



# BioTechnology

An Indian Journal

FULL PAPER

BTALJ, 10(5), 2014 [1074-1078]

## Comparison alone or combined with P-gp inhibitors on spinosin absorption using single-pass intestinal perfusion

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### ABSTRACT

This work determined the mechanisms of enhancing the oral bioavailability of Spinosin (SPI). The in situ perfusion method was used in rats to study the absorption mechanisms of SPI alone or combined with the P-gp inhibitors Verapamil (Ver) and cyclosporin A (CsA). The drug absorption rate constant ( $K_a$ ) and apparent absorption coefficient ( $P_{eff}$ ) had little significant effect on duodenum, jejunum, ileum and colon at  $40\mu\text{g}\cdot\text{mL}^{-1}$  concentration of Spinosin ( $P>0.05$ ). Compared with SPI group, SPI+Ver and SPI+CsA groups were increased significantly ( $P<0.05$ ). Ver and CsA remarkably enhanced the effect of intestinal absorption of SPI on duodenum ( $P<0.05$ ). The results of absorption in the intestines indicated that Efflux pump P-gp might exert some effects on the absorption of SPI. In conclusion, these data indicate that P-gp inhibitors are an effective and promising delivery system to enhance the oral bioavailability of poorly water-soluble drugs. © 2014 Trade Science Inc. - INDIA

### KEYWORDS

In situ perfusion method;  
Oral bioavailability;  
Spinosin;  
P-gp inhibitors.

### INTRODUCTION

Spinosin (2''- $\beta$ -O-glucopyranosyl swertisin,  $\text{C}_{28}\text{H}_{32}\text{O}_{15}$ ), a bioactive herbal ingredient isolated from Semen Ziziphi Spinosae, plays an important role in sedation and hypnosis<sup>[4,12]</sup>. It significantly potentiated the hypnotic effect of pentobarbital by decreasing sleep latency, increasing sleeping time, and enhancing the rate of sleep onset induced by subhypnotic doses of pentobarbital<sup>[6]</sup>. The mechanism of this action may be related to postsynaptic 5-HT<sub>1A</sub> receptors<sup>[5]</sup>. While, previous research showed that, as a single component, spinosin was poorly absorbed in rats after oral administration<sup>[11]</sup>.

In recent decades, the crucial role of efflux trans-

porters in drug absorption and disposition has gained considerable attention<sup>[10]</sup>. The inhibition or induction of transporters can lead to significant drug-drug interaction by affecting various pharmacokinetic parameters of the drug<sup>[8]</sup>. P-glycoprotein (P-gp), the most extensively studied ATP-binding cassette transporter, functions as a biological barrier by extruding toxic substances and xenobiotics out of cells<sup>[3]</sup>. The hypothesis that inhibition of P-gp improves the bioavailability of drugs that are substrate for this efflux transporter is gaining widespread recognition<sup>[2]</sup>. Up to date, the role of P-gp efflux transporter in spinosin metabolism rarely reported. To investigate the effect of P-gp on spinosin absorption, we used the in situ perfusion method to compare the

absorption rate and permeability at duodenum, jejunum, ileum and colon in rats.

## MATERIAL AND METHODS

**Reagents and chemicals:** Spinosin was purchased from Si Chuan Weikeqi Medical Technology Co., Ltd. (Chengdu, People's Republic of China). The purity of this compound was determined to be higher than 98% by normalization of the peak area detected by HPLC. CsA and Ver were purchased from Novartis Pharma Stein AG (Basel, Switzerland). Acetonitrile was of HPLC grade from Tedia Company, Inc (Fairfield, OH, USA). Purified water prepared by the Millipore system (Millipore, Bedford, MA, USA) was used for all the preparations. Other chemicals were of analytical grade.

### Instrumentation and chromatographic conditions

A ZORBAX SB-C18 column (250 mm\*4.6 mm, 5 $\mu$ m) analytical column from Agilent Co. was used. As the mobile phase, a binary mixture of acetonitrile-water (20:80, v/v) was delivered via a waters 2695 pump in isocratic mode at a flow rate of 1.0 ml/min. The waters 2487 detector was set at 335 nm and all measurements were performed at room temperature. An EMPORE-2000 workstation was used for data acquisition. **Animals:** Male Sprague Dawley rats (200-255g, Central Animal Laboratory of ZhengZhou University, ZhengZhou, People's Republic of China) were used to perform this study. They were acclimatized for at least 5 days prior to use. All the animal experimental procedures were approved by the institutional animal ethical committee and were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

### Single-pass intestinal perfusion studies

The surgical procedure for the single-pass perfusion studies was based on established methods as described previously<sup>7,9]</sup>. Briefly, eighteen male Sprague Dawley rats were divided randomly into three groups of six rats each. The rats were fasted overnight prior to the experiments with free access to water. Anesthesia was induced by the intraperitoneal administration of 20% urethane (0.6 mL/kg). The pre-disposal treatment of rats was the same as above described. After a 10 cm long midline incision was made, the duodenum (1 cm distal to the pyloric sphinc-

ter), jejunum (15 cm to the pyloric sphincter), ileum (20 cm proximal to the cecum), and colon (2 cm distal to cecum) of each rat were simultaneously cannulated with polyethylene tubes and ligated at both ends. Care was taken to perform the surgery gently to minimize the damage and maintain intact blood circulation. After being rinsed with physiological saline solution at 37°C, the selected segments were attached to the perfusion assembly, which included a peristaltic pump (BT100F-1; Baoding Longer Precision Pump Co, Ltd, Hebei, People's Republic of China). The entire surgical area was covered with a piece of sterilized absorbent gauze wetted with normal saline solution at 37°C. The perfusion solution was obtained by dispersing the Spinosin in Krebs-Rings perfusate solution at a drug concentration of 40 $\mu$ g/mL and was incubated in a 37°C water bath to maintain the temperature. Before the experiment, the intestinal segments were perfused with the test solution at a flow rate of 0.2 mL/minute for 30 minutes to achieve absorption equilibrium and stable outflow rates. Subsequently, intestinal perfusate samples were collected over 15 minute intervals for duration of 1 hour (15, 30, 45, 60 minutes) in 5 mL glass vials. At the end of the experiment, all samples, including the perfusion samples from both the inlet and outlet drug solutions at different time points, were immediately assayed by HPLC. The samples were prepared for HPLC analysis by adding 200  $\mu$ L of perfusion sample which was added to two volumes of absolute methanol, and after vortexing for 2 minutes, the mixture was centrifuged at 15,000 rpm for 20 minutes. A 10- $\mu$ L aliquot of the supernatant was injected into the HPLC system. All vials were weighed before and after perfusion. The perfused intestinal segments between two cannulas were excised without dragging, and their lengths were measured using silk thread. The absorption rate constant ( $K_a$ ) and apparent permeability coefficients ( $P_{eff}$ ) were calculated using the following equations:

$$K_a = \frac{(1 - \frac{C_{out}}{C_{in}} \times \frac{V_{out}}{V_{in}}) \times Q_{in}}{V}$$

$$P_{eff} = \frac{-Q_{in} \times \ln(\frac{C_{out}}{C_{in}} \times \frac{V_{out}}{V_{in}})}{A}$$

$$V = \pi R^2 L \quad A = 2\pi RL$$

$$V_{out} = m_{out} / \rho_{out} \quad V_{in} = m_{in} / \rho_{in}$$

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where  $m_{in}$  is the weight of the inlet solution,  $m_{out}$  is the weight of the exiting solution,  $\rho_{in}$  is the density of the inlet solution,  $\rho_{out}$  is the density of the exiting solution,  $V_{in}$  is the volume of the inlet solution,  $V_{out}$  is the volume of the exiting solution,  $Q_{in}$  is the flow rate (mL/minute) of the inlet solution,  $C_{in}$  is the concentration ( $\mu\text{g/mL}$ ) of the drug in the inlet solution,  $C_{out}$  is the concentration ( $\mu\text{g/mL}$ ) of the drug in the exiting solution,  $V$  is the volume of the perfused segment (mL),  $L$  is the length of the intestinal segment (cm), and  $R$  is the radius of the intestinal segment (cm).

Statistical analysis The results are reported as the mean  $\pm$  standard deviation (SD). Statistical analysis was performed by one-way analysis of variance. The results were considered significant if  $P < 0.05$ .

### METHOD VALIDATION

The method was fully validated according to the currently accepted Food and Drug Administration bioanalytical method validation guidance with respect to specificity, linearity, precision and accuracy, recovery and stability.

#### Specificity

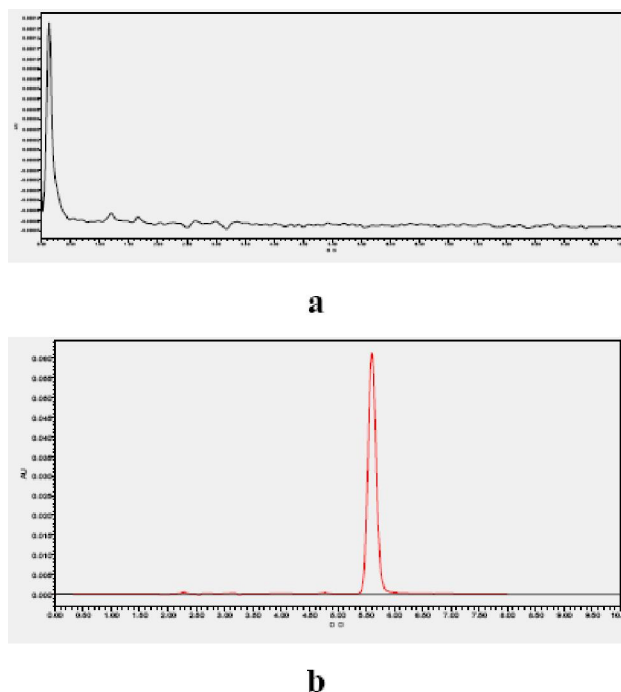
The Specificity was evaluated under the RP-HPLC conditions used, spinosin was eluted 5.8 min. Typical chromatograms of blank perfusion solution, blank perfusion solution spiked with spinosin shown in Figure 1, simple protein precipitation with acetonitrile was sufficient to isolate the analytes from the biological matrix without any interfering endogenous peaks.

#### Linearity

Different amounts of spinosin were added to 300 $\mu\text{L}$  of blank perfusion solution to obtain the spiked samples were then treated with the above extraction procedures and analyzed. The linear regression of the curve for the peak area (Y) versus concentration was  $Y = 18652x + 39332$ . The  $R^2$  for the standard curves are 0.9994. The calibration curve of spinosin in perfusion solution showed good linearity over the concentration range of 3–55 $\mu\text{g/mL}$ , and the detection limit of Spinosin was 1.5 $\mu\text{g/mL}$ .

#### Accuracy and precision

The accuracy and precision of intra-day and inter-



**Figure 1 : a RP-HPLC chromatograms of blank blank perfusion solution( —), b blank perfusion solution spiked with spinosin( —)**

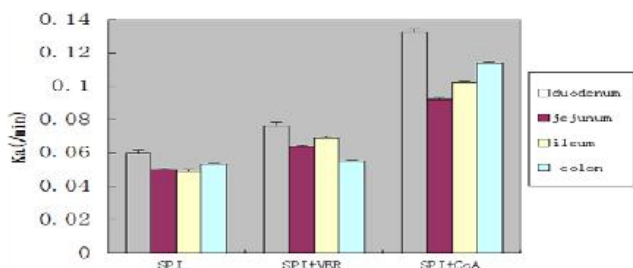
day determinations were assessed by analyzing QC samples in six replicates at three concentration levels (low, medium and high) on the same day and on three consecutive validation days. The intra- and inter precision for all samples was less than 6.4%. The extraction recoveries were evaluated at three concentration levels (low, medium and high) for analytes from six perfusion solution samples spiked before extraction to those after extraction. The mean recovery for all samples was greater than 95.4%.

#### Stability

The stability of spinosin in perfusion solution was determined through three freeze-thaw cycles in corresponding quality control samples. The percentage of deviation from the concentration observed was less than 5.8%.

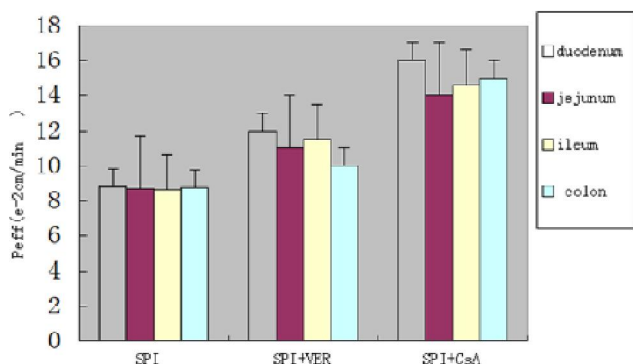
### RESULTS AND DISCUSSION

In the intestinal absorption studies, the absorption characteristics of the spinosin alone or combined with P-gp inhibitors were assessed in four different intestinal segments. The absorption rate constant ( $K_a$ ) and apparent permeability coefficients ( $P_{eff}$ ) obtained in situ



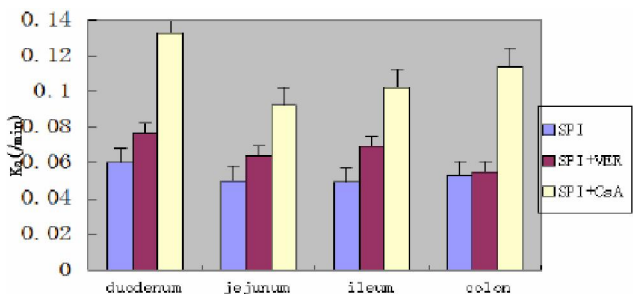
Abbreviations: *K<sub>a</sub>*, absorption rate constant; min, minute; SD, standard deviation; SPIP, single-pass intestinal perfusion

Figure 2 : The *K<sub>a</sub>* obtained for in situ perfusion in the SPIP model for four different intestinal segments. The data are presented as the mean± SD (n=6)



Abbreviations: *P<sub>eff</sub>*, apparent permeability coefficients; min, minute; SD, standard deviation; SPIP, single-pass intestinal perfusion.

Figure 3 : The *P<sub>eff</sub>* obtained for in situ perfusion in the SPIP model for four different intestinal segments. The data are presented as the mean± SD (n=6)

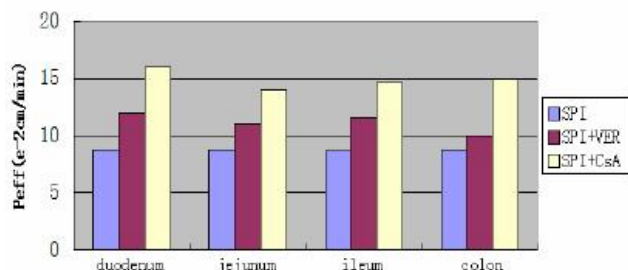


Abbreviations: *K<sub>a</sub>*, absorption rate constant; min, minute; SD, standard deviation; SPIP, single-pass intestinal perfusion

Figure 4 : The *K<sub>a</sub>* obtained for in situ perfusion in the SPIP model for four different intestinal segments. The data are presented as the mean± SD (n=6)

perfusion in the single-pass intestinal perfusion (SPIP) model for the four different intestinal segments are presented in Figures 2 and 3, respectively. The absorption rate constant (*K<sub>a</sub>*) in duodenum, jejunum, ileum and

colon were (0.062±0.002), (0.052±0.0009), (0.049±0.001), (0.053±0.0007)cm<sup>-1</sup>, respectively. Comparisons of the *K<sub>a</sub>* and *P<sub>eff</sub>* for spinosin alone or combined with P-gp inhibitors in the SPIP model are pre-sented in Figures 4 and 5. The data show that the coadministration with the P-gp inhibitors had higher *K<sub>a</sub>* and *P<sub>eff</sub>* values than the spinosin alone in all four intestinal segments, which could be due to the P-gp inhibition by CsA or Ver. In a previous study, our group observed that the absorption of spinosin in intestinal tract varied little indifferent PH conditions<sup>[1]</sup>. The results indicated that the absorption of spinosin could be affected by the P-gp, which widely distributed in intestinal tract. CsA and Ver inhibited the efflux of spinosin by P-gp located on the membrane of the intestinal tract barrier, which resulting in an increase of the spinosin absorption in the intestinal.



Abbreviations: *P<sub>eff</sub>*, apparent permeability coefficients; min, minute; SD, standard deviation; SPIP, single-pass intestinal perfusion

Figure 5 : The *P<sub>eff</sub>* obtained for in situ perfusion in the SPIP model for four different intestinal segments. The data are presented as the mean± SD (n=6)

### CONCLUSION

In conclusion, the results for the absorption mechanisms in the intestines indicated that efflux pump P-gp might exert some effects on the absorption of SPI. All of these results suggest that the enhanced oral bioavailability of spinosin can be attributed to combined with P-gp inhibitors. Additional studies are needed to determine the precise mechanism by which P-gp inhibitors enhance the oral bioavailability of drugs. Due to the change of the parameters of the bioavailability of substrate drugs caused by Chinese materia medica and its chemical compositions through acting on intestinal P-gp could influence the drug safety and efficacy, it is

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important that pharmacists should attach importance to the spinosin interactions in the concomitant use of Chinese traditional medicines and western drugs so as to promote rational drug use.

### ACKNOWLEDGMENTS

This work was supported by the National Major drug discovery, People's Republic of China (2012ZX09102201).

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