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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