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Research Paper

Econazole imprinted textiles with antifungal activity

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ABSTRACT

In this work, we propose pharmaceutical textiles imprinted with lipid microparticles of Econazole nitrate (ECN) as a mean to improve patient compliance while maintaining drug activity. Lipid microparticles were prepared and characterized by laser diffraction (3.5 ± 0.1 μm). Using an optimized screen-printing method, microparticles were deposited on textiles, as observed by Scanning Electron Microscopy. The drug content of textiles (97 ± 3 μg/cm^2) was reproducible and stable up to 4 months storage at 25 °C/65% Relative Humidity. Imprinted textiles exhibited a thermosensitive behavior, as witnessed by a fusion temperature of 34.8 °C, which enabled a larger drug release at 32 °C (temperature of the skin) than at room temperature. *In vitro* antifungal activity of ECN textiles was compared to commercial 1% (wt/wt) ECN cream Pevaryl®. ECN textiles maintained their antifungal activity against a broad range of *Candida* species as well as major dermatophyte species. *In vivo*, ECN textiles also preserved the antifungal efficacy of ECN on cutaneous candidiasis infection in mice. *Ex vivo* percutaneous absorption studies demonstrated that ECN released from pharmaceutical textiles concentrated more in the upper skin layers, where the fungal infections develop, as compared to dermal absorption of Pevaryl®. Overall, these results showed that this technology is promising to develop pharmaceutical garments textiles for the treatment of superficial fungal infections.

1. Introduction

Textile is a material that has been purposed to clothing for centuries. In recent years, the combined efforts of chemists, textile engineers and cosmetologists resulted in the development of biofunctional textiles that bring additional functions to garments than simple warmth and body protection. Also called cosmetotextiles, such textiles are defined as textile items containing substance or mixture that releases their active compounds when in contact with the human body [1]. Firstly focused on improved comfort, cosmetotextiles have since then been developed for slimming, moisturizing, and perfuming [2]. Innovative technologies have been incorporated into such fabrics, such as microencapsulated substances [1,3] or phase change materials that help the thermoregulation of the body [4]. Rapidly, various biofunctional textiles have been envisioned for the delivery of topical bioactive molecules, since the close and prolonged contact of fabric with the skin could make cloth an easy drug delivery system. Silver nanoparticles [5] and chitosan [6] were used as preservatives for antibacterial clothing. Fabrics with antioxidant properties were developed by incorporation of vitamin E [7] or gallic acid [8]. Some clinical indications have also been examined, such as venous insufficiency using aescin supported textiles [9] and atopic dermatitis with zinc oxide functionalized textiles [10]. Such examples show the evolution of cosmeto-textiles to pharmaceutical textiles, offering more than an improved comfort, but also a treatment for various skin diseases.

In particular, superficial fungal diseases are common worldwide and their incidence continues to increase. In 2010, they were the 4th most prevalent disease in the world, affecting more than 948 million people worldwide [11]. As compared to bacteria, fungal topical infections are longer in duration and require weeks and even months of fastidious treatment. Patient compliance would be greatly improved if a regular piece of textile (such as bandage or socks) could be used instead of applying a cream daily. Antifungal textiles have been prepared by soaking the fabric into a solution of antifungals [12,13], and promising clinical results

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have been obtained from a sock prototype to treat tinea pedis [14]. However, fabrication technology and controlled release of antifungal agents still need to be improved.

Econazole nitrate (ECN) is currently marketed for the treatment of vaginal candidiasis and topical fungal infections as a cream formulation [15,16]. It has demonstrated antifungal activity against *Candida* and dermatophytes species [15–17]. Encapsulation of ECN in lipid particles [18], microspheres [19], and micelles [20] has been reported to improve cutaneous efficacy of ECN. More precisely, comparing micro- and nano-solid lipid particles, nanoparticles were shown to improve transdermal administration whereas microparticles enhanced skin deposition [21]. Moreover, the lipid composition favored a good biocompatibility of the particles and improved skin penetration of the drug [22].

In this work, a novel ECN formulation on textile support was tested as a proof of concept for the treatment of topical fungal infections. The formulation is based on proprietary lipid microparticles exhibiting thermosensitivity in order to release the drug on contact with the skin [23]. Deposition of the microparticles on textile is achieved using an in-house modified screen-printing technique. The latter is a simple method where the microparticles are passed through a mesh with predefined openings to control the amount and the topology of the deposit [24,25]. This method allows for a physical uniform deposit of the microparticles at specific areas on textiles without addition of chemical binders. The solid microparticles (Dermotex®) and deposition method (On2™) are technologies proprietary to Biomod Concepts Inc., and have been used by the company to produce intelligent cosmetic textiles [23]. The objective of this study was to evaluate the potential of a pharmaceutical textile, namely a microparticle formulation of ECN deposited on textile. Its *in vitro* antifungal activity, percutaneous absorption, and *in vivo* pharmaceutical efficacy on a superficial fungal infection were compared to the commercial 1% (wt/wt) ECN cream Pevaryl®.

2. Experimental methods

2.1. Materials

ECN-loaded microparticles on textile and all placebo textile formulations were provided by Biomod Concepts Inc. (Ste-Julie, QC, Canada) and prepared according to their patented technology [23]. Laya™ textiles were provided by Biomod Concepts (Sainte-Julie, QC, Canada). ECN was purchased from AK Scientific (Union City, CA, USA, Lot# TC24717). Pevaryl® 1% (wt/wt) ECN formulation was purchased from Johnson & Johnson (France, Lot # DDB3400) and its generic version from Mylan Pharmaceuticals (Saint-Priest, France). Miconazole Nitrate was purchased from AK Scientific (Union City, CA, USA, Lot# TC25782). ECN standard disks were purchased from Rosco (Neo-sensitabs 10 μg disks, Denmark, Lot #1201-1). Prednisolone acetate was purchased from Sanofi Aventis (Paris, France). Polyethylene Glycol 400 (PEG-400) was purchased from Medisca Inc. (Montreal, QC, Canada). Sodium dodecyl sulfate (SDS) and semi-permeable polycarbonate membranes (Nucleopore Track-Etch Membrane, pores of 0.6 μm, 25 mm in diameter) were purchased from Sigma–Aldrich (Oakville, ON, Canada). Tape used for tape stripping was purchased from 3M tape (St-Paul, MN, USA). All samples were filtered using PTFE filters purchased from Fisher Scientific (EMD Millipore Millex, pores 0.45 μm, 13 mm in diameter, Ottawa, ON, Canada). All solvents (HPLC grade) were bought from Fisher Scientific (Ottawa, ON, Canada).

2.2. Organisms

Candida albicans strain SC5314 was originally isolated from a patient with disseminated candidiasis, and served as reference for the *C. albicans* genome sequencing project [26,27]. Thirteen clinical isolates of *Candida* spp. and *C. albicans* (CAAL93, CAAL121, CAAL123, CAAL124, CAAL294), *C. kefyr* (CAKE3, CAKE4), *C. krusei* (CAKR1, CAKR3), *C. glabrata* (CAGL1, CAGL5), and *C. lusitaniae* (CALU1, CALU2) were obtained from the Department of Parasitology and Medical Mycology, EA1155, at the University of Nantes, France. *Trichophyton rubrum* ($n = 2$) and *T. mentagrophytes* ($n = 2$) were obtained from the Laboratory of Parasitology and Medical Mycology at the Centre Hospitalier Universitaire of Nantes.

2.3. Preparation of ECN textiles

Intelligent textiles imprinted with ECN-loaded microparticles were prepared by Biomod Concepts Inc. using their patented technology [23]. Briefly, ECN lipid microparticles (1% wt/wt) were prepared under high shear using FDA-approved ingredients. The microparticles formulation was then applied onto textile surface using an adapted screen-printing method optimized for the microparticles deposition. A stencil with openings of more than 400 μm was used to apply the microparticles on 21.6 × 27.9 cm pieces of a polyester non-woven textile provided by Biomod Concepts Inc. ECN imprinted textiles were kept at 22 °C in sealed aluminum/acrylonitrile-coated packagings until analysis.

2.4. Characterization of microparticles

One hundred milligram (100 mg) of the ECN-loaded microparticles preparation used for screen-printing was diluted in 5 mL of milliQ water and analyzed for article size distribution at 22 °C by laser diffraction (LS 13 320, Beckman Coulter, Mississauga, ON, Canada). Pevaryl® particle size was measured by dynamic light scattering (Zetasizer Nano ZS, Malvern, Worcestershire, UK) using the automatic algorithm mode. Samples were prepared by diluting 100 mg of Pevaryl® in 5 mL of MilliQ water, position 4.65 and attenuator at 8. Measurements were recorded 3 times for each formulation.

Fusion temperature of the microparticles imprinted on textile was measured using thermal analysis based on heat-leak-modulus (TA-HLM) [28]. With TA-HLM, textile samples are wrapped around a sensor probe and heated. The samples of ECN-loaded textile (2.5 × 5 cm) were analyzed at a heating rate of 0.8 °C per second and heated from 0 °C to 100 °C. Measurement was repeated 3 times.

2.5. HPLC-UV analysis

High-performance liquid chromatography (HPLC) with ultraviolet (UV)-analysis was used for stability and quantification of samples.

The HPLC-UV system (Agilent 1100 Series, Mississauga, ON, Canada) consisted in a degasser, dual pumps, auto-sampler, column heater and photo-diode array detector. A C18 column (25 × 4.6 mm, 5 μm packing, Zorbax-C18, Agilent, Santa Clara, CA, USA) was used with a matching pre-column (Agilent Zorbax C18). Mobile phase was composed of methanol and water using the gradient detailed in Table 1.

The flow rate was 1.4 mL per minute. The column temperature was set to 35 °C. The injection volume was 20 μL. ECN was analyzed at 220 nm. ECN retention time was 8.7 min. The limit of quantification with this method is 9 μg/mL.

2.6. HPLC-MS/MS method

HPLC-Mass spectrometry (MS)/MS was used for *in vitro* release and *ex vivo* experiments on pig skin, which presented lower concentrations of ECN than the limit of quantification (LOQ) of