International Journal of Pharmaceutics 358 (2008) 198–204

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Enhancing effect of Labrafac Lipophile WL 1349 on oral bioavailability of hydroxysafflor yellow A in rats

Shujing Wang, Minjie Sun, Qineng Ping*

China Pharmaceutical University, Tong Jia Xiang No. 24, Nanjing, Jiangsu 210009, China

ARTICLE INFO

Article history:
Received 25 October 2007
Received in revised form 25 February 2008
Accepted 7 March 2008
Available online 13 March 2008

Keywords:
Hydroxysafflor yellow A
Labrafac Lipophile WL 1349
Bioavailability
Absorption enhancing mechanism

ABSTRACT

The objective of the present investigation was to clarify the mechanism by which Labrafac Lipophile WL 1349 (WL 1349) enhanced the oral bioavailability (BA) of hydroxysafflor yellow A (HSYA), the representative low permeable hydrophilic (biopharmaceutic classification system (BCS) Class III) drug. HSYA–phospholipid complex was prepared, and dissolved into WL 1349 with a certain surfactant to form a stable oil solution. Oral administration of HSYA aqueous solution at a dosage of 4.5 mg/kg resulted a low plasma HSYA concentration with C_{max} and $AUC_{0-8\,h}$ values of 0.105 μg/ml and 10.29 μg min/ml, respectively. HSYA–phospholipid complex oil solution with the same administration and dosage increased the plasma HSYA concentration significantly with C_{max} and $AUC_{0-8\,h}$ values of 2.063 μg/ml and 381.145 μg min/ml, respectively. The results showed that WL 1349 could improve oral absorption of HSYA remarkably. Bioavailability investigations were performed to show WL 1349 dosage independent from HSYA absorption within the dosage from 1 ml/kg to 9 ml/kg. The test of bile duct ligation in rats showed that the oil solution containing WL 1349 did not result in detectable plasma HSYA concentration, but HSYA aqueous solution had the same $AUC_{0-8\,h}$ as the bile duct was not ligated. The in vitro lipolysis experiments of WL 1349 showed that WL 1349 was emulsified by deoxycholate, and then was hydrolyzed to fatty acids and monoglycerides by pancreatic lipase rapidly. The lipolysis products of WL 1349, caprylic acid, capric acid and caprylic and capric acid monoglycerides all improved the BA of HSYA in vivo. The results above indicated the emulsifying by bile, and hydrolysis to fatty acids and monoglycerides by pancreatic lipase was one of the enhancing mechanisms of HSYA–phospholipid complex oil solution absorption.

1. Introduction

Many hydrophilic drugs such as bisphosphonate drugs, proteins, peptides, and peptide-like drugs are poorly absorbed from the gastrointestinal tract (GIT), among the various classes of biopharmaceutic classification system (BCS), peroral delivery of these Class III drugs is partially or completely hindered due to their poor intestinal permeability (Legen et al., 2005). Many drug molecules show poor permeability because of their unfavorable physicochemical and chemical features, which are difficult to change, thus an external excipient may be added to increase permeation transiently. It has been reported that association of hydrophilic drugs with oil carriers in the absence of aqueous phase can protect against intestinal proteases, improve stability and change cell permeability, and may improve absorption in GIT (New and Kirby, 1997). In present study, absorption enhancement of representative low permeable hydrophilic model drug of BCS class III, hydroxysafflor yellow A (HSYA), was investigated in bioavailability (BA) test by the preparation of HSYA–phospholipid complex oil solution.

The flower of the safflower plant, *Carthamus tinctorius* L., has been widely used in traditional Chinese medicine for treatment of cerebrovascular and cardiovascular diseases. The extracts of the flower contain yellow and red pigments including hydroxysafflor yellow A, safflor yellow B, safflomin A, safflomin C, and other chemicals (Liu et al., 2004, 2006). HSYA, the main chemical component of the safflower yellow pigments, has been demonstrated to antagonize platelet activating factor receptor binding and thus used to treat some dysaemia diseases, such as myocardial ischemia, cerebral ischemia, coronary heart disease, and cerebral thrombosis (Wang et al., 2006). HSYA is highly soluble in water (its water solubility is about 0.28 mg/ml, 25 °C) but slightly soluble in oil (Zhang et al., 2006), having very poor intestinal membrane permeability resulting in low oral BA. There is only injection of HSYA which has been reported (Shi, 2004; Wei et al., 2005) and no oral dosage forms have been mentioned by far. Labrafac Lipophile WL 1349 (WL 1349), caprylic and capric acid triglycerides, one of the medium chain triglycerides (MCT), have been used as oil system in emulsions (Devani et al., 2005). In the literature, MCT preferred in

* Corresponding author. Tel.: +86 25 83271098; fax: +86 25 83301606.
E-mail addresses: pingqn@cpu.edu.cn, wsj82101@163.com (Q. Ping).

0378-5173/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2008.03.006

lipid-based formulations was used to enhance the bioavailability of poorly water-soluble drug (Grove et al., 2006, 2007; Dahan and Hoffman, 2007; Hauss, 2007), however, the effect of water-soluble drug has not been studied, meanwhile, the absorption enhancing mechanism of MCT has not been studied clearly either. It has been reported that phospholipids complex can increase the lipophilicify of lipophilic drug (Yanyu et al., 2006), but the hydrophilic drug–phospholipid complex has not been studied. In this study, HSYA–phospholipid complex was prepared to increase its lipophilicify, and dissolved into WL 1349 with a certain surfactant to form a stable oil solution with the purpose of enhancing the oral BA of HSYA, and the possible enhancing mechanism of WL 1349 to HSYA was studied.

2. Materials and methods

2.1. Materials

WL 1349 was a gift from Gattefosse Corp., HSYA was purchased from Chang-sha-ke-luo-ma Medicine Technique Ltd., purity >90%, and phospholipids was purchased from Tai-wei-yao-ye Ltd., containing approximately 75–80% (w/w) of soybean phospholipids. The other chemical reagents were of analytical grade or better and were used as received.

2.2. Animals

Sprague–Dawley rats, male, weighing 200–250 g were fed standard laboratory chow and had free access to water. Food was withheld from the rats for 12 h before they used in BA and absorption enhancing mechanism experiments.

2.3. Preparations of HSYA oil solution

The required amount of HSYA and phospholipids (the weight ratio was 1:3) were placed in a 100 ml round-bottom flask and dissolved in tetrahydrofuran. After tetrahydrofuran was evaporated under vacuum at 40 °C, the dried residues of HSYA–phospholipid complex were collected and placed in the desiccator overnight, then stored in room temperature.

The sufficient quantum of HSYA–phospholipid complex was added into a required amount of WL 1349 with Span-80, which was taken as stabilizer and emulsifier. The oil solution was vibrated at 60 °C until it became clear, and then cooled to room temperature, and the HSYA–phospholipid complex oil solution was obtained.

2.4. Solubility determination of HSYA in WL 1349

Solubility determination of HSYA and phospholipids complex in WL 1349 was carried out by adding excessive HSYA, the physical mixture of HSYA and phospholipids or phospholipids complex to 5 ml WL 1349 in sealed glass containers. The liquid were vibrated for 24 h at 25 °C, and centrifuged to remove excessive HSYA (12,000 rpm, 10 min). 1 ml of the supernatant was mixed with 9 ml of methanol, 20 μl of the resulting solution was injected into a HPLC system. The stationary phase, C_{18} column (4.6 mm × 150 mm, 5 μm), was kept at 40 °C. The mobile phase was consisted of methanol:acetonitrile:0.05 M KH_2PO_4 solution = 25:2:73. The flow rate was 0.8 ml/min. The effluent was monitored at 403 nm.

2.5. In vitro lipolysis experiments of WL 1349

0.1 g of WL 1349 was added to 5 ml medium containing 5 mM deoxycholate and 1.25 mM L-α-phosphatidylcholine was continuously stirred gently (100 rpm) and heated (37 °C). Fresh pancreatin extract was prepared by adding 1 g of porcine pancreatin lipase to 5 ml distilled water, stirring for 15 min followed by centrifugation. About 3.5 ml of the pancreatin extract (1000 IU/ml) was added into the medium and stirred for 30 min and initiated the enzymatic digestion of the formulation. During the lipolysis process of WL 1349, free fatty acids were liberated and consequently the pH decreases. The enzymatic digestion process was observed by acid–base titration taking phenolphthalein as indicator, 0.05 M NaOH as titrant, and the solution turning red as the end point of titration.

2.6. BA studies in rats

2.6.1. Chromatography

The plasma concentrations of HSYA were determined by HPLC. A mobile phase consisting of methanol:acetonitrile:0.05 M KH_2PO_4 = 25:2:73 was pumped through the C_{18} column (4.6 mm × 150 mm, 5 μm) kept at 40 °C at a flow rate of 0.8 ml/min. The effluent was monitored at 403 nm.

2.6.2. Preparation of plasma samples and validity

The rats were anaesthetized with aether, and 300–400 μl blood was taken from the eyeground vein. The plasma was obtained after centrifugation (4000 rpm, 10 min) and stored at −20 °C before being analyzed.

100 μl of 6% perchloric acid was added to100 μl of the thawed plasma. The mixture was shaken for 3 min before centrifugation (12,000 rpm, 10 min). 20 μl of the supernatant was injected for HPLC analysis.

This method was validated by adding various quantities of HSYA to blank plasma of rats. The concentrations of HSYA were 0.03 μg/ml, 0.075 μg/ml, 0.3 μg/ml, 0.75 μg/ml, 1.5 μg/ml, and 3.0 μg/ml. The calibration was critical to the entire analytical procedure in testing the linearity, precision, and accuracy of the method.

2.6.3. Pharmacokinetic of HSYA in rats

The sufficient quantum of HSYA and the phospholipids complex were both diluted, by distilled water into 0.45 mg/ml (solution A) and 1.8 mg/ml (solution B, the concentration of HSYA equals to 0.45 mg/ml). In HSYA–phospholipid complex oil solution, the concentration of phospholipids complex in WL 1349 was 6 mg/ml (solution C, the concentration of HSYA equals to 1.5 mg/ml).

Pharmacokinetics studies were carried out after oral administration of the HSYA aqueous solution, HSYA–phospholipid complex aqueous solution and HSYA–phospholipid complex oil solution to rats. Eighteen male rats (body weight 200–250 g) divided randomly into three groups were fasted for 12 h with free access to water. The three groups of rats received each of the test preparations with the HSYA dosage of 4.5 mg/kg. All experiments were carried out at the same time of the day to exclude the influences by circadian rhythm. At 15 min before administration, a control blood sample (300–400 μl) was taken from the eyeground vein. After oral administration of the test solutions, 300–400 μl blood sample were collected from the eyeground vein at 8 min, 15 min, 30 min, 60 min, 90 min, 120 min, 180 min, 240 min, 300 min, 360 min, and 480 min. The plasma samples were detected according to the method in Section 2.6.2.

2.7. The influence of the dosage of WL 1349 on the absorption of HSYA

The HSYA–phospholipid complex oil solutions with different concentrations of HSYA, 0.5 mg/ml, 1.5 mg/ml, and 4.5 mg/ml, were prepared. Eighteen male rats (body weight 200–250 g) divided randomly into three groups, and each group was orally administered of HSYA–phospholipid complex oil solutions as above, with the HSYA dosage of 4.5 mg/kg and the WL 1349 dosages of 1 ml/kg, 3 ml/kg,