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Tadalafil-loaded nanostructured lipid carriers using permeation enhancers

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ABSTRACT

Tadalafil is a phosphodiesterase-5 inhibitor indicated for the treatment of erectile dysfunction. In this study, we prepared and evaluated transdermal nanostructured lipid carriers (NLC) to improve the skin permeability of tadalafil. Tadalafil-loaded NLC dispersions were prepared using glyceryl monostearate as a solid lipid, oleic acid as a liquid lipid, and Tween 80 as a surfactant. We characterized the dispersions according to particle size, polydispersity index, zeta potential, encapsulation efficiency, and transmission electron microscopy. *In vitro* skin permeation studies were carried out using Franz diffusion cells, and cytotoxicity was examined using HaCaT keratinocyte cell lines. Tadalafil skin permeability increased for all tadalafil-loaded NLC formulations. The tadalafil-loaded NLC dispersion with ethanol and limonene as skin permeation enhancers exhibited the highest flux (~4.8-fold) compared to that observed with tadalafil solution alone. Furthermore, a tadalafil-loaded NLC gel with selected permeation enhancers showed tolerance against toxicity in HaCaT cells. These results suggest that the NLC formulations with ethanol and limonene as skin permeation enhancers could be a promising dermal delivery carrier for tadalafil.

1. Introduction

Tadalafil (TAD) is the most potent phosphodiesterase (PDE) 5 inhibitor and at least 9000 times more selective than other PDE 5 inhibitors. Furthermore, TAD shows less inhibitory activity for PDE 6 compared to sildenafil and vardenafil and has less than a 0.1% occurrence of vision abnormalities (Brock et al., 2002; Carson et al., 2005; Coward and Carson, 2008). However, TAD has low-solubility and high-permeability, which leads to its classification as a class II drug within the FDA biopharmaceutical classification system (BCS) (Chavda et al., 2010) and poor dissolution in the gastrointestinal tract, resulting in variable bioavailability (Löbenberg and Amidon, 2000). TAD has the slowest absorption, reaching its maximum concentration (C_{max}) in 2 h compared to ~50 min for sildenafil and vardenafil (Saharan et al., 2009). Poor solubility and low bioavailability often result in limited or irreproducible clinical responses to the drug. Therefore, various techniques such as solid dispersion, microemulsion, lipid-based formulations, and complexation (El Maghraby et al., 2009) have been used to enhance bioavailability.

Transdermal drug delivery systems are an attractive alternative to traditional oral and hypodermic delivery, as they can overcome the limiting hepatic first-pass effect encountered by oral administration and are safe, painless, and easy to use (dos Anjos and Alonso, 2008). The effectiveness of transdermal drug delivery depends on the drug's ability to reach therapeutic levels by penetrating the skin sufficiently (Müller et al., 2002). For transdermal drug delivery, nanostructured lipid carriers (NLC) are an attractive strategy since the adhesion of an NLC to the skin surface provides an occlusive effect, which can eventually lead to an increase in skin hydration and promote the deposition of drugs by reducing corneocyte packing and widening inter-corneocyte gaps (Schäfer-Korting et al., 2007; Guo et al., 2012). Furthermore, the lipid and surfactant components can enhance permeation by reducing the barrier properties of the stratum corneum, thereby increasing drug permeation through the skin (Joshi and Patravale, 2006). However, NLC must be combined with topical formulations for easy application. Vitorino had published co-encapsulating of olanzapine and simvastatin nanostructured lipid carriers with permeation enhancers including ethanol and limonene (Vitorino et al., 2013; Vitorino et al., 2014). Therefore, this study aims to

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prepare and evaluate a novel TAD-loaded NLC gel and establish an optimal formulation for efficient skin permeation of TAD.

2. Materials and methods

2.1. Materials

TAD was obtained from Korea United Pharm., Inc. (Seoul, Korea). Dynasan 118 was purchased from Sigma–Aldrich (Steinheim, Germany). Stearic acid, Miglyol 812, Tween 80, oleic acid, glyceryl monostearate (GMS), and triethanolamine were purchased from Samchun Chemical Co., Ltd. (Pyungtaek, Korea). HPLC-grade acetonitrile was obtained from JT Baker (Phillipsburg, NJ, USA). Carbomer 940 was used as received without further purification. All solvents were analytical grade and used without further purification.

2.2. Solubility of TAD in lipids

To design formulations, the solubility of TAD was determined in stearic acid, GMS, or Dynasan 118 as solid lipids and soybean oil, Miglyol 812, or oleic acid as liquid lipids. For solid lipids, 2 g lipid was melted 5 °C above the melting point of each solid lipid in a water bath, and 2 mg TAD was added to the melted lipid until saturation was achieved (Li et al., 2008). For liquid lipids, excess TAD was dispersed in a tube containing the liquid lipid (5 mL) and stirred for 48 h at room temperature. The samples were then centrifuged at 12,000 rpm for 5 min, and 0.5 mL of the supernatant was suitably diluted with methanol and analyzed by HPLC. Each determination was carried out in triplicate.

2.3. Preparation of TAD-loaded NLC formulations

NLC was prepared by a hot-melted ultrasonic method to enhance the encapsulation of lipophilic drugs in the nanoparticles (Doktorovova and Souto, 2009). GMS was selected as the solid lipid matrix and mixed with oleic acid as the liquid matrix and TAD at 70 °C to form a lipid phase. The aqueous phase was composed of distilled water and 1.5% (w/v) or 3% (w/v) Tween 80 (surfactant), which was utilized as an emulsifier for the nanoparticles. After the two phases were separately heated to 70 °C, the aqueous phase was added to the lipid phase, and the mixture was homogenized at 70 °C in a water bath for 3 min to obtain the coarse emulsion. Then, this emulsion was sonicated with a probe-type sonicator for 20 min and quickly cooled in an ice bath to form NLC, hereafter referred to as the TAD-loaded NLC dispersion.

Subsequently, a gel was introduced to obtain viscosity levels suitable for transdermal application. Briefly, 0.5% (w/v) of Carbomer 940 and 30% (v/v) ethanol were added to the TAD-loaded NLC dispersion and hydrated under gentle stirring for 1 h with triethanolamine, hereafter called the TAD-loaded NLC gel.

The TAD gel was prepared by dispersing Carbomer 940 to a solution of TAD in water containing 30% (v/v) ethanol under stirring followed by neutralization with triethanolamine. As a control, a blank NLC gel without TAD was prepared using the process described for the TAD-loaded NLC gel.

2.4. Determination of particle size, polydispersity, zeta potential, and morphology

Particle size and polydispersity (PDI) were determined by photon correlation spectroscopy (PCS) using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). All samples were diluted with distilled water to gain optimal intensity, and measurements were performed at room temperature. Each value was measured in triplicate, and the results are presented as mean ± standard deviation.

The zeta potential is a physical property exhibited by particles in suspension and is related to the respective surface charge. It is a useful parameter to predict the physical stability of colloidal systems (de Vringer and de Ronde, 1995). Zeta potential analysis was carried out using a dynamic light scattering analyzer (ELS-8000, Otsuka Electronics, Japan). All samples were diluted with distilled water, and experiments were performed in triplicate to ensure a suitable conductivity for analysis.

The surface morphology of the TAD-loaded NLC dispersion was evaluated by transmission electron microscopy (TEM) (Zeiss 902A, Zeiss, Germany) operating at 80 kV. Before loading into the microscope (direct deposition), the samples were drop-cast onto a carbon-coated copper grip and dried for 1 min at room temperature.

2.5. Determination of occlusion factor

The occlusive properties of the TAD-loaded NLC gel, blank NLC gel, and TAD gel were evaluated by an *in vitro* occlusion test (Farthing et al., 2010). Beakers (100 mL) with a diameter of 5.0 cm were filled with 50 mL distilled water and covered with a filter paper (Whatman number 6, cutoff size: 3 μm, USA). Each formulation (250 mg) was applied uniformly with a spatula on the filter paper surface (18.8 cm^2), and the beakers were subsequently stored at 32 °C (to mimic the temperature of the skin surface) for 48 h. The water remaining in the beaker was weighed after 6, 12, 24, and 48 h. The occlusion factor (F) was calculated by the following equation:

$$F = \frac{(A - B)}{A} \times 100$$

where A and B represent the water loss in the absence or presence, respectively, of TAD-loaded NLC gel, blank NLC gel, or TAD gel. An F value of 0 indicates no occlusive effect, whereas an F value of 100 indicates maximum occlusiveness.

2.6. HPLC analysis

HPLC analysis of TAD from buffer and rat plasma was performed as described previously with slight modification (Williams and Barry, 2004). The mobile phase composed of acetonitrile and water (30:70, v/v) was filtered using a 0.45-μm filter, degassed, and delivered at a flow rate of 1.2 mL/min. The column temperature was maintained at 25 °C, the injection volume was 20 μL, and TAD was detected at 285 nm.

2.7. In vitro skin permeation study

All animal studies were conducted in accordance with the "Guiding Principles in the use of Animals in Toxicology" adopted by the Society of Toxicology (USA), and the experimental protocols were approved by the Animal Care Committee of Chungnam National University. Dorsal skin was excised from Sprague-Dawley rats whose hair had been previously removed. All adhering fat and other visceral tissue was removed carefully with forceps and scissors. The excised rat skin was stored at −20 °C prior to use. Franz diffusion cells with an effective diffusion area of 1.81 cm^2, a diameter of 16 mm, and a receptor volume of 11.8 mL were used to assess *in vitro* skin permeation. The receptor compartment was maintained at 37 °C, and a surface temperature of 32 °C was assured to mimic human skin conditions. The receptor medium was selected as pH 7.4 phosphate buffered solution containing 1% Tween 80 and stirred continuously with a magnetic stirrer at 500 rpm. Each TAD-loaded NLC formulation (500 μL) was placed in the donor compartment. Permeation experiments were carried out