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A novel vesicular carrier, transethosome, for enhanced skin delivery of voriconazole: Characterization and *in vitro/in vivo* evaluation

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ABSTRACT

This study describes a novel carrier, transethosome, for enhanced skin delivery of voriconazole. Transethosomes (TELs) are composed of phospholipid, ethanol, water and edge activator (surfactants) or permeation enhancer (oleic acid). Characterization of the TELs was based on results from recovery, particle size, transmission electron microscopy (TEM), zeta potential and elasticity studies. In addition, skin permeation profile was obtained using static vertical diffusion Franz cells and hairless mouse skin treated with TELs containing 0.3% (w/w) voriconazole, and compared with those of ethosomes (ELs), deformable liposomes (DLs), conventional liposomes (CLs) and control (polyethylene glycol, PG) solutions. The recovery of the studied vesicles was above 90% in all vesicles, as all of them contained ethanol (7–30%). There was no significant difference in the particles size of all vesicles. The TEM study revealed that the TELs were in irregular spherical shape, implying higher fluidity due to perturbed lipid bilayer compared to that of other vesicles which were of spherical shape. The zeta potential of vesicles containing sodium taurocholate or oleic acid showed higher negative value compared to other vesicles. The elasticities of ELs and TELs were much higher than that of CLs and DLs. Moreover, TELs dramatically enhanced the skin permeation of voriconazole compared to the control and other vesicles ($p < 0.05$). Moreover, the TELs enhanced both *in vitro* and *in vivo* skin deposition of voriconazole in the dermis/epidermis region compared to DLs, CLs and control. Therefore, based on the current study, the novel carrier TELs could serve as an effective dermal delivery for voriconazole.

1. Introduction

Voriconazole, a synthetic derivative of fluconazole, is a new second-generation triazole agent approved by the US Food and Drug Administration (FDA) in May 2002. This anti fungal agent is used for the systemic treatment of filamentous fungal infections [1–4]. Moreover, it is not only active against all *Candida* species, but also fluconazole-resistant *Candida albicans* and *Cryptococcus neoformans* [5,6]. Recently, its efficacy against cutaneous aspergillosis, and skin and nail infections by Scytalidium was reported [7,8]. However, the commercial voriconazole prescription for fungal infection is generally in the form of oral tablets, oral suspensions or intravenous injections. Yet, although the bioavailability of voriconazole is estimated to be 96%, physicians usually do not opt for oral therapy of voriconazole for topical infections due to side effects including visual disturbances, skin rashes, drug interactions, elevated hepatic enzyme levels, abdominal pain, nausea and vomiting [3,9]. Hence, topical delivery system could be a better alternative for voriconazole treatment.

Liposomal carriers for topical drug delivery have been studied since the 1980s and have evoked a considerable interest. However, the conventional liposomes (CLs) do not deeply penetrate the skin and remain confined to the outer layer of stratum corneum [10,11]. Thus, over the past decade, new classes of lipid vesicles such as deformable liposomes (DLs) and ethosomes (ELs) have been developed as an enhanced type of liposomes [12–14].

DLs, also known as transfersomes, are liposomes that contain edge activators including Tween 20, Tween 60, Tween 80, Span 60, Span 65, Span 80, dipotassium glycyrrhizinate, sodium cholate or sodium deoxycholate. These edge activators destabilize the lipid bilayer of liposomes and increase the flexibility of liposomes [15,16]. Numerous reports have shown that the drug delivery across the skin from DLs was more effective than that of rigid liposomes such as CLs [17,18]. Nevertheless, several studies showed that the DLs were not able to penetrate the low layers of stratum corneum although they could improve the skin deposition of hydrophobic drugs such as 5-fluorouracil [12,19].

Ethosomes (ELs), on the other hand, are novel lipid carriers developed by Touito et al. [13] which are composed of phospholipid, ethanol and water. ELs were reported to enhance the skin permeation of drugs due to the interdigitation effect of ethanol on the lipid bilayer of liposomes and increasing fluidity of

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stratum corneum lipids [20]. Therefore a novel liposome, which can encompass the advantages of both DLs and ELs, would be desirable as an elastic carrier in order to deliver drugs to dermis layer through stratum corneum barriers. Herein we describe the preparation and characterization of this new type of carrier, "transethosomes (TELs)", which is derived from transfersomes and ethosomes, which contains high content of ethanol together with an edge activator or permeation enhancer. The feasibility of improving the skin deposition of voriconazole with TELs will also be reported.

2. Materials and methods

2.1. Materials

Voriconazole was purchased from Beijing HuaFeng United Technology (Bejing, China). Lipoid S100 (Phosphatidylcholine (PC) from soybean lecithin) was kindly provided by Lipoid GmbH (Ludwigshafen, Germany). Cholesterol (CHOL) was purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA). Polyoxyethylene sorbitan monooleate (Tween 80) and taurocholic acid sodium salt (sodium taurocholate) were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). All other agents were analytical grade or better and used without further purification.

2.2. Preparation of liposome formulations

CLs containing voriconazole were prepared by the film-hydration method [21]. The prepared liposomes and their composition are shown in Table 1. Briefly, Lipoid S100 (final PC concentration of 3% (w/v)), cholesterol (final concentration of 1% (w/v)) and voriconazole (final concentration of 0.3% (w/v)) were dissolved in chloroform. The solvent was removed under reduced pressure at 50 °C, followed by evaporation with a rotary evaporator (Buchi Rotavapor R-200, Switzerland) under high vacuum to form a thin lipid film. The lipid film was hydrated with 7% ethanol by gently mixing for 30 min. The hydrated solution was left for 2 h at room temperature and sonicated for 20 min to obtain the liposomes, which were filtered through a membrane filter (0.2 μm, Minisart, Sartorius stedim, Germany) to remove any undissolved voriconazole. Similar procedure was carried out to prepare the DLs, which comprised of lipoid S100 (for final PC concentration of 3% (w/v) in 7% ethanol), edge activator (1–30 molar ratio of lipoid S100) and voriconazole. All products were kept at 4 °C until used.

The drug loaded ELs were prepared as reported earlier [13]. The composition of the prepared vesicles is shown in Table 1. Briefly, Lipoid S100 (for final PC concentration of 3% (w/v) in 30% ethanol) and voriconazole (final concentration of 0.3% w/v) were dissolved in ethanol. DDW was added to the ethanol solution under mixing at 700 rpm with a magnetic stirrer after which the mixture was homogenized at 10,000 rpm for 1 min. The preparation was then continuously mixed for 10 min and filtered through a membrane filter (0.2 μm, Minisart, Sartorius stedim, Germany) to obtain the ELs formulation. Similar procedure was carried out to prepare the TELs, which were prepared by adding edge activator at 1–30 molar ratio concentrations to the lipoid S100 in ELs formulation. ELs and TELs were kept at room temperature until used.

2.3. Characterization of liposomes

The particle diameter and zeta-potential of the prepared vesicles were measured by light scattering with a particle size analyzer (ELS-8000, Zeta-potential and particle size analyzer, Otsuka, Japan). The morphology of liposomes was examined by transmission electron microscopy (TEM) (JEM-1010, JEOL, Japan) after positively stained with uranyl acetate. The amount of voriconazole recovered in the vesicle preparation was determined by HPLC (Shimadzu, Japan) after removing the undissolved voriconazole by a membrane filter (0.2 μm, Minisart, Sartorius stedim, Germany). The vesicles were then ruptured with 20% Triton X-100 aqueous solution. The total recovery of voriconazole in the prepared vesicle formulations was calculated by the following Eq. (1):

$$\text{Recovery}\,(\%) = \frac{F_t}{F_i} \times 100 \quad (1)$$

where F_t is the total amount of voriconazole in the vesicle preparations and F_i is the initially added amount of voriconazole.

2.4. Measurement of elasticity

The elasticity of the prepared vesicle bilayer was measured by the extrusion method as reported earlier [22–24]. Briefly, the vesicle carriers were extruded for 5 min through a 50 nm pore size cellulose membrane filter (Millipore, The Ireland) by applying a pressure of 0.5 MPa. The elasticity of the vesicles was calculated from the following Eq. (2):

$$E = j\left(\frac{r_v}{r_p}\right)^2 \quad (2)$$

where E is the elasticity index of the vesicles bilayer; j is the rate of penetration through a membrane filter (the weight of suspension extruded in 5 min); r_v is vesicle size (after extrusion); and r_p is pore size of membrane.

2.5. In vitro skin permeation and deposition studies

Franz diffusion cells with an effective diffusion area of 2 cm^2 were used at 37 °C to perform the *in vitro* skin permeation and deposition studies. Six-week-old hairless male mice were purchased from HanLim Animal (Hwasung-gun, Gyunggi-do, Korea). The excised skin samples of dorsal side were clamped between the donor and the receptor chamber of Franz diffusion cells with the stratum corneum facing the donor chamber. The vesicle formulations (1 ml equivalent to 3 mg of voriconazole) containing 0.3% of vorinconazole were applied on the donor compartment.

Table 1
The composition of elastic liposomes in 1 ml aqueous solution.

Ingredient (mg)	CLs	DL1	DL2	DL3	ELs	TEL1	TEL2	TEL3
Lipoid S100	30	30	30	30	30	30	30	30
Cholesterol	10							
Tween 80		5.3				5.3		
Sodium taurocholate			5.3				5.3	
Oleic acid				5.3				5.3
Voriconazole	3	3	3	3	3	3	3	3
Ethanol (μL)	70	70	70	70	300	300	300	300
Water (μL)	930	930	930	930	700	700	700	700

CLs: conventional liposomes; DLs: deformable liposomes; ELs: ethosomes and TELs: transethosomes (ethosome containing edge activator (TEL1 and TEL2) or permeation enhancer(TEL3))