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Complexation of tanshinone IIA with 2-hydroxypropyl-β-cyclodextrin: Effect on aqueous solubility, dissolution rate, and intestinal absorption behavior in rats

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Abstract

In this paper, the effect of 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) on the aqueous solubility, dissolution rate, and intestinal permeability of the tanshinone IIA (TS IIA) was investigated. The corresponding inclusion complex of TS IIA/HP-β-CD at the molar ratio of 1:1 was obtained by coevaporation and characterized by differential scanning calorimetry, and X-ray diffraction. The solubility of complexed TS IIA in water at $37 \pm 0.1\,^{\circ}C$ was 17 times greater than that for the uncomplexed drug. The dissolution rate of TS IIA was significantly increased by the complexation with HP-β-CD, due to its solubilizing activity. The everted intestinal sac technique in rats was used to study the absorption behavior of TS IIA and this complexation through the intestinal tissues. The permeability rates of TS IIA across the intestinal epithelial membrane were enhanced by the formation of inclusion complex with HP-β-CD about 5.2, 5.8 and 4.8 times of the uncomplexed TS IIA in duodenum, jejunum and ileum, respectively. It was revealed that the absorption rate-limiting step of TS IIA might be the dissolution process. The present results indicate the potential use of HP-β-CD to improve the gastrointestinal tract absorption of TS IIA.

Keywords: Tanshinone IIA; Hydroxypropyl-β-cyclodextrin; Inclusion complex; Dissolution study; Solubility study; Intestinal absorption

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides, containing six, seven, or eight D-(+)-glucopyranose units (α-, β-, and γ-cyclodextrin) attached by α-1, 4-linkage. CDs can trap the lipophilic drug as guest in a cage-like meshwork, thereby enhancing its solubility and dissolution rate (Blanco et al., 1991; Palmieri et al., 1997; Castillo et al., 1999). β-cyclodextrin appears to be the best natural cyclodextrin due to its efficient drug complexation and availability in pure form (Cheng and Li, 2001). However, natural β-cyclodextrin can be modified to improve the low aqueous solubility ($18.5\,g\,l^{-1}$ at $25\,^{\circ}C$). One of the pharmaceutically important β-cyclodextrin derivatives is 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), which has higher water solubility and greater solubilizing capacity and complexing property than the parent compound and improved safety feature (Albers and Müller, 1992; Loftsson and Brewster, 1996). More and more studies also applied HP-β-CD to improve the solubility and dissolution rate, physical and chemical stability and the oral bioavailability of drugs, especially when the rate-limiting step in drug absorption is poor dissolution (Hovgaard and Brondsted, 1995; Castillo et al., 1999).

Danshen, the root of the Chinese herbal plant-*Salvia miltiorrhiza Bunge*, is classified as "Blood invigorating" in traditional Chinese medicine. The chief bioactive ingredients of *Danshen* are the phenanthrofurane quinone derivatives, particularly known as tanshinones (Lin et al., 1988; Li et al., 1991; Lin and Chang, 2000; Tian et al., 2002). The pharmacological tests revealed that tanshinones can dilate coronary arteries, increase coronary flow, and protect the myocardium against ischemia. In addition, tanshinones have attracted particular attention because they exhibit significant antibacterial, antioxidant, antitumor, anti-inflammatory, and anti-platelet aggregation activities (Shi et al., 2004). Among them, the most abundant, effective and structurally representative bioactive constituent in the fraction

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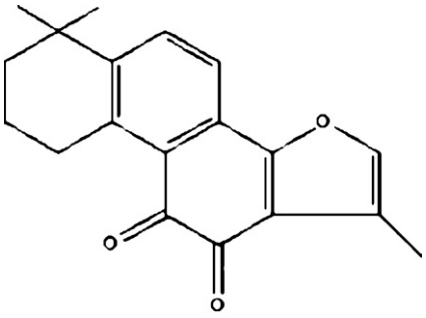

Fig. 1. Chemical structure of TS IIA.

is tanshinone IIA (TS IIA) (Fig. 1) (Tian et al., 2000), which is selected as the marker component for the quality control of containing *Danshen* preparations in the Chinese Pharmacopoeia (2005 Edition). However, its oral bioavailability is very low, and the plasma concentration is well below 10 ng ml^{-1} (Tian et al., 2003; Li et al., 2004, 2005). This is probably due to TS IIA's poor solubility in water and insufficient dissolution rate (Blanchard et al., 2000; Arima et al., 2001). As a result, to find an approach to increase its oral bioavailability by enhancing its dissolubility using a pharmaceutical carrier seems necessary and urgent.

The main objective of the present study was to evaluate the effect of HP-β-CD on the solubility, dissolution rate and intestinal absorption of TS IIA. So, 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 phase solubility, dissolution rates and absorption properties of TS IIA in rats were examined and comparatively evaluated. Till now a number of research work about TS IIA has concentrated on the pharmacology and quantitative analytical methods, and there are few reports concerning the inclusion complex of TS IIA with HP-β-CD but no literature about the intestinal permeability of TS IIA in humans or in laboratory animals (Fan et al., 2005; Yan et al., 2006).

2. Materials and methods

2.1. 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 (PR 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. All other reagents and solvents used in this experiment were of the highest purity commercially available. The water used was purified by ion exchange and removal of organic material using the Elgat Maxima (Bucks, UK) water purification system.

2.2. Animals

Healthy male Sprague-Dawley rats (250–300 g) were purchased from Laboratory Animal Center of Sichuan University, Sichuan (PR China). Prior to the experiments, the rats were housed in a temperature and humidity controlled room (23 °C, 55% air humidity) with free access to water and standard rat chow. The rats were acclimated for at least 5 days and fasted overnight but supplied with water ad libitum before the experiments. All experiments were approved by the Institutional Animal Care and Use Committee of Sichuan University.

2.3. Determination of TS IIA

Concentrations of TS IIA were determined by high-pressure liquid chromatography (HPLC). The HPLC measurements were carried out by using a Shimadzu assembly equipped with a LC-10AT model pump, a Diamonsil-ODS column (150 mm × 4.6 mm, 5 μm, Dikma, Canada), a SPD-LC10A model UV–vis detector at 270 nm. The mobile phase used was methanol: water (85:15), and delivered at a flow rate of 1.0 ml min^{-1}. The injection volume was 20 μl. Mobile phase was filtered through a type HA, 0.45 μm membrane filter (Millipore Corporation) and deaerated under reduced pressure.

2.4. Preparation of the solid complexes

Inclusion complex (IC): The solid TS IIA/HP-β-CD complex were prepared by coevaporation with the weights taken for 1.06 g of TS IIA and 5.37 g of HP-β-CD (i.e. molar ratio of 1:1). The required stoichiometric amount of TS IIA and HP-β-CD were dissolved in the minimum amount of 60% ethanol to obtain a solution, and agitated for 1 h at 30 °C. Then, the suspension was filtered through 0.45 μm Millipore filter. The filtrate was evaporated under vacuum at 40 °C and 100 rpm in a rotary evaporator (ShenSheng Corp., China) until dryness.

Physical mixture (PM): A physical mixture of TS IIA and HP-β-CD in the same weight ratio as the coevaporated complex was prepared. PM was previously sieved individual components through a 315 μm mesh in a mortar and pestle for 3 min.

2.5. Characterization of the solid complexes

2.5.1. Differential scanning calorimetry (DSC)

Approximately 5 mg of TS IIA, HP-β-CD, PM and IC 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. Duplicate determinations were carried out for each sample.

2.5.2. Powder X-ray diffractometry (XRD)

Powder X-ray diffraction patterns were obtained from a Philips X'Pert, model PW 3040/00 diffractometer. Samples were irradiated with monochromatized Cu/Kα radiation and analyzed between a 2θ range of 5–70° (where theta is the scattering angle) with a step size of 0.05° (2θ). Duplicate determinations were made for each sample.

2.6. Phase solubility studies

Solubility measurements were carried out according to a modification of the method of Higuchi (Higuchi and Connors, 1965). Excess amounts of TS IIA were added to 10 ml of aqueous solutions containing various concentration of HP-β-CD (0–24.14 mM). The suspensions were shaken for 3 days at 37 ± 0.1 °C on a shaker (150 r min^{-1}) and protected from light to equilibrate. After equilibration, the suspensions were filtered through 0.45 μm Millipore membrane filters. The first 15% of the filtrate was discarded to avoid any potential loss of the drug, because of absorption by the filter until and the subsequent filtrate was collected. All procedures were conducted at the test temperature to avoid any precipitation of the drug. The filtrate was appropriately diluted by ethanol and the concentration of the TS IIA in the filtrate was determined by HPLC. Earlier experiments showed that the presence of HP-β-CD did not interfere with the assay at the concentration employed.

2.7. Dissolution studies

In vitro the dissolution studies were conducted according to the CP2005 in the deionized water at 37 ± 0.5 °C by the paddle method at a rotation speed of 100 ± 2 rpm using a six-vessel dissolution apparatus (Erweka, DT-D6, Germany). Powdered samples containing 25 mg of TS IIA or its equivalent in complexed or PM with HP-β-CD were added to the dissolution medium (250 ml). At predetermined time intervals (2, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, and 60 min), aliquots (0.5 ml) were withdrawn for HPLC determination of TS IIA concentration following filtration (0.45 μm) and replaced by an equal volume of the same dissolution medium kept at 37 ± 0.5 °C. Samples should be withdrawn from a zone roughly midway between the surface of dissolution medium and the top of the rotating blade. Each experiment was carried out in triplicate.

2.8. In vitro intestinal absorption studies: everted intestinal sacs of rats

2.8.1. Everted intestinal sacs of rats: experimental setup

Everted intestinal sac experiments were performed based on the method described by Barr and Riegelman (1970) and Barthe et al. (1998). The rats were anaesthetized by intraperitoneal administration of sodium pentobarbital solution (40 mg kg^{-1}). After the abdominal area had been shaved, the abdominal cavity was cut opened with a midline incision (2–3 cm). The intestinal segment of interest was isolated carefully and removed quickly, including 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) (Rama Prasad et al., 2004). Rats were sacrificed with an overdose of sodium pentobarbital by injection into the heart before recovering from anaesthesia. The excised pieces of intestinal segments were immediately flushed with ice-cold PBS (Bio Whittaker, USA) to clean it from intestinal contents and removed the underlying mesenterium. The intestinal segments of approximately 10 cm in length were tied at one end, everted and blotted dry to remove most of the mucus present. Then the sacs (i.e. a receiver compartment) were filled with 3.5 ml of clean Krebs-Ringer Bicarbonate (KR) buffer (i.e. a serosal fluid) (Sigma, St. Louis, USA), and the other end was ligated with needle for the sampling.

At time equal to zero, the everted intestinal sac was submerged in 50 ml of the experimental test suspension (i.e. mucosal fluid). The mucosal fluid (donor compartment) was saturated with 5% CO_2 and 95% O_2 (carbogen) gas in 37 ± 0.1 °C waterbath (Stuart Scientific, England). The whole sample solution from the receiver compartment was taken every 10 min for 3 h, then equivalent fresh KR buffer was added to serve as the serosal solution at the time intervals and incubation was continued. The process of the absorption study must be away from light. Each experiment was performed six times. After each experiment the mucosal integrity of the everted intestinal sac was inspected microscopically. The samples were prepared for HPLC analysis by filtering through 0.45 μm membrane filters. Appropriate dilutions were made with KR buffer such that the final concentration was within the linear portion of the standard curve.

2.8.2. Validation of the integrity of the everted rat intestinal sacs

Intestinal segment samples were fixed in 12% (v/v) formaldehyde-saline solution for 24 h. Then they were serially dehydrated by increasing concentrations of ethanol, embedded in paraffin. Two to 4 μm sections were cut and stained with hematoxylin and eosin for histological examination (Masahiro et al., 2002; Ruan et al., 2006). All slides were observed under 200× in Olympus C-7070. Quantitative parameters (Nucleo-apical distance, NPA and Villi index, Vi) were determined as described by Polentarutti et al. (1999).

2.8.3. Intestinal absorption study protocol

2.8.3.1. Stability studies. The stabilities of TS IIA and its complex were studied in the blank KR buffer and the transport KR buffer. Especially, the preparation of the transport KR buffer was that the blank KR buffer was recirculating perfused in the intestinal lumen and collected at 37 ± 0.1 °C after 3 h. The solutions of TS IIA and its complex (10.8 μg ml^{-1}) were incubated in blank and transport KR buffer in a total volume of 50 ml at 37 ± 0.1 °C for 3 h, respectively. Samples (0.4 ml) were withdrawn at 0, 30, 60, 90, 120, and 180 min. The samples were pretreated and assayed as described above.

In addition, we had also simultaneously compared the influence of light on TS IIA content in above-mentioned conditions.

2.8.3.2. The absorption behavior of free and complexed TS IIA in rat intestinal tissues. Uncomplexed TS IIA and TS IIA/HP-β-CD inclusion complex dispersed in blank KR buffer solution, equivalent to 0.25 mg ml^{-1} of TS IIA, were used as experimental test suspensions. Information on the absorption of TS IIA in rat various intestinal regions could be obtained from the apparent permeability coefficient (P_{app}) and the amount absorbed (Q_s).

2.8.4. Calculation of in situ absorption parameters

Apparent permeability coefficient (P_{app}) was calculated according to the following equation (Zheng et al., 1999):

$$P_{app} = \left(\frac{dQ}{dT}\right)\left(\frac{1}{A \times 60 \times C_d}\right) \quad (1)$$

where P_{app} is the apparent permeability coefficient ($cm\,s^{-1}$), Q the amount of drug transport in time t (min), A the area of exposed tissue, and C_d represents the initial concentration of soluble drug on the donor side and 60 represents 60 s/min. In this study, C_d were determined to be 11.2 and 188.7 $\mu g\,ml^{-1}$ for uncomplexed TS IIA and complexed TS IIA, respectively.

The perfusion area of the intestine (cm^2) was measured after each experiment. The excised intestinal sac was cut along the mesenteric line and spread on a flat surface. The length and width was electronically measured with a Vernier Caliper and the area was calculated mathematically.

In the usual way, the calculating method of the accumulated absorptive amount in the serosal compartment (Q_s) was quantified using the following equation:

$$Q_s = 3.5 \times (C_{s1} + C_{s2} + \cdots\cdots + C_{si}) \quad (2)$$

where C_{s1} is TS IIA concentration in the serosal compartment at the first time interval, 3.5 is the volume of the serosal compartment. C_{si} is the final TS IIA concentration in the serosal compartment.

2.8.5. Statistical analysis

The results were expressed as the mean ± S.D. of at least six independent experiments, unless otherwise stated. The P_{app} average values found at the different intestinal segments were compared using a one-way analysis of variance (ANOVA) test followed by a multiple-range post hoc test (Ruiz-Balaguer et al., 2002). In addition, statistical analysis was performed using the Student's t-test to compare two data sets, i.e. the difference of P_{app} values of uncomplexed and complexed TS IIA. A p-value <0.05 was considered statistically significant.

3. Results and discussion

3.1. Determination of TS IIA

Content determination for TS IIA was carried by HPLC by in ethanol ranging from 0.05 to 25.65 $\mu g\,ml^{-1}$ was $A = 1.5519 \times 10^5 C - 1110.22$ ($r^2 = 0.9999$). Based on the concentration of TS IIA at 0.05, 8.55, and 25.65 $\mu g\,ml^{-1}$, the assay intra-day precision were 2.46, 1.93, and 0.78% with a mean recovery of 98.42 ± 3.79, 98.81 ± 3.16, and 97.95 ± 3.91% and inter-day precision were 3.11, 2.27 and 1.29% ($n = 3$).

The regression equation for TS IIA concentration in KR solution versus response of peak area ranging from 0.042 to 8.40 $\mu g\,ml^{-1}$ was $A = 1.1312 \times 10^5 C - 333.09$ ($r^2 = 0.9998$). From the obtained analytical parameters it can be concluded that the method fulfils analytical requirements with an adequate repeatability (<2%). The mean recovery of TS IIA in KR solution at concentrations of 0.042, 5.40, and 8.40 $\mu g\,ml^{-1}$ were 100.88 ± 4.85, 102.51 ± 6.10, and 99.26 ± 5.32% ($n = 3$).

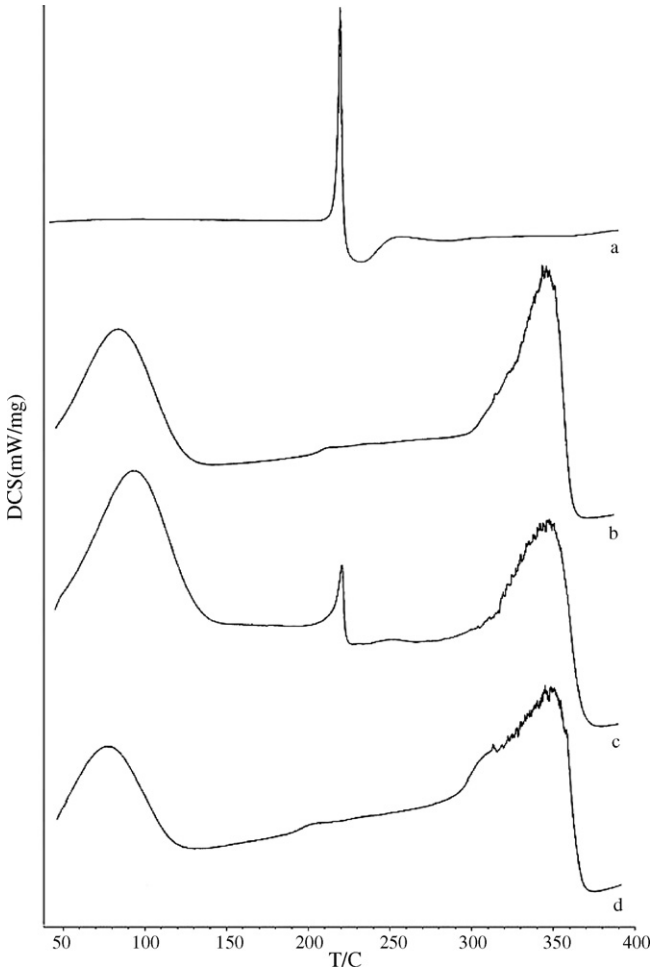

Fig. 2. DSC thermograms of (a) TS IIA, (b) HP-β-CD, (c) physical mixture (PM), and (d) inclusion complex (IC).

3.2. Characterization of the solid complexes

Some evidence of inclusion complexation was obtained from thermal analysis. When guest molecules were embedded in HP-β-CD cavities, their melting, boiling or sublimation point generally could shift to a different temperature or disappear within the temperature range where HP-β-CD was decomposed (Cabral Marques et al., 1990). The thermograms of TS IIA and HP-β-CD corresponding binary systems were shown in Fig. 2. The DSC curve of TS IIA exhibited a sharp endothermic peak at 219.0 °C, corresponding to the melting point of the drug (Fig. 2a). The thermogram of HP-β-CD showed a very broad endothermic effect, which attained a maximum around 70 °C, due to the release of bound water in the cavity (Fig. 2b). Besides, the small base shift around 200 °C resulted from the transconformation of the molecule (i.e. glass transition) and a decomposition process took place at around 300 °C (Marini et al., 1993; Muñoz-Ruiz and Paronen, 1997; Suihko et al., 2001; Liu et al., 2006; Liu and Zhu, 2006;). Concerning the physical mixture of TS IIA with HP-β-CD, the drug endother-

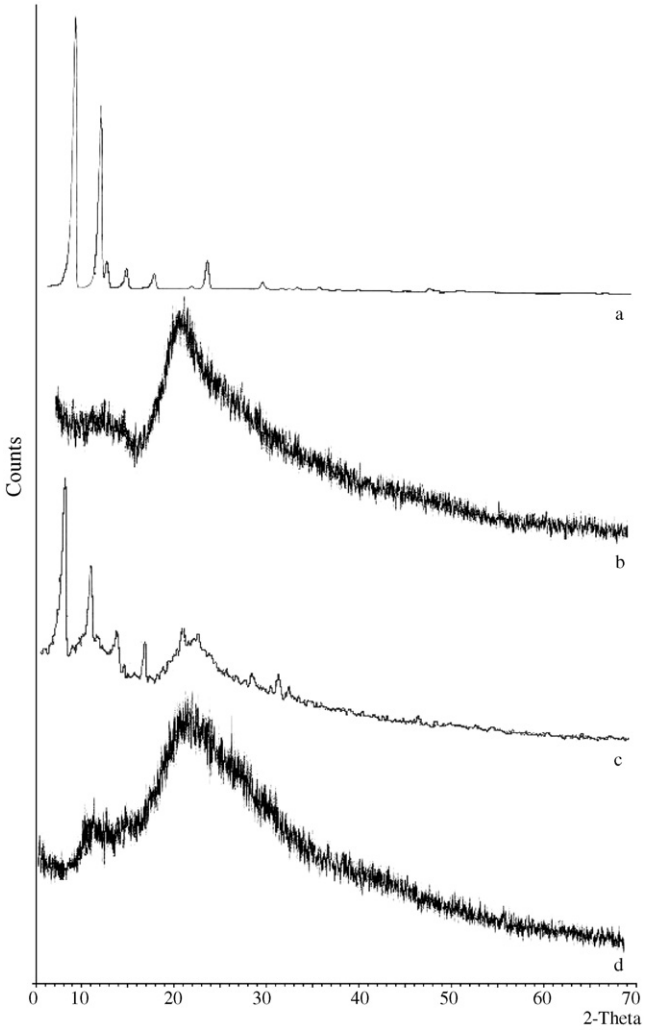

Fig. 3. X-ray diffractograms of (a) TS IIA, (b) HP-β-CD, (c) physical mixture (PM), and (d) inclusion complex (IC).

mic peak was found at 218.6 °C. The HP-β-CD dehydration peaks were also present, as if the thermogram was the sum of those of the components analyzed separately. This thermogram indicated that the absence of interaction between TS IIA and HP-β-CD in PM system and an inclusion complex could not be obtained by simple blending the drug and HP-β-CD. As can be seen in Fig. 2a and d, the complete disappearance of the TS IIA endothermic peak was observed for the complex since TS IIA molecule was contained within the cavity of the HP-β-CD ring molecule (Williams et al., 1998; Fernandes et al., 2002). This demonstrated that an inclusion complex could be obtained by coevaporation method, in agreement with previous studies reported by Blanco et al. (1991) and Castillo et al. (1999).

Further evidence of TS IIA and corresponding complexes with HP-β-CD formation was obtained by X-ray diffraction patterns as demonstrated in Fig. 3. In the X-ray diffractogram of TS IIA powder (Fig. 3a), crystalline peaks at a diffraction angle of 2θ 7.205°, 9.532°, 9.689°, 12.049°, 14.488°, 17.859°, 19.336°, 24.128°, 26.542°, 50.072°, and 53.787° were present and it suggested that the drug was present as a crystalline material. The absence of any peak in the HP-β-CD diffractogram revealed the

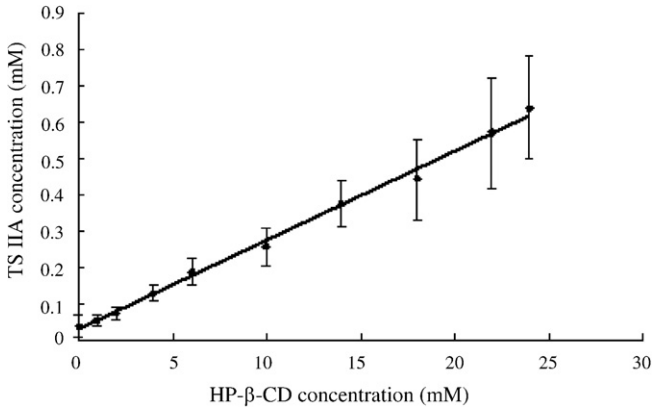

Fig. 4. Phase solubility diagrams of TS IIA/HP-β-CD system in water at 37 ± 0.1 °C.

amorphous nature of this compound as shown in Fig. 3b. The diffraction profile of PM was found to be the simple superimposition of each component, with the crystalline peaks of TS IIA emerging on the diffuse background of the amorphous carrier and having a lower intensity. This was due to a reduction in particle size during the preparation of the physical mixture and to the dilution of the TS IIA in the physical mixture. The complex system did not exhibit peaks corresponding to TS IIA and displayed a completely diffuse diffraction pattern, indicating the entirely amorphous nature of TS IIA in the complex (Fig. 3d) (Latrofa et al., 2001; Calabrò et al., 2004). X-ray powder diffraction patterns confirmed the results of DSC analysis, showing that an inclusion complex between TS IIA and HP-β-CD was formed by the coevaporation method.

3.3. Phase solubility studies

The solubility method is useful for studying inclusion compound of poorly soluble drugs with CDs in water because it gives not only the solubilizing ability of CDs but also the stability constant (K_s) of the complexes by analyzing the solubility curves (Higuchi and Connors, 1965). The phase solubility profiles for the TS IIA/HP-β-CD systems were presented in Fig. 4. The diagram showed that the aqueous solubility of the drug increased linearly as a function of HP-β-CD concentration, over the entire concentration range studied. The solubility curve with correlation coefficient squared values (r^2) >0.990 ($r^2 = 0.995$) was regarded as a straight line (A_L type) (Higuchi and Connors, 1965). Because this linear host-guest correlation with slope of less than 1 (i.e. 0.0246), it was suggested the complexes formed were of the first order with regards to the host molecule concentrations (Fernandes et al., 2002). The stoichiometry 1:1 apparent stability constant of the complex was calculated from the slope of the straight line of the phase-solubility diagram according to the equation $K_s = \text{slope}/S_0\ (1 - \text{slope})$, where S_0 is the solubility of the pure drug at the same temperature in water (38.06 μM). The K_s was calculated to be 631.4 M^{-1} in our study and 569.9 M^{-1} in pH 7.4 by Yan et al. (2006). The author in that article added excess Tanshinone IIA into phosphoric acid buffer solution with different concentration of HP-β-CD to carry out

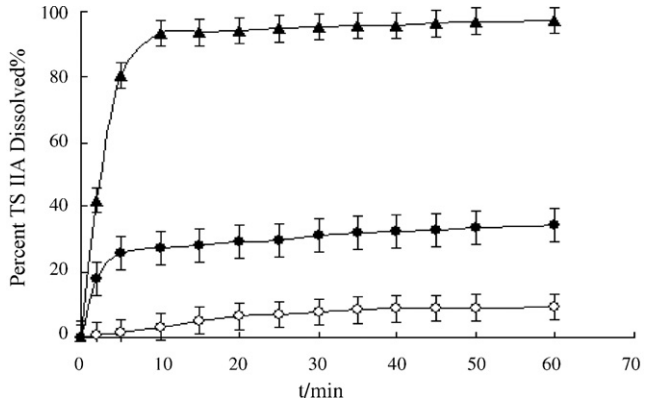

Fig. 5. Dissolution profiles of (◯) free TS IIA, (●) physical mixture (PM), and (▲) inclusion complex (IC).

the phase solubility study. Nevertheless, the medium used in our experiment was water. The difference in K_s may result from different medium.

3.4. Dissolution studies

The dissolution data were shown in Fig. 5. The dissolution efficiency parameter was evaluated by the mean percentage of dissolved TS IIA at 60 min, according to the regulations of CP2005. As could be seen from the figure, it was evident that the HP-β-CD complex exhibited faster dissolution than the corresponding PM and the free TS IIA, being immediately dispersed and completely dissolved within 10 min. The uncomplexed TS IIA dissolved only to the extent of 9.26% at the end of 60 min. The percentage of TS IIA dissolved from the PM and the coevaporated product was 34.3 and 97.2%, respectively. These facts were consistent with the results of the solubilizing activity of HP-β-CD, suggesting that the enhancing effect of HP-β-CD on the dissolution rate of TS IIA could be explained from the enhanced aqueous solubility of TS IIA after the formation of complexation. In addition, according to literature suggestions, a decrease in crystallinity of the drug might be the other factor in the enhanced dissolution by the complex besides an increase in solubility (Stzejtli, 1994).

3.5. Absorption behavior of free and complexed TS IIA in everted intestinal sacs of rats

3.5.1. The integrity of the everted rat intestinal sacs

In order to verify the integrity and viability of the gut sacs at the *in vitro* model set-up stage, the everted intestinal sac was inspected by light microscopy. The effect of various experimental solutions on these morphometric parameters of intestine were shown in Fig. 6. NPA is an important morphological parameter, which is defined as the distance between the nucleus and the apical membrane of enterocytes determined using an eyepiece micrometer. The extent of decrease in NPA by treatment with KR buffer solutions with TS IIA was not statistically significant ($p > 0.05$) when compared to NPA in control group (Fig. 6A). Vi was determined by measuring the ratio of height and width (at one half of height of villus) of enterocytes. In the present study,

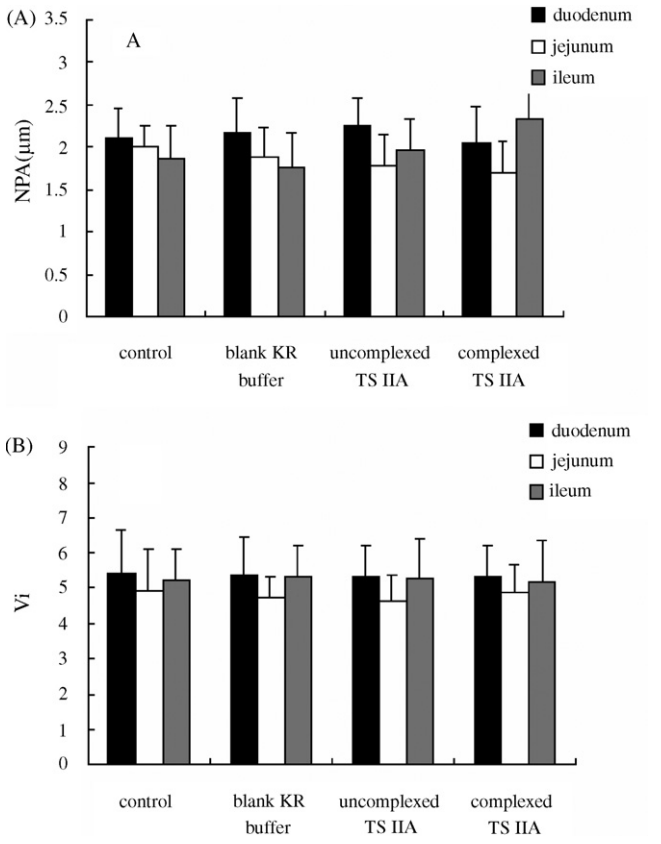

Fig. 6. Morphological parameters in different intestinal segments of rat when incubated with different experimental solutions *in vitro*. (A) NPA and (B) Vi ($n = 3$, values were shown as mean ± S.D.).

absence of any significant decrease in Vi of intestinal villus exposed to KR buffer solutions with or without TS IIA confirmed that experimental solutions did not influence the integrity of intestine (Fig. 6B).

The light photomicrographs of the intestinal sacs exposed to different solutions were presented in Fig. 7. The three lines from left to right refer to duodenum, jejunum and ileum, respectively. Fig. 7A showed the normal intestinal sacs before experiment as the control group and Fig. 7B–D showed the intestinal sacs in blank KR solution, KR solution with free and complexed TS IIA after experiment as the tested groups. From the light photomicrographs, it was observed that the villi at the mucosal surfaces were clear and the paracellular junction was intact. No significant presence of inflammatory cells was observed in these samples. No destruction of the epithelium was observed. Histological studies showed the treating process and drugs (TS IIA and complexed TS IIA) had no influence on the integrity of intestinal sacs.

3.5.2. Stability studies

Preliminary studies were necessary before commencing the absorption studies to ensure that the amount of drug disappearing resulted from the absorption rather than other losses. The upper boundary of the concentration was limited at 10.8 μg ml^{-1} because of the low aqueous solubility of free TS IIA.

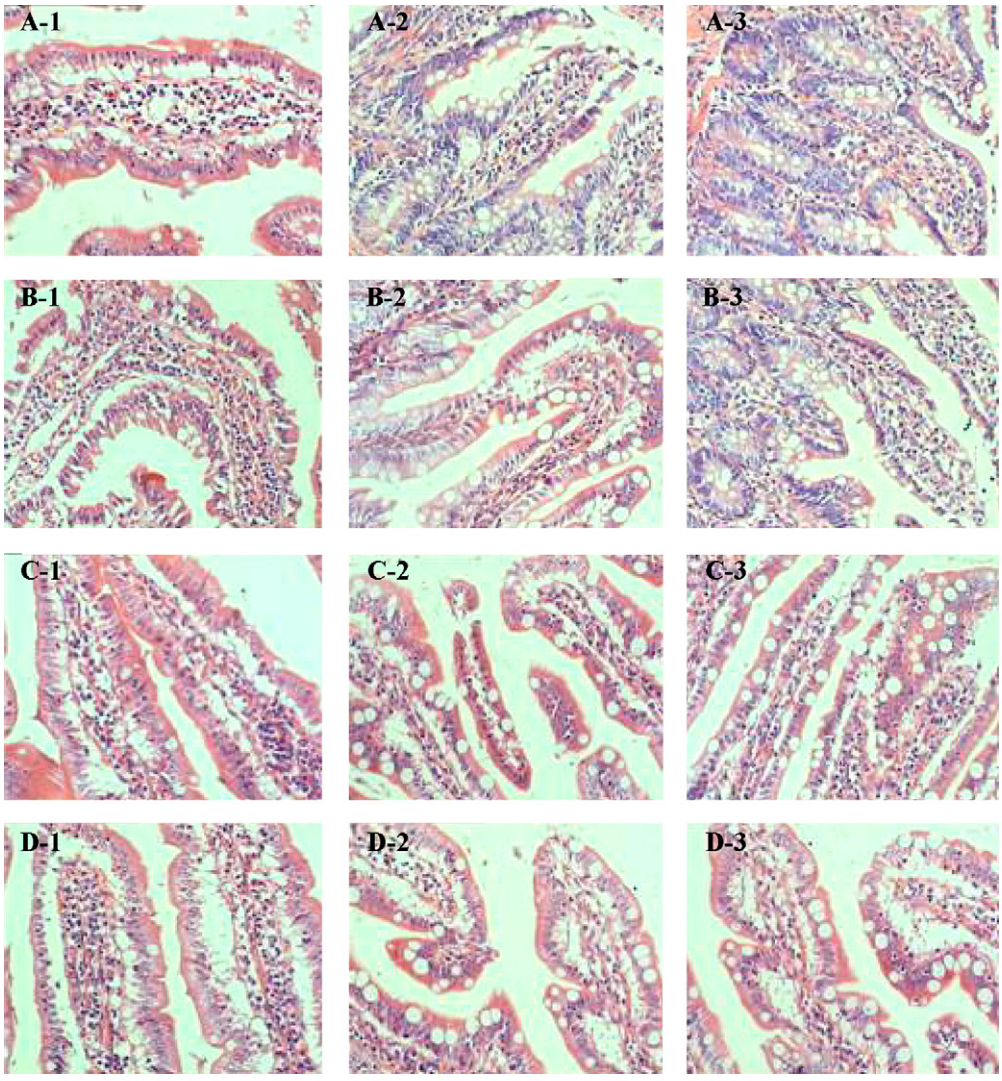

Fig. 7. Representative photomicrographs (200×) of intestinal segments of rat in everted intestinal sac model. (A) Control, (B) blank KR solution, (C) uncomplexed TS IIA, and (D) complexed TS IIA.

The stabilities of free TS IIA in the blank KR buffer were observed on the decrease of TS IIA as quantitated by HPLC. However, in both blank and transport KR buffer solution, the results indicated that content of uncomplexed and complexed TS IIA had no change in view of away from light (Fig. 8). It was estimated that the solution containing free TS IIA existed photolysis. Therefore, all experiments were carried out in a dark-room under yellow light (Philips Powertone SON E27) to avoid

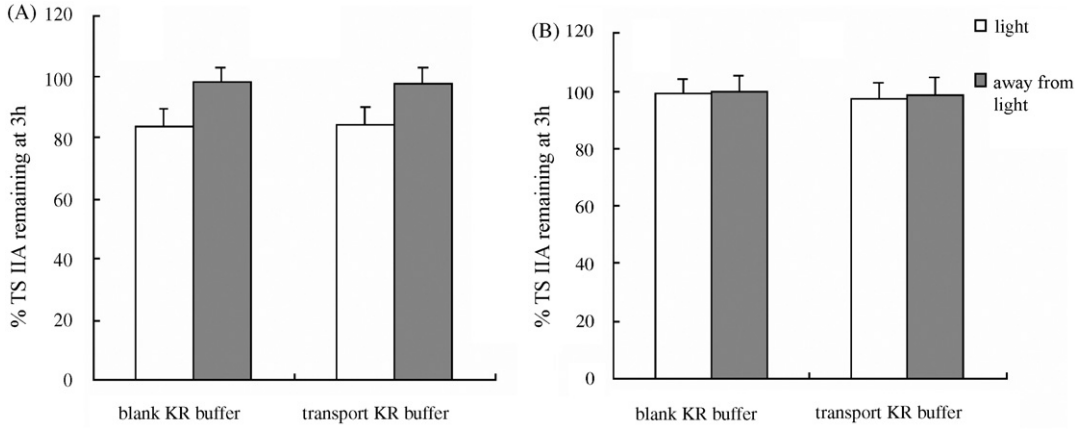

Fig. 8. Stability studies of (A) uncomplexed TS IIA and (B) complexed TS IIA in different test conditions.

Table 1
P_{app} values of TS IIA following administration of TS IIA and TS IIA complexation with HP-β-CD (250 μg ml^{-1}) in different intestinal segments tested

Intestinal segment	$P_{app} \times 10^{-5}$ (cm s^{-1})[a]	
	Uncomplexed TS IIA	Complexed TS IIA
Duodenum	0.58 ± 0.07	3.04 ± 0.34
Jejunum	0.47 ± 0.11	2.74 ± 0.23
Ileum	0.54 ± 0.13	2.61 ± 0.26

[a] Each value represents the mean ± S.D. of six rats.

photodecomposition. Meanwhile, as shown in the Fig. 8B, the complexed TS IIA was sufficiently stable (>95% remaining) in the experimental solutions. This demonstrated that the stability of free TS IIA could be improved via the formation of the inclusion complex with HP-β-CD.

3.5.3. The intestinal absorption behavior of free and complexed TS IIA in rats

The P_{app} values of uncomplexed TS IIA at duodenum, jejunum and ileum were 0.58×10^{-5}, 0.47×10^{-5}, and 0.54×10^{-5} cm s^{-1}, respectively. In comparison, the respective P_{app} values of complexed TS IIA at each segment were 3.04×10^{-5}, 2.74×10^{-5}, and 2.61×10^{-5} cm s^{-1}, approximately 5.2, 5.8 and 4.8 times greater than the uncomplexed drug (Table 1). The complexation of TS IIA with HP-β-CD resulted in the enhancement of the TS IIA transport. The maximum amounts of TS IIA transported across intestinal epithelial membrane from the complexation (0.679–0.705 mg) were higher than those from the uncomplexed drug (0.007–0.008 mg, Fig. 9). In the present study, all samples in the donor compartment (mucosal side), either uncomplexed or complexed TS IIA, were present in a suspension form. The amount of TS IIA incorporated in the donor compartment (250 μg ml^{-1}) exceeded the apparent solubilities of uncomplexed and complexed TS IIA in water at 37 ± 0.1 °C, i.e. 11.2 and 188.7 mg/l, respectively. The amount of TS IIA present in solution form for complex in the donor compartment was 17-fold greater than that for the uncomplexed drug. A species available in molecular dispersion was capable of passing through the intestinal epithelial membrane. The higher absorbed amount of TS IIA observed in the complex through the intestinal sacs might be attributed to the higher percentages of drug available in mucosal fluid (donor compartment). The enhanced absorption rate of TS IIA via the formation of an inclusion complex with HP-β-CD might be due to the increased dissolution rate of TS IIA in water. Thus, the absorption pattern of TS IIA through intestinal epithelial membrane might be a dissolution-rate-limiting process. On the other hand, based on the ANOVA-test results, permeability values of TS IIA following using either free TS IIA or inclusion complex for the experimental concentration in different segments were no statistical differences ($p > 0.05$), although the P_{app} value was higher in the duodenum compared with that of in the other segments. The results indicated that TS IIA could be non-specific absorption site in the small intestine.

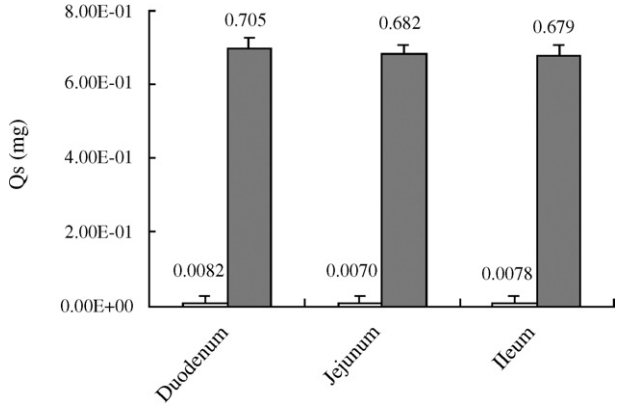

Fig. 9. Amount of TS IIA absorbed following administration of (□) uncomplexed TS IIA and (■) complexed TS IIA (250 μg ml^{-1}) in different intestinal segments tested.

In the preliminary experiment, we considered to study the effect of complexation on TS IIA absorption by *in situ* intestinal perfusion model, which had the advantages of experimental control (e.g. compound concentration, pH and osmolality), an intact intestinal blood supply and innervations of the animal. However, this model was only available to the drugs, which must dissolve in the perfusate (Fagerholm et al., 1996; Chen et al., 2003). On one hand, the perfusate was hard to prepare by virtue of the poor solubility of TS IIA in KR buffer solution. On the other hand, once free TS IIA and complexed TS IIA dissolved in KR suffer solution, respectively, the influence of dissolution on TS IIA absorption could not be indicated. So the everted intestinal sac technique was performed, which was a simple and useful *in vitro* model to study the drug absorption (Barthe et al., 1999). The system could provide information on drug absorption mechanisms by testing the drug content absorbed through the intestinal tissue. It had been used to study the uptake of lipid vesicles, proteins and macromolecules with oral drug delivery potential, synthetic nondegradable polymers and the effect of pharmaceutical excipients on drug absorption (Chaing and Weiner, 1987; Jonker et al., 2002; Lo, 2003). In the model, at predetermined time intervals, the samples were taken from the receiver compartment (i.e. serosal compartment). The method of sampling did not rely on the form of drug in donor side, which meant drug could be present in suspension form in donor compartment. As a result, the accumulated amount of drug across the intestinal tissue appearing into the receiver side could quantitatively reflect the uptake and absorption extent of tested drug during the experiment period. Further pharmacokinetic studies on the whole animal level are under investigation to provide more useful information with respect to the overall application of TS IIA and its complexation.

In conclusion, the dissolution rate of the TS IIA/HP-β-CD complex was much faster than that of TS IIA alone as a consequence of the increased solubility and decrease in crystallinity cased by complexation. It was also demonstrated that the permeabilities of TS IIA through different intestinal segments were enhanced via complexation with HP-β-CD by *in vitro* model studies. The present results suggest the potential use of HP-β-CD for improving the gastrointestinal tract absorption of TS IIA as oral preparations.

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