European Journal of Pharmaceutics and Biopharmaceutics 99 (2016) 65–72

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research Paper

Dual strategies to improve oral bioavailability of oleanolic acid: Enhancing water-solubility, permeability and inhibiting cytochrome P450 isozymes

Qikun Jiang, Xiaoxu Yang, Ping Du, Huifen Zhang *, Tianhong Zhang *

Department of Pharmaceutical Analysis, Shenyang Pharmaceutical University, Shenyang, China

ARTICLE INFO

Article history:
Received 23 September 2015
Revised 8 November 2015
Accepted in revised form 18 November 2015
Available online 27 November 2015

Keywords:
Oleanolic acid
Phospholipid complex
Hydroxyapatite
Oral bioavailability
Cytochrome P450 isozymes

ABSTRACT

Oleanolic acid (OA) is a typical BCS IV drug with low water-solubility and poor permeability, metabolized by cytochrome P450 (CYP) isozymes in the intestinal tract, such as CYP3A. These are the reasons for the low oral bioavailability of OA which have restricted its wide application. In this study, a solidified phospholipid complex (OPCH) composed of OA–phospholipid complex (OPC) and hydroxyapatite (HA) was prepared by simple solvent evaporation. OPC was used to improve the liposolubility of OA, and HA was used to improve the flowability of OPC. Ketoconazole (KCZ, inhibitor of CYP3A) was co-administrated with OPCH to inhibit the metabolism of OA by CYP3A in the intestine. DSC, PXRD, SEM and IR analysis confirmed the formation of OPC and OPCH. Compared with the water-solubility and *n*-octanol solubility of OA, that of OPCH was increased nearly 15.3-fold and 3.19-fold, respectively. An in vitro dissolution study showed that the cumulative dissolution rate of OPCH was nearly 2.23-fold and 4.57-fold higher than that of OA and OPC at 2 h. Single-pass intestinal perfusion studies showed that the absorption of OA from OPCH was increased nearly 1.6–2.6-fold compared with that of pure OA and this was mainly due to the improved permeability and was further increased by OPCH with KCZ 1.2–2.4-fold compared with that of OPCH because KCZ inhibited metabolism of OA by CYP3A. A pharmacokinetic study of OPCH in rats following co-administration of KCZ was investigated. The C_{max} was increased markedly from 59.5 to 78.7 and 131.3 ng/mL in case of OA alone, OPCH alone and OPCH with KCZ. In parallel with the C_{max}, the AUC_{0-24h} was increased from 259.6 to 306.6 and 707.7 ng h/mL, respectively. All the results obtained demonstrated that formulation of OPCH and co-administration of KCZ significantly improved the bioavailability of OA by increasing the solubility and permeability in combination with inhibiting the metabolism of OA.

1. Introduction

Oleanolic acid (OA) is a pentacyclic triterpenoid, widely found in many Asian herbs, such as Fructus ligustri lucidi, Mile swertia, and Vitisvinifera [1]. OA has multiple pharmacological actions including hepatoprotective [2], anti-inflammatory [3], and antidiabetic effects [4]. OA is commercially available and has been used in clinical situations as an adjunct therapy for hepatitis for several decades [5]. Despite its promising biological effects, OA is poorly absorbed from the gastrointestinal (GI) tract, leading to low bioavailability after oral administration. It has been reported that OA has an absolute oral bioavailability of only 0.7% and this is because OA has very low aqueous solubility (20 °C, 4.61 mg/L) [6] and permeability (P_{app} = 1.1–1.3 × 10^{-6} cm/s in the apical-to-basolateral direction at 10 and 20 μM) [7].

At present, many researchers are focusing on increasing the water-solubility of OA to increase its bioavailability. Several methods have been attempted, including the preparation of a solid dispersion [8], a β-cyclodextrin compound [9], and different crystal forms of OA by spray drying [10]. Although the water-solubility

Abbreviations: OA, oleanolic acid; OPC, OA–phospholipid complex; HA, hydroxyapatite; OPCH, OA–phospholipid complex and hydroxyapatite; KCZ, ketoconazole; CYP, cytochrome P450; GI, gastrointestinal; PC, phospholipid; DDS, drug delivery system; SD, Sprague–Dawley; DSC, differential scanning calorimetric; PXRD, powder X-ray diffraction; SEM, scanning electron microscopy; IR, infrared spectroscopy; SDS, sodium dodecyl sulfate; SPIP, single-pass intestinal perfusion study; K_a, constant of drug absorption; P_{eff}, effective permeability; C_{max}, maximum concentration; T_{max}, maximum time; MTBE, Methyl Tertiary Butyl Ether; UPLC–MS/MS, ultra performance liquid chromatography–dual mass spectrometry; Log*P*, oil–water partition coefficient; SIR, selective ion reaction; α, angle of repose; *r*, diameter; *H*, height of the mount; g, gram; mg, milligram.

* Corresponding authors. Tel.: +86 24 23984159; fax: +86 24 23986321.
E-mail address: zhangth_student@aliyun.com (T. Zhang).

http://dx.doi.org/10.1016/j.ejpb.2015.11.013
0939-6411/

has been increased by the above methods, the poor permeability of OA has remained unchanged, and this is another major cause of its low bioavailability. Recently, phospholipid complex has been shown to have a positive effect on the limitations associated with drugs belonging to biopharmaceutics classification system IV (BCS IV) and their oral bioavailability [11,12].

Phospholipids (PCs) are biocompatible surfactants with high solubility in aqueous and oily media. In addition, PCs are important components of cell membranes, which make them highly compatible with the human physiological system and they can also penetrate cell membranes and enter the cytoplasm of living mammalian cells [13]. Most importantly, PCs have a protective effect against liver injury without any toxicity and mutability [14], which can improve the pharmacological functions of OA. In our study, we tried to prepare an OA–PC complex (OPC) in order to increase the permeability of OA. However, the prepared OPC was observed to be very sticky which was a disadvantage to increase the dissolution rate and for production on an industrial scale. Thus, suitable carriers are needed to solidify OPC in order to reduce its stickiness and to improve dissolution rate and industrialization.

Hydroxyapatite (HA) is a major component of hard tissues like bone and teeth [15] and HA has been used as a compatible and non-toxic material in a variety of applications, including bone repair and tissue engineering. In recent years, research into the use of HA as a drug delivery system (DDS) has attracted much attention because it offers several attractive advantages, such as good flowability, relatively large surface area, and high strength, as well as being chemically inert and highly biocompatible [16]. Furthermore, poorly water-soluble drugs are usually in an amorphous state and tend to have a higher solubility and dissolution rate when they are dispersed into HA, and this may significantly improve bioavailability [17]. With this in mind, we used HA as a carrier to solidify OPC, with the aim of creating free-flow solidified OPC with a high and rapid dissolution rate [18,19].

Regarding the poor bioavailability of OA, research has demonstrated that OA is metabolized by a variety of enzymes in the intestine and liver, especially by cytochrome P450 (CYP) isozymes, such as CYP3A. CYP3A is significantly involved in the metabolism of many different drugs given by the oral route. Moreover, its localization in high amounts in both the small intestinal epithelium and liver means that it mainly undergoes systemic elimination after oral drug administration [20]. There are many compounds that can inhibit these enzymes and several studies have shown that ketoconazole (KCZ) is a noncompetitive inhibitor of CYP3A enzymes [21]. It has been reported that KCZ, acting as an inhibitor of CYP 3A, can inhibit the metabolism of OA and it was found that it could markedly increase the bioavailability of OA after co-administration in Sprague–Dawley (SD) rats [22].

In light of the above information, a novel solidified powder of OPC–HA (OPCH) was prepared using the solvent evaporation method in our study. OPCH was characterized by a series of parameters including differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), scanning electron microscopy (SEM), infrared spectroscopy (IR), solubility, oil–water partition coefficient and dissolution profile. In addition, we also compared the intestinal absorption and pharmacokinetics of OA alone, OPCH alone and OPCH in combination with KCZ in SD rats.

2. Materials and methods

2.1. Materials

OA (purity 98.0%) was purchased from Shikun Sichuan Pharmaceutical Co., Ltd. Lipoid E200 (trade name PC, purity 80%) was purchased from Shanghai Advanced Vehicle Technology Co., Ltd. Glycyrrhetinic acid was used as an internal standard and purchased from National Institute for the Control of Pharmaceutical and Biological Products. HA was supplied by Shanghai Yan Tuo Biological Technology Co., Ltd. Ammonium acetate (chromatographic grade), methanol, and phosphoric acid were supplied by Concord Technology (Tianjin) Co., Ltd. Sodium dodecyl sulfate was purchased by Fengli Technology (Beijing) Co., Ltd. And KCZ was obtained from Jiangsu EnHua Pharmaceutical Co., Ltd.

2.2. Preparation of solidified OPC by the solvent-evaporation method

OA and PC at a molar ratio of 1:1 (account to 1:2, w/w) were placed in a 100 mL round bottom flask and dissolved in 20 mL anhydrous ethanol. The mixture was then refluxed at 60 °C for 2 h in accordance with the conditions under which our research group prepared OPC [23]. HA in different mass ratios (weighed ratios of OPC:HA = 1:3; 1:5; 1:7; 1:9, w/w) was mixed with the above solution and constantly stirred for 2 h and then the anhydrous ethanol was removed by a rotary evaporator at 40 °C. The dried residues were placed in desiccators for 12 h, then passed through a 100 mesh sieve and stored at room temperature. Based on the dissolution rate and flowability results, we were able to determine the optimal proportion of OPC and HA.

2.3. Flowability of OPCH

The angle of repose (α) is an important parameter for evaluating the flowability which was determined by the funnel method [24]. For this, 2.0 g OPCH was allowed to flow slowly from two successive funnels into a flat Petri dish with a known diameter (r). The height of the samples (H) formed on the dish was measured and the α was calculated on the basis of the following formula:

$$\alpha = \arctan\frac{H}{r}$$

2.4. Characterization of OPCH

2.4.1. DSC

DSC curves were obtained using a Mettler DSC 30S (Mettler Toledo, Switzerland). The samples including pure OA, PC, OPC, HA, OPCH, a physical mixture of OA, PC and HA were sealed into aluminum pans separately and heated from 40 to 360 °C at a heating rate of 10 °C/min.

2.4.2. PXRD

PXRD patterns of pure OA, PC, OPC, HA, OPCH, a physical mixture of OA, PC and HA were measured on a D/max-r A (Rigaku Denki, Japan) equipped with a Cu Kα radiation source at a voltage of 45 kV and a current of 30 mA in the region $5° \leqslant 2\theta \leqslant 45°$.

2.4.3. SEM

The morphology of pure OA, PC, OPC, HA, OPCH, a physical mixture of OA, PC and HA was investigated by SEM (SU 8000, Hitachi, Japan). In brief, samples were fixed on an aluminum stub with conductive double-sided-adhesive tape and then coated with gold. The surface morphology of the samples was then examined.

2.4.4. IR

The infrared spectra of OA, PC, OPC, a physical mixture of OA, PC and HA were obtained by IR Spectrometry (Avatar 360, Nicolet, USA). The samples were scanned over the range 4000–400 cm^{-1}.