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Formulation and in vitro absorption analysis of *Rhizoma paridis* steroidal saponins

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ABSTRACT

Rhizoma paridis steroidal saponins (RPS) have been prepared and identified as the active compounds for antitumor activity in our previous study. However, the low oral bioavailability of the steroidal saponins restricted its using. In the present research, solid dispersion (SD) and phytosome (PHY) formulation of RPS were prepared, and the physicochemical parameters as well as the intestinal absorption in rat everted gut sac model were investigated. Seven agents were selected as the carriers of SD, and poloxamer 407 (P 407) was the most suitable one. SD reduced the particle size of saponins in the water solution, enhanced the solubility of the saponins by about 3.5 folds, and significantly improved the absorption transport of saponins from 48 to 104 μg in everted gut sac of the rat system. PHY significantly enhanced the hydrophilic of saponins but showed little effect on the absorption in small intestine. Jejunum and ileum part absorbed more absolute contents of total saponins than duodenum parts. Six saponins, the main contents of RPS, used as the index of comparing the three forms, were also further investigated in the physico-chemical properties and the absorption tests. *n*-Octanol/water partition coefficients of the six saponins ordered in RPS, SD and PHY were Chonglouoside H > Dioscin > Polyphyllin D > Gracillin > Paris-VII > Formosanin C. All the saponins possessed the higher absorptive characteristics in SD formulation. The absorption rate of diosgenyl saponins in intestine was more than the pennogenyl saponins.

1. Introduction

The dried rhizoma of *Paris polyphylla* Smith var. *yunnanensis*, commonly known as "Chonglou" in China, have been used in treating the diseases such as snakebite and abscess in folk medicine for a long time. Steroidal saponins of *Rhizoma paridis* are the main active components for anti-tumor, immunity adjustment, antiviral and anti-inflammation (Deng et al., 2008; Lee et al., 2005; Man et al., 2009a). A lot of diosgenyl saponins and pennogenyl saponins have been separated from the genus *Paris* (Man et al., 2009a; Zhang et al., 2010). Saponins of Chonglouside H, Polyphyllin D, Dioscin and Formosanin C showed potent effect on antitumor activity in vitro (Chan et al., 2011; Cong et al., 2012; Man et al., 2011; Sun et al., 2011; Wen et al., 2012; Yan et al., 2009), and by affecting the platelet, Paris-VII was a potential hemostyptic drug (Siu et al., 2008). However, saponins failed to fulfill their therapeutic potential owing to poor bioavailability as a result of low intestinal permeability. The plasma level of Dioscin was very low after giving a high oral dose (90 mg/kg) in rat, and the extremely oral bioavailability was 0.2% (Li et al., 2005a, 2005b). In our previous work, *Rhizoma paridis* saponins extracts (RPS) could not be detected in plasma after administration at high dose, which indirectly indicated the poor absorption of RPS.

Generally, bioavailability of drugs following oral administration is determined by several factors such as solubility, gastric-intestinal stability, intestinal permeability and first-pass extraction in the gut and by the liver. The poor solubility of drugs could be changed using dosage forms such as solid dispersions (SD) or phytosome (PHY). SD formulation with hydrophilic carriers has been a promising technique for improving the solubility, dissolution rate and the absorption of many substances as they overcome the limitations of previous approaches (Fernandez et al., 1992; Han et al., 2011; Hirasawa et al., 2003). PHY as a dosage form enhances the ability of a drug to pass across the lipid-rich biological membranes and reach circulation (Maiti et al., 2007; Marczylo et al., 2007).

In the present study, RPS was extracted as previously reported (Man et al., 2009b), and two formulations of SD and PHY of RPS were prepared. The physico-chemical properties of the two formulations

Abbreviations: SD, solid dispersions; PHY, phytosome; RPS, *Rhizoma paridis* steroidal saponin extract; PK 30, Povidone K-30; P 407, poloxamer 407; P 188, poloxamer 188; PEG 4000, polyethylene glycol 4000; PEG 6000, polyethylene glycol 6000.

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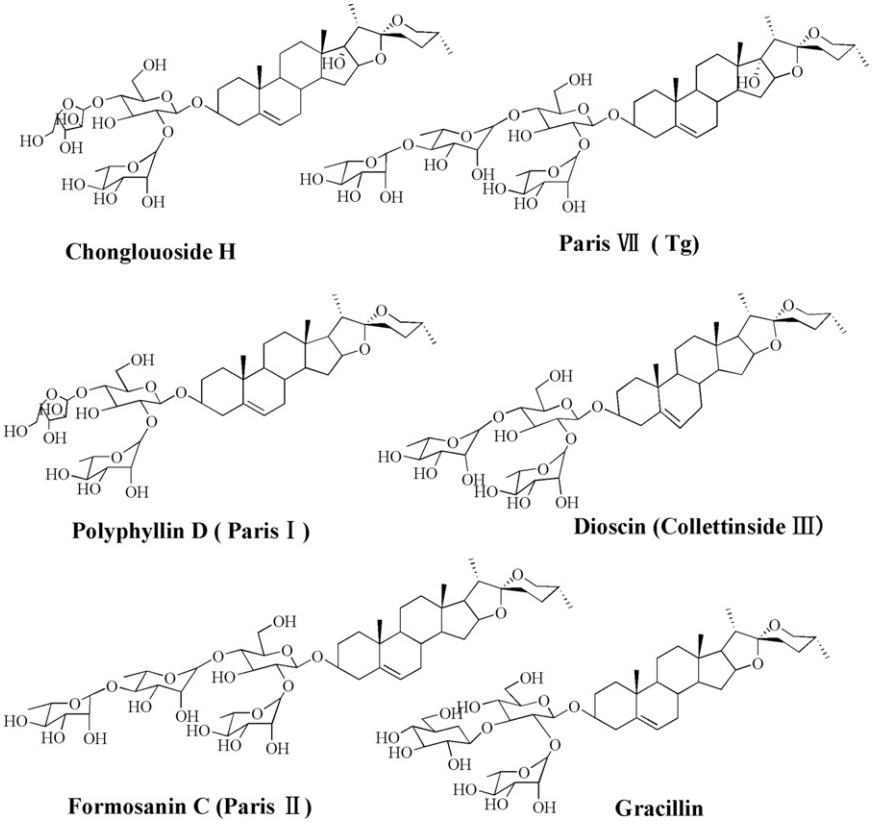

Fig. 1. Structures of six steroidal saponins used in this study.

were compared by determining the changes of six *Rhizoma paridis* steroidal saponins. The intestinal absorptions of these dosage forms were observed in the everted gut sac of the rat to obtain the absorption site, and the absorption capacity of six saponins was also investigated.

2. Materials and methods

2.1. Materials

The dried rhizomes of *P. polyphylla* Smith var. *yunnanensis* were collected from Lijiang, Yunan Province, China, and identified by Prof. Gao (Tianjin University, China). RPS was purified by macroporous resin after extracting by 70% ethanol–water as previously reported (Man et al., 2009b). The main compounds were the saponins, and the structures were described in Fig. 1. The standard references of Paris-VII, Formosanin C, Dioscin and Polyphyllin D were purchased from National Institute for the Control of Pharmaceutical and Biological Products (purity >99%). The authentic standards of Chonglouoside H and Gracillin were prepared in our laboratory, and confirmed by ESI-MS, and ^{1}H, ^{13}C NMR (purity >98%, HPLC). Povidone K-30 (PK 30), poloxamer 407 (P 407), poloxamer 188 (P 188), mannitol, dextrin, polyethylene glycol 4000 (PEG 4000), polyethylene glycol 6000 (PEG 6000) and phosphatidyl choline were from Yunhong Pharmaceutical Excipients & Technology Co. Ltd. (Shanghai, China). HPLC-grade methanol and acetonitrile were purchased from Tedia (USA). The other reagents were commercially available and of analytical purity.

2.2. Preparation of solid dispersions and phytosome formulations

The effects of carriers including PK 30, P 407, P 188, mannitol, dextrin, PEG 4000 and PEG 6000 on the dissolutions of RPS were examined to prepare SD formulation. RPS and these excipients at the drug–carrier ratio of 1:5 were prepared using the solvent method. First, RPS and each excipient were dissolved in 90% ethanol under continuous stirring and heated at 60 °C in a water bath for 1 h until a homogeneous solution is formed, and then SD was prepared after all the solvents were removed under vacuum.

RPS and phosphatidyl choline at a weight ratio of 1:4 were placed in a 100 ml round-bottom flask and dissolved in anhydrous ethanol. The mixture was refluxed at 55 °C for 1 h. After evaporation of ethanol under vacuum, 20 ml of dichloromethane was added to it with continuous stirring. The RPS–phospholipid complex was gathered and dried. The resultant RPS–phospholipid complex (yield 90%, w/w) was kept in an amber colored glass bottle, flushed with nitrogen and stored at room temperature.

2.3. Physicochemical analysis

2.3.1. HPLC–UV analysis

For quantitative determination of the six steroidal saponins, all experiments were carried out by using a high speed liquid chromatography (HPLC) system (Agilent, USA) equipped with a quaternary pump with on-line degasser, and a VWD detector at 203 nm. The chromatographic separation was achieved at 35 °C on a Kromasil RP-C18 HPLC column (4.6 mm × 250 mm). Mobile phases A and B were water and acetonitrile, respectively. The operating condition for the gradient elution was as follows: 0–5 min, 33–37% B; 5–35 min, 37–38% B; 35–38 min, 38–47% B; 38–60 min, 47–45% B; 60–68 min, 45–50% B. The flow rate was 1 ml/min and the sample injection volume was 20 μl. A mixed standard stock solution was prepared with methanol, containing the following constituents: Paris-VII, Chonglouoside H, Formosanin C, Gracillin, Dioscin and Polyphyllin D. The standard solutions were obtained by diluting the mixed standard solution with methanol to a series of proper