1000 mL distilled water.

2.2. Preparation and physicochemical characterization of ISL-NLC

ISL-NLCs were prepared by the solvent diffusion method as described previously [19,32]. MS and Miglyol® 812 were used as the solid lipid material and the liquid lipid material, respectively. Briefly, weighed MS, Miglyol® 812 and the drug (20 mg) were dissolved into 5 mL of mixed organic solvent of ethanol and acetone (1:1, v/v) in a water bath at 55 °C. The resultant organic solution was quickly dispersed into 20 mL of aqueous solution of Tween80 (1% (W/V)) and Poloxamer188 (1% (W/V)) at room temperature (25 °C) under mechanical agitation (DC-40, Hangzhou Electrical Engineering Instruments, China) with 3000 rpm for 30 min until NLC suspensions were obtained. The mean particle size of ISL-NLC was (160.73 ± 6.08) nm with a zeta potential of (−26.18 ± 3.22) mV.

The obtained ISL-NLCs were ultra-centrifuged for 1 h at 80,000*g* (4 °C) using a super-speed refrigerated centrifuge (MIKR022, HEETTICH, Germany). The bottom pellet after centrifugation was re-suspended in double distilled water containing 5% (w/v) lactose and 5% (w/v) glucose. Lactose and glucose were used in the freeze-drying process as cryoprotectants. The addition of lactose and glucose in the lyophilization process was to prevent the coagulation between NLC. The NLC suspensions were fast frozen in an aqueous lactose and glucose solution under −80 °C in a ULT 2586-5-A14 freezer (Revco scientific, Asheville NC, USA) for 5 h and then the samples were moved to the freeze-drier (LGJ0.5-II, Beijing, China) and lyophilized at −50 °C for 48 h. The NLC dried powders were collected and stored at 4 °C for further experiments.

ISL-NLCs were diluted in Krebs–Rings buffer to produce different concentrations of test formulations for gastrointestinal (GI) absorption at a dose of 5, 25, 50, 100, 150, and 200 μg/mL, respectively.

2.3. Preparation of ISL solution (ISL-Sol)

During *in situ* intestinal perfusion experiments, ISL was dissolved in the K–R buffer solution to yield different concentrations of test solutions (5, 25, 50, 100, 150, and 200 μg/mL). Owing to low solubility of ISL, ISL was difficult to formulate into perfusion solution and thus, DMSO (2%, w/v) was used to overcome the solubility barrier.

2.4. Animals

Male Sprague–Dawley (SD) rats (220 ± 20 g), obtained from the Laboratory Animal Center of Lanzhou University (Lanzhou, China), were used for Gastrointestinal (GI) absorption experiments and pharmacokinetic studies. They were kept in an environmentally controlled breeding room for 5 days and fasted for 12 h with free access to water before experiments. All protocols and procedures were approved by Lanzhou University Animal Care and Use Committee.

2.5. Determination of ISL by HPLC

Perfusate samples were analyzed by HPLC as described previously [18–20]. The HPLC system was performed on an Agilent 1200 HPLC (Boeblingen, Germany) system consisting of G1322A Vacuum Degasser, G1311A Quat Gradient Pump, G1316A Thermostatted Column Compartment, G1329A Autosampler, G1315B Diode Array Detector and LC 3D instrument Chem Station. Chromatographic separation was achieved on a YMC-packed ODS-A C_{18} column, 150 × 4.6 mm, 5 μm (YMC Co. Ltd., Kyoto, Japan) preceded by an Agilent Zorbax Reliance Cartridge guard column (Eclipse XDB-C_{18}, 12.5 mm × 4.6 mm, 5-Micron). The mobile phase was composed of acetonitrile, 0.05 mol/L potassium dihydrogen phosphate and triethylamine in a volume ratio of 50:50:0.5. The pH of the mobile phase was adjusted to 2.0 with 85% phosphoric acid. Prior to use, the mobile phase was filtered through a 0.45 μm hydrophilic membrane filter. Detection wavelength was set at 242 nm while reference wavelength was set at 360 nm from 0 to 5 min, and 360 nm and 700 nm from 5 to 9 min, respectively. The mobile phase was delivered at a flow rate of 1.0 mL/min. The temperature of the thermostated oven containing the column was set at 25 °C and the injection volume was 20 μL.

Prior to analysis, 20 μL of Acetanilide (ACE, internal standard: IS) solution (50 μg/mL) were added to the perfusate samples and