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Evaluation of the cytotoxicity and intestinal absorption of a self-emulsifying drug delivery system containing sodium taurocholate

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Sodium taurocholate (PubChem CID: 23666345)
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Verapamil (PubChem CID: 2520)
2,4-dinitrophenol (PubChem CID: 1493)
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ABSTRACT

Currently, many surfactants used in self-emulsifying drug delivery systems (SMEDDS) can cause gastrointestinal mucosal irritation and systemic toxicity. In the present study, SMEDDS were loaded with pueraria flavones, using sodium taurocholate to replace polyoxyl 40 dydrogenated castor oil (Cremophor® RH 40) as the surfactant (PF-SMEDDS$_{NR}$) to reduce the toxicity of SMEDDS using Cremophor® RH 40 as the surfactant (PF-SMEDDS$_R$). The absorption rate constants (K_a) and intestinal permeability coefficients (P_{eff}) were measured. The effects of P-glycoprotein inhibitor (verapamil), adenosine triphosphate (ATP) inhibitor (2,4-dinitrophenol), and carrier inhibitor on K_a and P_{eff} values in the ileum were determined. Biological safety was also evaluated. The K_a and P_{eff} values increased for PF-solution concentrations of 200 μg/ml > 100 μg/ml > 400 μg/ml in individual segments of the intestines. The results indicated that P_{eff} values of PF-SMEDDS$_{NR}$ were distinctly higher than those of SMEDDS loaded with pueraria flavones using Cremophor®RH 40 as the surfactant (PF-SMEDDS$_R$) and PF-solution in four intestinal segments. However, the K_a values of PF-SMEDDS$_{NR}$ were higher only in the jejunum and ileum segments compared with those of PF-SMEDDS$_R$ and PF-solution. The K_a and P_{eff} values without verapamil were significantly lower than those with verapamil. 2,4-Dinitrophenol had no effect on K_a and P_{eff} values. The K_a and P_{eff} values of PF-SMEDDS$_{NR}$ significantly decreased after perfusing B-SMEDDS$_{NR}$ for 1 h prior to the study. The cell viabilities after exposure to SMEDDS$_{NR}$ were higher than those of SMEDDS$_R$ in the range of 81–324 μg/ml. Lactate dehydrogenase release from cells treated with PF-SMEDDS$_{NR}$ or B-SMEDDS$_{NR}$ was significantly lower than that from cells treated with PF-SMEDDS$_R$ or B-SMEDDS$_R$ at surfactant concentrations of 243 and 324 μg/ml. However, there were no differences with SMEDDS treatment at surfactant concentrations of 0–162 μg/ml. Hence, we conclude that SMEDDS using sodium taurocholate as the surfactant can reduce the toxicity of SMEDDS, meanwhile, maintain the characteristics of SMEDDS, and enhance intestinal absorption.

1. Introduction

Self-emulsifying drug delivery systems (SMEDDS) are colloidal systems, comprising oils, surfactants, and cosurfactants, which form oil-in-water microemulsions in aqueous media with gentle agitation (Agrawal et al. 2015; Wang et al. 2015; Yeom et al. 2016). Recently, much attention has been focused on the development of SMEDDS for their potential to enhance solubility, membrane permeability, and bioavailability of water-insoluble drugs (Agrawal et al. 2015; McConville and Friend 2013; Yeom et al. 2016). SMEDDS have been widely used in the pharmaceutical industry, benefiting from the success of cyclosporin A softgel formulations (*e.g.*, Sandimmune® and Neoral®) (Agrawal et al. 2015; Gao and Morozowich 2006; Yeom et al. 2016).

Unfortunately, SMEDDS require a number of surfactants during their preparation. Many surfactants used in SMEDDS can cause gastrointestinal mucosa irritation and systemic toxicity (Chen et al. 2011; Huang et al. 2014). Some strategies have been explored to minimise the toxicity of surfactants. Tian et al. prepared a supersaturated SMEDDS by adding polyvinylpyrrolidoneK30 into SMEDDS composed of biphenyl dimethyl dicarboxylate. The *in vivo* pharmacokinetic data confirmed that these supersaturated SMEDDS could enhance oral drug availability and reduce toxicity (Tian and Quan 2011). Huang et al. developed a novel puerarin self-microemulsion drug delivery system by replacing polysorbate 80 with natural emulsifiers to reduce surfactant-derived toxicity in SMEDDS (Huang et al. 2012).

In this study, pueraria flavones (PF) were selected as the model drug. PF are major active ingredients extracted from *Radix Puerariae*, traditional Chinese herb medicines that are effective in treating diseases, such as hypertension, hypercholesterolemia, coronary heart disease, and angiocardiopathy (Fan et al. 2014; Guo et al. 2009). However,

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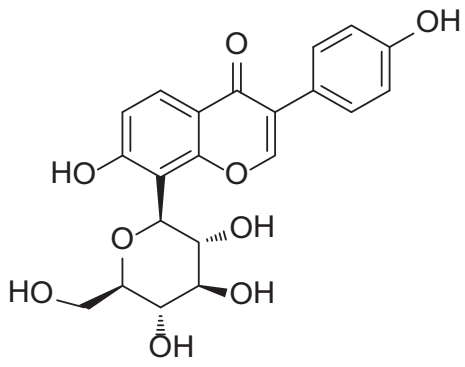

Fig. 1. Chemical structure of puerarin.

the effectiveness of PF as cardiovascular disease remedies is restricted by their poor solubility (Guo et al. 1998). In the present study, we prepared PF-SMEDDS using either Cremophor® RH 40 (PF-SMEDDS$_R$) or sodium taurocholate and Cremophor® RH 40 (PF-SMEDDS$_{NR}$) as surfactants. We then evaluated these drug carrier systems as follows. (i)The intestinal absorption of PF-SMEDDS$_{NR}$ in four individual intestinal segments (*i.e.*, duodenum, jejunum, ileum, and colon) was determined *via in situ* single-pass intestinal perfusion (SPIP) and compared to that of PF-SMEDDS$_R$ and PF-solution. (ii)The intestinal absorption mechanism of PF-SMEDDS$_{NR}$ was investigated preliminarily with three absorptive inhibitors (*i.e.*, P-glycoprotein (P-gp) inhibitor, carrier inhibitor, and ATP inhibitor), and (iii) the cellular toxicity of the SMEDDS was studied by MTT method and LDH release (LDH) assay.

2. Materials and methods

2.1. Materials

Puerarin (Fig. 1, purity > 99.0%) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (No. 110752-200912, Beijing, P.R. China).PF were purchased from Xi'an SaiBang Pharmaceuticals and Technology Co., Ltd. (Xi'an, P.R. China, purity: 66.7%). Maisine™ 35-1 was obtained from Gattefossé (Saint-Priest, France). Propane-1, 2-diol was provided by Tianjin Guangfu Fine Chemical Research Institute (Tianjin, P.R. China). Polyoxyl 40 dydrogenated castor oil (Cremophor® RH 40) was provided by BASF (Ludwigshafen, Germany). Sodium taurocholate was purchased from Shanghai Ryon Biological Technology Co., Ltd. (Shanghai, P.R. China). Dulbecco's modified Eagle medium (DMEM), foetal bovine serum (FBS), trypsin, methylthiazolyldiphenyl-tetrazolium bromide (MTT), and HPLC-grade methanol were all purchased from Thermo Fisher Scientific Inc. (Madison, USA). F-12 K medium was provided by Wisent Bio products (Montreal, Canada).Endothelial cell growth supplement was purchased from ScienCell (USA). Lactate dehydrogenase (LDH) kit was obtained from Nanjing Jiancheng Bioengineering Institute. All the other chemicals used in the study were of analytical grade and obtained commercially.

2.2. Preparation of self-microemulsion formulation

Blank self-microemulsion drug delivery systems consisted of Maisine™ 35-1, Cremophor® RH 40, and propane-1,2-diol at mass ratios of 1:3:6 (B-SMEDDS$_R$), and Maisine™ 35-1, sodium taurocholate, Cremophor® RH 40, and propane-1,2-diol at mass ratios of 1:1.5:1.5:6 (B-SMEDDS$_{NR}$). The mixtures were thoroughly equilibrated at 37 ± 0.5 °C with 100 rpm magnetic stirring. PF-SMEDDS$_R$ and PF-SMEDDS$_{NR}$ were obtained by adding PF into B-SMEDDS$_R$ and B-SMEDDS$_{NR}$ at a mass ratio of 1:4.5, respectively, and mixing with a water bath shaker at 37 ± 0.5 °C for 72 h.

2.3. Characterisation of SMEDDS

The particle size, polydispersity index (PDI), and *zeta* potential of SMEDDS were measured on a dynamic light scattering particle size analyser (Zetasizer Nano ZS, Malvern Instruments, UK) at 25 °C. All samples were diluted 100-fold with distilled water, stirred at 100 rpm for 1 min and measured. Each experiment comprised 10 to 120 runs, which depended on the acquisition of a stable reading.

In order to estimate the physical stability of SMEDDS after using sodium taurocholate to Cremophor® RH 40 as surfactants, particle size, PDI and *zeta* potential of SMEDDS were measured at different time points and compared under room temperature. Moreover, transparency was observed and absorbance diluted by 100-fold distilled water before or after centrifuge was measured at 500 nm by Utlraviolet spectrophotometer. Centrifugal stability coefficient (Ke) values were calculated according to eq. (1). All experiments were performed in triplicate. All data were calculated as mean ± S.D.

$$Ke = [(A_0 - A)/A_0] \times 100\% \quad (1)$$

where A_0 represents absorbance before centrifuge, A represents absorbance after centrifuge.

2.4. Release study in vitro

Briefly, *in vitro* release studies were carried out using a dialysis method in 45 mLof pH 6.8 phosphate buffer saline (PBS) on a horizontal stirring instrument. The temperature was maintained at (37 ± 0.5) °C and the stirring speed was set to 100 rpm. PF-SMEEDS$_R$ and PF-SMEEDS$_{NR}$ were diluted with pH 6.8 PBS respectively with a final concentration up to 500 μg/ml respectively. Then 5 mL of PF-SMEEDS$_R$ or PF-SMEEDS$_{NR}$ solution were transferred into a cellophane membrane dialysis bag (8–12 kDa) respectively. At definite time intervals (0, 0.5, 1, 2, 4, 8, 12, 22 and 24 h), An aliquot of 5 ml sample was withdrawn and the same temperature and equivalent volume of fresh release medium was immediately compensated. All samples were filtrated through 0.45 μm membrane and concentration of puerarin was analysed using HPLC method.

2.5. Single-pass intestinal perfusion studies

2.5.1. Preparation of solutions

The Krebs-Ringer solution was composed of NaCl (133 mM), KCl (4.7 mM), NaH_2PO_4 (2.7 mM), $NaHCO_3$ (16.3 mM), $MgCl_2$ (0.2 mM), D-glucose (7.7 mM), and $CaCl_2$ (3.3 mM). Perfusate solutions of PF (100, 200, and 400 μg/ml) were obtained by dissolving PF in Krebs-Ringer solution under ultrasound bath. Perfusate solutions of PF-SMEDDS$_{NR}$ and PF-SMEDDS$_R$ were prepared by dissolving PF-SMEDDS$_{NR}$ or PF-SMEDDS$_R$, respectively, in Krebs-Ringer solution with a final PF concentration of 200 μg/ml. All sample solutions were prepared at 37 ± 0.5 °C.

2.5.2. Perfusion experiments

All experiments were conducted according to the Guidelines for Care and Use of Laboratory Animals of Jilin University. Male Wistar rats (230–270 g) were supplied by the Central Animal Laboratory of Jilin University, P.R. China, and were randomly divided into six groups. The rats were housed and handled at 23 ± 1 °C and a humidity of 65–75% with a 12 h/12 h light/dark cycle. Rats were fasted for 24 h with free access to water prior to experiments.

The procedure for the *in situ* SPIP was performed as previously reported (Dahan et al. 2009; Ho et al. 2008; Mora et al. 2015; Reis et al. 2013). Rats were anaesthetised and affixed supine on a heated surface (37 °C) under suitable lighting. The abdomen was opened through a midline incision of 3–4 cm. The duodenum (4 cm distance from pylorus), jejunum (15 cm distance from pylorus), ileum (20 cm upward from ileocecal valve), and colon (3 cm downward from ileocecal valve)

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