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Preparation of isoliquiritigenin-loaded nanostructured lipid carrier and the *in vivo* evaluation in tumor-bearing mice

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ABSTRACT

Isoliquiritigenin-loaded nanostructured lipid carrier (ISL-NLC) was constructed and characterized. *In vivo* antitumor efficacy and immuno-modulation effects of ISL-NLC were evaluated in sarcoma 180 (S180)-bearing and murine hepatoma 22 (H22)-bearing mice model through intraperitoneal (i.p.) administration. The ISL-NLC biodistribution was also investigated in H22-bearing mice. Results demonstrated that the ISL-NLC had a spherical shape with a mean size of (160.73 ± 6.08) nm and encapsulation efficiency of (96.74 ± 1.81)%. ISL released from the nanoparticles was in a sustained manner with an initial burst release. ISL-NLC significantly inhibit tumor growth at 10, 20 and 40 mg/kg levels, and inhibition rates were 75.70%, 82.27% and 83.90% in the S180-bearing mice and 71.49%, 81.11% and 85.62% in the H22-bearing mice, respectively. The biodistribution study showed that ISL concentration of ISL-NLC in tumor is higher 2.5-fold than ISL suspension. The elimination half-life ($t1/2$), area under the curve (AUC) and the mean residence times (MRTs) of the ISL-NLC was much longer than that of the ISL suspension. As a whole, anticancer effect of ISL encapsulated in NLC was superior to ISL in suspension on H22-bearing and S180-bearing mice at the same dose and was a dose-dependent way, and ISL-NLC improved immunity of ISL. It can be inferred that nanostructured lipid carriers are a promising carrier for cancer therapy using ISL.

1. Introduction

Isoliquiritigenin (ISL), a flavonoid with chalcone structure (2,4,4′-trihydroxychalcone), is found in licorice, shallot and bean sprouts (Kitagawa et al., 1994, 1998; Hayashi et al., 1996, 1998). Pharmacological studies showed that ISL has wide pharmacological effects including antioxidant (Vaya et al., 1997; Yu et al., 2007), anti-flammatory and analgesic (Semnani et al., 2004), cytoprotective effects (Kim et al., 2004), antiplatelet aggregation (Chisato et al., 2008), chemopreventive agent (Hsu et al., 2005), anti-angiogenic effect (Kobayashi et al., 1995) and radical-scavenging activity (Takahashi et al., 2004). Recently, there has been a growing interest in antitumor researches of ISL due to its broad antitumor spectrum and potent antitumor activities (Iwashita et al., 2000; Ma et al., 2001; Baba et al., 2002; Maggiolini et al., 2002; Yamazaki et al., 2002; Kanazawa et al., 2003). ISL has been reported to inhibit the proliferation of many different types of cancer cells and to reduce the growth and metastasis of hepatoma cells (Hsu et al., 2005), gastric cells (Ma et al., 2001), melanoma cancer cells (Iwashita et al., 2000) and prostate cancer cells (Jung et al., 2006). In particular, the antiproliferative activity of isoliquiritigenin against a variety of cancer cells is mediated by the cell cycle arrest at G2/M phase via upregulation of p53, p21 or GADD153 in Hep G2 hepatoma cells, DU145 and LNCaP prostate cancer cells, A549 human lung cancer cells and uterine leiomyoma cells (Kanazawa et al., 2003; Ii et al., 2004; Hsu et al., 2005; Kim et al., 2008) or via the inhibition of topoisomerase II activity and by blocking the metaphase/anaphase transition in HeLa cells (Park et al., 2009). In order to improve therapeutic response against tumors, nanostructured lipid nanoparticle (NLC) was developed as drug carrier. Nanoparticles, which can provide a controlled and targeted way to deliver the encapsulated anticancer drugs and thus result in high efficacy with low side effects (Smets, 1994; Langer, 2000; Lonning, 2003), has become attraction. Lipid nanoparticles used as drug vehicles composed of physiological lipids such as phospholipids, cholesterol, cholesterol esters and triglycerides (Fenske and Cullis, 2008). These biologically originated nanocarrier materials offer a number of advantages such as less toxic as compared to polymeric nanoparticles due to their bioacceptable and biodegradable nature, which makes lipid nanoparticles as one of the ideal drug delivery vehicles (Rawat et al., 2008). The nanostructured lipid carrier, as a new generation of lipid nanoparticles, which can control drug release as well as drug targeting, has been developed to overcome the limitations of solid lipid nanoparticles (SLNs) in recent years, such as drug leakage during storage, drug expulsion and low loading capacity (Müller et al., 2002; Müller and Keck, 2004;

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Schäfer-Korting et al., 2007; Liu et al., 2011; Tarl et al., 2011; Cirri et al., 2012). Conventionally, NLC is produced by controlled mixing of solid lipids with spatially incompatible liquid lipids which leads to special nanostructure with improved properties for drug loading, modulation of the drug release profile and stable drug incorporation during storage. Depending on the preparation method and the composition of lipid blend, NLCs with different structures were obtained, i.e., the imperfect, amorphous and multiple components (Mitri et al., 2011). These NLCs have low crystallinity, thereby leading to highest incompatibility and higher drug loading and the minimum incidence of drug expulsion during storage (Souto et al., 2004; Jenning and Gohla, 2001; Jenning et al., 2000). Admixture of liquid lipids with solid lipids leads to a less ordered inner structure. Thus, the drug molecules can be accommodated in between lipid layers and/or fatty acid chains (Müller et al., 2002). Therefore, NLCs are promising carrier to increase the drug loading efficiency and prolong exposure of tumor cells to antitumor drug, enhance permeability and retention (EPR) effect (Maeda et al., 2000), which can be taken as an advantage to effectively improve the drug concentration in tumor tissues after systemic administration (intravenous i.v., intraperitoneal i.p.) (Vlerken et al., 2008) and subsequently increase the therapeutic effect of antitumor drug.

In the present research, a solvent diffusion method in aqueous system was applied to prepare nanostructured lipid carriers. The ISL was used as model drug. Glycerol monostearate (MS) and Miglyol 812 were chosen as the solid and liquid lipid materials of lipid nanoparticles, respectively. The physicochemical characteristics of ISL-NLC were investigated. In addition, we investigated the *in vivo* antitumor effect in Kunming mice bearing H22 and S180 and biodistribution of ISL-NLC and ISL suspension in H22-bearing mice after i.p. administration. Simultaneously, the influence of ISL-NLC on the immunity of tumor-bearing mice was also evaluated. We postulate that this new drug vehicle might become a promising high-performance delivery system for ISL.

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). The molecular structure of isoliquiritigenin is shown in Fig. 1. The excipients, used in the preparation of the nanoparticles, were as the followings: 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 kindly donated by Beijing Fengli Jingqiu Commerce and Trade Co., Ltd., China. Tween80 (CRODA, Great Britain) was purchased from Shanghai Chemical Reagent Co., Ltd., China. Lactose, glucose and *n*-octanol were all purchased from Sinopharm 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 Co., Ltd. (Beijing, China). Other commercial reagents and solvents were of analytical grade.

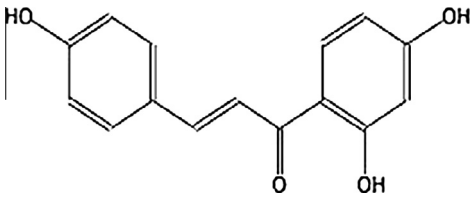

Fig. 1. Molecular structures of isoliquiritigenin.

2.2. Physicochemical properties of ISL

2.2.1. Solubility

Excess amount of ISL was placed in three microtubes which contained 1.0 mL double distilled water, 1.0 mL 0.9% NaCl and 1.0 mL phosphate butter solution (PBS, pH 7.4), respectively, and shaken at 25 °C, 100 rpm for 12 h to ensure the solubility equilibrium. At the end of this step, solution was centrifuged at 80,000*g* for 10 min and the supernatant was filtered through 0.45 μm filter membrane (Millipose). And 50 μL of the filtered supernatant was analyzed by HPLC.

2.2.2. Apparent partition coefficient

Accurately weighed amount of ISL were partitioned between double distilled water and *n*-octanol for 12 h at room temperature by the shake flask method. ISL concentrations in the aqueous phase before and after partitioning were measured through HPLC analysis, respectively, and the partition coefficients from the results were calculated as $\log P = \log(C_o - C_w)/C_w$.

2.3. Preparation of ISL-NLC nanoparticles

ISL-NLC was prepared by previously reported method (Zhang et al., 2010). Briefly, MS, Miglyol® 812 and ISL 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 drug-free NLC nanoparticles were prepared with exactly the same procedures except the drug. Prepared NLCs were placed in a vacuum desiccator for 24 h at room temperature to eliminate the residual organic solvent.

The obtained ISL-NLCs were ultra-centrifuged for 1 h at 80,000*g* (4 °C) using a superspeed 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.

2.4. Characterization of nanoparticles

2.4.1. Size and zeta potential analysis of the nanoparticles

Particle size analysis was performed by dynamic light scattering (DLS) with a Malvern Zetasizer 3000 HSA (Malvern Instruments, UK). DLS yields the mean diameter and the polydispersity index (PI) which is a measure of the width of the size distribution. The mean diameter and PI values were obtained at an angle of 90° in 10 mm diameter cells at 25 °C. Prior to the measurements all samples were diluted with double distilled water to produce a suitable scattering intensity. The zeta potential, reflecting the electric charge on the particle surface and indicating the physical stability of colloidal systems, was measured using the Malvern Zetasizer