Intestinal Permeability of Forskolin by *In Situ* Single Pass Perfusion in Rats

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Abstract

The intestinal permeability of forskolin was investigated using a single pass intestinal perfusion (SPIP) technique in rats. SPIP was performed in different intestinal segments (duodenum, jejunum, ileum, and colon) with three concentrations of forskolin (11.90, 29.75, and 59.90 µg/mL). The investigations of adsorption and stability were performed to ensure that the disappearance of forskolin from the perfusate was due to intestinal absorption. The results of the SPIP study indicated that forskolin could be absorbed in all segments of the intestine. The effective permeability (P_{eff}) of forskolin was in the range of drugs with high intestinal permeability. The P_{eff} was highest in the duodenum as compared to other intestinal segments. The decreases of P_{eff} in the duodenum and ileum at the highest forskolin concentration suggested a saturable transport process. The addition of verapamil, a P-glycoprotein inhibitor, significantly enhanced the permeability of forskolin across the rat jejunum. The absorbed fraction of dissolved forskolin after oral administration in humans was estimated to be 100% calculated from rat P_{eff}. In conclusion, dissolved forskolin can be absorbed readily in the intestine. The low aqueous solubility of forskolin might be a crucial factor for its poor oral bioavailability.

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

Introduction

Forskolin (**Fig. 1**) is a diterpenoid isolated from *Plectranthus barbatus* Andr. (also named as *Coleus forskohlii* Briq., family Lamiaceae), which has been used as an herbal medicine in India, Brazil, tropical Africa, and China [1]. Forskolin can directly activate adenylate cyclase and thus increases intracellular cAMP levels greatly. The main pharmacological effects of forskolin such as lowering blood pressure, inhibiting platelet aggregation, and blocking bronchospasm are mediated by the increase of cAMP [2,3]. For many years, forskolin extract has been incorporated into pharmaceutical preparations and dietary supplements for heart health, blood vessel relaxation, and weight loss [2,3]. Despite a large body of literature describing the pharmacology of forskolin, relatively few articles address its biopharmaceutical properties. A recent study indicates that its absolute bioavailability after oral administration to rats is only 0.53% [4]. Therefore, it is important to study the intestinal permeability of forskolin, which would be helpful to assess the rate-limiting processes of oral absorption and to provide basic information for its further application.

Single pass intestinal perfusion (SPIP) is regarded as a classic technique for estimating the intestinal absorption of chemical compounds after oral administration [5]. Compared with other techniques, SPIP provides the advantages of precise experimental control, intact blood supply, and ability to investigate regional factors influencing intestinal absorption of compounds [6]. It has been widely utilized to predict the extent of oral drug absorption and to clarify absorption mechanisms [5,7,8].

The objective of this study was to investigate the intestinal permeability of forskolin employing a rat SPIP technique. In this model, forskolin was perfused through intestinal segments with an intact blood supply. Disappearance of forskolin from the perfusate could be attributed to intestinal absorption. Its effective permeability (P_{eff}) was calculated. Verapamil, a P-glycoprotein (P-gp) in-

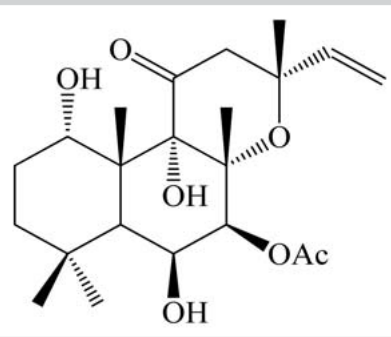

Fig. 1 Chemical structure of forskolin.

hibitor, was added to the perfusate to delineate P-gp's role in forskolin intestinal absorption.

Materials and Methods

Materials

Forskolin was purified by our team from forskolin extract which was purchased from Changsha Huacheng Biotechnology, Inc., and its structure was identified by ^{1}H NMR, ^{13}C NMR, and MS spectra (see Supporting Information). The purity of forskolin was assessed by HPLC–ELSD (Evaporative Light Scattering Detector) as > 98%. Verapamil hydrochloride (purity > 98.5%) was supplied by Jiangsu Hengrui Medicine Co. Ltd. Other chemicals and reagents were of analytical or HPLC grade as appropriate.

Male Sprague-Dawley rats, weighing 250 ± 20 g, were supplied by Shanghai Laboratory Animal Resources Center. The animal experimental protocol was approved by the Ethics Committee of the School of Pharmacy, Fudan University. The date of approval was January 17, 2011, and the approval number was 2011–10. All animal studies were performed according to the Guide for the Care and Use of Laboratory Animals [9]. Animals were acclimated for at least a week and fasted overnight before use in the SPIP study.

Perfusion solution

The intestinal perfusion solutions were obtained by dissolving forskolin in modified Krebs-Ringer's buffer (Krebs-Ringer's buffer supplemented with 2% ethanol to ensure the complete dissolution of forskolin). The pH values of the perfusates were controlled at 6.5 ± 0.05. SPIP was performed with three concentrations of forskolin (11.90, 29.75, and 59.90 µg/mL).

Adsorption and stability studies

These studies were carried out to ensure that the loss of forskolin during SPIP is due to absorption only and not due to other losses such as physical binding to the tubing and chemical degradation. In the adsorption study, the intestinal perfusion solution of forskolin was incubated with the tubing for 2 h at 37 °C. Samples were collected at appropriate time intervals (15 min) and analyzed by HPLC. A stability study was conducted in the blank intestinal perfusate after having been perfused in the intestine for 2 h. Forskolin was incubated in the blank intestinal perfusate for 2 h at 37 °C. Samples were collected every 15 min and analyzed by HPLC to monitor any degradation.

Rat single pass intestinal perfusion study

SPIP study was performed as previously described [6, 10, 11], with minor modifications. Rats were anesthetized with an intraperitoneal injection of urethane at a dose of 1.5 g/kg and were placed on a warming pad to maintain the body temperature during surgery and perfusion. Upon verification of the loss of pain reflex, a midline abdominal incision was made. The intestine is divided into different segments as duodenum, jejunum, ileum, and colon [12]. Each intestinal segment of approximately 10 cm was isolated and cannulated with silicone tubing. Care was taken to avoid injury of the local circulatory system. The exposed segment was covered with a cotton pad soaked in normal saline solution at 37 °C. Intestinal segments were rinsed with Krebs-Ringer's buffer maintained at 37 °C for approximately 30 min until the outlet solution was visually clear. At the start of the study, the perfusion solution was perfused through the intestinal segment at 0.28 mL/min for 45 min using a peristaltic pump (Instrument Plant of Shazhou County, Jiangsu Province). After reaching steady state, the perfusate samples were collected at 15 min intervals in preweighed glass tubes. All the perfusate solutions collected were weighed and centrifuged at 8000 g for 5 min. The supernatants were assayed immediately by a validated HPLC method. At the end of the experiment, the length and radius of perfused intestinal segments were measured accurately. In order to delineate P-gp's role in forskolin intestinal absorption, verapamil hydrochloride (20 µM) was added to the perfusion solution of forskolin (11.90 µg/mL), and jejunum perfusion was performed at a flow rate of 0.28 mL/min.

Determination of forskolin by HPLC

The concentrations of forskolin in perfusate samples were analyzed by a reversed-phase HPLC method. A Shimadzu HPLC system consisting of an LC-10ADvp pump, an SPD-10Avp UV detector, and an SCL-10Avp system controller was used. The chromatographic separation was performed on a Promosil C_{18} (5 µm, 4.6 mm × 150 mm; Agela Technologies, Inc.) analytical column coupled with a Phenomenex C_{18} guard column (5 µm, 4.0 mm × 3.0 mm). The temperature was set at 37 °C. The mobile phase, delivered at 1.0 mL/min, consisted of methanol-water (70 : 30, v/v). The detection wavelength was set at 210 nm. The elution time for forskolin was approximately 5.5 min. No interfering peaks were observed at the retention time of forskolin. The calibration curves were constructed by plotting the peak areas of forskolin versus the concentrations of calibration standards. The solvent used for calibrator preparation was modified Krebs-Ringer's buffer. A good linearity was observed over the concentration range from 5.95 to 59.50 µg/mL (r > 0.999). The intra- and inter-day precision values were less than 1.59% and 1.71%, respectively. The accuracy of the method was between 94.2% and 103.3%. The recoveries of forskolin from the perfusate having been perfused in the intestine for 2 h ranged from 96.3% to 102.8%. The limit of quantification (LOQ) was 5.95 µg/mL (S/N > 5), and the limit of detection (LOD) was 2 µg/mL (S/N > 3).

Permeability calculations

SPIP was based on reaching steady state with respect to the diffusion of forskolin across the intestine. Steady state was confirmed by plotting the ratio of the outlet concentration corrected for water flux to the inlet concentration ($\frac{C_{out\,(corr)}}{C_{in}}$, equation 1) versus time. The P_{eff} of forskolin was calculated from equation 2.

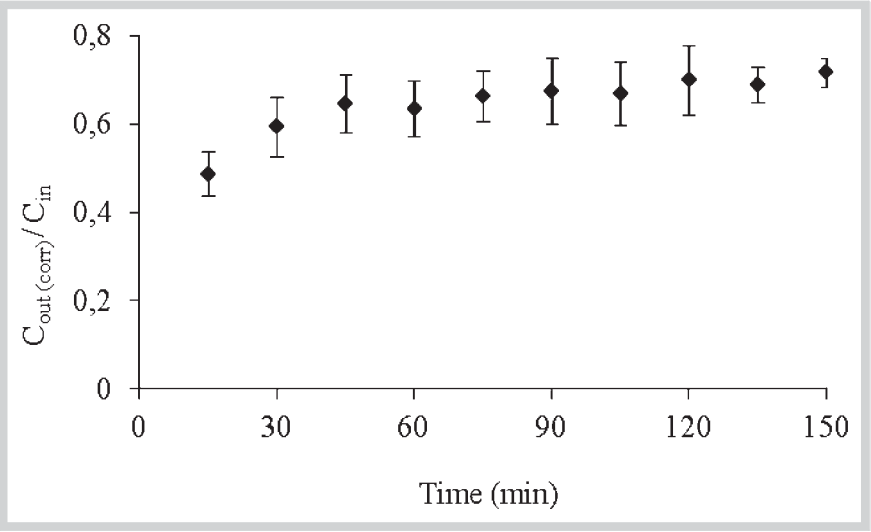

Fig. 2 Representative plot of $\frac{C_{(corr)}}{C_{in}}$ vs. time (n = 5).

Table 1 Region and concentration dependence of forskolin permeability (n = 5).

Concentration (μg/mL)	$P_{eff} \times 10^4$ (cm/s)			
	Duodenum	Jejunum	Ileum	Colon
11.90	2.27 ± 0.14♦	1.54 ± 0.11	1.54 ± 0.27	1.32 ± 0.11♦
29.75	2.26 ± 0.17♦	1.51 ± 0.20	1.59 ± 0.20	
59.50	1.75 ± 0.08*♦	1.58 ± 0.07	1.21 ± 0.08*♦	

* P < 0.01, compared with either of the two lower concentrations at the same intestinal section; ♦ p < 0.01, compared with jejunum at the same concentration.

$$\frac{C_{out\,(corr)}}{C_{in}} = \frac{Q_{out}C_{out}}{Q_{in}C_{in}} \quad (1)$$

$$P_{eff} = -\frac{Q_{in}}{2\pi RL}\ln\left(\frac{Q_{out}C_{out}}{Q_{in}C_{in}}\right) \quad (2)$$

where C_{in} and C_{out} are forskolin concentrations (μg/mL) in the inflow and outflow perfusate, respectively; Q_{in} is the measured flow rate (mL/min) of entering intestinal perfusate; Q_{out} is the measured flow (mL/min) of exiting intestinal perfusate for the specified time interval calculated from the actual intestinal perfusate density (g/mL), which is determined by weighing the contents of a known volume of perfusate; R is the radius (cm) of the intestine; and L is the length (cm) of the perfused intestinal segment.

Statistical analysis

All experiments were conducted in quintuplicate, and results are expressed as mean ± SD. Statistical comparison of mean values was performed with one-way analysis of variance (ANOVA) (SPSS 13.0 for Windows).

Supporting information

Details of extraction, isolation, and structure identification of forskolin are available as Supporting Information.

Results and Discussion

Adsorption of forskolin to the silicone tubing was negligible during the incubation of the intestinal perfusate with the tubing. The stability of forskolin was based on the decrease of the parent compound as quantitated by HPLC. After 2 h incubation at 37 °C, at least 97.1% forskolin remained in the blank intestinal perfusate. No appreciable degradation of forskolin was noticed. Estimated time to reach steady state of diffusion was 30–45 min after the beginning of the perfusion. Representative results are plotted in **Fig. 2**. The P_{eff} of forskolin was calculated only from experiments where steady state was achieved.

In the SPIP experimental procedure, the influencing factors of intestinal absorption such as flow rate, length of perfused intestine, and concentration of the compound of interest can be precisely controlled. The intestinal permeability of forskolin was studied as a function of concentration in each segment of the intestine. The upper boundary of the concentration range (11.90–59.50 μg/mL) was limited because of the low aqueous solubility of forskolin. The P_{eff} values for each section at different concentrations are listed in **Table 1**.

Results indicated that forskolin could be absorbed across the intestine. The P_{eff} of forskolin was highest in the duodenum as compared to other intestinal segments especially at the two lower concentrations. There was no statistical difference in P_{eff} values for the three concentrations in the jejunum. However, concentration-dependent changes in P_{eff} were evident in the duodenum and ileum where the P_{eff} at 59.50 μg/mL was significantly different ($p < 0.01$) from the values at either of the two lower concentrations (**Table 1**). The decreases in P_{eff} at the highest concentration in the duodenum and ileum suggested that forskolin might be transported across the intestinal epithelium via a saturable transcellular mechanism in these two segments.

Ethanol is the most commonly used organic solvent to help increase the solubility of drugs and can be tolerated in relatively high doses. In order to assess the influence of 2% ethanol on the permeability of forskolin, the perfusion solution (containing 11.90 μg/

Table 2 Effect of perfusion flow rate on the P_{eff} of forskolin.

Flow rate (mL/min)	P_{eff} ($\times 10^4$, cm/s)
0.28	1.54 ± 0.11
0.56	1.77 ± 0.23
1.12	1.68 ± 0.33

mL of forskolin) without ethanol was perfused through the rat jejunum at 0.28 mL/min. The P_{eff} was $1.54 \times 10^{-4} \pm 0.41 \times 10^{-4}$ cm/s. There was no statistical difference ($p > 0.05$) in the P_{eff} values between the perfusate with ethanol and the one without ethanol, suggesting that 2% ethanol had a negligible effect on the permeability of forskolin.

Diffusion through the unstirred water layer (UWL) adjacent to the mucosal cell might be rate limiting for the absorption of a lipophilic drug-like forskolin. The most common way to evaluate the thickness and importance of the UWL on intestinal absorption is to determine the P_{eff} at different perfusion flow rates [13]. Jejunum perfusion with forskolin (11.90 µg/mL) was carried out with three perfusion flow rates (0.28, 0.56, and 1.12 mL/min). There was no significant difference ($p > 0.05$) in P_{eff} among the three flow rates (**Table 2**) suggesting that UWL was not a main diffusion barrier for dissolved forskolin. The P_{eff} of forskolin was mainly determined by the membrane permeability but not the aqueous permeability.

Forskolin has been implicated as a compound to interact with P-gp [14, 15], an efflux protein expressed primarily in certain cell types in the liver, pancreas, kidney, colon, and jejunum [16]. Since P-gp mediates a saturable drug efflux and works effectively under low concentrations of substrates [17], the lowest concentration (11.90 µg/mL) was used to investigate the influence of P-gp on the permeability of forskolin. In the presence of verapamil, the P_{eff} of forskolin in jejunum increased significantly ($p < 0.01$) from $1.54 \times 10^{-4} \pm 0.11 \times 10^{-4}$ cm/s to $1.99 \times 10^{-4} \pm 0.31 \times 10^{-4}$ cm/s. The result indicated that forskolin was a P-gp substrate and that ATP-dependent efflux might be a reason for the low oral bioavailability of forskolin. Since P-gp increases along the intestinal axis from proximal to distal regions [18], the low duodenal expression of P-gp might be a reason for the high permeability observed in the duodenum.

For passively absorbed solutes, the rat and human jejunum P_{eff} correlate highly. The human permeability value ($P_{eff\ human}$) and the fraction of dose absorbed in humans ($f_{a\ human}$) can be predicted from rat SPIP experiments using the following equations [19]:

$$P_{eff\ human} = 3.6 P_{eff\ rat} + 0.03 \times 10^{-4} \quad (3)$$

$$f_{a\ human} = 1 - e^{-(2 \times P_{eff\ human} \times t_{res} \times 2.8/r)} \quad (4)$$

where t_{res} is the average small intestine transit time (3 h) and r is the average human small intestine radius (1.75 cm).

The absorption of forskolin in the jejunum is most likely via passive transport, since no statistical difference in P_{eff} values was observed for the concentration ranges from 11.90 to 59.50 µg/mL. Therefore, the P_{eff} values obtained in the jejunum were used to calculate $P_{eff\ human}$ and $f_{a\ human}$. The $P_{eff\ human}$ of forskolin was estimated to be $5.46 \times 10^{-4} - 5.73 \times 10^{-4}$ cm/s, which is in the range of drugs with high intestinal permeability. The calculated $f_{a\ human}$ of dissolved forskolin was 100%.

In order to monitor the viability of the perfusion model in our study, the rat P_{eff} of verapamil hydrochloride, used as a marker compound, was investigated adopting the same SPIP method as forskolin. The $P_{eff\ human}$ of verapamil, calculated from the rat P_{eff} via equation 3, was compared with the published data. Both verapamil and forskolin are lipophilic drugs with high and complete intestinal absorption even though they are P-gp substrates [20]. Therefore, verapamil is a good marker compound to validate the results of the forskolin perfusion study. The P_{eff} of verapamil (40 µg/mL) in rat jejunum at a flow rate of 0.28 mL/min was $1.40 \times 10^{-4} \pm 0.27 \times 10^{-4}$ cm/s. The average calculated $P_{eff\ human}$ was 5.09×10^{-4} cm/s, which was comparable to the published data (6.8×10^{-4} cm/s [20] or $3.11 \times 10^{-4} - 6.27 \times 10^{-4}$ cm/s [21]). The result of the verapamil SPIP study proved that the perfusion model in our study was reliable and suitable for predicting the oral absorption of forskolin in human.

Oral bioavailability is mainly dependent on the fraction of dose absorbed (f_a) and the first-pass extraction in the gut wall and liver [20]. The f_a from oral solid preparations is governed by several factors such as drug release, stability, gastrointestinal transit time, and P_{eff} [20]. The results of our study indicate that dissolved forskolin can be absorbed readily in the intestine even though it is a substrate of P-gp. Therefore, the low oral bioavailability of forskolin is probably due to its low aqueous solubility or metabolism *in vivo*. Since forskolin is a lipophilic drug with high and complete intestinal absorption, it is of high importance to come up with innovative strategies to increase the solubility and dissolution rate to avoid losing an interesting drug candidate. Metabolism is another key factor governing the bioavailability. Unfortunately, no studies describing the metabolism of forskolin *in vivo* have been reported so far.

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Conflict of Interest

Herewith we declare the absence of any conflict of interest, financial or personal, for all authors.

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