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Ethosomes for enhanced skin delivery of griseofulvin

Joana Marto, Catarina Vitor, Ana Guerreiro, Cristiana Severino, Carla Eleutério, Andreia Ascenso, Sandra Simões *

Universidade de Lisboa, Faculdade de Farmácia, Nano2B Group of Research Institute for Medicines (iMed.ULisboa), Av. Professor Gama Pinto, 1649-003 Lisboa, Portugal

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ABSTRACT

Griseofulvin (GRF) is an important antifungal drug with low bioavailability and, for this reason, a topical formulation with a targeted action and minimal systemic effects, appears to be a preferable solution. GRF poor solubility has limited the development of topical formulations and their release to the market.

The aim of this work was to prepare a new GRF formulation for topical application using lipid-based nanosystems; to study its permeation and penetration, cell viability and to evaluate its therapeutic action.

Ethosomal systems composed of soy bean phosphatidylcholine, ethanol and water were prepared for incorporating GRF. After the characterization of the vesicles in terms of size, charge and penetrability, permeation through newborn pig using Franz diffusion cells was conducted. Cell viability at different concentrations of the chosen formulation was determined. At last, skin adapted agar diffusion test was performed to assess the therapeutic efficacy of the formulation.

GRF vesicles had mean size of 130 nm. Permeation and penetration assays revealed that GRF-loaded ethosomes have an adequate profile to be used in a topical formulation since drug retention in the *stratum corneum* was achieved. Cell viability tests proved this formulation presented no cytotoxicity to HaCaT cells for concentrations below 50 μg/mL. The skin diffusion test evidenced the potential of developed formulation to target skin dermatophytes.

The results obtained in this study contribute to a new perspective in topical treatment of fungal infections.

1. Introduction

Dermatophytoses are among the most prevalent infections in the world. Although they can be persistent and troublesome, they are not debilitating or life-threatening – yet millions of dollars are expended annually in their treatment [1], configuring an important health concern.

Griseofulvin (GRF), a classical oral antibiotic first isolated as a metabolic product from a culture of *Penicillium griseofulvum* in 1939 by Oxford et al. [2], is active against *Microsporum*, *Trichophyton*, and *Epidermophyton floccosum*, the causative fungi of *Tinea Corporis* [3]. GRF is practically insoluble in water, 8.64 mg/L (at 25 °C), making it preferable to be administered orally [4]. GRF has long been a concern in its systemic side effects, namely its interaction with drugs mostly related to cytochrome p450 metabolism [5]. Also, the oral route of administration requires long-term treatments. For these reasons, topical administration is an advantage.

Moreover, it has also been reported that the GRF concentration in the skin resulting from a single topical application is much higher than that obtained after prolonged oral administration and persists in measurable amounts for 4 or more days [6]. In addition, the application of GRF topically required a much lower amount of drug to achieve similar integumentary levels compared with the amount required orally [6,7].

Even though there is a large variety of antifungal agents, dermatophytosis are difficult to heal since long term treatments are often required until a complete cure is achieved and a treatment of election for each type of dermatophytosis still does not exist [3]. However, it is known that GRF is the drug of choice to use in childhood mycoses, such as tinea capitis and tinea corporis, because many of the newer antifungal agents are not indicated for this age group [8,5].

Despite efforts to develop a topical formulation of GRF and to enhance its topical bioavailability, this route of administration remains within the area of experimental therapeutics [7].

* Corresponding author.
E-mail addresses: jmmarto@ff.ulisboa.pt (J. Marto), catarina.vitor@campus.ul.pt (C. Vitor), analuisacoelhoguerreiro@yahoo.com (A. Guerreiro), cristianaseverino@hotmail.com (C. Severino), carlavania@ff.ul.pt (C. Eleutério), andreiaascenso@ff.ulisboa.pt (A. Ascenso), ssimoes@ff.ulisboa.pt (S. Simões).

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Several drug delivery strategies have been attempted to develop GRF topical formulations, such as emulsions [9], suspensions [9], hydrogels [7], niosomal gels and liposomal gels [3], deformable membrane vesicles [10], microemulsions [11] and solid lipid nanoparticles (SLNs) [12]. However, most of the studies do not present a consistent proof of efficient dermal delivery. There is a recent trend for producing nanocarriers loaded with GRF and co-solvents, to increase its solubility and allow topical application. In the literature, nanocarriers loaded with GRF with concentrations of 1.0% [3], 0.1% and 1.0% [9], 0.2% can be found [5,11,13].

In this study, a topical GRF formulation based on deformable lipid-vesicles was prepared. It is generally accepted that classic liposomes are of little value as carriers for transdermal drug delivery because they do not penetrate into the deeper layers of the skin, remaining confined in the upper layer of *stratum corneum* (SC). Ethosomes have been described as efficient systems for dermal and transdermal drug delivery composed of phospholipid, ethanol and water. This vesicular system contains ethanol in relatively high concentration, which enhances topical drug delivery [14,15]. Therefore, ethosomes are able to enhance permeation through the SC barrier into the deep layers of the skin. These effects are related not only to the action of ethanol on the SC, but also to the higher deformability and malleability of these vesicles and to the interaction of these vesicles with skin lipids [14,16].

The Kirby-Bauer method or disc diffusion antibiotic sensitivity test is generally accepted for selecting antibiotic therapy as a measure of drug efficacy [17]. In this study, instead of using antibiotic impregnated discs, new born pig skin discs containing the antifungal formulation on the SC were used to evaluate the efficacy of GRF-ethosomes.

The aim of this study was to develop an effective and safe treatment for dermatophytoses based on a GRF ethosomal system. The drug-carrier systems were characterized and the drug skin targeting was evaluated as well.

2. Material and methods

2.1. Material

GRF was purchased from Sigma (Sigma, St. Louis, USA). Soybean phosphatidylcholine (SPC), S100, was purchased from Lipoid (Lipoid AG, Ludwigshafen, Germany). All other reagents were of analytical grade.

Fresh skin of newborn domestic pigs was obtained from a local abattoir.

3. Methods

3.1. Preparation of GRF-ethosomes

GRF-ethosomes were prepared according to Touitou et al. [14]. Ethosomes were prepared with 5% (w/v) SPC, 45% (v/v) ethanol and different amounts of GRF (0.01–0.5%). Briefly, SPC and GRF were dissolved in ethanol, under constant stirring, in a water bath at 30 °C. Water was then added slowly in a fine stream under constant stirring, in a well-sealed container. Stirring was continued for additional 5 min. After, the suspension was left to cool down at room temperature for 30 min. Finally, the suspension was dimensioned by pressure filtration through track-etched polycarbonate (PCTE) membranes of 100 nm pore size under nitrogen pressure.

For penetration studies, 0.4% dye free as well as Nile red-loaded ethosomes were prepared, according to the method described above, where 0.4% Nile red was used instead of GRF. After preparation, the formulations were protected from the light with aluminium foil.

3.2. Vesicles physico-chemical characterization

Macroscopic organoleptic characteristics (color, odor, texture and appearance) were analyzed. The pH was determined using a LLViscotrode combined glass electrode from Metrohm® (Germany) and a 713 pH meter from Metrohm® (Germany). Measurements were done in triplicate. The lipid content of the samples was determined using a colorimetric technique described by Rouser et al. [18]. Ethosomes were characterized in terms of lipid composition and by the following incorporation parameters: initial and final GRF to lipid ratios ((GRF/Lip)i and (GRF/Lip)f, respectively), and Incorporation Efficiency (I.E.) defined as the percentage of [(GRF/Lip)f]/[(GRF/Lip)i].

Non-incorporated GRF was separated from the incorporated one using a gel chromatography column (BIO RAD Econo-Pac® 10DG Desalting Chromatography Column; exclusion limit (nominal) 6000 Da; column material: polypropylene) using water as the mobile phase. Incorporated GRF was determined by high-performance liquid chromatography (HPLC) according to Yang et al. [19] with adaptations. The HPLC system consisted of 32 Karat Software (Beckman Coulter Inc., Fullerton, USA), a Midas Spark 1.1 autoinjector and a Diode-Array 168 detector (Beckman Coulter Inc., Fullerton, USA). The detector wavelength was set to 290 nm. The analytical column was Nuceosil® 100 5 μm C18 (150 × 4.6 mm) (Macherey-Nagel, Düren, Germany). The mobile phase consisted of acetonitrile: 0.1% trifluoroacetic acid (60:40, v/v) with a flow rate of 1 mL/min at 25 °C. The experimental validation of the assay method was performed in accordance with the recommendation of ICH Guideline [20].

GRF-loaded vesicles mean size was determined by dynamic light scattering (DLS) in a Malvern Nano-S (Malvern Instruments, Malvern, UK). Polydispersity index was also measured to analyze the size distribution homogeneity of the sample. The zeta potential was determined by Laser Doppler Anemometry in a Malvern Nano-Z (Malvern Instruments, Malvern, UK).

3.3. Penetration of lipid vesicles: pressure-driven transport

The flux of vesicle suspension (diluted with water to the final concentration of 2% (w/w) SPC) through a microporous filter (PCTE) with 30 nm pore diameter was driven by different external pressures (0.7 and 1.0 MPa), created by a nitrogen stream, and measured as a function of time. These were done with a 1 mL-filtration unit that miniaturizes the commercial available pressure filtration device (home-built). The suspension was collected into a container on a Sartorius LA620P scale (Sartorius, Göttingen, Germany) to determine automatically the weight of filtered suspension. The data were collected with the Wedge software for Windows (TAL Technologies Inc., Philadelphia, USA).

3.4. In vitro *release studies*

The *in vitro* release profile was determined by using vertical Franz diffusion cells with a diffusion area of 1.0 cm^2. About 0.3 mL of freshly prepared sample (n = 3 for each formulation) was spread over the donor side of the cellulose nitrate membrane (47 mm, 0.2 μm, Sartorius, Germany) impregnated with isopropyl myristate (during 30 min, to make the membrane more hydrophobic). The receptor phase contained a mixture of saline phosphate buffer (pH = 7.4) and 0.1% TAGAT® CH 40. TAGAT® CH 40 was used to solubilize GRF in the receptor solution maintained at 32–37 °C. Care was taken to remove any bubbles between the underside of the diffusion membrane and the solution in the receiver compartment.

At pre-determined times, samples (200 μL) were collected and the same volume was replaced with fresh solution. The GRF amount in the receptor phase was quantified by HPLC. Data was expressed