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Roxithromycin-loaded lipid nanoparticles for follicular targeting

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ABSTRACT

Particulate drug carriers *e.g.* nanoparticles (NPs) have been shown to penetrate and accumulate preferentially in skin hair follicles creating high local concentration of a drug. In order to develop such a follicle targeting system we obtained and characterized solid lipid nanoparticles (SLN) loaded with roxithromycin (ROX). The mean particle size (172 ± 2 nm), polydisperisty index (0.237 ± 0.007), zeta potential (−31.68 ± 3.10 mV) and incorporation efficiency (82.1 ± 3.0%) were measured. The long term stability of ROX-loaded SLN suspensions was proved up to 26 weeks. *In vitro* drug release study was performed using apparatus 4 dialysis adapters. Skin irritation test conducted using the EpiDerm™ tissue model demonstrated no irritation potential for ROX-loaded SLN. *Ex vivo* human skin penetration studies, employing rhodamine B hexyl ester perchlorate (RBHE) as a fluorescent dye to label the particles, revealed fluorescence deep in the skin, specifically around the hair follicles up to over 1 mm depth. The comparison of fluorescence intensities after application of RBHE solution and RBHE-labelled ROX-loaded SLN was done. Then cyanoacrylate follicular biopsies were obtained *in vivo* and analyzed for ROX content, proving the possibility of penetration to human pilosebaceous units and delivering ROX by using SLN with the size below 200 nm.

1. Introduction

Follicular penetration of active substances applied topically has for years been undervalued because of the minute area that hair follicles occupy compared to the skin area of the entire body. However, there are body areas where hair density is high enough for the follicles to offer a major drug penetration path, or for the targeted delivery to the specific sites in the follicle. The hair follicle density and the volume of the follicular openings on the forehead and head are the highest on the human body. The combined area of the follicular openings in these locations can be as much as 10% of the total skin area in these body regions. The same body sites frequently suffer from dermatological disorders such as androgenic hair loss and common acne, the etiology of which is closely related to the pilosebaceous apparatus. In such cases targeted drug delivery to the hair follicles becomes the key element of the therapy (Blume-Peytavi and Vogt, 2011; Knorr et al., 2009; Wosicka and Cal, 2010). The pilosebaceous unit is a complex structure that includes the hair follicle, hair shaft, adjoining arrector pili muscle and associated sebaceous gland(s) (Fig. 1).

Pilosebaceous units can be utilized as reservoirs for localized therapy or as a transport pathway for systemic drug delivery. Various attempts to increase the distribution of active pharmaceutical ingredient (API) in that region have already been made for such active substances as adapalene, erythromycin–zinc complexes, isotretinoin, anti-androgens, diphencyprone and minoxidil (Aljuffali et al., 2014; Morgen et al., 2011; Patzelt et al., 2011; Tschan et al., 1997). Preferential follicular deposition is highly dependent on a type of the vehicle used in the formulation, application way and physicochemical properties of the drug itself. What is even more important this transport pathway especially favors particulate drug carriers *e.g.* nanoparticles (NPs) (Główka et al., 2014;

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Fig. 1. Fluorescence imaging of a hair follicle stained with Nile red, magnification 40× (diagonal view at an angle of about 45°; *x*-axis bar is not suitable for *y*-axis data estimation). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Wosicka and Cal, 2010). Some research studies suggest that follicular delivery is more efficient with smaller nanoparticles (<300 nm) and lipophilicity of the material applied on the skin improves the penetration rate (Alvarez-Roman et al., 2004; Desai et al., 2013; Iannuccelli et al., 2014). NPs surface charge is another parameter affecting follicular penetration, although the published results are ambiguous (Desai et al., 2013; Lee et al., 2013). Encapsulation of drugs into particulate carriers may allow to omit the transepidermal pathway and increase the drug concentration in the hair follicles as particles have the tendency to penetrate and accumulate preferentially in the hair follicle orifices. In this way, pilosebaceous units represent a reservoir where particles create high local concentration of API and the particle depot in the follicular duct also ensures a prolonged drug release enabling the reduction of the applied dose and the frequency of applications. The particles are depleted from the follicles only by slow processes such as hair growth, shedding and sebum flow (Lademann et al., 2011; Rancan et al., 2014). The role of massage during application has to be emphasized since many experiments proved that it improves follicular penetration of nano- and microcarriers. This can be explained by the fact that massage *in vitro* imitates the hair movement occurring *in vivo* (Lademann et al., 2008; Toll et al., 2004).

During the last decade follicular penetration of lipid NPs has been studied, showing that the lipophilicity of the carriers improves drug uptake by the hair follicles. It is possible to detect solid lipid nanoparticles (SLN) in the hair follicles 24 h after application, which presents SLN as a slow-release system (Lin et al., 2013; Munster et al., 2005).

Roxithromycin (ROX) is a semi-synthetic macrolide antibiotic derivative of erythromycin. It is characterized by molecular weight: 837 g/mol and lipophilicity: logP = 3.1 (PubChem CID: 9604450). The antibiotic is administered orally in treating infections with Gram-positive bacteria, and, though less frequently, Gram-negative bacteria. The mechanism of ROX's action involves blocking bacterial protein biosynthesis (Ostrowski et al., 2010).

ROX, on top of its typical anti-bacterial action with respect to *Propionibacterium acnes*, inhibits the bacterial production of pro-inflammatory compounds (lipase, NCF – neutrophil chemotactic factor) which also contribute to the development of acne. Thanks to the above, ROX demonstrates very potent anti-inflammatory properties. There are also reports on its inhibition of free radicals formation in the neutrophils and on inhibition of keratinocyte apoptosis (Akamatsu et al., 2002; Takahashi et al., 2004). Ito et al., (2009) showed that ROX prevented apoptosis of hair follicle keratinocytes in mice and human. Androgenetic alopecia, characterized by shortened anagen stage, is caused by dihydrotestosterone binding to androgen receptors in dermal papilla, resulting in apoptosis induction. Anti-apoptotic effect of ROX implies its potential role as a hair restoration agent.

The aim of the work was to efficiently incorporate ROX into SLN in order to evaluate it as a follicular targeting system having the potential to treat acne or androgenetic alopecia. To our knowledge, there are no data reporting on ROX incorporation into the lipid nanoparticles.

2. Materials and methods

2.1. Materials

Compritol ATO 888 (glyceryl behenate) was obtained from Gattefosse (Gennevilliers, France), Poloxamer 188 from BASF SE (Ludwigshafen, Germany). Rhodamine B hexyl ester perchlorate (RBHE) was purchased from Invitrogen (Carlsbad, USA), ROX from POL-NIL SA (Warsaw, Poland). Spectra/Por Biotech (10 kDa molecular weight cut off) regenerated cellulose ester dialysis membrane was obtained from Spectrum Labs (Rancho Dominguez, USA). The EpiDerm™ System along with the MTT kit were supplied by MatTek Corporation (Ashland, USA). All other solvents and chemicals used were of the highest purity available. Ultrapure water was used throughout all experiments (Millipore® Simplicity UV Water Purification System; Millipore, Bedford, USA).

2.2. Preparation of solid lipid nanoparticles

The composition of SLN aqueous suspension was the following: glyceryl behenate 5%, ROX 1%, poloxamer 2.5% (w/w). 1 g of ROX was used to prepare 100 g of the formulation (ROX comprised 16,67% of the lipid phase). The dispersion was prepared using ultrasonication technique. The lipid (glyceryl behenate, ROX) and aqueous (ultrapure water, poloxamer) phases were heated separately to 80 °C. The aqueous phase was added to the lipid phase and the mixture was treated using a probe-type sonicator (VCX130, Sonics and Materials, Newtown, USA) for 10 min (20 kHz), then cooled in an ice–water bath. RBHE-labelled particles were prepared using the same procedure. The fluorescent dye was added into the lipid phase. For blank SLN, no ROX was added.

2.3. Microscopic studies

Atomic force microscopy (AFM) imaging was carried out using an Agilent 5500 AFM (Agilent, Santa Clara, USA) at a tapping mode and silicon nitride probes (PPP-FM, $k = 2.8$ N/m Nanosensors, Neuchatel, Switzerland). Topography, amplitude and phase data were collected simultaneously. The samples were prepared by appropriate dilution and drying in a nitrogen stream.