Drug Development and Industrial Pharmacy, 34:164–170, 2008

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040701484668

informa
healthcare

Assessment of Intestinal Absorption of Vitexin-2″-*O*-Rhamnoside in Hawthorn Leaves Flavonoids in Rat Using In Situ and In Vitro Absorption Models

Ying-ai Xu, Guorong Fan, Shen Gao, and Zhanying Hong

Shanghai Key Laboratory for Pharmaceutical Metabolite Research, School of Pharmacy, Second Military Medical University, China

The purpose of this study was to investigate the absorption mechanism of vitexin-2″-*O*-rhamnoside, the index component in hawthorn leaves flavonoids (HLF) in the rat intestine, using two different absorption models, the in situ single-pass intestinal perfusion and the in vitro everted gut sac model. The effective permeability coefficients (P_{eff}) in the in situ single-pass intestinal perfusion experiments and the apparent permeability coefficients (P_{app}) in the in vitro everted gut sac experiments were calculated. Furthermore, the influences of the P-glycoprotein inhibitors, verapamil and digoxin, on the intestinal absorption of vitexin-2″-*O*-rhamnoside in HLF were studied using the above-mentioned two models. Results showed that there were no significant differences in the absorption of vitexin-2″-*O*-rhamnoside in HLF in four segments of the rat intestine, duodenum, jejunum, ileum, and colon, and at different concentrations of HLF ranging from 0.05 mg/ml to 0.5 mg/ml ($P > 0.05$). The P_{eff} values for vitexin-2″-*O*-rhamnoside in the rat jejunal perfusion at the concentration of 0.05, 0.1, 0.25, and 0.5 mg/ml were $(2.48 \pm 0.33) \times 10^{-5}$; $(2.23 \pm 0.67) \times 10^{-5}$; $(2.18 \pm 0.48) \times 10^{-5}$; and $(2.25 \pm 0.17) \times 10^{-5}$ cm/s, respectively. But there was significant difference between absence and presence of verapamil or digoxin ($P < 0.05$). P_{eff} and P_{app} values of vitexin-2″-*O*-rhamnoside in HLF were increased in the presence of verapamil or digoxin. In conclusion, vitexin-2″-*O*-rhamnoside can be classified into high permeability class drug according to the biopharmaceutical classification system. Passive diffusion dominates the absorptive transport behavior of vitexin-2″-*O*-rhamnoside in HLF. The absorption and secretion are mediated by the efflux transport system, P-gp. The absorption of vitexin-2″-*O*-rhamnoside in HLF can be enhanced administered together with P-gp inhibitors.

Keywords hawthorn leaves flavonoids; vitexin-2″-*O*-rhamnoside; intestinal absorption; single-pass perfusion; everted gut sac; P-glycoprotein

Address correspondence to Guorong Fan, School of Pharmacy, Second Military Medical University, 325 Guohe Road, Shanghai 200433, P. R., China. E-mail: guorfan@yahoo.com.cn

INTRODUCTION

Crataegus (hawthorn) is a medicinal plant that has been widely used for a long time. It has numerous mild but well-documented pharmacological activities, especially in the cardiovascular system, with few side effects (Ammon & Händel, 1981a, 1981b) In China, there are about 16 species of hawthorn. *Crataegus pinnatifida* Bge. is one of the two major species, called Shanzha in Chinese. The extract of hawthorn leaves is reported to improve the heart function deficiency of the coronary blood supply and mild forms of arrhythmias (Christian, Wilson, & Thomas, 2000; Tauchert, 2002). Hawthorn leaf flavonoids (HLF) are the main active constituent of hawthorn leaves, in which more than 30 components were isolated, including vitexin, vitexin-2″-*O*-rhamnoside, quercetin, rutin, and hyperoside, and so on. (Ding, Jiang, Zhong, & Zuo, 1990). The content of vitexin-2″-*O*-rhamnoside is the highest in HLF (Ying, Lu, Li, & Li, 2007).

HLF preparations are marketed mainly as tablets and capsules in China. For these oral drug delivery formulations, it is required to characterize the intestinal absorption, including the transport mechanisms and the transport rate. So studying the intestinal absorption of HLF is warranted. In vitro and in situ absorption models, such as Caco-2 cell monolayers, everted gut sacs, and perfused animal intestine, are commonly used to investigate transport mechanisms, classify permeability, and predict in vivo absorption of drugs in humans (Amidon, Lennernäs, Shah, & Crison, 1995; Lennernäs, Nylander, & Ungell, 1997). It has been reported previously that these models are well correlated to the in vivo permeability and fraction of the dose absorbed in humans, and therefore have a proper predictive value (Fagerholm, Johansson, & Lennernäs, 1996; Lennernäs, Nylander, & Ungell, 1997; Lennernäs, Palm, Fagerholm, & Artursson, 1996). P-glycoprotein (P-gp), a 170 kDa transmembrane protein, is an ATP-dependent efflux membrane transporter apically expressed throughout the gastrointestinal tract and acting as major biochemical barrier to oral bioavailability. P-gp

also plays a critical role in the distribution, metabolism, and elimination of many clinically important therapeutic substrates (Varma, Ashokra, Dey, & Panchagnula, 2003). Even if passive transcellular diffusion is the dominant transport mechanism for intestinal absorption of many drugs, it has been reported that carrier-mediated efflux transport by P-glycoprotein (P-gp) and other efflux proteins may affect the overall rate and extent of drug absorption and first-pass metabolism in rat and human intestines (Gan et al., 1996; Kruijtzer et al., 2002; Lown et al., 1997; Terao, Hisanaga, Sai, Tamai, & Tsuji, 1996; Zhang & Benet, 2001). An understanding of the physiologic regulation of P-gp is a key to design strategies for the improvement of therapeutic efficacy of these drugs. Verapamil and digoxin are P-gp substrates that have been increasingly used for understanding mechanistic aspects of intestinal P-gp (Berggren, Hoogstraate, Fagerholm, & Lennernäs, 2004; Varma, Kapoor, Sarkar, & Panchagnula, 2004; Varma, Sarkar, Kapoor, & Panchagnula, 2005).

The main advantage of the in situ single-pass intestinal perfusion technique is the presence of an intact blood and nerve supply in the experimental animals. This methodology is found to be simple and highly accurate for predicting intestinal absorption in humans (Fagerholm, Johansson, & Lennernäs, 1996). Because water absorption and secretion during the perfusion may cause errors in the calculated P_{eff} values, a non-absorbable marker to correct water flux is needed (Amidon et al., 1995). For this purpose, phenol red is co-perfused with drug compounds. The everted gut sac technique is a simple and useful in vitro model to study drug transport (Barthe, Woodley, & Houin, 1999).

To develop a theoretical basis for understanding the oral absorption of the main component, vitexin-2″-*O*-rhamnoside (Figure 1) in HLF, characterization of its intestinal transport mechanism and contribution of putative secretory/efflux is imperative. In this study, we used the above-mentioned two intestinal absorption models in rats, in situ single-pass intestinal perfusion model and in vitro everted gut sac model, to investigate and characterize the intestinal absorption of vitexin-2″-*O*-rhamnoside in HLF in different intestinal segments, at different concentrations and P-gp–mediated intestinal transport.

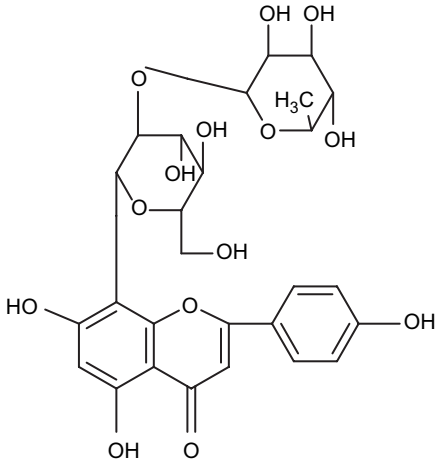

FIGURE 1. Chemical structure of vitexin-2″-*O*-rhamnoside.

MATERIALS AND METHODS

Drugs and Materials

HLF was purchased from Jinjia Pharmacy Co. Ltd. (Shanxi, China). Digoxin and vitexin-2″-*O*-rhamnoside were obtained from Sigma (St. Louis, Missouri, United States). Verapamil was obtained from the National Control Office of Pharmaceutical and Biological Products (Beijing, China). High-performance liquid chromatography (HPLC) grade solvents such as glacial acetic acid and tetrahydrofuran methanol, acetonitrile were obtained from Caldon Laboratories Ltd. (Ont., Canada). Phenol red was purchased from Sanaisi Reagents Co. Ltd. (Shanghai, China). All other chemicals were of analytical grade and were used as such.

Single-Pass Perfusion Experiments

Sprague-Dawley rats of both sexes weighing 230 to 250 g were obtained from Slac Laboratory Animal Co., Ltd. (Shanghai, China). The surgical procedure and the jejunal perfusion of a 10 cm isolated intestinal segment were performed as previously described (Lindahl, Sandström, Ungell, & Lennernäs, 1998). In brief, the animals fasted for 16 to 18 hours but with free access to water, and were anaesthetized 1 hour before the experiment by an intraperitoneal injection of ethyl carbamate (20%, w/v) at a dose of 0.6 ml/100 g body weight. The rats were placed under a lamp to maintain a body temperature of 37 ± 1 °C . The abdomen was opened by a midline longitudinal incision; a segment of the jejunum (10 cm) was cannulated with plastic tubing (4 mm o.d.) and perfused (0.2 ml/min) for 90 minutes. The effective jejunal permeability (P_{eff}) of vitexin-2″-*O*-rhamnoside in HLF was determined at concentrations of 0.05, 0.1, 0.25, and 0.5 mg/ml in separate experiments (n = 4). Each perfusion experiment lasted for 90 minutes and the outlet perfusate was quantitatively collected on ice at steady state at 40, 50, 60, 70, 80, and 90 minutes. The intestinal topographic study of vitexin-2″-*O*-rhamnoside in HLF at the concentration of 0.25 mg/ml was also performed in four separate groups (n = 4). The intestine was divided into four fractions of equal lengths (10 cm): the duodenum segment (1 cm from the pylorus), the jejunum segment (15 cm from the pylorus), the ileum segment (20 cm above the cecum) and the colon segment (from the end of the cecum). The efflux substrates/inhibitors, verapamil or digoxin, were added at a concentration of 0.05 mM at the concentration of 0.1 mg/ml of HLF. Both the control and inhibition perfusion experiments were performed in separate groups (n = 4). The samples were immediately frozen and stored at –20 °C until analysis.

The perfusion solution (pH 7.4) consisted of 48 mM NaCl, 5.4 mM KCl, 2.8 mM Na_2HPO_4, 4mM NaH_2PO_4 and 1 g/l D-glucose, 20 μg/ml phenol red, and HLF in the absence and presence of verapamil or digoxin as a P-gp inhibitor. Phenol red was included as a marker for transmucosal water flux. The current animal study was approved by the animal ethics committee in Second Military Medical University, Shanghai.

Intestinal Everted-Sac Experiments

Sprague-Dawley rats of both sexes weighing 230 to 250 g, provided by Shanghai Slac Laboratory Animal Co., Ltd. (China), were anesthetized by an intraperitoneal injection of ethyl carbamate (20%, w/v) at a dose of 0.6 ml/100 g body weight. A 20 cm segment of jejunum was quickly removed, rinsed with Tyrode buffer and everted (Barthe et al., 1999; Guo et al., 2004). This segment was tied at one end with a cotton thread, filled with oxygenated Tyrode buffer, and then tied at the other end. The resultant large sac was divided into four 3 to 4 cm sacs by tying at intervals. Each sac contained about 0.5 ml oxygenated Tyrode buffer.

Each sac was individually placed in a 15 cm high glass tube containing 2 ml oxygenated Tyrode buffer kept in a 37 °C water bath. After equilibration at 37 °C for 5 minutes, 2 ml of drug solution was added to each tube to initiate the experiment. The whole samples of inside medium were taken at 20, 30, 45, 60, 75, and 90 minutes, then fresh oxygenated Tyrode buffer was added to the sacs and incubation was continued. At the end of the 90-minute experiment, the everted sacs were removed from the tube. The area of each sac was measured. Different concentrations of HLF were tested. In addition, the effect of verapamil and digoxin on the absorption of vitexin-2″-*O*-rhamnoside in HLF was investigated. All the samples collected were extracted by mixing with methanol. The mixtures were centrifuged at 6,000 rpm for 10 minutes and the supernatants were analyzed by HPLC. The current animal study was approved by the animal ethics committee in Second Military Medical University, Shanghai.

Analytical Methods

Samples were assayed for vitexin-2″-*O*-rhamnoside content by HPLC. The Dionex HPLC system (Dionex, Sunnyvale, California, United States) was used including P680 pump, thermostatted column compartment, and PDA-100 photodiode array detector. Chromeleon software (Version 6.50) was used for evaluation and quantification. Vitexin-2″-*O*-rhamnoside and phenol red were all quantified with reversed phase HPLC using a Diamonsil-C_{18} column (5 μm, 4.6 mm × 200 mm) (Dikma, Beijing, China) preceded by a C_{18} guard column (Dikma, Beijing, China). The mobile phase consisted of 80% solvent A (tetrahydrofuran-methanol-acetonitrile [80:8:12, v:v:v]) and 20% solvent B (2.5% acetic acid). The samples were thawed, mixed, and centrifuged at 5,000 rpm for 10 minutes, whereupon 20 μl was injected into the column at a flow rate of 1.0 ml/min and the column temperature was maintained at 30 °C. The detector was set at 340 nm and 430 nm for determination of vitexin-2″-*O*-rhamnoside and phenol red, respectively.

Data Analysis

Effective permeability coefficient (P_{eff}) was calculated from the steady-state concentrations of vitexin-2″-*O*-rhamnoside in the perfusate collected. Permeability values were calculated using the following equation (Berggren et al., 2004):

$$P_{\text{eff}} = \frac{Q_{\text{in}} \ln(C_{\text{in}} / C_{\text{out (corrected)}})}{2\,rL}$$

where C_{in} is the inlet concentration and $C_{out\ (corrected)}$ is outlet concentration of vitexin-2″-*O*-rhamnoside, which is corrected for volume change in segment using phenol red concentration in inlet and outlet tubing. Q_{in} is the flow rate (0.2 ml/min), r is the rat intestinal radius, and L is the length of the segment. It has been demonstrated that in humans, at a Q_{in} of 2 to 3 ml/min, P_{eff} is membrane-controlled. In the rat model the Q_{in} is scaled to 0.2 ml/min, since the radius of the rat intestine is about 10 times less than that of human. $C_{out(corrected)}$ was calculated according to the following equation (Cummins, Salphati, & Reid, 2003; Singhal, Ho, & Anderson, 1998):

$$C_{\text{out (corrected)}} = C_{\text{out}} \cdot \frac{PR_{\text{in}}}{PR_{\text{out}}}$$

where PR_{in} and PR_{out} are the inlet and outlet concentrations of the non-absorbable water flux marker, phenol red.

Apparent permeability coefficients (P_{app}) of vitexin-2″-*O*-rhamnoside in HLF were calculated according to the following equation:

$$P_{\text{app}} = \frac{dQ/dt}{A \cdot C_0}$$

where the dQ/dt (μg/min) is the drug permeation rate, A is the cross-sectional area (4.2 cm^2), and C_0 (μg/ml) is the initial vitexin-2″-*O*-rhamnoside concentration in the donor compartment at t = 0 min.

Statistical Analysis

The results reported are the means (± SD). The statistical difference between treatment groups was evaluated using analysis of variance (ANOVA) and the identification of significances was carried out with Student's t test; $P < 0.05$ was considered to be statistically different.

RESULTS AND DISCUSSION

Determination of Vitexin-2″-*O*-rhamnoside

Calibration curves covering the entire range of vitexin-2″-*O*-rhamnoside and phenol red concentrations in the perfusate were prepared. Excellent linear plots relating the peak area and the concentrations of vitexin-2″-*O*-rhamnoside and phenol red were obtained. The mean recovery for determination of vitexin-2″-*O*-rhamnoside and for phenol red was 100.8% ± 3.6% and 100.3% ± 2.5%, respectively. Precision assay showed the averages of the relative standard deviations within 1 day for vitexin-2″-*O*-rhamnoside and for phenol red were 2.4% and 1.7%, respectively, and between days for vitexin-2″-*O*-rhamnoside and phenol red was 4.1% and 3.3%, respectively.

Excellent linear plots relating the peak area and the concentrations of vitexin-2″-*O*-rhamnoside red covering the entire range in the intestinal sac were also obtained. The mean recovery for determination of vitexin-2″-*O*-rhamnoside was 101.3 ± 3.4%. Precision assay showed that the average of the relative standard deviations within 1 day was 2.5% and between day was 4.6%.

The analytic method fit the requirement of content determination.

In Situ Single-Pass Perfusion Experiments

Intestinal Absorption of Vitexin-2″-O-Rhamnoside in Different Segments and at Different Concentrations of HLF

In situ perfusion of intestinal segments of rodents (mice, rats, and rabbits) is frequently used to study the permeability and absorption kinetics of drugs (Stewart, Chan, Jezyk, & Fleisher, 1997; Varma, Kapoor, et al., 2004). In vivo studies permit determination of absolute or relative bioavailability, but are also complex in terms of plasma assay and assessing rate-limiting process in drug absorption. Meaningful feedback to the drug discovery efforts generally requires a balance between the higher throughput of in vitro or in vivo studies. In vitro or in situ models are of particular utility when absorption is rate-limiting to systemic availability and permeability is rate-limiting to absorption. Of all permeability screening methods, in situ rat single-pass intestinal perfusion study offers a simple and relevant method of permeability assessment and correlates very much with the true absorption properties in human beings (Grassi & Cadelli, 2001; Salphati, Childers, Pan, Tsutsui, & Takahashi, 2001). Single-pass perfusion of the rat's different intestinal segments showed that the effective permeability coefficients (P_{eff}) of vitexin-2″-*O*-rhamnoside were not significantly different in duodenum, jejunum, ileum, and colon segments (Figure 2). There were no significant differences at different concentrations of HLF ranging from 0.05 to 0.5 mg/ml (Figure 3).

We conducted a topographic study to assess drug absorption in the duodenum, jejunum, ileum, and colon segments. These experiments allowed us to detect or discard possible preferential absorption at some point along the intestine attributable to localization of the iminoacid carrier or simply to differences in permeability along the intestinal canal, as has been reported for different marker molecules (Artursson, Ungell, & Löfroth, 1993). The results did not reveal any statistically significant differences among the P_{eff} tested in different intestinal segments, duodenum, jejunum, ileum, and colon. Thus, P_{eff} values seem to be invariable along the rat small intestine. Based on these results, the existence of a preferential absorption zone in the small intestine can be discarded. Since P_{eff} values did not differ along the small intestine, we have chosen the jejunum segment for the experiments at different concentrations and the influences of P-gp inhibitors on the absorption of vitexin-2″-*O*-rhamnoside in HLF. The results also showed that there were no significant

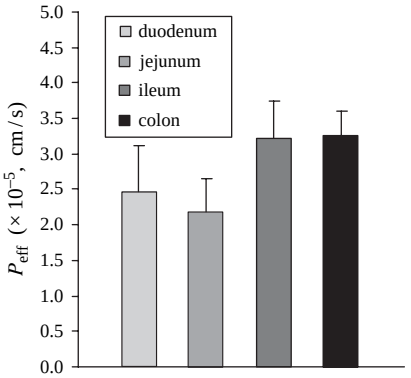

FIGURE 2. Effective permeability (P_{eff} [cm/s]) of vitexin-2″-*o*-rhamnoside using intestinal single-pass perfusion at intestinal segments: duodenum, jejunum, ileum, and colon. The standard deviation of each mean value is also presented (n = 4 in each group).

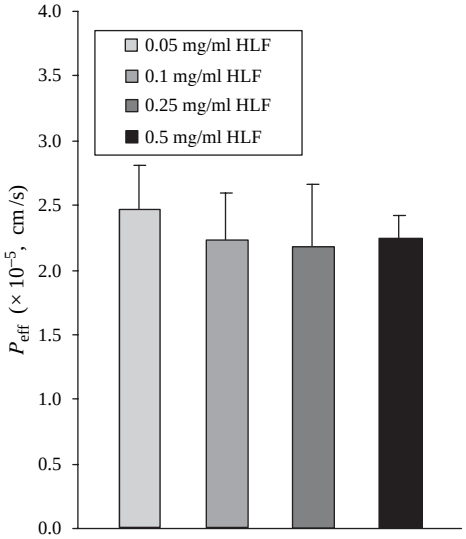

FIGURE 3. Effective permeability (P_{eff} [cm/s]) of vitexin-2″-*o*-rhamnoside using intestinal single-pass perfusion at the HLF concentrations of 0.05 mg/ml, 0.1 mg/ml, 0.25 mg/ml, and 0.5 mg/ml. The standard deviation of each mean value is also presented (n = 4 in each group).

differences in the permeability at different concentrations of HLF ranging from 0.05 to 0.5 mg/ml, which clearly indicates that potential saturable carrier-mediated intestinal efflux did not largely affect the rate or extent of the intestinal absorption of vitexin-2″-*O*- rhamnoside. These combined observations render it likely that passive membrane diffusion dominates the absorptive transport behavior of vitexin-2″-*O*-rhamnoside in HLF.

The P_{eff} values for vitexin-2″-*O*- rhamnoside in the rat jejunal perfusion at the concentration of 0.05, 0.1, 0.25, and 0.5 mg/ml were $(2.48 \pm 0.33) \times 10^{-5}$; $(2.23 \pm 0.67) \times 10^{-5}$; $(2.18 \pm 0.48) \times 10^{-5}$; and $(2.25 \pm 0.17) \times 10^{-5}$ cm/s, respectively. Three different classes of in vivo absorption may be defined in man: (a) poor, 0% to 30%; (b) intermediate, 30% to 90%; and (c) complete, 90% to 100%. According to this classification, compounds with an average P_{eff} in humans of approximately $< 0.1 \times 10^{-4}$ cm/s are poorly absorbed, whereas those with P_{eff} of about $> 0.7 \times 10^{-4}$ cm/s are completely absorbed. The P_{eff} for passively absorbed compounds is on average 3.6 times higher in humans compared with rats ($P_{eff,man} = 3.6P_{eff,rat} + 0.03 \times 10^{-4}$, $r^2 = 1.00$). So corresponding estimates in rats are $< 0.03 \times 10^{-4}$ cm/s and $> 0.2 \times 10^{-4}$ cm/s, respectively (Fagerholm et al., 1996). Based on these permeability data, vitexin-2″-*O*-rhamnoside can be classified as a high permeability class drug according to the biopharmaceutical classification system (BCS) (Amidon et al., 1995).

Influence of P-gp Inhibitors on P_{eff} of Vitexin-2″-O-Rhamnoside in HLF

The P-gp efflux inhibitors, verapamil and digoxin, significantly increased the measured jejunal P_{eff} for vitexin-2″-*O*-rhamnoside at the HLF concentration of 0.1 mg/ml (Figure 4) ($P < 0.05$). The permeability (P_{eff}) of vitexin-2″-*O*-rhamnoside absence of P-gp inhibitor, presence of verapamil, and presence of digoxin as P-gp inhibitor were $(2.25 \pm 0.67) \times 10^{-5}$; $(4.10 \pm 0.34) \times 10^{-5}$; and $(10.59 \pm 0.87) \times 10^{-5}$ cm/s, respectively. To evaluate the quantitative functional role of P-gp, the intestinal efflux inhibition ratio (EIR), that is, the ratio of permeability due to P-gp–mediated efflux activity ($P_{P\text{-}gp}$) and the passive permeability (P_{PD}), was calculated. $P_{P\text{-}gp}$ was obtained by subtracting $P_{eff,control}$ (absence of P-gp inhibitor) from $P_{eff,inh}$ (presence of P-gp inhibitor) while P_{PD} is equal to $P_{eff,inh}$ (Troutman & Thakker, 2003). EIR values using verapamil and digoxin as P-gp inhibitors were found to be 0.45 and 0.79, respectively, indicating that about 45% and 79% of passive transport of vitexin-2″-*O*-rhamnoside was attenuated by P-gp–mediated efflux, respectively. The absorption and secretion of vitexin-2″-*O*-rhamnoside in HLF are mediated by P-gp.

In Vitro Everted Gut Sac Experiments

Intestinal Absorption of Vitexin-2″-O-Rhamnoside at Different Concentrations of HLF

The everted gut sac technique is a simple and effective method of studying absorptive transport and the action of intestinal P-gp on intestinal drug absorption in vitro (Barthe, Bessouet, Woodley, & Houin, 1998). The system provides information on drug absorption mechanisms through testing the drug content in the intestinal sac. The everted sac provides quantitative information on the intestinal absorption of tested drug (Guo et al., 2004; Sha & Fang, 2004).

The mucosal side (bulk solution) to the serosal side (inside sac) transport of vitexin-2″-*O*-rhamnoside was investigated at concentrations ranging from 0.1 to 0.5 mg/ml. Figure 5 shows

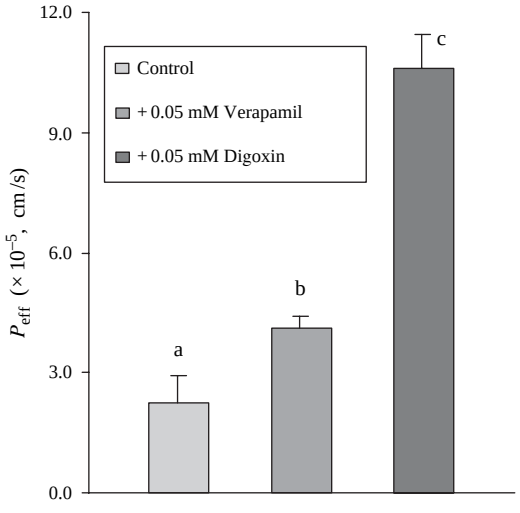

FIGURE 4. Effective permeability (P_{eff} [cm/s]) of vitexin-2″-*o*-rhamnoside using intestinal single-pass perfusion at the HLF concentration of 0.1 mg/ml (control), 0.1 mg/ml with addition of verapamil, and 0.1 mg/ml with addition of digoxin. Groups with different letters are statistically different at a 5% significance. The standard deviation of each mean value is also presented ($n = 4$ in each group).

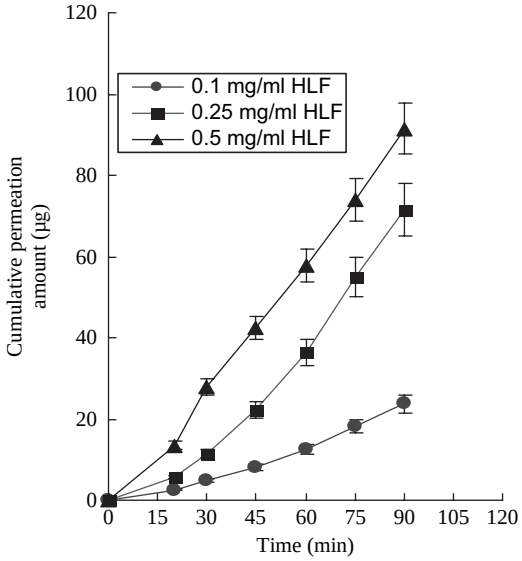

FIGURE 5. Cumulative permeation of vitexin-2″-*O*-rhamnoside across everted gut of rat ($n = 4$, $M \pm SD$).

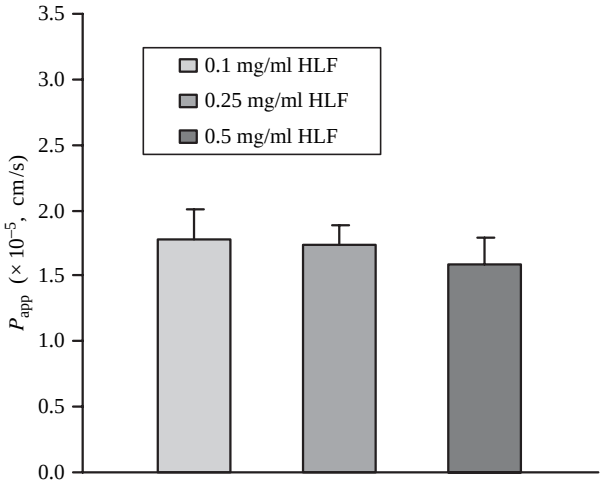

FIGURE 6. Apparent permeability (P_{app} [cm/s]), of vitexin-2″-*O*-rhamnoside using the everted rat sac model at HLF concentrations of 0.1 mg/ml, 0.25 mg/ml, and 0.5 mg/ml. The standard deviation of each mean value is also presented (n = 4 in each group).

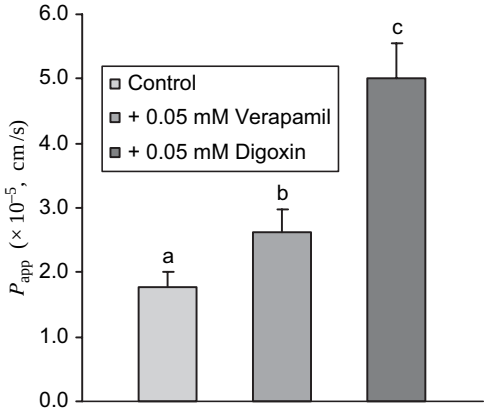

FIGURE 7. Apparent permeability, (P_{app} [cm/s]) of vitexin-2″-*O*-rhamnoside using the everted gut sac model at HLF concentrations of 0.1 mg/ml (control), 0.1 mg/ml with addition of verapamil, and 0.1 mg/ml with addition of digoxin. Groups with different letters are statistically different at a 5% significance. The standard deviation of each mean value is also presented (n = 4 in each group).

the cumulative permeation of vitexin-2″-*O*-rhamnoside across the everted gut of rat. It can be seen that the cumulative permeation amount of drug increased proportionally with time. No concentration dependence or saturation was observed for the absorptive transport of vitexin-2″-*O*-rhamnoside at concentrations ranging from 0.1 to 0.5 mg/ml. Figure 6 shows the apparent permeation coefficients (P_{app}) of vitexin-2″-*O*-rhamnoside in rat gut at different concentrations of HLF. There were no significant differences at different concentrations of HLF ranging from 0.1 to 0.5 mg/ml. Similar to the results of in situ single-pass perfusion, the absorption of vitexin-2″-*O*-rhamnoside was not concentration dependent, suggesting that passive diffusion dominates in the absorptive process.

Influence of P-gp Inhibitors on P_{app} of Vitexin-2″-O-Rhamnoside in HLF

P-gp is an energy-dependant efflux pump associated with multidrug resistance in tumor cells, but is also expressed in a variety of normal human tissues including the liver, brain, kidney, and gastrointestinal tract (Thiebault, Tsuruo, Hamada, Gottesman, & Pastan, 1987). At the intestinal level, P-gp is located on the apical membrane of the mature intestinal cells and acts as a pump that transports drugs back into the lumen as they are absorbed across the intestinal mucosa (Hebert, 1997; Watkins, 1997). There is currently considerable interest in intestinal P-gp and the central role it plays in limiting the oral bioavailability of a wide class of drugs (Benet, Wu, Hebert, & Wacher, 1996). The everted gut sac method is a simple and efficient in vitro model for studying the intestinal role of P-gp (Barthe et al., 1998).

In this study we investigated the influence of P-gp inhibitors on P_{app} of vitexin-2″-*O*-rhamnoside in HLF by using everted gut sac method. Figure 7 shows the apparent permeation coefficients of vitexin-2″-*O*-rhamnoside in rat gut in absence and in presence of P-gp inhibitors verapamil or digoxin. The P-gp efflux inhibitors verapamil and digoxin significantly affected the measured jejunal P_{app} of vitexin-2″-*O*-rhamnoside. It was found that the addition of verapamil or digoxin significantly increased the absorption of vitexin-2″-*O*-rhamnoside ($P < 0.05$). One likely explanation was that vitexin-2″-*O*-rhamnoside absorption was lowered through interaction with P-gp. These results are similar and in agreement with those obtained using the in situ intestinal single-pass perfusion technique.

CONCLUSIONS

In conclusion, the results presented here show vitexin-2″-*O*-rhamnoside in HLF can be classified as a high permeability class drug according to the biopharmaceutical classification system. The existence of a preferential absorption zone in the small intestine for vitexin-2″-*O*-rhamnoside can be discarded. Passive membrane diffusion dominates the absorptive transport behavior of vitexin-2″-*O*-rhamnoside. The absorption and secretion are mediated by the efflux transport system, P-gp. The absorption of vitexin-2″-*O*-rhamnoside is enhanced when administered together with P-gp inhibitors.

ACKNOWLEDGMENTS

This work was supported by Research Key Project, founded by the Science & Technology Department of Shanghai, P. R. China, Grant NO. 03JC14005 and Grant NO. 06DZ19702.

REFERENCES

Amidon, G. L., Lennernäs, H., Shah, V. P., & Crison, A. (1995). A theoretical basis for a biopharmaceutic drug classification: The correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm. Res., 12*, 413–420.

Ammon, H. P. T., & Händel, M. (1981a). *Crataegus*, toxicology and pharmacology: I. Toxicity. *Planta Med., 43*, 105–120.

Ammon, H. P. T., & Händel, M. (1981b). *Crataegus*, toxicology and pharmacology: II. Pharmacodynamics. *Planta Med., 43*, 209–239.

Artursson, P., Ungell, A. L., & Löfroth, J. E. (1993). Selective paracellular permeability in two models of intestinal absorption: Cultured monolayers of human intestinal epithelial cells and rat intestinal segments. *Pharm. Res., 10*, 1123–1129.

Barthe, L., Bessouet, M., Woodley, J. F., & Houin, G. (1998). The improved everted gut sac: A simple method to study intestinal P-glycoprotein. *Int. J. Pharm., 173*, 255–258.

Barthe, L., Woodley, J., & Houin, G. (1999). Gastrointestinal absorption of drugs: Methods and studies. *Fundam. Clin. Pharmacol., 13*, 154–168.

Benet, L. Z., Wu, C.-Y., Hebert, M. F., & Wacher, V. J. (1996). Intestinal drug metabolism and antitransport processes: A potential paradigm shift in oral drug delivery. *J. Control Release, 39*, 139–143.

Berggren, S., Hoogstraate, J., Fagerholm U., & Lennernäs, H. (2004). Characterization of jejunal absorption and apical efflux of orpivacaine, lidocaine and bupivacaine in the rat using in situ and in vitro absorption models. *Eur. J. Pharm. Sci., 21*, 553–560.

Christian, J. F. H., Wilson, S. C., & Thomas, M. (2000). Survival and prognosis: Investigation of *Crataegus* extract WS 1442 in congestive heart failure SPICE rationale—Study design and study protocol. *Eur. J. Heart. Fail., 2*, 431–437.

Cummins, C. L., Salphati, L., & Reid, M. J. (2003). In vivo modulation of intestinal CYP3A metabolism by P-glycoprotein: Studies using the rat single-pass intestinal perfusion model. *J. Pharmacol. Exp. Ther., 305*, 306–314.

Ding, X. B., Jiang, Y. Q., Zhong, Y., & Zuo, C. X. (1990). Study on chemical constituents in hawthorn leaves. *China J. Chin. Materia Medica., 15*, 295–297.

Fagerholm, U., Johansson, M., & Lennernäs, H. (1996). Comparison between permeability coefficients in rat and human jejunum. *Pharm. Res., 13*, 1336–1342.

Gan, L.-S. L., Moseley, M. A., Khosla, B., Augustijns, P. F., Bradshaw, T. P., Hendren, R.W., et al. (1996). CYP3A-like cytochrome P450-mediated metabolism and polarized efflux of cyclosporin A in Caco-2 cells: Interaction between the two biochemical barriers to intestinal transport. *Drug Metab. Dispos., 24*, 344–349.

Grassi, M., & Cadelli, G. (2001). Theoretical considerations on the in vivo intestinal permeability determination by means of the single pass and recirculation techniques. *Int. J. Pharm., 229*, 95–105.

Guo, J., Ping, Q., Dong, J., Qi, S., Feng, L., Li, Z., et al. (2004). Transport of leuprolide across rat intestine, rabbit intestine and Caco-2 cell monolayer. *Inter. J. Pharm., 278*, 415–422.

Hebert, M. F. (1997). Contribution of hepatic and intestinal metabolism and P-glycoprotein to cyclosporine and tacrolimus oral drug delivery. *Adv. Drug Deliv. Rev., 27*, 201–214.

Kruijtzer, C. M. F., Beijnen, J. H., Rosing, H., ten Bokkel Huinink, W. W., Schot, M., Jewell, R.C., et al. (2002). Increased oral bioavailability of topotecan in combination with the breast cancer resistance protein and P-glycoprotein inhibitor GF120918. *J. Clin. Oncol., 20*, 2943–2950.

Lennernäs, H., Nylander, S., & Ungell, A. L. (1997). Jejunal permeability: A comparison between the Ussing chamber technique and the single-pass perfusion in humans. *Pharm. Res., 14*, 667–671.

Lennernäs, H., Palm, K., Fagerholm, U., & Artursson, P. (1996). Comparison between active and passive drug transport in human intestinal epithelium (Caco-2) cells in vitro and human jejunum in vivo. *Int. J. Pharm., 127*, 103–107.

Lindahl, A., Sandström, R., Ungell, A. L., & Lennernäs, H. (1998). Concentration- and region-dependent intestinal permeability of fluvastatin in the rat. *J. Pharm. Pharmacol., 50*, 737–744.

Lown, K. S., Mayo, R. R., Leichtman, A. B., Hsiao, H.-L. Turgeon, D. K., Schmiedlin-Ren, P., et al. (1997). Role of intestinal P-glycoprotein (mdr1) in interpatient variation in the oral bioavailability of cyclosporin. *Clin. Pharmacol. Ther., 62*, 248–260.

Salphati, L., Childers, K., Pan, L., Tsutsui, K., & Takahashi, L. (2001). Evaluation of a single-pass intestinal-perfusion method in rat for the prediction of absorption in man. *J. Pharm. Pharmacol., 53*, 1007–1013.

Sha, X. Y., & Fang, X. L. (2004). Transport characteristics of 9-nitrocamptothecin in the human intestinal cell line Caco-2 and everted gut sacs. *Inter. J. Pharm., 272*, 161–171.

Singhal, D., Ho, N. F., & Anderson, B. D. (1998). Absorption and intestinal metabolism of purine dideoxynucleosides and an adenosine deaminase-activated prodrug of 2',3'-dideoxyinosine in the mesenteric vein cannulated rat ileum. *J. Pharm. Sci., 87*, 569–577.

Stewart, B. H., Chan, O. H., Jezyk, N., & Fleisher, D. (1997). Discrimination between drug candidates using models for evaluation of intestinal absorption. *Adv. Drug Deliv. Rev., 23*, 27–45.

Tauchert, M. (2002). Efficacy and safety of *crataegus* extract WS 1442 in comparison with placebo in patients with chronic stable New York Heart Association class-III heart failure. *Am. Heart J., 143*(5), 910–915.

Terao, T., Hisanaga, E., Sai, Y., Tamai, I., & Tsuji, A. (1996). Active secretion of drugs from the small intestinal epithelium in rats by P-glycoprotein functioning as an absorption barrier. *J. Pharm. Pharmacol., 48*, 1083–1089.

Thiebault, F., Tsuruo, T., Hamada, H., Gottesman, M. M., & Pastan, I. (1987). Cellular localisation of the multidrug resistance gene product P-glycoprotein in normal human tissues. *Proc. Natl. Acad. Sci. U.S.A., 84*, 7735–7738.

Troutman, M. D., & Thakker, D. R. (2003). Novel experimental parameters to quantify the modulation of absorption and secretary transport of compounds by P-glycoprotein in cell culture models of intestinal epithelium. *Pharm. Res., 20*, 1200–1209.

Varma, M. V. S., Ashokra, Y. J, Dey, C. S., & Panchagnula, R. (2003). P-glycoprotein inhibitors and their screening: A perspective from bioavailability enhancement. *Pharmacol. Res., 48*, 347–356.

Varma, M. V. S., Kapoor, N., Sarkar, M., & Panchagnula, R. (2004). Simultaneous determination of digoxin and permeability markers in rat in situ intestinal perfusion samples by RP-HPLC. *J. Chromatogr. B., 813*, 347–352.

Varma, M. V. S., Khandavilli, S., Ashokraj, Y., Jain A., Dhanikula, A., Sood, A., et al. (2004). Biopharmaceutic classification system: A scientific framework for pharmacokinetic optimization in drug research. *Curr. Drug Metab., 5*, 375–388.

Varma, M. V. S., Sarkar, M., Kapoor, N., & Panchagnula, R. (2005). pH-dependent functional activity of P-glycoprotein in limiting intestinal absorption of protic drugs 1: Simultaneous determination of quinidine and permeability markers in rat in situ perfusion samples. *J. Chromatogr. B., 816*, 243–249.

Watkins, P. B. (1997). The barrier function of CYP3A4 and P-glycoprotein in the small bowel. *Adv. Drug Deliv. Rev., 27*, 161–170.

Ying, X. X., Lu, X. M., Sun, X. H., Li, X. Q., & Li, F. M. (2007). Determination of vitexin-2-O-rhamnoside in rat plasma by ultra-performance liquid chromatography electrospray ionization tandem mass spectrometry and its application to pharmacokinetic study. *Talanta, 72*, 1500–1506.

Zhang, Y., & Benet, L. Z. (2001). The gut as a barrier to drug absorption: Combined role of cytochrome P450 3A and P-glycoprotein. *Clin. Pharmacokinet., 40*, 159–168.