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# Development of Liposome containing sodium deoxycholate to enhance oral bioavailability of itraconazole

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ABSTRACT

The aim of this study was to enhance oral bioavailability of itraconazole (ITZ) by developing Liposome containing sodium deoxycholate (ITZ-Lip-NaDC). The liposome, consisting of egg yolk lecithin and sodium deoxycholate, was prepared by thin-film dispersion method. Differential Scanning Calorimetry (DSC) results indicated an amorphous state in the liposome. The physicochemical characteristics including particle size, morphology, entrapment efficiency, dissolution properties were also investigated. The performance of single-pass intestinal infusion exhibited that the transport order of intestinal segment was jejunum, duodenum, colon and ileum, and that all the segments participated in the absorption of ITZ in intestinal tract. The bioavailability study in rats showed that the $AUC_{0\text{-}72}$ of the liposome was nearly 1.67-fold higher than that of commercial capsules (SPORANOX) in terms of oral administration, and the RSD of $AUC_{0\text{-}72}$ of ITZ-Lip-NaDC was also decreased. Our results indicated that ITZ-Lip-NaDC liposome was facilitated to improve dissolution efficiency, augment transmembrane absorption, and then enhance the oral bioavailability of ITZ, successfully.



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## 1. Introduction

Itraconazole (ITZ) is widely employed in the treatment of fungal infections, especially in the cure of histoplasmosis, blastomycosis and refractory aspergillosis [1–3]. Despite effective antifungal therapy, oral bioavailability of ITZ is still restricted by its extremely poor water solubility, which hinders the further clinical application of ITZ [4–7].

In order to address these challenges, much effort about preparations has been paid on ITZ to enhance its aqueous solubility, dissolution/release, and ultimately improving bioavailability, such as intravenous emulsion, microemulsion, nanoemulsion, solid dispersion, gelatin microcapsule, flocculated amorphous nanoparticles, nanosuspensions, nanocrystal, cyclodextrin complexes, polymeric micelles, itraconazole/Soluplus extrudate and liposomes [1–3,5,6,8–18]. Among these, itraconazole/Soluplus extrudate formulated by Zhang et al. demonstrated that the $AUC_{0\text{-}48h}$ were 6.9-times higher than those of pure ITZ. However, the oral bioavailability of ITZ/Soluplus was similar to commercial Sporanox(R) capsule. Maria et al. utilized flocculated amorphous itraconazole nanoparticles to enhance *in vitro* supersaturation and improved nearly 2-fold high bioavailability than Sporanox capsules, but the particle size was about 1000 nm upon dispersion at pH 6.8, which might hinder its absorption transportation across intestinal membrane. Meanwhile, cyclodextrin–water soluble polymer ternary complexes were also emerging, which revealed advanced solubility and dissolution behavior; however, the increased bioavailability of itraconazole was only predicted based on pharmacokinetic in silico model and the usage of cyclodextrins might only be slightly suitable for patients who suffered from kidney failure or renal insufficiency. To correspond to the predicted pharmacokinetic results, nanocrystal-based per-oral itraconazole prepared by Sarnes et al. also exhibited superior dissolution behavior by means of nanosized formulations, but the effective *in vivo* drug absorption was not realized in comparison with Commercial oral Sporanox® capsules. In spite of all of the approaches performed to overcome the unsatisfied bioavailability, the oral liposome encapsulating ITZ has been poorly reported.

Liposomes, exhibiting the advantage of encapsulating various drug entities, excellent bioavailability/non-immunogenicity and intrinsic biocompatibility, represent as an important delivery system to package drugs in lipid bilayer with a range of several nanometers to micrometers [19]. Sodium deoxycholate has been utilized as a pharmaceutical penetration enhancer for drugs administered via many routes, including the oral route and it is generally considered that the ability of bile salts to act as penetration enhancers is due to the membrane destabilizing activities of these agents [20–22]. Guan et al. evaluate liposomes containing sodium deoxycholate (SDC), as oral drug delivery systems to enhance the oral bioavailability, and indicated that SDC facilitated the absorption of liposomes vehicle [23].

Based on the above considerations, the study mainly aimed to enhance oral bioavailability of itraconazole (ITZ) by developing Deoxycholate-Modified Liposome (ITZ-Lip-NaDC), which consisted of egg yolk lecithin and sodium deoxycholate. We formulated the liposomes by thin-film dispersion method, and DSC was applied to demonstrate the state of ITZ in the liposomes. Then the physicochemical properties, stability, dissolution properties *in vitro* and pharmacokinetic behavior were also evaluated.

## 2. Materials and methods

### 2.1. Materials

ITZ was supplied from Wan'an Shanghai biological technology Co., LTD (Shanghai, China). Egg yolk lecithin and sodium deoxycholate were provided by Beijing AOBOX biological technology Co., LTD (Beijing, China). Cholesterol was obtained by Tianjin Bodi chemical Co., LTD (Tianjin, China). All other chemicals were of reagent grade. All solvents were of HPLC grade without purification.

### 2.2. Preparation of ITZ-Lip-NaDC

ITZ-Lip-NaDC was prepared by thin-film dispersion method [18]. Egg yolk lecithin, 300 mg, cholesterol, 37.5 mg, ITZ, 30 mg and Vitamin E, 3 mg, were mixed and dissolved in dehydrated dichloromethane. Then, the organic solvent was removed by rotary vacuum evaporation (E-52A, Yarong, Shanghai, China) at a 30 °C water bath (HH-2, Guohua, Changzhou, China). 5 mg/mL sodium deoxycholate solution was employed to hydrate the thin films, stirring for 4 h at 40 °C. The hydrated liposomes were homogenized by a miniprobe sonography (JY92-2D, Xinzhi, Ningbo, China) for 5 min at 300 W and filtered through a 0.22 μm filter to obtain the ultimate concentration of about 3 mg/mL formulations. Blank liposomes were prepared in the same way except ITZ [24]. The liposome formulation was lyophilized to facilitate the storage under the protection of maltose.

### 2.3. Characterizations of ITZ-Lip-NaDC

#### 2.3.1. Particle size and Zeta potential measurement

The particle size and Zeta potential of liposome formulation and re-suspended frozen formulation were measured by dynamic light scattering (DLS) method with a zetasizer instrument (Nano ZS, Malvern Co., UK) [4,25].

#### 2.3.2. Morphologic observation

The morphological image of ITZ-Lip-NaDC was obtained by a transmission electron microscope (TEM) (H-600, Hitachi, Japan). A drop of ITZ-Lip-NaDC solution was deposited on a carbon-coated copper grid, and excess solution was tapped with filter papers. Then the thin-film solution was dried at room temperature, stained with 0.2% phosphotungstic acid aqueous solutions for 1 min before observation under TEM [4,26].

### 2.4. Entrapment efficiency

Entrapment efficiency of ITZ-Lip-NaDC was determined with UV-vis at absorption wavelength of 262 nm. Briefly, SephadexG-50 was swelled overnight and packed in a 2 mL syringe with filter paper in the bottom to obtain gel column. Then 200 μL

ITZ-Lip-NaDC was added onto the gel column and centrifuged (13,000 rpm, 20 min) to collect the elution. Next, the eluent and 200 μL ITZ-Lip-NaDC were dissolved in a 25 mL volumetric flask with methanol to detect the content of ITZ in liposome and total ITZ weight in the liposome formulation, respectively. The entrapment efficiency (*EE*%) was calculated as:

$$EE\% = W_{in}/W_{tot} \times 100\%$$

where $W_{in}$ and $W_{tot}$ are the content of ITZ in liposome and total ITZ weight in the liposome formulation, respectively.

### 2.5. *DSC analysis*

DSC curves of raw material, Lip-NaDC, ITZ-Lip-NaDC as well as the physical mixtures were performed using a DSC-60 (Mettler-ToledoInternational Inc., Switzerland). Aluminum oxide was used as a reference standard. Samples (about 2 mg) were accurately weighed and sealed in aluminum pans. Then, the samples were heated over the range 30–200 °C at a rate of 10 °C/min [27].

### 2.6. *Stability of of ITZ-Lip-NaDC*

The stability study of ITZ-Lip-NaDC was evaluated at different temperatures including 4 °C and 37 °C for five days. Changes in particle size and encapsulated efficiency were selected to assess the stability of liposomes [28].

### 2.7. *Dissolution test*

Dissolution of ITZ-Lip-NaDC lyophilized powder was performed using a ZRS-G8 instrument (Tianjin Tianda Tianfa Technology Co.,Ltd., Tianjin, China), taking the commercial capsules (SPORANOX, Xian-Janssen Pharmaceutical Ltd., China) as control [29]. The dissolution media were 900 mL phosphate buffers with different pH values, including pH 1.2, pH 2.5, pH 4.0, pH 5.5 and pH 6.8. The rotary speed of the paddles was set to 75 rpm. Five milliliters of samples were withdrawn at 5, 10, 15, 20, 30, 45, 60 and 90 min, and replaced with 5 mL of fresh medium to keep the volume constant. After being completely dissolved in methanol, the concentration of dissolution samples of ITZ-Lip-NaDC lyophilized powder was performed using UV-vis at an absorption wavelength of 262 nm.

### 2.8. In situ *single-pass intestinal perfusion of ITZ-Lip-NaDC liposome in rats*

Healthy male Sprague–Dawley rats were obtained from the animal center of Shenyang Pharmaceutical University. The animal studies were approved by the Shenyang Pharmaceutical University Animal Care and Use Committee. Sprague–Dawley rats of about 220 g were made to fast, having access to water for 12 h before perfusion experiment. After intraperitoneal injection of 20% urethane (1 g/kg), the rats were restrained on a warming pad to keep body temperature. Then a gentle incision along midline was opened, and intestinal segments including duodenum, jejunum, ileum and colon were pulled out carefully, which were rinsed with freshly prepared Krebs Ringer's (KR) buffer solution (7.8 g of NaCl, 0.35 g of KCl, 1.37 g of $NaHCO_3$, 0.02 g of $MgCl_2$, 0.32 g of $NaH_2PO_4$, 1.4 g of glucose, and 0.32 g of $CaCl_2$ in 1000 mL of purified water) and balanced at a constant flow rate (Q) of 0.2 mL/min using peristaltic pump. ITZ-Lip-NaDC liposome solution and ITZ-HP-β-CD solution (dissolved in 30 g/L HP-β-CD KR buffer solution) were dispersed in KR buffer solution to achieve a final concentration equivalent to 40 μg/mL of ITZ. The exposed incision was covered with sterilized cotton to maintain intestinal segments moist. Finally the animal was executed; the length and radius of the infused segments were measured precisely. The absorption rate (Ka) and apparent permeability (Papp) of ITZ-Lip-NaDC liposome solution and ITZ-HP-β-CD solution in the intestinal segments are given by the following equations:

$$K_a = \left(1 - \frac{C_{out}}{C_{in}} \cdot \frac{V_{out}}{V_{in}}\right) \cdot \frac{Q}{\pi r^2 L}$$

$$P_{app} = \frac{-QLn\left(\frac{C_{out}}{C_{in}} \cdot \frac{V_{out}}{V_{in}}\right)}{2\pi r L}$$

where $C_{out}$ is the ITZ concentration in the receptor tube, $V_{out}$ is the ITZ volume in the receptor tube, $C_{in}$ is the ITZ concentration in the donor solution, $V_{in}$ is the ITZ volume in the donor solution and Q is the perfusion flow rate, and *r* is the intestinal radius and *L* is the length of infusion segment [30].

In addition, to investigate the effect of ITZ concentration on intestinal perfusion, 20 μg/mL and 80 μg/mL ITZ-Lip-NaDC liposome formulations were also evaluated.

### 2.9. *Pharmacokinetic study* in vivo

The rats, with weights of 180–220 g, were randomly divided into four groups and fasted overnight prior to the experiment with access to water. Two groups of rats were orally administrated with ITZ-Lip-NaDC solution and commercial capsules suspension (dispersed into saline) at a dose of 20 mg/kg. At predetermined time points, 0.3 mL of blood samples was collected and centrifuged at 13,000 rpm for 10 min, then plasma was frozen at –80 °C. The concentration of ITZ was determined by UPLC-MS/MS(HPLC-ZQ2000 MS, Waters, USA), coupled with ACQUITY UPLCTM BEH $C_{18}$ chromatographic column (50 mm × 2.1 mm, 1.7 μm, Waters Corp, Milford, MA, USA). The mobile phase consisted of water containing 0.2% formic acid and acetonitrile containing 0.2% formic acid (70:30, v/v) at a flow rate of 0.3 mL/min, and the column temperature was sustained at 40 °C. Quantitation of ITZ was performed using multiple reactions monitoring of the transition of m/z: 705.78-392.40 for ITZ and 285.07-193.03 for Diazepam (internal standard) during investigation. The concentration of each sample that was calculated referred to a calibration curve with the concentration range from 10 to 3000 ng/mL with a correlation coefficient of 0.997. The related pharmacokinetic parameters were achieved using DAS 2.0 software [4,31].

### 2.10. *Statistical data analysis*

The results were listed as mean or mean± standard deviation (SD). Statistical data analysis was carried out using

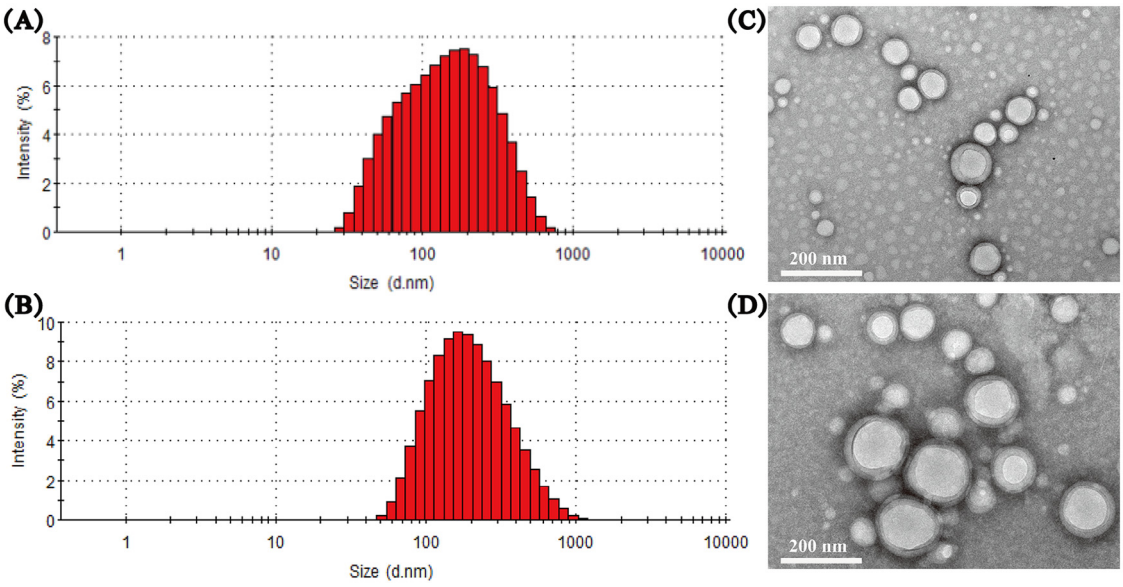


**Fig. 1 – Intensity-size distribution of ITZ-Lip-NaDC (A) re-suspended ITZ-LIP-DC (B) and transmission electron microscopy (TEM) image of ITZ-Lip-NaDC (C) re-suspended ITZ-LIP-DC (D), respectively.**

one-way ANOVA. Difference was set as significance at a level of $P < 0.05$, and a high significance was considered as $P < 0.01$.

## 3. Results and discussions

### 3.1. *Preparation and characterization of ITZ-Lip-NaDC*

ITZ-Lip-NaDC was prepared by thin-film dispersion method in this study, where sodium deoxycholate was applied to modify ITZ-Lip to improve oral bioavailability of ITZ [23]. As shown in Fig. 1 and Table 1, the liposome formulation was in good spherical shape with 118.1 ± 2.0 nm size and –21.5 ± 1.3 mV zeta potential. However, the study of stability indicated that the liposome was unstable either at 4 °C or room temperature. As demonstrated in Fig. 2, the size of ITZ-Lip-NaDC increased to around 3-fold of the initial size and the entrapment efficiency decreased from 92.7% to 79% after 5 days. To address the unsatisfied stability, maltose was employed as cryoprotectant in frozen drying process, resulting in about 165.0 ± 2.5 nm obtained liposome size, which was more uniform (slightly larger) after re-suspended in PBS than fresh preparation, as illustrated in Fig. 1. The size distribution and encapsulated efficiency of lyophilizated ITZ-Lip-NaDC after sealed storage in darkness for 1 month were nearly the same with the evaluation of its re-dissolving without reservation.

**Table 1 – Size, zeta potential and encapsulation efficiency of ITZ-Lip-NaDC and re-dissolved ITZ-Lip-NaDC ($n = 3$).**

| Formulations | ITZ-LIP-NaDC | Re-dissolved ITZ-LIP-NaDC |
|---|---|---|
| Particle size (nm) | 118.1 ± 2.0 | 165.0 ± 2.5 |
| Zeta potential (mV) | –21.5 ± 1.3 | –25.1 ± 1.6 |
| Entrapment efficiency (%) | 90.61 ± 0.66 | 88.4 ± 0.4 |

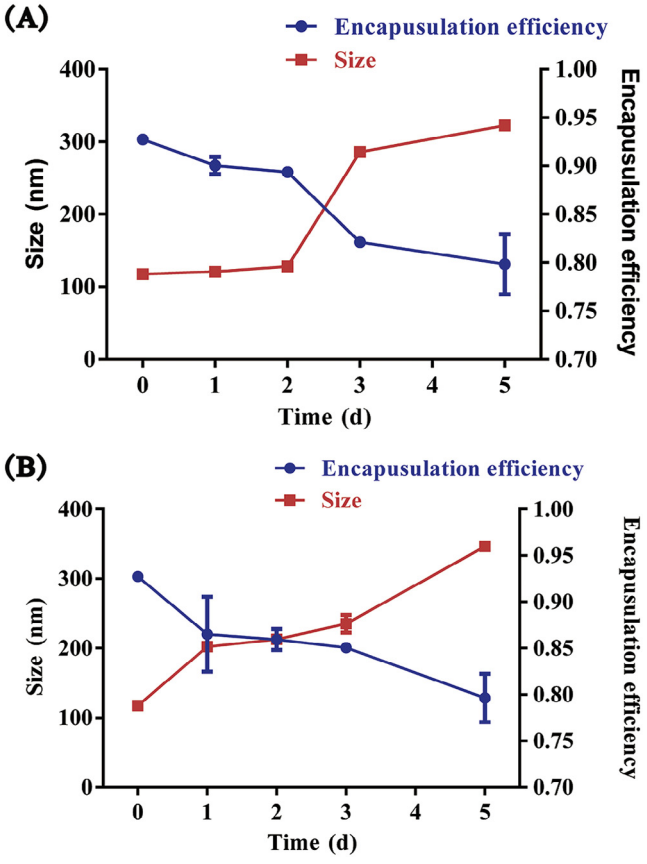


**Fig. 2 – Size and encapsulation efficiency changes of ITZ-Lip-NaDC in PBS 7.4 at 4 °C (A) and 37 °C (B) for 5 d ($n = 3$).**

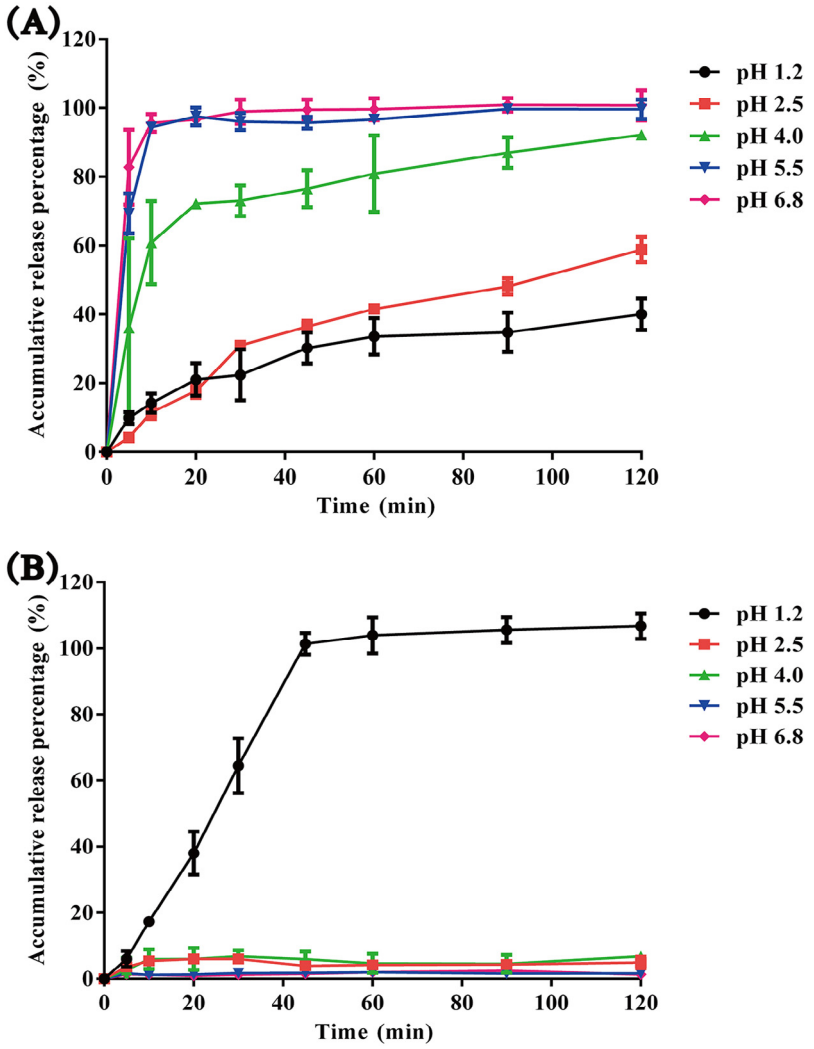


**Fig. 3 – Dissolution profiles of ITZ-Lip-NaDC liposomes (A) and commercial capsules (B) in pH1.2, pH2.5, pH 4.0, pH 5.5, and pH 6.8 PBS ($n$ = 3).**

### 3.2. *Dissolution properties* in vitro

Fig. 3 shows the different dissolution profiles of commercial capsules and ITZ-Lip-NaDC lyophilized powder. At pH 1.2, commercial capsules appeared to have a faster dissolution rate than ITZ-Lip-NaDC lyophilized powder. The cumulative released percentage of commercial capsules was nearly 100% after 45 min, while corresponding values were less than 40% in 120 min. Interesting, ITZ amounts dissolved from ITZ-Lip-NaDC lyophilized powder were significantly higher than commercial capsules with the improvement of pH, as indicated in Fig. 3. When pH was up to 5.5 or 6.8, the ITZ of ITZ-Lip-NaDC was released completely in 15 min, but the released amount of the commercial capsules was only 2% after 120 min. ITZ is a lipophilic alkaline exhibiting insoluble characteristic in phosphate buffer (pH 4.0), but it could form hydrochloride in lower pH to achieve a higher solubility [32]. Solid dispersion technique was utilized in the preparation of commercial capsule by spraying the mixture of ITZ and HPMC to blank sugar pills, thus its dissolution could be easily changed according to pH [33,34]. Compared with commercial capsules, the encapsulated ITZ in ITZ-Lip-NaDC was less affected because of the external shell protection of ITZ-Lip-NaDC. Meanwhile, the DSC results from Fig. 4 revealed that ITZ exist in the form of amorphous state in the liposome. Hence, it maybe speculated that the

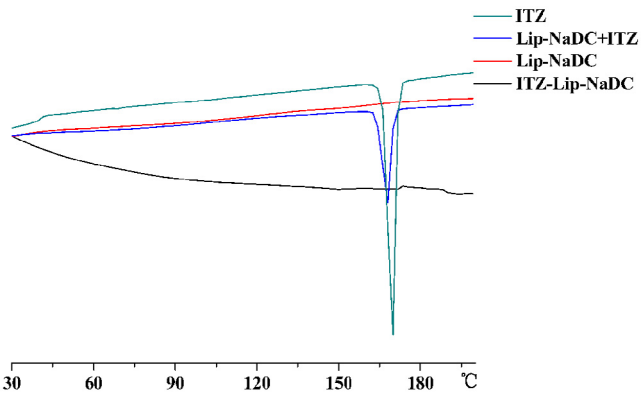


**Fig. 4 – DSC thermograms of ITZ, ITZ-Lip-NaDC, Lip-DC and the physical mixture of ITZ and Lip-DC.**

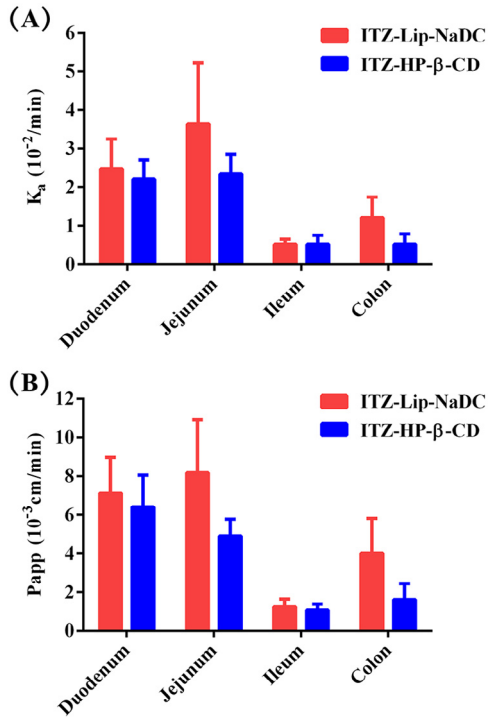


**Fig. 5 – *In situ* absorption of ITZ-Lip-NaDC in different rat intestinal segments compared with ITZ HP-β-CD complex solution. (A) The absorption rate ($K_a$), (B) The apparent permeability ($P_{app}$). Data were shown as mean + SD, *n* = 3.**

significant improvement of the dissolution efficiency of ITZ-Lip-NaDC might be ascribed to amorphous form of ITZ in liposome.

### 3.3. In situ *single-pass intestinal infusion of ITZ-Lip-NaDC in rats*

The *in situ* single-pass intestinal infusion model in rats was used to evaluate the membrane permeability of ITZ-Lip-NaDC. As shown in Fig. 5 and Fig. S1, the absorption rate constant and apparent permeability coefficient of ITZ-Lip-NaDC were generally higher than that of ITZ HP-β-CD in the whole intestinal segments, where HP-β-CD was utilized to improve ITZ solubility. Compared with 40 μg/mL and 20 μg/mL ITZ-Lip-NaDC, the permeability of 80 μg/mL liposome is reduced to 0.3-fold and 0.5-fold, respectively. Therefore, it is reasonable to attribute the absorption phenomenon of ITZ-Lip-NaDC to passive transport involved in active endocytosis in the intestinal tract. Moreover, the passive diffusion of released ITZ might also participate in the absorption process. The performance of single-pass intestinal infusion exhibited that the transport order of intestinal segment was jejunum, duodenum, colon and ileum, and that all the segments participated in the absorption of ITZ in intestinal tract.

### 3.4. *Bioavailability study by UPLC-MS/MS*

Two groups of rats were orally administrated with ITZ-Lip-NaDC suspension and commercial capsules suspension (dispersed into saline) at a dose of 20 mg/kg. The methodology of ITZ *in vivo* by UPLC-MS/MS was conformed to the standards, and the tested linearity ranged from 10 to 3000 ng/mL with a correlation coefficient of 0.9965 with the standard curve being $Y = 0.03510X + 0.05150$.

As shown in Table 2 and Fig. 6, the $AUC_{0\text{-}72}$ of ITZ-Lip-NaDC and commercial capsules were 3484.6 ± 1658.2 ng/mL·h and 5547.8 ± 1951.8 ng/mL·h, respectively. Moreover, the RSD of $AUC_{0\text{-}72}$ of ITZ-Lip-NaDC was around 35.1%, a decrease in comparison with commercial capsules (about 47.6%). Therefore, it might infer that ITZ-Lip-NaDC can improve oral bioavailability of ITZ and reduce the variability of gastrointestinal absorption, which might lie in gastrointestinal pH, liposome protection and endocytosis pathways. In comparison with ITZ-Lip-NaDC formulation, commercial capsules might reveal a poorer drug release behavior except pH 1.2 as estimated from dissolution study, and the ionization of ITZ at pH 1.2 also restrains its transport across membrane. In addition, the commercial capsules might be liable to the complex intestinal condition including the enzymes and mechanical contraction because of lack of the shell protection of liposome [35,36]. Moreover, ITZ-Lip-NaDC might be endocytosed through nanoparticle endocytosis pathways on account of involvement in the active endocytosis in intestinal tract due to the penetration-enhancing effect of sodium deoxycholate, which could be inferred from the results of *in situ* single-pass intestinal infusion.

## 4. Conclusions

The liposome, mainly consisting of egg yolk lecithin and sodium deoxycholate, was prepared by thin-film dispersion method, and DSC demonstrated an amorphous state in the liposome. The bioavailability study in rats showed that the AUC of the liposome was nearly 1.67-fold higher than commercial capsules (SPORANOX) in terms of oral administration. Our results indicated that ITZ-Lip-NaDC liposome could be a potential oral formulation of itraconazole, and the performance might shed light on the design or development of oral delivery of liposome preparations.

**Table 2 – Pharmacokinetic parameters of ITZ in rats after oral administration of commercial capsules and ITZ-Lip-NaDC solution at a dose of 20 mg/kg ITZ, respectively (mean + SD, *n* = 6).**

| Preparations | $t_{1/2}$ (h) | $C_{max}$ (ng/mL) | $T_{max}$ (h) | $AUC_{0\text{-}72}$ (ng/mL·h) |
|---|---|---|---|---|
| Commercial capsules | 13.5 ± 8.0 | 270.9 ± 133.3 | 4.5 ± 2.3 | 3484.6 ± 1658.2 |
| ITZ-Lip-NaDC | 12.7 ± 5.7 | 416.5 ± 134.9 | 5.5 ± 2.1 | 5547.8 ± 1951.8 |

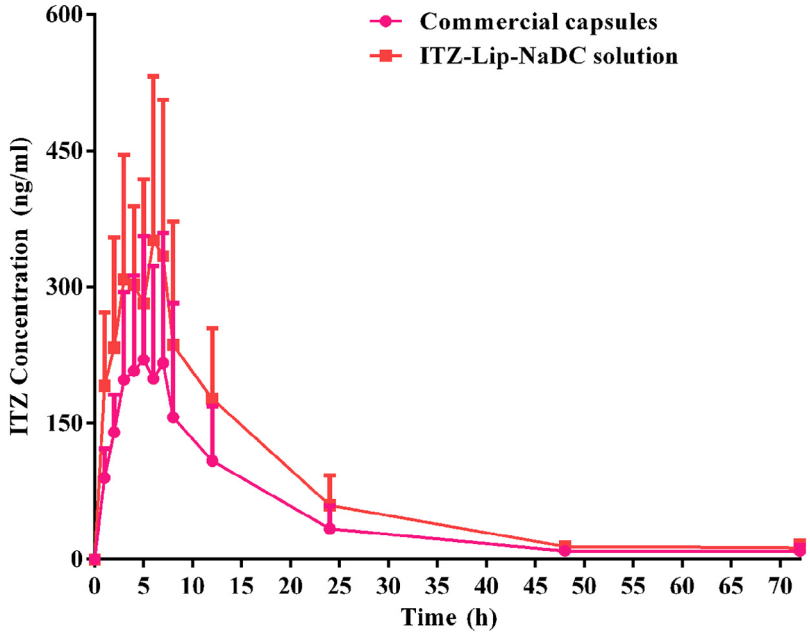


**Fig. 6 – Mean plasma concentration–time curves of ITZ in rats after oral administration of commercial capsules and ITZ-Lip-NaDC solution at a dose of 20 mg/kg ITZ, respectively (mean + SD, *n* = 6).**

## Acknowledgments

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## Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.ajps.2016.05.006.

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