Journal of Drug Delivery Science and Technology 31 (2016) 1–10

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

journal homepage: www.elsevier.com/locate/jddst

Research paper

In-vitro evaluation of griseofulvin loaded lipid nanoparticles for topical delivery

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ARTICLE INFO

Article history:
Received 30 May 2015
Received in revised form
4 November 2015
Accepted 4 November 2015
Available online 10 November 2015

Keywords:
Lipid nanoparticles
Griseofulvin
Topical
In-vitro

ABSTRACT

In this work, the potential of targeting griseofulvin (GF) into the skin by topical application was evaluated by *in-vitro* studies. Griseofulvin loaded lipid nanoparticles (GF-LN) were prepared using high pressure homogenization method without any penetration enhancer or solubilizing solvent. The formulations were freeze dried to increase stability of the lipid nanoparticles (LN). The optimum formulation showed a mean particle size of 179.8 nm with polydispersity index of 0.306 and zeta potential of −27.9 mV and the loading of griseofulvin was 0.77%. Morphology and the degree of crystallinity of the LN were investigated using transmission electron microscope (TEM) and differential scanning calorimetry (DSC). *In-vitro* cytotoxicity studies showed the LN has better safety profile in human keratinocyte cells (HaCaT) with four-folds reduction in cytotoxicity (IC_{50} 30 ± 2.37 μg/mL). In the *in-vitro* antifungal susceptibility tests, the GF-LN demonstrated comparable antifungal activity against *Trichophyton rubrum* (MIC 0.5 μg/mL) and *Trichophyton mentagrophytes* (MIC 0.25 μg/mL). *In-vitro* skin penetration and retention studies were carried out on porcine skin. The GF-LN demonstrated sufficient skin permeation with penetration flux of 0.067 ± 0.003 μg/cm^2/hr. The retention of griseofulvin was found four-folds higher in the epidermis (14.9 ± 0.483 μg/cm^2) on comparison to the dermis.

1. Introduction

Delivery of drug via the skin is an interesting area of research. Skin, the largest organ in humans [1] serves as the first line barrier that protects the body from exogeneous factors. With such barrier, delivery of drug via the skin is never easy. In order to facilitate the delivery of drug via the skin, colloidal carriers such as liposomes [2,3] and lipid nanoparticles (LN) [4–7] have been actively investigated. Among the colloidal carriers, LN is more superior as it shows some clear advantages in controlled drug release, targeted delivery, increased drug stability, safe without producing toxic metabolites, ease of large scale production and suitable for lipophilic and hydrophilic drugs [8].

The reported positive features of LN such as occlusive effect and skin hydration property [9], shorter duration of treatment [7], modified release profile [4], increased photostability of compound [10], increased penetration and targeting effect without going into the systemic circulation [11,12] have made them the carrier of choice for a number of cosmetic, nutraceutical and pharmaceutical products in the market. Numerous cosmetic products formulated with LN have successfully reached the market [13,14]. These reports are very encouraging thus the present study has encapsulated a poorly aqueous soluble drug, griseofulvin (GF) into the LN. Besides, the present study has converted the long standing antifungal agent available only in the oral preparation into a topical formulation.

Griseofulvin ($C_{17}H_{17}ClO_6$) is a fungistatic agent that is effective against dermatophytosis (tinea infection) caused by *Epidermophyton*, *Trichophyton* and *Microsporum* [15]. It is the 'curling

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http://dx.doi.org/10.1016/j.jddst.2015.11.002
1773-2247/

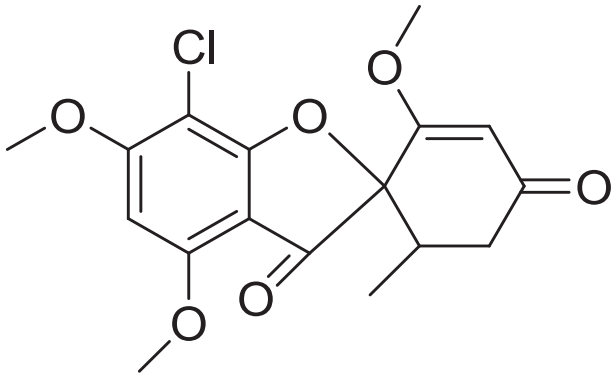

Fig. 1. Chemical structure of griseofulvin.

factor' to fungi as it is able to cause stunting, excessive branching and characteristic distortions of fungi [16]. Generally, it inhibits the division process by disrupting the mitotic spindle of the dermatophyte [15]. The chemical structure of griseofulvin is as shown in Fig. 1. It is a Biopharmaceutical Classification System (BCS) Class II drug with high permeability but low solubility [17], hence causing it to has highly variable bioavailability. Oral form of griseofulvin also has been known of its long period of treatment [15], unfavourable systemic side effects [18] and drug interactions [15]. All these disadvantages have urged the need for a topical griseofulvin formulation. Besides, dermatophytosis is a superficial fungal infection on skin, hence targeted treatment via topical route would be beneficial [19].

Griseofulvin has been investigated in various topical dosage forms, namely ethosomes [20], microemulsion [21], thermogelling microemulsions [22], polymer micelles [23] and hydrogel [17]. These topical dosage forms have been reported for their stability and promising results in delivery of griseofulvin. For instance, the application of griseofulvin-loaded microemulsion and ethosomes formulations on guinea pigs induced with dermatophytosos showed complete recovering in 7 and 8 days upon daily topical application [20,21]. In another report, griseofulvin prepared in anhydrous solvent system showed higher skin concentration from a single topical application (detectable even after 4 days) when compared to prolonged oral administration [24]. In recent year, griseofulvin-loaded lipid nanoparticles (GF-LN) had been prepared by hot microemulsion technique [25]. The *in-vitro* permeation study demonstrated that lipid nanoparticles possessed higher permeation effect (more than 5-folds) through the excised mice skin as compared to conventional cream base. At present, there is no topical griseofulvin formulation product in the market despite the reported studies [20–26]. Further exploration on the potential of delivering griseofulvin via topical route is no doubt essential to bring it a step closer to the market. In this work, we aimed to assess the feasibility of targeting griseofulvin by topical delivery via a series of *in-vitro* studies. The GF-LN were prepared using palm triglycerides and solvent-free high pressure homogenization technique. *In-vitro* cytotoxicity tests were carried out to assess the toxicity of the ingredients used in preparing the LN. *In-vitro* antifungal susceptibility tests were performed to evaluate the efficacy of the prepared GF-LN as antifungal agent. With the aim to prepare a topical formulation of griseofulvin, *in-vitro* penetration and retention through porcine skin were also evaluated.

2. Material and methods

2.1. Material

Griseofulvin, dimethyl sulfoxide (cell culture grade), thiazolyl blue tetrazolium bromide (MTT) were purchased from Sigma–Aldrich (US). Palm triglyceride was obtained as gift from Malaysia Palm Oil Board (Malaysia). Glycerol, methanol (HPLC grade), and Tween surfactants were purchased from Merck (Germany). Dulbecco's Modified Eagle Medium (DMEM), foetal bovine serum (FBS), Penicillin-Streptomycin were purchased from Life Technologies (US). Sabouraud dextrose agar (SDA) was purchased from Fisher Scientific (US). Human keratinocyte cell line (HaCaT) was from CLS-Cell Line Services (Germany). Roswell Park Memorial Institute medium-1640 (RPMI-1640) was from i-DNA Biotechnology (Singapore). *Trichophyton mentagrophytes* ATCC® 9533 and *Trichophyton rubrum* ATCC® 28188 were from MBL (US). All other reagents were of analytical grade.

2.2. Preparation of lipid nanoparticles

GF-LN were prepared using palm triglycerides as the core lipid and Tween 80 as the surfactant. The selection of lipid, Tween surfactant and lipid to Tween surfactant ratio were reported in another paper. Briefly, the lipid phase (lipid and griseofulvin) and aqueous phase (Tween 80, glycerol and deionised water) were heated to the same temperature (70 °C) separately and mixed together. Both glycerol and Tween 80 were set at 1%w/w and griseofulvin was set at 0.05%w/w. The LN formulations were prepared by different ratios of lipid and tween surfactant ranged from 1:4, 1:2, 1:1, 2:1 to 3:1. Ultrapure water was added to 100%w/w and the formulation was prepared into a total weight of 50 g. The premix was subjected to the Emulsiflex-C3 high pressure homogenizer (Avestin, Canada) at 1500 bar and 5 cycles. The mixture was set aside at room temperature for the lipid to recrystallise and form the lipid nanoparticles. GF-LN were separated from the excess griseofulvin by diluting 10 times with deionised water prior to ultracentrifugation (Beckman Coulter, US) at 40 K RPM for 3 h. The harvested GF-LN were stored in a serum bottle at −80 °C and subjected to freeze drying for 24 h to improve its stability. Blank LN were prepared in the same procedure and undergoing freeze drying process. The freeze dried GF-LN and blank LN were used for all the subsequent studies.

2.3. Stability study

The stability of GF-LN formulations were evaluated by photon correlation spectroscopy and drug loading capacity study after one month of storage at room temperature (25 °C) and high temperature (45 °C).

2.3.1. Photon correlation spectroscopy (PCS)

Zetasizer Nano ZS (Malvern Instruments, UK) with particle size limitation of 0.3 nm–10 μm was used in this study. The LN were suitably diluted with deionized water and then subjected to the zetasizer to measure the particle size, polydispersity index (PDI) and zeta potential.

2.3.2. Drug loading capacity study

The freeze dried LN were melted at 70 °C and the griseofulvin was extracted with warm mobile phase (70% methanol). Griseofulvin was quantified by High Performance Liquid Chromatography