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Itraconazole-hydroxypropyl-β-cyclodextrin loaded deformable liposomes: *In vitro* skin penetration studies and antifungal efficacy using *Candida albicans* as model

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ABSTRACT

The study aimed to develop novel ITZ-loaded deformable liposomes (DL) in presence of hydroxypropyl-β-cyclodextrin (HPβCD) (DL-CD) to enhance antifungal activity. These formulations have been reported as conceivable vesicles to deliver drug molecules to the skin layers. The efficiency of the prepared systems was compared with conventional liposomes (CL) and ITZ solution. The developed liposomes were characterized for particle size, entrapment efficiency (EE %), deformability, stability, and morphology of the vesicles. In addition, *ex vivo* penetration and antifungal activity were evaluated. It was found that the presence of HPβCD played a significant role in reducing the vesicle size to nano range. The deformability study and TEM images revealed that membrane deformability of DL and DL–CD was much higher than that of CL. Moreover, DL–CD enhanced the amount of ITZ in SC and deeper skin layers compared to DL and CL. The antifungal activity of ITZ-loaded deformable liposomes remained intact compared to ITZ solution. It can be concluded that deformable liposomes in the presence of HPβCD may be a promising carrier for effective cutaneous delivery of ITZ.

1. Introduction

Itraconazole (ITZ) is one of the antifungal agents, which is widely prescribed for patients with systemic or superficial fungal infections. ITZ has high lipophilicity ($\log p > 5.5$), low water solubility (<1 μg/ml) and relatively large molecular weight (705.64) [1]. These properties of ITZ, as well as the barrier nature of the stratum corneum (SC) of the skin, are the main problems that may face transdermal delivery of ITZ. It was reported that the transdermal delivery of drugs using liposomes as a carrier system has been enhanced. Many researchers reported that the conventional liposomes are not the proper carrier system for transdermal delivery, because they remain confined to the upper layers of the SC [2]. Hence, a series of liposomes with elastic (*i.e.* deformable) membranes were developed to improve the skin delivery [3]. Particularly, edge activators like sodium cholate or deoxy cholate, Spans and Tweens were used to produce the deformable liposomes [4]. It was stated that edge activators destabilize lipid bilayers of the vesicles and increase deformability of the membrane [5]. The deformable vesicles have ability to penetrate intact skin efficiently [6]. This was attributed to its ability to squeeze themselves through cells of the SC [6]. Furthermore, these vesicles can disrupt the intercellular lipid of the SC forming channel like penetration pathways which allow the drug molecules to penetrate [4]. The liposomes have capacity to entrap hydrophilic and lipophilic drugs in their aqueous core and lipid bilayer, respectively. However, some of the lipophilic drugs can interfere with lipid bilayer of the liposomes leading to decrease its stability [5]. To avoid such destabilizing effect, some researchers found that the uploading of drug–cyclodextrin complex into liposomes has a positive impact in liposomes stability [7]. Cyclodextrins (CD) are cyclic (β-1,4)-linked oligosaccharides of D-glucopyranose containing a relatively hydrophobic central cavity and a hydrophilic outer surface. Cyclodextrins are able to form inclusion complexes

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with poorly water-soluble drugs. Hydroxypropyl-β-cyclodexterin (HPβCD) is one of the safest used cyclodextrins. HPβCD has the ability to form hydrophilic inclusion complexes with a number of hydrophobic drugs in aqueous solution. HPβCD can improve the solubility of the hydrophobic drugs without shifting their intrinsic ability to permeate lipophilic membranes [8]. HPβCD has presented as a solubilizer and penetration enhancer for transdermal application [9]. ITZ is commercialized as Sporanox® oral solution (ITZ/HPβCD inclusion complex containing 10 mg/ml of ITZ and 400 mg/ml of HPβCD) [10]. For skin fungal infections, oral antifungal agents need time and show inter-individual variation and numerous systemic side effects [11]. So, the developed topical antifungal formulations will contribute in avoiding these oral problems.

In the current study, ITZ and HPβCD loaded deformable liposomes were prepared with purpose of enhancing the topical activity of ITZ. The physicochemical characters like particle size, zeta potential, entrapment efficiency, deformability and vesicle morphology were investigated. Moreover, *ex vivo* skin penetration and antifungal activity were evaluated.

2. Materials and methods

2.1. Materials

Itraconazole was purchased from Betapharma, China. Phospholipids, Lipoid S100 was purchased from Lipoid KG, Germany. Sodium deoxycholate (SDC) was purchased from Fisher Scientific Co., UK. Tween 20 and 80 were purchased from BDH, Organics, England. Hydroxypropyl-β-cyclodextrin (HPβCD) was purchased from ARCOS, USA. Methanol and acetonitrile (HPLC grade) were obtained from Fisher Scientific Co., UK. Sodium chloride, potassium dihydrogen phosphate and disodium hydrogen phosphate were obtained from Merck, Darmstadt, Germany. ITZ solution (control) was prepared by dissolving ITZ in solvent mixture of propylene glycol, water and ethanol (75:15:10, v/v, respectively).

2.2. Preparation of liposomes

Conventional liposomes (CL) and deformable liposomes (DL) containing ITZ were prepared. The composition of CL and DL are presented in Table 1. ITZ-loaded liposomes were prepared using film hydration method [12]. Briefly, phospholipids, cholesterol and ITZ in the presence and absence of surfactant(s) in weight ratios (making total amount of 100 mg of lipids) were dissolved in a mixture of chloroform:methanol (2:1, v/v) in a round bottom flask. The lipid film was obtained by removing the organic solvents under vacuum at 55 °C using a rotary evaporator (Buchi Rotavapor R-200, Switzerland). The formed film was then flashed with nitrogen gas to remove any possible traces of organic solvent. The dry lipid film was then rehydrated and dispersed with water by gently mixing for 30 min. Probe sonicator at 40% power for 2 min (Badnelin, Germany) was used to obtain liposomes in nano size range. The formed liposomes were light centrifuged at 1000 rpm for 5 min to remove any undissolved ITZ. All formulations were kept at 4 °C for further investigation.

2.3. Preparation of liposomes containing ITZ and HPβCD

Previous study revealed that the solubility of ITZ in purified water was increased by HPβCD (stoichiometric molar ratio of 1:3; ITZ:HPβCD) [10]. Based on that, 1:3 molar ratio of ITZ:HPβCD was selected in the current study to prepare deformable liposomes.

For CL and DL containing ITZ and HPβCD, the same preparation method previously described was employed (film hydration method). ITZ and HPβCD were added to the organic phase [7,13].

2.4. Physicochemical characterization

2.4.1. Particle size and zeta potential of liposomes

The particle size, polydispersity index (PDI) and zeta-potential of the liposomes were measured by Brookhaven 90 Plus (Brookhaven Instruments Corporation, USA). Dynamic light scattering (DLS) mode was used to measure the vesicular size and size distribution of liposomes (polydispersity index; PDI) at 25 °C after proper dilution. Laser Doppler Velocimetry (LDV) mode of the same instrument was used for the determination of zeta potential (represented as mean of the zeta potential (mV)) of liposomes after an appropriate dilution with distilled water at 25 °C. All experiments were performed in triplicate.

2.4.2. Morphology of liposomes

Transmission electron microscopy (TEM) was used to study the shape and lamellarity of the liposomes. They were negatively stained with a 1% (w/v) aqueous solution of uranyl acetate on the grid. The excess of uranyl acetate solution was removed and the samples were examined by TEM (JEM-1011, JEOL, Tokyo, Japan) at 60 kV.

2.4.3. Deformability study

The deformability of the liposomal membranes was evaluated by adopting vesicular size changed method used by Aggarwal and Goindi [14]. This method is based on measuring the change in the particle size upon passing through artificial membranes. Liposomes samples (500 μl) were extruded, using an extruder (Avestin Co., Canada), through a 100 nm of double polycarbonate membrane (Nucleopore®, Whatman). The size of the vesicles was monitored

Table 1
Composition of the different types of liposomes containing ITZ or HPβCD-ITZ.

Ingredients (weigh ratio)	CL	DL1	DL2	DL3	CL-CD	DL1-CD	DL2-CD	DL3-CD
Lipoid S100	10	10	10	10	10	10	10	10
Cholesterol	5	–	–	–	5	–	–	–
Tween 20	–	2	–	–	–	2	–	–
Tween 80	–	–	2	–	–	–	2	–
SDC	–	–	–	2	–	–	–	2
ITZ[a]	√	√	√	√	–	–	–	–
HPβCD:ITZ[b]	–	–	–	–	√	√	√	√

CL: conventional liposomes; DL: deformable liposomes; CL-CD: conventional liposomes with HPβCD; DL-CD: deformable liposomes with HPβCD; SDC: sodium deoxycholate; ITZ: itraconazole.
[a] ITZ was added in an amount of 50 mg per 100 mg of plain liposomes.
[b] HPβCD-ITZ* (3:1 molar ratio) was added in an amount equivalent to 50 mg of ITZ per 100 mg of plain liposomes.