Colloids and Surfaces B: Biointerfaces 170 (2018) 341–346

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

The role of formulation and follicular pathway in voriconazole cutaneous delivery from liposomes and nanostructured lipid carriers

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ARTICLE INFO

Keywords:
Skin permeation *in vitro*
Iposome
Nanostructured lipid carrier
Follicular pathway
Dermatomycosis

ABSTRACT

In general, colloids provide increased cutaneous permeation of drugs. Still, skin interaction and main pathways for drug diffusion may vary depending on system and formulation characteristics. The knowledge of how different colloidal systems interact with biological membranes and the formulation impact on delivery is especially relevant for drugs that can be encapsulated in multiple nanosystems, as voriconazole (VOR). In here, we compared VOR release and permeation profile from liposomes (LP) and nanostructured lipid carriers (NLC) in aqueous colloidal dispersions and in gel formulations. Despite the controlled drug release provided by gel formulations, formulation only had a significant impact on drug skin accumulation from LP. The reduced mobility in gel formulations compromised follicle deposition and drug retention in the skin. Such a hypothesis was confirmed by permeation experiments evaluating follicle pathway influence. Follicular route also had an influence on delivery from NLC, which was only significant for total drug that reached the acceptor medium. These differences could be attributed to the mechanisms of colloid interaction with the skin and subsequent drug release. Follicle LP deposition and slow drug release leads to higher cutaneous amounts whilst NLC interaction with skin and fast drug release leads to fast drug diffusion and deeper penetration. By the low MIC_{50} values encountered against *Trichophyton rubrum* (~ 0.001 μg/mL), permeated amounts could inhibit fungal growth, regardless the system. In conclusion, both LP and NLC seem to be valuable systems for cutaneous VOR delivery. Fluidic formulations could provide better efficiency for cutaneous drug delivery from LP.

1. Introduction

Cutaneous topical treatment of skin diseases, targeting the drug directly to the site of action, can significantly reduce adverse effects compared to oral and parenteral routes. However, the skin barrier function is a recognizable impediment for the effectiveness of such treatments [1,2]. Colloidal systems in general have emerged as valuable option to overcome this issue and other physicochemical problems, as they are able to protect the drug from degradation, enhance loading dose and promote skin interaction, increasing bioavailability [3,4]. Still, drug skin delivery performance could be strongly affected by colloidal systems, according to their specific interaction mechanisms with the skin, and by pharmaceutical vehicles and their influence on colloidal behavior. When the drug physicochemical properties allow it to be encapsulated in more than one type of colloidal system, formulators must select an optimum nanosystem in a proper pharmaceutical vehicle. Such decision may not always be possible by simply assessing studies from different research groups, because of differences in models and protocols used.

This is the exact case of voriconazole (VOR), a second-generation triazole agent, which has emerged as a potent option in the management of several resistant fungal infections [5–9]. Since its approval by the US Food and Drug Administration (FDA) in 2002, systemic side effects have become evident, varying from minor reactions as skin rashes, abdominal pain, nausea and vomiting, to more serious and persistent problems as severe photosensitivity, exfoliative dermatitis, accelerated photo-aging, increased predisposal for cutaneous squamous cell carcinoma, besides drug interactions [6,10]. Recent reports have demonstrated colloidal lipid systems efficiently incorporate VOR enhancing formulations loading dose (VOR aqueous solubility is approximately 600 μg/mL) and improving drug interaction with biological membranes [10–15]. Transethosomes, ethosomes, deformable and conventional liposomes (LP) have shown to enhance skin permeation compared to control solution [16]. A nanostructured lipid carrier

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https://doi.org/10.1016/j.colsurfb.2018.06.037
Received 19 April 2018; Received in revised form 5 June 2018; Accepted 18 June 2018
Available online 19 June 2018

(NLC)-based hydrogel formulation has also shown superior delivery into hairless mouse skin *in vitro* when compared to conventional cream and microemulsion-based gel formulations [17]. When comparing the results from these two studies, VOR amount permeated through the skin was approximately 5 to 10-times higher from NLC than from transethosomes or conventional LP. Conversely, our group has observed a 3-fold higher ocular delivery of VOR from LP compared to NLC [12,13]. Still, results obtained with cornea may not directly relate to skin, because of the particular organization of the stratum corneum and the presence of an additional penetration pathway, the follicular route, particularly important for colloidal systems. In addition, mouse skin is not the most appropriate model to study drug penetration, as the stratum corneum of the mouse is less than half as thick as that of the human tissue, presenting lower barrier properties [18]. Hairless skin is even more inadequate for the examination of colloidal drug delivery systems behavior as such model lacks the follicular route.

Hence, in here, we used VOR as a model drug to compare its skin deposition behavior from NLC and LP as aqueous colloidal dispersions or incorporated in gel formulations as a way to select which nanosystem and pharmaceutical vehicle could provide better local cutaneous delivery. Porcine skin, recognized as an optimal model for percutaneous experimentation [19–21], was used to perform *in vitro* experiments, evaluating the impact of the follicular route on epidermal deposition. Nanosystems antifungal activity against *Trichophyton rubrum*, a common etiological agent of dermatophytosis, was also assessed. Based on such experiments, the possible interaction mechanisms of these colloids with the skin surface are discussed.

2. Material and methods

2.1. Materials

VOR (99.85%) was purchased from Hangzhou Dayang chem Co. Limited (Hangzhou, China). Solvents used for extraction and chromatographic analysis were all of HPLC grade. Soybean phosphatidylcholine was purchased from Avanti Polar Lipids (Alabaster, USA). Polysorbate 80 (Tween 80), sorbitantrioleate (Span 85), poloxamer 407 (POL), 3-(N-morpholino) propanesulfonic acid, RPMI-1640 medium, resazurin sodium salt and cetylpyridinium chloride (CPC) were purchased from Sigma Aldrich (St. Louis, MO). Glyceryl behenate (Compritol ATO 888) was purchased from Gattefossé (Weil am Rhein, Germany). Mixture of capric and caprylic triglycerides (Miglyol 812 N) were purchased from Stallergenes SA (Antony, France).

Skin was obtained from porcine ears kindly provided from a local abattoir (Bonasa Alimentos, São Sebastião, Brazil) shortly after animal sacrifice. Samples were stored at −20 °C for a maximum of 1 month before use.

2.2. Preparation of lipid-based nanosystems

LPs were produced by thin film hydration method as previously described [12]. Briefly, VOR (11.2 mg) was solubilized in methanol and added to a round bottom flask with 800 µL of a stock solution of PC in chloroform (200 mmol/L) to form a lipid film after solvent evaporation followed by hydration with 4 mL of HEPES buffer (pH 7.4). To obtain the desired size, the suspension was submitted to10 cycles of extrusion through 600 nm pore polycarbonate membranes and 6 cycles through 100 nm pore membranes (Lipex, Northern Lipids Inc., Canada).

NLC were preparated using a microemulsion technique [13]. Briefly, the lipid phase (350 mg of Compritol® and 50 µL of Miglyol®) abd the surfactants (140 µL of Tween® 80 and 60 µL of Span® 85) were heated at 70 °C under stirring. Then, 100 mg of CPC and 7 mg of VOR were added to these melted materials. Subsequently, 250 µL of water, at 80 °C, were added under stirring to the mixture under magnetic stirring to obtain a microemulsion. Hot microemulsion was dispersed into cold HEPES buffer at (2–4 °C) under vigorous stirring (13,400 rpm for 10 min, IKA T25 Ultra-Turrax; Staufen, Germany). This final colloidal dispersion was sonicated for 20 pulsed duty cycles of 30 s (60 s off) with power delivery of 30% (Sonics, Connecticut, U.S.A). During this process, samples were kept in ice bath to prevent heat damage.

2.3. Incorporation of lipid-based nanosystems in hydrogel

LP or NLC were added into poloxamer 16% (w/w) and maintained at 4 °C overnight for gel polymerization.

2.4. Physical characterization of lipid-based nanosystems

Formulations of LP and NLC were characterized by means of hydrodynamic diameter, polydispersity index (PdI), zeta potential and entrapment efficiency (EE), as previously described [12,13].

LP and NLC morphological analysis was performed and colloidal suspensions were compared to nanosystems incorporated in gel-based formulations. For this, diluted samples (1:200) were analyzed by transmission electron microscopy (TEM; JEM 1011 Transmission Electron Microscope, JEOL, Tokyo, Japan – 100 kV) and the images were captured with a GATAN BioScan camera (model 820, GATAN, PA, USA) using the Digital Micrograph 3.6.5 software (GATAN, PA, USA). To prepare the samples, 20 µL aliquots of diluted samples were deposited on a Formvar-coated copper grid (Electron Microscopy Sciences, PA, USA) and air-dried for 10 min. After that, 20 µL of uranyl acetate solution 3% (w/v) was added and air-dried for 10 min, and the excess was removed with filter paper.

2.5. In vitro release studies

The release of VOR from LP and NLC (400 µL) and from colloidal dispersions incorporated in poloxamer gel (LP-POL or NLC-POL) were determined using a modified Franz-type diffusion cells (diffusional area = 1.6 cm^2). A hydrophilic cellulose membrane (Fisherbrand, Nepean, Canada) (12,000–14,000 MWCO) was placed between the donor and acceptor compartments. The acceptor compartment was filled with 15 mL of HEPES buffer (pH 7.4) and maintained under stirring at 300 rpm. The system temperature was kept at 32 °C, by water bath, over a period of 6 h.

2.6. In vitro skin permeation

Modified Franz-type diffusion cells were used with full-thickeness porcine skin between the donor and acceptor compartments in order to evaluate *in vitro* cutaneous permeation.

In the donor compartment, 400 µL of each colloidal dispersion was added (LP or NLC) and their formulations incorporated in poloxamer gel (LP-POL or NLC-POL). The acceptor compartment was filled with 15 mL of HEPES buffer (pH 7.4) and maintained under stirring at 300 rpm. System temperature was controlled at 32 °C, by water bath, over a period of 6 h.

The acceptor solution was collected at the end of the experiment for quantification of the permeated drug. Extraction of the retained drug in the stratum corneum was performed using tape stripping technique (15-tapes) and the drug amount retained in the remaining skin, comprising the lower epidermis and part of the dermis was extracted by cutting the tissue in small pieces, followed by methanol extraction for 3 h under magnetic stirring at 300 rpm. Samples were then filtered and quantified by HPLC, according to section 2.7.

VOR has an intermediary lipophilicity (log P 1.8 and aqueous solubility of approximately 700 µg/mL). As so, liposomes presented higher VOR entrapment efficiency and consequently higher loading dose than NLC with donor formulations presenting different VOR concentrations (2800 µg/mL in LP, 2352 µg/mL in LP-POL, 500 µg/mL in NLC and 420 µg/mL in NLC-POL). To allow the comparison between the colloidal systems permeated drug amounts were presented as