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Formulation, characterization and evaluation of an optimized microemulsion formulation of griseofulvin for topical application

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ABSTRACT

The main objective of the study was to develop a microemulsion (ME) formulation of griseofulvin for the treatment of dermatophytosis (Indian Patent Application 208/DEL/2009). The oil phase was selected on the basis of drug solubility whereas the surfactant and cosurfactant were screened on the basis of their oil solubilizing capacity as well as their efficiency to form ME from pseudo-ternary phase diagrams. The influence of surfactant and cosurfactant mass ratio (Smix) on the ME formation and its permeation through male Laca mice skin was studied. The optimized formulation (ME V) consisting of 0.2% (w/w) griseofulvin, 5% (w/w) oleic acid, 40% (w/w) Smix (1:1, Tween 80 and ethanol) possessed globule size of 12.21 nm, polydispersity index of 0.109 and zeta potential value of −0.139 mV. ME V exhibited 7, 5 and almost 3-fold higher drug permeation as compared to aqueous suspension, oily solution and conventional cream respectively. Besides this the formulation was also evaluated for drug content, pH, stability, dermatopharmacokinetics and antifungal activity against *Microsporum canis* using guinea pig model for dermatophytosis. Treatment of guinea pigs with ME V resulted in a complete clinical and mycological cure in 7 days. The formulation was observed to be non-sensitizing, histopathologically safe, and stable at 5 ± 3 °C, 25 ± 2 °C and 40 ± 2 °C for a period of six months.

1. Introduction

Griseofulvin is a heterocyclic benzofuran extracted from *Penicillium griseofulvum*. It is BCS class II drug having a log *P* value of 2.17 and is practically insoluble in water [1]. The conventional oral route of administration of griseofulvin is associated with issues of poor and highly variable bioavailability, numerous systemic side effects and long duration of treatment. The analysis of physicochemical characteristics reveals that the molecule possesses high melting point of 218–220 °C. This indicates that high energy is required to break the crystal lattice of the molecule in order to dissolve the drug. Literature reveals that despite possessing all the favorable molecular characteristics like the molecular weight (352.77 Da), lipophilicity (log *P* = 2.17), hydrogen bond donors (0) and acceptors (6), polar surface area (71.06 Å) and molar refractivity (87.85) [1,2]; the clinical performance of the drug is compromised just because of poor aqueous solubility. There are numerous reports pertaining to solubility and bioavailability enhancement of griseofulvin out of which micronized, ultramicronized and Gris-PEG™ (solid dispersion of griseofulvin and polyethylene glycol 8000) ultramicrosize tablets proved to be commercial success [3].

Literature also cites the usage of aprotic solvent(s), fugitive solvent(s) or their combination and some conventional formulations of griseofulvin for topical application [4]. In the case of topical drug delivery, the diffusion takes place mainly through the stratum corneum (lipoidal barrier). The drug follows different paths to permeate through the stratum corneum. Existing experimental models explain the existence of parallel path (lipid-only, aqueous-only) and series path (alternating lipid and aqueous). Owing to poor aqueous solubility griseofulvin cannot permeate through the skin due to its less solubility in water than required for crossing the skin barrier. Moreover the possibility of dependence of flux through parallel path exists only when log *P* < 0.8 [5]. Thus, for griseofulvin the optimum solubility in both aqueous and lipid phase is vital in order to maximize its flux through the series path.

Therefore, with an aim to enhance the solubility and eventually the dermal bioavailability of griseofulvin, microemulsion (ME) formulations were designed to increase the dermal penetration and permeation of the drug. Owing to the facile and low cost preparation ME system was opted over the other colloidal counterparts such as liposomes, niosomes, nanoparticles [6]. MEs are transparent, optically isotropic and thermodynamically stable liquid solutions; comprising of oil, water and amphiphile(s), in which either the oil globules are dispersed in water (o/w) or water globules are dispersed in oil (w/o). The globule size typically varies in the range of 10–100 nm [7]. Numerous investigations have revealed the pharmaceutical significance of MEs for dermal [8] as well as

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transdermal [9] administration of a wide variety of drug molecules. The components of microemulsion can interact with the lipid layers of stratum corneum and change its structural integrity leading to enhanced permeation of drug(s) without the need of any specific penetration enhancer(s) [10]. In view of all the above mentioned features of MEs, this system was explored for topical delivery of griseofulvin. Further, the optimized ME formulation was evaluated for *ex vivo* permeation, dermatopharmacokinetics and pharmacodynamic performance using *Microsporum canis* induced guinea pig model for dermatophytosis.

2. Materials and methods

2.1. Materials

Griseofulvin (Wallace Pharmaceuticals Ltd., Mumbai, India), Isopropyl palmitate, Eutanol GPH, Cetiol LC PH and Myritol 318 (Cognis GmbH, Düsseldorf, Germany), Captex 200, Captex 300, Captex 355 and Captex 1000 (Abitec, Janesville, WI, US), Labrafac CC and Labrafac Lipophile 1349 (Gattefossé, USA) and Carbopol® 980 NF (Lubrizol Advanced Materials India Pvt. Ltd., Mumbai, India) were received as gift samples. RPMI 1640 medium (Sigma–Aldrich Inc., MO, USA); HPLC-grade acetonitrile, acetic acid and methanol (Merck KGaA, Darmstadt, Germany) were also used in the study. Triple distilled water (TDW) was used throughout the study. All other chemicals and reagents were of analytical grade and were used without further purification.

2.2. Fungal strains

The standard strains of dermatophytes, *Microsporum gypseum* (MTCC no. 2830), *M. canis* (MTCC no. 2820), *Trichophyton mentagrophytes* (MTCC no. 7250) and *Trichophyton rubrum* (MTCC no. 296) were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India.

2.3. Animals

Male Laca mice 8–9 weeks old, weighing 30–35 g was obtained from Central Animal House, Panjab University, Chandigarh, India. These were housed in polypropylene cages and employed for performing *ex vivo* permeation, histopathology and dermatopharmacokinetic studies. Male albino guinea pigs (Duncan Hartley strain) 8–9 weeks old weighing between 350 and 400 g were obtained from disease free small animal house of College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India. Guinea pigs were housed in stainless steel metabolic cages and allowed to acclimatize for a minimum of 15 days before initiating the experiment. All the animals were kept at ambient temperature with a 12-h night/day cycle, and supplied with a standard pellet diet and water *ad libitum*. The protocols for animal use and care were approved by the Institutional Animal Ethics Committee (IAEC), Panjab University, Chandigarh, India (IAEC/97 dated 24.03.2011).

2.4. Screening of formulation ingredients

2.4.1. Screening of oils

The oil phase for developing MEs of griseofulvin was selected on the basis of solubility, surfactant efficiency; Smin [11] and water solubilization capacity; Wmax [12]. The solubility of griseofulvin in various oils (Table 1) was determined employing shake flask method [13] and drug content was analyzed using UV–visible spectrophotometer at 293 nm.

Smin % (w/w) was determined as the minimum amount of surfactant required for completely homogenizing equal masses of oil and TDW to form a single phase. Wmax % (w/w) was determined by titrating equal masses of oil and surfactant with TDW until the system became turbid.

2.4.2. Screening and selection of surfactants

Four different surfactants namely, Tween 20, Tween 40, Tween 60 and Tween 80 were screened. The solubilization capacity of surfactants for oleic acid was studied using 3 mL of 15% (w/v) aqueous solution of surfactants to which aliquots of 5 μL of oil was added with vigorous vortexing until the solution became cloudy [10]. Also, emulsification ability of above mentioned surfactants was screened. 500 mg of surfactant was added to 500 mg of oleic acid. The mixture was homogenized and then 100 mg of this isotropic mixture was accurately weighed and diluted with TDW (500 times) to yield fine emulsion. The emulsions were allowed to stand for 2 h and their transmittance was assessed at 650 nm by UV spectrophotometer using TDW as blank [14].

2.4.3. Screening and selection of cosurfactants

The selection of cosurfactants was done on the basis of ME region. Tween 80 was mixed with four types of cosurfactants, namely, ethanol, isopropyl alcohol, *n*-butanol and isobutyl alcohol. Smix ratio (1:1) was kept constant and pseudoternary phase diagrams were constructed. Twelve different combinations in different weight ratios of oil and Smix, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 1:0.7, 1:0.43 and 1:0.11 were taken so that maximum ratios were covered to explain the boundaries of phases formed in phase diagrams [15].

2.5. Influence of surfactant and cosurfactant mass ratio on ME formation

The selected surfactant and cosurfactant (Smix) were blended in the weight ratios of 3:1, 2:1, 1:0; 1:1, 1:2, and 1:3. Smix, ratios were chosen in decreasing concentration of surfactant with respect to cosurfactant and *vice versa* for a detailed insight into the phase diagrams. Different combinations in different weight ratios of oil and Smix, 1:9, 1:8, 1:7, 1:6, 1:5, 2:9, 1:4, 2:7, 1:3, 3:7, 1:2, 1:1, 1:0.7, 1:0.43, 1:0.25 and 1:0.11 were taken. Aqueous titration method was employed for the construction of the pseudoternary phase diagrams. Subsequently the mixtures were evaluated visually and ME phase was identified as the region in the phase diagram where clear, easily flowable, and transparent formulations were obtained.

2.6. Preparation and optimization of ME formulation

Griseofulvin loaded o/w ME was prepared by dissolving 0.2% (w/w) griseofulvin in 5% (w/w) oleic acid. Then required quantity of different Smix (Tween 80 and ethanol) ratios was added to oil phase and mixed with the aid of vortex mixer (Table 2). The mixture was made up to 100% (w/w) with slow addition of TDW with continuous stirring.

Microemulsion was optimized with respect to Smix ratios and effect of its concentration on *ex vivo* permeation characteristics. In order to alleviate the influence of composition of MEs and take into account the effect of Smix only, all other formulation and process variables were kept constant.

Optimized ME gel of griseofulvin was prepared using 0.2% (w/w) griseofulvin, 5% (w/w) oleic acid, 40% (w/w) mixture of Smix (1:1). This mixture was slowly added to 0.5% (w/w) Carbopol previously gelled in TDW and neutralized with triethanolamine and then TDW was added to make it 100% (w/w).

Also, griseofulvin (0.2% (w/w)) was incorporated in oleic acid to prepare oily solution, in aqueous dispersion (comprising of 0.5% (w/v) Carbopol in water) and o/w conventional cream (comprising of 6% sorbitan mono-oleate, 3% white bees wax, 36% white soft