In Situ Intestinal Absorption Behaviors of Tanshinone IIA from Its Inclusion Complex with Hydroxypropyl-β-cyclodextrin

Wang LING,[a,b] Li Chen RUI,[b] and Jiang Xue HUA*,[b]

[a] *Institution of Clinical Pharmacological, West China Second University Hospital, Sichuan University; and* [b] *West China School of Pharmacy, Sichuan University; Chengdu 610041, P. R. China.* Received May 15, 2007; accepted July 14, 2007

In this paper, the intestinal permeability of the inclusion complex of tanshinone IIA (TS IIA) with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) was investigated. The corresponding complexation of TS IIA–HP-β-CD was obtained by coevaporation and characterized by differential scanning calorimetry and X-ray diffraction. The recirculation intestinal perfusion technique in rats was used to study the absorption behavior of free and complexed TS IIA. The change of concentration of TS IIA was separately calculated according to Michaelis–Menten and the Fick's equation to investigate its absorption rate-limiting step. Using the mathematical models above, it was concluded that the limit step to absorption of TS IIA was the dissolution process. Different concentrations of complexed TS IIA were administrated to three intestinal segments, with the intestinal permeability ranging from 3.16×10^{-5} cm·s^{-1} in the duodenum (50 μg·ml^{-1}) to 4.11×10^{-5} cm·s^{-1} in the jejunum (100 μg·ml^{-1}). With the increase of dosage of complex, TS IIA's absorption did not show saturated phenomenon, suggesting its transport mechanism *in vivo* might primary be passive transport. Besides, the permeability of TS IIA was not apparently influenced by the perfusion section studied, which indicated that there might not exist specific absorption site for TS IIA.

Key words tanshinone IIA; inclusion complex; hydroxypropyl-β-cyclodextrin; recirculation intestinal perfusion; intestinal absorption

The dried root of *Salvia miltiorrhiza* (Chinese name: *Danshen*) listed in the Chinese Pharmacopoeia (CP) is widely used traditional Chinese medicine to treat coronary heart disease, cerebrovascular disease, hepatocirrhosis and against bacteria. The chemical constituents of *Salvia miltiorrhiza* have been studied extensively and well documented.[1)] More than 30 diterpenoid quinone pigments, particularly known as tanshinones have been isolated and identified from *Danshen*.[2—4)] Tanshinone I, tanshinone IIA, and cryptotanshinone are the main abietane-type diterpense contained in *Danshen*. The pharmacological tests revealed that tanshinones can dilate coronary arteries, increase coronary flow and protect the myocardium against ischaemia. Some of them have also been used to treat neurasthenic insomnia. In addition, tanshinones have attracted particular attention because they exhibit significant antibacterial, antioxidant, and antitumor activities.[5)] And among them, the most abundant and structurally representative bioactive component in the fraction is tanshinone IIA (TS IIA) (Fig. 1).[6)] Then TS IIA is selected as the marker component for the quality control of *Danshen* and *Fufang Danshen* tablet in the Chinese Pharmacopoeia (2005). However, TS IIA's bioavailability is very low, which results in difficulties in its gastrointestinal formulation.

Cyclodextrins are cyclic oligosaccharides, containing at least 6 d-(+)-glucopyranose units attached by α-1,4-linkage.

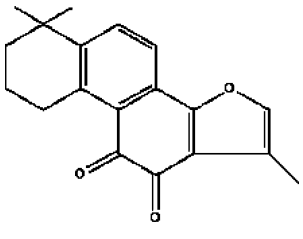

Fig. 1. Chemical Structure of TS IIA

β-Cyclodextrin appears to be the best natural cyclodextrin due to its cavity size, efficient drug complexation, availability in pure form, and relatively low cost.[7)] Natural cyclodextrins can be modified to improve the low aqueous solubility. One of the pharmaceutically important cyclodextrin derivatives is 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), which has greater solubilizing capacity and improved safety feature than the parent compound.[8,9)] More and more studies also applied HP-β-CD to improve the solubility, dissolution rate and the oral bioavailability of drugs.[10—13)]

The present study is to find the absorptive rate-limiting step of TS IIA *via* investigating its absorption behavior and to gain a reasonable explanation for the cause leading to low oral bioavailability. Thereafter, we prepared complexation of TS IIA with HP-β-CD by coevaporation and characterized them by differential scanning calorimetry (DSC) and X-ray diffractometry (XRD). The absorption properties of TS IIA from its inclusion complex in rats were investigated. We hope that this research could establish a base for investigating the absorption of the complexation of TS IIA with HP-β-CD *in vivo* and finding an optimum pharmaceutical carrier for developing the oral preparation of TS IIA.

MATERIALS AND METHODS

Materials TS IIA (98.63% $C_{19}H_{18}O_3$ MW=294.3) was purchased from Sichuan Huakang Medicine Raw Material Factory, and TS IIA standard was purchased from the National Institute for the Control of Biological and Pharmaceutical Drugs (P. R. China). HP-β-CD (average MW=1450), degree of substitution (DS)=0.8, was purchased from Sigma Chemicals Corporation.

Methanol (Fisher) was of HPLC grade. The water was double-distilled. All other reagents and solvents used in this experiment were of the highest purity commercially available. All water used was purified by ion exchange and re-

* To whom correspondence should be addressed. e-mail: strawberry_baby@126.com
© 2007 Pharmaceutical Society of Japan

moval of organic material using the Elgat Maxima (Bucks, U.K.) water purification system.

Animals Male Sprague-Dawley rats weighting 220—250 g were purchased from Laboratory Animal Center of Sichuan University, Sichuan, China. All experiments were approved by the Sichuan University Medical Research Ethical Committee for Animal Experiments. Prior to the experiments, the rats were housed in a temperature and humidity controlled room (23 °C, 55% air humidity, 12-h light cycle) with free access to water and standard rat chow. The rats were acclimated for at least 5 d and fasted overnight before the experiments.

HPLC Analysis The HPLC measurements were carried out by using a Shimadzu assembly equipped with a LC-10AT model pump, a SPD-LC10A model UV–VIS detector and CTO-10A model column oven. The mobile phase used was methanol : water (85 : 15); the flow rate was 1.0 ml·min^{-1}; the detection wavelength was 270 nm; the column temperature was 30 °C. Mobile phase was filtered through a type HA, 0.45 μm membrane filter (Millipore Corporation) and deaerated under reduced pressure.

Preparation of Solid Complexes All the complexes as well as physical mixture were prepared with 1 : 1 molar ratios. The weights taken for 1 : 1 ratio were 1.06 g of the TS IIA and 5.37 g of HP-β-CD.

The inclusion complex (IC) between the TS IIA and HP-β-CD was prepared by freeze-drying method. The required stoichiometric amount of the TS IIA was added, under magnetic stirring, to an aqueous solution of HP-β-CD. After 1 h at 30 °C of agitation, the resulting solution was subjected to a process of lyophilization in a freeze-dryer (Lyph-lock 6 apparatus, Labconco) for 3 d.

The physical mixture (PM) of the TS IIA and HP-β-CD was in the same weight ratio of the inclusion complex and prepared by simple mechanical mixing.

Differential Scanning Calorimetry (DSC) Approximately 5 mg of the TS IIA, HP-β-CD and inclusion complexes were subjected to DSC analysis, using a SEIKO EXSTAR6000 TGIDTA6300. Alumina was used as a reference material and the scanning rate was 10.00 °C·min^{-1}, with the scanning temperature range of 50 and 400 °C.

X-Ray Diffractometry (XRD) Powder X-ray diffraction patterns were obtained from a Philips MRD diffractometer. Samples were irradiated with monochromatized Cu/C radiation and analyzed between 2θ angles of 5 and 75° at a scan rate of 1 min^{-1}. Duplicate determinations were made for each of the samples.

***In Situ* Rat Intestinal Perfusion Experiment. Composition of Perfusion Solutions** Freshly prepared Krebs bicarbonate Ringer (KR) buffer was prepared with 114.3 mM NaCl, 4.2 mM KCl, 24.9 mM $NaHCO_3$, 12.2 mM glucose, 1.2 mM $MgCl_2$, 1.25 mM $CaCl_2$, 1.65 mM Na_2HPO_4 and 0.3 mM NaH_2PO_4, and adjusted to pH 7.4 with carbogen (95% O_2+5% CO_2) bubbling.[14]

In addition, in this experiment, the phenolsulfonphthalein (psp) was added into the blank KR buffer to act as a nonabsorbable marker to help identify any transfer of water into or out of the intestine and to monitor the structural integrity of the intestinal segment.[15]

Recirculating Intestinal Perfusion of Rats: Experimental Setup The rats were anaesthetized by intraperitoneal administration of sodium pentobarbital solution (40 mg·kg^{-1}). To maintain normal body temperature, the rats were placed under a surgical lamp. The temperature was measured in the segment and was found to be between 36 and 37 °C. The abdominal cavity was cut opened with a midline incision and upper small intestine was isolated. Intestinal (10 cm) segments were inserted by an inlet cannula and tied-off at the duodenum (2 cm from the pyloric sphincter), the jejunum (just distal to the ligament of Treitz) or the ileum (immediately proximal to the cecum).[16] Initially, the intestinal segment was rinsed with isotonic saline (37 °C) until the outlet solution was clear. The preparation time took approximately 15 min. The cannula was then connected to two plastic tubes that were connected to a constant-flow pump (HL-2, XiangShan Lid., China).[17—19] As soon as the experiment began, the perfused intestinal segment was repositioned in the abdominal cavity, unimpeded axial flow was maintained, and the abdominal incision closed with wound clips. The recirculation fluid flowed into the segment at a rate of 1.0 ml·min^{-1}. Each perfusion experiment lasted for 120 min, and 0.5 ml perfusion was quantitatively taken at 15 min intervals. Then the equal volume of fresh blank psp KR buffer was added into the recirculation fluid. The samples were filtered through 0.45 μm membrane filters. The first 20% of the filtrate was discarded, and then the total concentration of the TS IIA in the subsequent filtrate was analyzed by HPLC. Each experiment was replicated on 6 rats.

Intestinal Absorption Study Protocol. Comparison of the Absorption Behavior of Free and Complexed TS IIA Uncomplexed and complexed TS IIA dispersed in KR buffer, equivalent to 5 μg·ml^{-1} of TS IIA, were used as perfusate I and perfusate II. The intestinal behavior of free and complexed TS IIA was comparatively studied at the whole small intestine. The results were calculated and fitted *via* the following both mathematical models.

The Intestinal Absorption of Complexed TS IIA Complexed TS IIA of three different concentrations from low to high (50, 100, 150 μg·ml^{-1}) were prepared. The absorption parameters of TS IIA at each concentration in the three intestinal segments (duodenum, jejunum, ileum) were tested by the *in situ* method. Information on the absorption of TS IIA in rat intestinal could be obtained from the absorptive rate constant (K_a), apparent permeability coefficient (P_{app}) and absorptive fraction in unit time (P%).

Data Analysis The absorption rate of oral administration depended on the rate-limiting step in the drug dissolution and in the drug mucosal permeability.

When the rate-limiting step is drug dissolution, the drug absorption follows a nonlinear kinetics. The time course for the concentration of drug in the intestinal absorption can be estimated from the Michaelis–Menten expression[20]:

$$-\frac{dC}{dt}=\frac{V_{max}C}{K_m+C} \quad (1)$$

Where V_{max} is the maximum rate of absorption and K_m is the Michaelis constant. $-dC/dt$ is the rate of absorption.

The rate of change in perfusate solution concentration, together with the drug concentration at the midpoint of each sampling period, C_m, can be incorporated into a number of expressions to solve for V_{max} and K_m. From the Eq. 1 the following typical expression can be derived:

$$-\frac{\Delta t}{\Delta C}=\frac{K_m}{V_{max}}\times\frac{1}{C_m}+\frac{1}{V_{max}} \quad (2)$$

When the rate-limiting step is drug mucosal permeability, the time course for the concentration of drug in the intestinal absorption can be estimated from the Fick's equation[21]:

$$-\frac{dC}{dt}=\frac{DkA}{h}(C-C_b) \quad (3)$$

where, $-dC/dt$ is absorptive rate, D is the permeability coefficient in membrane, k is the distribution coefficient, A is the surface area of the absorbing membrane, h is the thickness of the absorbing membrane, $(C-C_b)$ represents the concentration difference of the drug diffusion direction, *i.e.*, concentration difference between absorbing membrane thickness (between inside and outside of membrane).

When C is much greater than C_b and K_a is equal to DkA/h, from the Eq. 3 the following equation can be derived:

$$-\frac{dC}{dt}=K_aC \quad (4)$$

Where, K_a is the absorptive rate constant, Eq. 4 can be integrated, then be written in a form which includes concentration at time 0 and concentration at any given time t. At time 0, concentration is the initial concentration. Thus, Eq. 4 becomes

$$\ln C_t=\ln C_0-K_at \quad (5)$$

There are two kinds of methods to assess the absorptive rate-limiting step.[22] One of which is to choose the model with the larger correlation coefficient, another assessment is to utilize the maximum R^2 method. The equation expression is:

$$R^2=\frac{\sum_{i=1}^{n}y_i^2-\sum_{i=1}^{n}(y_i-\hat{y}_i)^2}{\sum_{i=1}^{n}y_i^2} \quad (6)$$

Where, y_i is the actuality value, $\hat{y}_i$ is the computed value obtained by the two Eqs. 2 and 5. The model with the larger R^2 value indicates to accord with the actual state.

RESULTS AND DISCUSSION

Determination of TS IIA Content determination for TS IIA was carried by HPLC by in ethanol ranging from 0.023 to 5.65 $\mu g\cdot ml^{-1}$ was $A=1.5519\times10^5C-1110.22$ ($r^2=0.9999$). Precision assay showed the average of the relative standard deviations (RSD) within 1 d was 1.7% and intraday was 2.2%. The mean recovery was 98.39±3.68% ($n=3$).

The regression equation for TS IIA concentration in perfusate *versus* response of peak area ranging from 0.050 to 2.01 $\mu g\cdot ml^{-1}$ was $A=1.1312\times10^5C-333.09$ ($r^2=0.9998$). From the obtained analytical parameters it can be concluded that the method fulfils analytical requirements with an adequate repeatability (<2%). The mean recovery was 100.87±5.45% ($n=3$).

Characterization of Solid Complexes Figure 2 showed the DSC thermograms of TS IIA and HP-β-CD corresponding binary systems. The thermogram of the TS IIA revealed an endothermic peak at 219.0 °C, corresponding to its melting point. The thermogram of HP-β-CD showed a very broad endothermic effect, which attained a maximum around 100 °C and 50 °C, due to the dehydration effect. The thermogram of PM still demonstrated the melting point of the TS IIA at 218.6 °C, indicating that an inclusion complex could not be obtained by simply blending the drug and HP-β-CD. The complete disappearance of the TS IIA endothermic peak was observed for the complex, indicating that the TS IIA was incorporated in the HP-β-CD cavity. Further evidence of inclusion complex formation was observed from X-ray powder diffractograms. Figure 3 showed the X-ray diffractograms of the TS IIA, HP-β-CD and corresponding complexes. The diffraction pattern of the TS IIA displayed crystallinity, which was shown by the sharp peaks, whereas HP-β-CD was amorphous in the solid state. The diffraction pattern of PM system was found to be the simple sum of the TS IIA and HP-β-CD. The diffractogram of IC did not exhibit peaks corresponding to the TS IIA and was practically identical to those of the amorphous HP-β-CD. Thus, the X-ray diffraction analysis confirmed the DSC results: the diffraction peaks relevant to crystalline TS IIA were no longer detectable in the inclusion complex, and the reduction of the degree of crystallinity of the drug could be taken as an indication of complexation. The complex system displayed diffuse diffraction pattern (identical to that of HP-β-CD without drug peaks), indicating the entirely amorphous nature of TS IIA in the complex.[23,24]

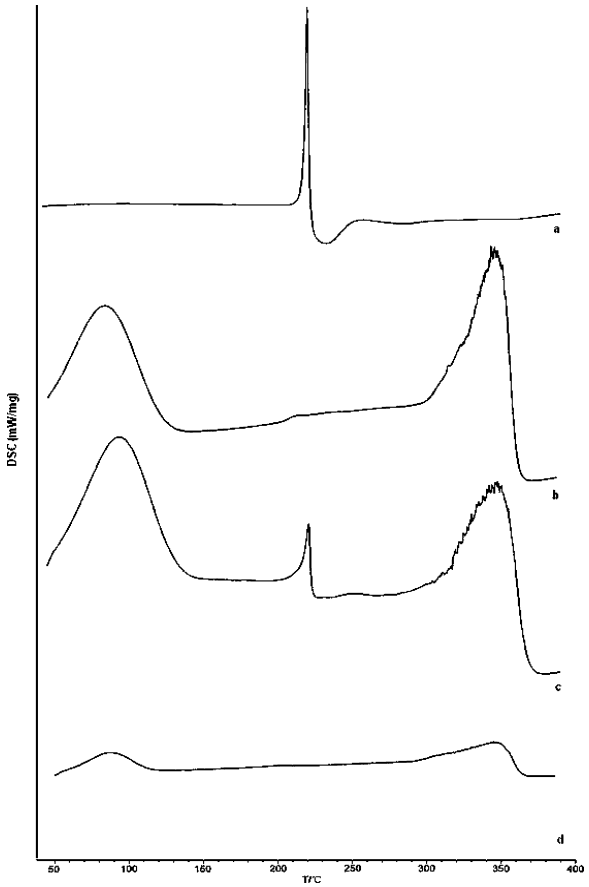

Fig. 2. DSC Thermograms of (a) TS IIA, (b) HP-β-CD, (c) Physical Mixture (PM), and (d) Inclusion Complex (IC)

Comparison of the Absorption Behavior of Free and Complexed TS IIA The upper boundary of the concentration range ($5\,\mu g\cdot ml^{-1}$) was limited because of the low aqueous solubility of free TS IIA. In 2 h *in situ* rat intestinal perfusion experiments, the average remaining concentration values of TS IIA in the perfusate I, II were showed in Table 1. Fitting the data in the table by the above two models, the correlation coefficients and the R^2 of the regression equations were showed in Table 2. The results indicated the absorption process of the uncomplexed TS IIA was consistent with the first model and dissolution was the absorptive rate-limiting step, however, the absorption process of the inclusion complex was more suitable to the second model and the absorptive rate-limiting step was the intestine membrane permeation process. In fact, some reports indicated that CDs had been investigated the interaction between CDs with intestinal membrane and demonstrated the mechanism of the enhancement of intestinal absorption of drugs.[25,26] Sharma *et al.* came to the conclusion that perturbation of intestinal membrane by β-CD as well as HP-β-CD would result into release of proteins from enterocytes into the lumen. This was due to extraction of lipoidal constituents by phenomenon of inclusion complex formation leading to disorder in membrane lipid and/or interacting with hydrophilic domains of membrane. This maybe resulted in increased permeability across intestinal membrane and showed the potential to improve oral delivery.

The Absorption Parameters of the Complexed TS IIA According to the absorption process, which fits the Fick's formula, the absorption parameter K_a was calculated by Eq. 5. In the usual way, the calculating method of the absorptive fraction in unit time (P%) was quantified using Eq. 7.

$$P\%\,(h^{-1}) = \frac{(C_0V_0 - C_tV_t)}{C_0V_0} \times 100\% \tag{7}$$

Where C_0 is the initial TS IIA concentration in perfusate, V_0 is the initial volume of perfusate. C_t is the final TS IIA concentration in perfusate; V_t is the final volume of perfusate. t is the time of circulation of perfusate.

Intestinal permeability, directly reflecting the interactions of the molecule with the tissue was affected by the physiological properties of the tissue as well as the physicochemical properties of the transported compound. Thus, permeability measurement is important to predict absorption of a compound. Apparent permeability coefficient (P_{app}) was calculated according to the Eq. 8

$$P_{app} = \frac{K_a}{3600 \times A} \tag{8}$$

Where K_a is the absorption rate constant according to the second model, A is the area of exposed tissue and 3600 represents 3600 seconds per hour.

In 2 h *in situ* intestinal perfusion experiments, after the administrations of three concentrations of complexed TS IIA in each small intestinal tissue, including duodenum, jejunum and ileum, the average parameters were listed in Table 3. Complexed TS IIA exhibited an intestinal permeability ranging from $3.16\times10^{-5}\,cm\cdot s^{-1}$ in the duodenum ($50\,\mu g\cdot ml^{-1}$) to $4.11\times10^{-5}\,cm\cdot s^{-1}$ in the jejunum ($100\,\mu g\cdot ml^{-1}$). A one-way ANOVA test was used to assess significant difference between P_{app} values for TS IIA in different concentrations at each segment. Statistical analysis results showed the TS IIA permeability had no significant difference regardless of in the proximal, mean and distal segments when the initial

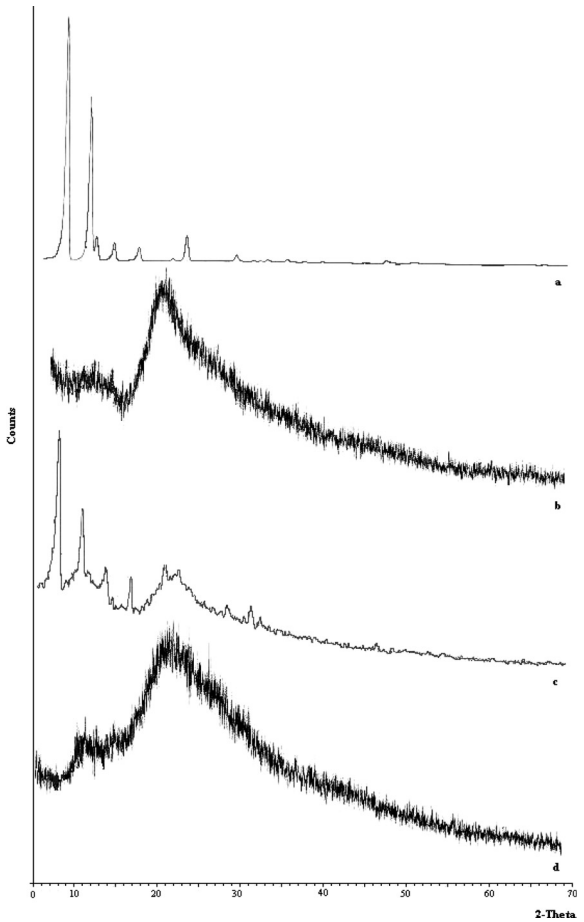

Fig. 3. X-Ray Diffractograms of (a) TS IIA, (b) HP-β-CD, (c) Physical Mixture (PM), and (d) Inclusion Complex (IC)

Table 2. Stimulated Results in Accordance with Two Mathematical Models

Sample	Correlative index		Fitting index	
	Model 1	Model 2	Model 1	Model 2
Perfusate I	0.9954	0.9862	0.9999	0.9988
Perfusate II	0.4319	0.9837	0.7380	0.9723

Model 1 represents the relationship of remaining concentration of TS IIA and time is calculated *via* the Michaelis–Menten mathematical model. Model 2 represents their relationship is calculated *via* the Fick's equation.

Table 1. The Remaining Concentration of TS IIA ($\mu g\cdot ml^{-1}$) in Two Perfusates at Each Time Interval

Time (h)	0	0.25	0.5	0.75	1	1.25	1.5	1.75	2
Perfusate I	4.98±0.14	4.53±0.22	4.09±0.31	3.66±0.09	3.24±0.51	2.83±0.36	2.43±0.19	2.05±0.21	1.69±0.16
Perfusate II	4.97±2.17	3.44±1.27	2.89±0.94	1.73±0.78	1.39±0.44	1.03±0.21	0.89±0.26	0.78±0.23	0.28±0.13

Table 3. Absorption Parameters of TS IIA from Its Complex at a Series of Concentration in Three Tested Intestinal Segments

Intestinal segment	TS IIA concentration ($\mu g \cdot ml^{-1}$)	Complexed TS IIA		
		P_{app}, $\times 10^{-5} cm \cdot s^{-1}$	K_a/h^{-1}	$P\%/h^{-1}$
Duodenum	50	3.16±0.52	1.37±0.27	56.63±12.55
	100	3.32±1.06	1.43±0.46	59.30±17.41
	150	3.63±0.61	1.57±0.26	64.47±11.12
Jejunum	50	3.27±0.71	1.41±0.31	58.09±13.37
	100	4.11±0.87	1.77±0.36	70.23±14.76
	150	3.54±0.73	1.53±0.37	61.54±16.28
Ileum	50	3.37±0.54	1.45±0.23	59.75±10.54
	100	3.76±0.74	1.63±0.32	67.89±13.71
	150	3.25±1.11	1.40±0.47	57.71±18.93

perfusion solution concentration increased from 50 to 150 $\mu g \cdot ml^{-1}$ ($p>0.05$). The data suggested that the absorption process of TS IIA from the inclusion complex was linear for the range of perfused concentration. The ways of drug absorbed from gastrointestinal tract into system circulation usually can be divided as passive transport, facilitated diffusion and active transport.[27] Unlike the active process, the passive diffusion of a compound through the intestinal cell membrane was driven by the concentration gradient from the intestinal lumen to the blood supply with a constant permeability coefficient. In this study, varying the donor concentration of TS IIA did not lead to a significant statistical deviation in the permeability coefficient at various intestinal segments, which indicated the absorption processes of TS IIA had no saturation phenomenon. This suggested that a passive diffusion process might not be excluded for transport of TS IIA from the complex through the intestinal membrane. The statistical test of P_{app} at the same concentration in different segments was performed the same as above. Comparisons across the different segments showed that the permeability at 100 $\mu g \cdot ml^{-1}$ was higher in the jejunum compared with the other segments, but did not reach statistical significance ($p>0.05$). In the other concentration, the permeability of TS IIA was not apparently influenced by the perfusion section studied. The results indicated that the absorption of TS IIA had not the specific site in the small intestine.

Intestinal absorption is a complex process where not all underlying mechanisms are fully understood, though the *in situ* technique provides the advantages of experimental control (*e.g.* compound concentration, pH, osmolality), ability to study regional differences, and an intact intestinal blood supply and innervations of the animal.[28] Several factors, such as, intestinal disease, surgery and the choice of anaesthetic might influence the absorption parameters. For instance, it was recently reported that surgery and anaesthetics might cause anoxia and decease in intestinal blood flow, motility and energy supply, which consequent decreases of both passive and active transport.[17] Therefore, it is likely that no single experimental method will be ideal to study absorption process and that maximum information will often require corroborative evidence from more than one method.[29] Further studies on the human Caco-2 cells (the human colon carcinoma cells) and *in vivo* animal experiments are in progress to verify the present results.[30] The correlations are being established with data generated from the *in situ* rat perfusion and the Caco-2 culture model.

Acknowledgements This research work is granted by the National Natural Science Foundation of China, with project number 30070935 and 30271614.

REFERENCES

1) Lin H. C., Chang W. L., *Phytochemistry*, **8**, 951—953 (2000).
2) Lin L. Z., Wang X. M., Huang X. L., Huang Y., Yang B. J., *Acta Pharm. Sin.*, **4**, 273—275 (1988).
3) Li Z. T., Yang B. J., Ma G. E., *Acta Pharm. Sin.*, **3**, 209—213 (1991).
4) Tian G. L., Zhang T. Y., Zhang Y. B., Ito Y., *J. Chromatogr. A*, **1–2**, 281—285 (2002).
5) Shi Z. H., He J. T., Chang W. B., *Talanta*, **2**, 401—407 (2004).
6) Tian G. L., Zhang Y. B., Zhang T. Y., Yang F. Q., Ito Y., *J. Chromatogr. A*, **1**, 107—111 (2000).
7) Cheng K. W., Li X. H., *J. Shenyang Ag. Univ.*, **4**, 313—316 (2001).
8) Albers E., Müller B. W., *J. Pharm. Sci.*, **81**, 756—761 (1992).
9) Loftsson T., Brewster M. E., *J. Pharm. Sci.*, **85**, 1017—1025 (1996).
10) Hovgaard L., Brondsted H., *Pharm. Res.*, **12**, 1328—1332 (1995).
11) Castillo J. A., Canales J. P., Garcia J. J., Lastres J. L., Bolas F., Torrado J. J., *Drug Dev. Ind. Pharm.*, **25**, 1241—1248 (1999).
12) Rawat S., Jain S. K., *Eur. J. Pharm. Biopharm.*, **2**, 263—267 (2004).
13) Shi J. M., He Z. G., Xing N., Geng G. X., Sun Y. H., Li H. B., *Chin. Pharm. J.*, **5**, 353—355 (2004).
14) Zheng Y. Q., Qiu Y. H., Fu L. M. Y., Hoffman D., Reiland T. L., *Int. J. Pharm.*, **185**, 83—92 (1999).
15) Cui S. M., Zhao C. S., He Z. G., Xu W., *J. Shenyang Pharm. Univ.*, **19**, 161—164 (2002).
16) Rama Prasad Y. V., Minamimotoa T., Yoshikawa Y., Shibata N., Mori S., Matsuura A., Takada K., *Int. J. Pharm.*, **271**, 225—232 (2004).
17) Fagerholm U., Johansson M., Lennernäs H., *Pharm. Res.*, **9**, 1336—1342 (1996).
18) Annaert P., Tukker J. J., van Gelder J., Naesens L., de Clercq E., van Den Mooter G., *J. Pharm. Sci.*, **8**, 1054—1062 (2000).
19) Manosroi J., Apriyani M. G., Foe K., Manosroi A., *Int. J. Pharm.*, **1–2**, 235—240 (2005).
20) Welling P. G., "Pharmacokinetics Processes and Mathematics," 1st ed., Chap. 16, American Chemical Society, Washington, D.C., 1986, pp. 255—263.
21) Madan P. L., "Biopharmaceutics and Pharmacokinetics," 1st ed., Chap. 5, Jaypee Brothers Medical Pub., New Delhi, 2000, pp. 99—100.
22) Lv W. L., Tu X. D., Zheng F. X., Cao W. J., *J. China Pharm. Univ.*, **4**, 210—214 (1997).
23) Esclusa-Dýaz M., Torres-Labandeira J., Kata M., Vila-Jato J., *Eur. J. Pharm. Sci.*, **1**, 291—296 (1994).
24) Mura P., Adragna E., Rabasco A., Moyano J., Perez-Martinez J., Arias M., Gines J., *Drug Dev. Ind. Pharm.*, **25**, 279—287 (1999).
25) Sharma P., Varma M. V. S., Chawla H. P. S., Panchagnula R., *IL Farmaco*, **60**, 884—893 (2005).
26) Yamamoto A., Okagawa T., Kotani A., Uchiyama T., Shimura T., Tabata S., Muranishi S., *J. Pharm. Pharmacol.*, **49**, 1057—1061 (1997).
27) Lipinski C. A., Lombardo F., Dominy B. W., Feeney P. J., *Adv. Drug Delivery Rev.*, **46**, 3—26 (2001).
28) Chen Y., Ping Q. N., Guo J. X., Lv W. L., Gao J., *Int. J. Pharm.*, **261**, 21—26 (2003).
29) Lipka E., Amidon G. L., *J. Controlled Release*, **62**, 41—49 (1999).
30) Saha P., Jim H. K., *Eur. J. Pharm. Biopharm.*, **50**, 403—411 (2000).