International Journal of Pharmaceutics 479 (2015) 349–356

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical nanotechnology

Dramatic improvement of the solubility of pseudolaric acid B by cyclodextrin complexation: Preparation, characterization and validation

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ARTICLE INFO

Article history:
Received 8 October 2014
Received in revised form 29 December 2014
Accepted 5 January 2015
Available online 6 January 2015

Keywords:
Cyclodextrins (CDs)
Pseudolaric acid B (PAB)
Solubilization
Molecular simulation
Validation

ABSTRACT

As one of the most important technologies to improve the solubility of poorly water-soluble drugs, the solubilization effects of cyclodextrins (CDs) complexation are, on occasions, not as large as expected, which tends to detract from the wider application of CDs. In this study, a dramatic improvement of the solubility of pseudolaric acid B (PAB) by CDs has been found with a 600 fold increase by HP-β-CD complexation. In addition, the solubility enhancement of PAB by various CDs, including α-CD, β-CD, γ-CD, HP-β-CD and SBE-β-CD was investigated by phase solubility studies. The inclusion complex of PAB/HP-β-CD was prepared by different methods and characterized by differential scanning calorimetry (DSC), powder X-ray diffractometry (XRD), nuclear magnetic resonance spectroscopy (^{1}H NMR) together with molecular simulation. The results indicated that the solubility of PAB was increased to 15.78 mg mL^{-1} in the presence of 30% HP-β-CD, which is a 600 fold increase compared with that in pure water. And the chemical stability of PAB in PBS (pH 7.4) can be enhanced. The results of DSC and XRD showed the absence of crystallinity in the PAB/HP-β-CD inclusion complex prepared by the saturated water solution method. The results of ^{1}H NMR together with molecular simulation indicated the conjugated diene side-chain of PAB was included into the cavity of HP-β-CD, with the free energy of -20.34 ± 4.69 kJ mol^{-1}. While the enzymatic degradation site of the carboxyl polar bond is located in the hydrophilic outer of HP-β-CD resulted in no significant difference for the enzymatic degradation rate between PAB and PAB/HP-β-CD complexes in rat plasma. In summary, the PAB/HP-β-CD inclusion complex prepared in this study can greatly improve the solubility and chemical stability of PAB, which will result in the *in vivo* administration of PAB as a liquid solution.

1. Introduction

The aqueous solubility of molecules is one of the key determinants in developing new chemical entities into successful medicines. However, modern drug discovery technologies, such as combinatorial chemistry and high throughput screening, are usually based on the basic principles of medicinal chemistry, considering that the most efficient method to improve the potency of compounds is to add a lipophilic moiety at an appropriate position of the lead structure. This has led to an increase in the number of lipophilic and poorly soluble molecules being investigated for their therapeutic activity (Lipinski, 2000).

Therefore, various formulation techniques are applied to enhance the aqueous solubility of poorly water-soluble drugs, including formulation of amorphous solid forms, nanoparticles, microemulsions, solid dispersions, melt extrusion, salt formation and formation of water-soluble complexes (Murtaza, 2012; Kawabata et al., 2011). Cyclodextrin (CDs) complexation is one

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http://dx.doi.org/10.1016/j.ijpharm.2015.01.005
0378-5173/

of the technologies used to improve the solubility of poorly water-soluble drugs. The most remarkable property of CDs is their ability to modify the physicochemical characteristics of molecules that are accommodated within the internal cavity of CDs to form the so-called inclusion complexes (Carrier et al., 2007; Loftsson et al., 2005; Rao and Stella, 2003).

However, the solubilization effects of CDs complexation are limited for many drugs as evidenced by the fact that there were only about thirty products marketed since the first CD-containing sublingual tablet (Prostarmon E^{TM}, prostaglandin E2/βCD) launched in Japan in 1976 (Brewster and Loftsson, 2007). In this publication, the solubility enhancement of 59 drugs have been summarized according to the relevant literature (Brewster and Loftsson, 2007; Carrier et al., 2007; Loftsson and Brewster, 2012) (Fig. 1), among which 50 drugs were less than 100 fold enhanced (about 85%) and 32 drugs less than 20 fold enhanced (about 54%). Therefore, for poorly water-soluble drugs with aqueous solubility $<100\ \mu g\ mL^{-1}$, the apparent solubility of drugs after inclusion by CDs with a solubility enhancement of less than 100 fold is not sufficient for pharmaceutical injections (usually at least 5–10 mg/mL) (http://www.fda.gov/Drugs/default.htm). This is due to to the fact that the desirable solubilization effect can only be achieved when the guest molecules fit completely into the CD cavity, or the hydrophobic groups in the drug molecules fit within the CD cavity.

In this study, a particularly effective improvement of solubility of pseudolaric acid B (PAB) by cyclodextrin complexation has been found, with the solubility increase of about 600 fold by HP-β-CD. PAB is the most active constituent extracted from the root bark of *Pseudolarix kaempferi* Gordon tree (Pinaceae) (Trost et al., 2007). It displays multiple biological and pharmacological activities including antifungal (Li et al., 1995; Yang et al., 2003), antifertility (Wang et al., 1982) and antitumor properties in various tumor lines (U87 cells, L929 cells, MDA-MB-468 cells and MCF-7 cells, *etc.*) through anti-angiogenesis (Li et al., 2004; Tan et al., 2004) and inducing apoptosis (Khan et al., 2012; Sarkar et al., 2012; Wong et al., 2005; Yu et al., 2013). For the stability of PAB, there is no report about the investigation of its chemical stability and just some indications can be found in two references. One is about the extraction, a pure PAB compound has been obtained after the H_2O-soluble potassium and sodium salts of PAB powder acidified with diluted hydrochloric acid and extracted with $CHCl_3$ (Li et al., 1995). Another one is about the bioactivity studies (Chiu et al., 2010), in which PAB as well as its monopotassium and monosodium salts are active in antimicrobial activity. Therefore, PAB is stable in acidic solution and alkaline salt solution. However, PAB is quickly metabolized into pseudolaric acid C_2 (PC_2), once absorbed into blood almost no PAB could be detected in rat blood (Liu et al., 2011). PAB is poorly soluble in water with solubility of 26.25 $\mu g\ mL^{-1}$ at pH 5.24 and 25 °C. Therefore, solubility enhancement of PAB is essential for parenteral delivery of this drug.

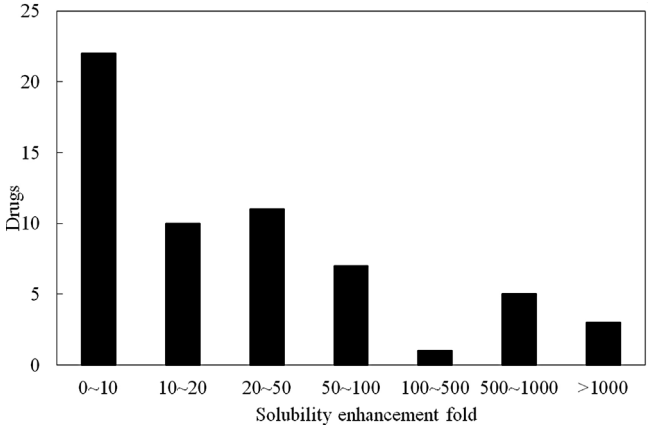

Fig. 1. Summary of the solubility enhancement folds for 59 drugs (Brewster and Loftsson, 2007; Carrier et al., 2007; Loftsson and Brewster, 2012).

From a literature search, it is apparent that there is no report available about the solubilization of PAB by CDs. In this study, pre-formulation studies including the solubility, chemical stability at different temperature and pH were carried out to assess the suitable conditions to prepare PAB/CDs complexes. Then, the solubilization effects of different CDs (α-CD, β-CD, γ-CD, HP-β-CD and SBE-β-CD) on PAB were investigated. Furthermore, the complex composed of PAB and optimized CD was prepared and characterized by various methods, including differential scanning calorimetry (DSC), powder X-ray diffractometry (XRD), nuclear magnetic resonance spectroscopy (^{1}H NMR). Molecular simulation was employed to elucidate the formation mechanism of the inclusion complex. In addition, *in vitro* stability of PAB in porcine esterase, rat plasma and human plasma were performed to verify whether or not the methyl ester group in the molecular structure of PAB had been included into the cavity of HP-β-CD.

2. Materials and experimental methods

2.1. Materials

PAB (purity > 98%) was obtained from Anhui University of Chinese Medicine (Anhui, China). Ketoprofen was obtained from Shanghai Hanhong Chemical Co., Ltd. (Shanghai, China) and used as internal standard (IS) for the degradation experiment. The porcine esterase was the esterase from porcine liver (PLCE, ≥15 U/mL, Sigma). Rat blank plasma was obtained from Fudan University (Shanghai, China). Human blank blood was supplied by the Shanghai Institute of Materia Medica, Chinese Academy of Sciences (Shanghai, China). Hydroxypropyl-β-cyclodextrin (HP-β-CD, degree of substitution = 4.5) was obtained from Shijiazhuang Pharmaceutical Group Co., Ltd. (Shijiazhuang, Hebei, China). The α-cyclodextrin (α-CD), β-cyclodextrin (β-CD), γ-cyclodextrin (γ-CD) and sulfobutylether-β-cyclodextrin (SBE-β-CD) were obtained from China Kunshan Tusk Chemical Co., Ltd. (Kunshan, Jiangsu, China). Acetic acid and methanol were HPLC grade from Merck Co., Ltd. (Germany). The propylene glycol was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Water used for solutions was purified by Milli-Q system (Millipore). Other reagents were of analytical grade.

2.2. Experimental methods

2.2.1. HPLC analysis

Quantitative analysis of PAB was performed using an HPLC method. The HPLC instrument (Agilent, Palo Alto, USA) consisted of a binary pump (G1311C), an autosampler (G1329B) and a diode-array detector (G4212B). The separation was achieved on the Apollo C18 analytical column (150 mm × 4.6 mm i.d., 5 μm; Grace, USA) equipped with an EasyGuard II C18 guard column (Grace, USA) maintained at 40 °C. The mobile phase consisted of methanol: 0.3% glacial acetic acid aqueous solution (53:47, v/v) and the flow rate set to 1.0 mL min^{-1}. The detection wavelength of the detector was set to 260 nm.

2.2.2. Solubility studies of PAB

The solubility of PAB in different media at different pH values was investigated. Excess amounts of PAB were dispersed in different media (5 mL), including distilled water, 5% $NaHCO_3$ solution, 0.1 mol L^{-1} hydrochloric acid (HCl) solution (pH 1.03) and phosphate buffer solution (pH of 2.52, 5.05, 6.76, 7.38),