Journal of Controlled Release 242 (2016) 16–24

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Improved topical delivery of tacrolimus: A novel composite hydrogel formulation for the treatment of psoriasis

Doris Gabriel [a,*], Thibault Mugnier [a], Herve Courthion [a], Ksanthi Kranidioti [b], Niki Karagianni [b], Maria C. Denis [b], Maria Lapteva [c], Yogeshvar Kalia [c], Michael Möller [a], Robert Gurny [a]

[a] Apidel SA, 29 Quai du Mont Blanc, 1201 Geneva, Switzerland
[b] Biomedcode Hellas SA, 34 Al Fleming Street, 16672 Vari, Athens, Greece
[c] School of Pharmaceutical Sciences, University of Geneva and University of Lausanne, CMU-1, Rue Michel Servet, 1211 Geneva 4, Switzerland

ARTICLE INFO

Article history:
Received 11 June 2016
Received in revised form 30 August 2016
Accepted 8 September 2016
Available online 14 September 2016

Keywords:
Polymeric nanocarriers
Tacrolimus
Skin topical drug delivery
Methoxy poly (ethylen glycol) hexyl-substituted poly (lactic acid)
Hydrogel
Dermatology
Immune-mediated skin disease

ABSTRACT

We have developed a composite hydrogel for improved topical delivery of the poorly soluble drug Tacrolimus (TAC) to psoriasis lesions. TAC is efficiently solubilized in methoxy poly- (ethylene glycol) hexyl substituted poly-(lactic acid) (mPEGhexPLA) based nanocarriers. For convenient and patient-friendly topical administration, TAC loaded polymeric nanocarriers were incorporated in a Carbopol® based hydrogel, to yield a composite hydrogel formulation (TAC composite hydrogel). TAC composite hydrogel was designed to have superior pharmaceutical formulation properties, delivery efficiency and local bioavailability, compared to currently available paraffin-based TAC ointments. Composite hydrogel formulations had good local tolerance and showed no signs of immediate toxicity after repeated topical administration in healthy mice. Skin delivery of TAC composite hydrogel in an imiquimod-induced psoriasis mouse model was found to be twice as high as for the commercial formulation Protopic™, used as benchmark. TAC composite hydrogel showed significant improvement in the in vivo and histopathological features of the imiquimod-induced psoriasis model.

1. Introduction

Psoriasis is a chronic immune-mediated inflammatory skin disease affecting approximately 1% to 3% of the world's population. Disease manifestation usually occurs as highly inflamed and sharply demarcated erythemateous oval plaques with adherent silvery scales [1]. Skin scaling is a consequence of epidermal hyper-proliferation, incomplete cornification and retention of nuclei in the stratum corneum [2]. Histopathological hallmarks are epidermal hyperplasia with dysregulated keratinocyte differentiation, prominent inflammatory infiltrate, and increased vascularization [3]. Disease etiology is multi-factorial with a combination of environmental and genetic factors triggering the immuno-histological changes observed in the skin [4]. A broad spectrum of anti-psoriatic treatments, both topical and systemic are available for psoriasis management. While mild disease is generally treated by topical agents used as a first line treatment, for patients with higher percentage of body surface area affected, or with disease that significantly impacts quality of life, systemic therapy or phototherapy is indicated [5]. Systemic psoriasis medication involves small molecule immunosuppressive and/or anti-proliferative drugs such as methotrexate, cyclosporine or acitretin, as well as biologics which often target the TNF pathway [6]. Combinations of several topicals or a topical along with a systemic treatment are common practice. While systemic immunosuppressive therapy can be associated with undesirable side effects [7], patient adherence to topical treatment is limited and improved dosage forms are needed [8,9]. Here we describe the development of a nanocarrier composite hydrogel formulation for improved patient compliance and topical drug delivery to psoriasis lesions. Nanocarriers are based on methoxy – poly (ethylene glycol) – hexyl substituted poly (lactic acid) (mPEGhexPLA). mPEGhexPLA is an amphiphilic polymer which self-assembles to drug-loaded spherical nanostructures in an aqueous environment. These nanostructures have an inert, PEG-based surface with neutral to slightly negative net charge and a particle size <60 nm [10]. Nanocarriers contain a high payload of the hydrophobic Tacrolimus (TAC), which is a potent immunosuppressive drug. TAC ointments are currently approved for the topical treatment of atopic dermatitis [11,12]. Off-label use in other inflammatory skin diseases including psoriasis is common practice, with treatment of facial and intertriginous psoriasis in particular recommended by the American Academy of Dermatology [13]. With the development of a novel TAC hydrogel composite formulation, a major step towards a drug product with improved patient acceptability and compliance has been made, as major disadvantages of commercial formulations including persistent residence of a sticky, greasy film, have been overcome. In nanocarrier hydrogel composite formulations, TAC is temporarily solubilized in the

* Corresponding author.
E-mail address: doris00gabriel@gmail.com (D. Gabriel).

http://dx.doi.org/10.1016/j.jconrel.2016.09.007
0168-3659/

amphiphilic carrier and incorporated into a hydrogel for easy topical administration, resulting in the following advantages: (i) the hydrogel rapidly evaporates leaving no residual formulation on the skin and (ii) compared to commercially available ointments, in which high affinity between drug and formulation base compromises an efficient drug transfer into the skin, nanocarrier-based formulations facilitate partitioning of the drug into the skin and can therefore achieve higher local drug concentrations. Dilution of the nanocarrier below the critical micellar concentration leads to drug release and formation of an *in situ* tissue drug depot. In the present work we show that TAC hydrogel composite formulations safely and efficiently deliver a high drug payload to inflammatory lesions after local application. Using the imiquimod-induced psoriasis-like inflammation model, we demonstrate that TAC hydrogel composite formulations have therapeutic efficacy equivalent to the positive treatment control clobetasol propionate. Importantly, TAC nanocarrier hydrogel composite formulation delivered significantly higher drug levels into inflamed skin enabling next generation products with reduced drug dose and/or treatment frequency.

2. Materials & methods

2.1. Materials

Acetonitrile HPLC grade and water HPLC grade (Biosolve®), sterile water (Corning®) were purchased from Brunschwig, Switzerland. Acetone Ph.Eur., trifluoroacetic acid (TFA) HPLC grade, Rhodamine B octadecyl ester (RhB), 6-Diamidino-2-phenylindole hydrochloride (DAPI), sodium hydroxide (NaOH) pellets and Superfrost® microscope slides were obtained from Sigma, Switzerland. Tacrolimus (TAC) >99% and Sirolimus (SIR) > 99% were purchased from LC-labs, USA. Sodium citrate dihydrate and citric acid Ph.Eur. grade were purchased from Haenseler, Switzerland. Carbopol® ETD 2020 was obtained from Lubrizol, Belgium. Optimum cutting temperature (OCT) compound and 100 mL sterile beakers were obtained from VWR, Switzerland. mPEGhexPLA (M_w 5.5 kDa) was supplied by Apidel SA, Switzerland.

2.2. Preparation of polymeric nanocarriers

Placebo and 0.2% TAC mPEGhexPLA nanocarrier formulations were prepared in citrate buffer 20 mM, pH 5.5, at a 15 mL batch scale. Briefly, 618.8 mg of polymer and 41.3 mg of TAC were dissolved in 2 mL of acetone. The organic phase was added dropwise (6 mL/h) to the citrate buffer (20 mM, pH 5.5) under sonication (amplitude 20%, SD450, Branson, USA). Acetone was evaporated under reduced pressure (58 °C, 180 mbar, Rotovapor R210, Buechi, Switzerland) and TAC dose was adjusted to 0.2% by addition of citrate buffer. Placebo mPEGhexPLA nanocarrier formulations were prepared as described, but without TAC.

2.3. Preparation of composite hydrogels

Concentrated Carbopol® gels (1.2% (w/w)) were prepared at a batch size of 40 g. Carbopol® ETD 2020 (480 mg) was weighed and dispersed into water for injectables to obtain a partially swollen polymer. pH was adjusted to 5.5 ± 0.2 with a NaOH solution (10% w/w). The gel was stirred until the Carbopol® polymer was in its fully swollen state. If required, weight of the final gel was adjusted to 40 g with sterile water. 0.1% TAC composite hydrogels were prepared at a batch size of 40 g, by slow addition of 0.2% TAC mPEGhexPLA nanocarrier formulation (20 g) to the concentrated Carbopol® gel (20 g) under magnetic stirring. The final TAC composite hydrogel was analyzed for pH, TAC dose and viscosity. Placebo composite hydrogel formulations were prepared accordingly, but using placebo instead of TAC loaded nanocarriers.

2.4. Drug assay by high performance liquid chromatography (HPLC)

TAC was quantified by HPLC (Agilent 1100, USA) with a Kinetex Phenomenex® C18 column (75 × 3 mm I.D., 2.6 μm) thermostated to 50 °C. The analysis was carried out in the gradient mode at a flow rate of 1 mL/min over 7 min. The mobile phase consisted of a mixture of water with 0.1% v/v TFA (A) and acetonitrile with 0.1% v/v TFA (B). A linear gradient from 45% to 55% B was applied for 4 min, followed by 2 min at 55% B and an equilibration step down to 45% B in 1 min. The injection volume was set to 20 μL and UV detection at 205 nm. Limit of quantification (LOQ) and limit of detection (LOD) were found to be 115 ng/mL and 38 ng/mL, respectively. The method was considered as robust and reproducible (Relative Standard Deviation (RSD) < 2%, $n = 3$) over the linear range (10–100 μg/mL, $R^2 = 1.00$).

2.5. Particle size measurement by dynamic light scattering (DLS)

Particle size was characterized by DLS with a Zetasizer Nano-ZS, (Malvern Instruments, UK) using the DTS Nano software. Colloidal solutions were filled into disposable plastic cuvettes and were analyzed using back scattering light (173°). Samples were measured at a polymer concentration of 20 mg/mL and samples were equilibrated at 25 °C for 60 s before the first measurement. Micelle size and distribution were measured in triplicates and expressed as size distribution by number, *Z*-average diameter and polydispersity index (PDI).

2.6. Transmission electron microscopy (TEM)

Morphology of micellar nanocarriers was evaluated using a TEM FEI TecnaiTM G2 Sphera (FEI, USA). Carbon disc ultrathin grids were ionized under vacuum (0.3 Torr, 400 V, 20 s). 5 μL of aqueous formulation (diluted to 5 mg/mL mPEGhexPLA) was deposited onto the disc and incubated for 5 s, followed by staining with 0.2% uranyl acetate for 30 s. Imaging was performed at a tension of 120 keV and a magnification ranging from 5000× to 30,000×.

2.7. Viscosity measurement

Viscosity was measured with a Haake Rheostress 1 rheometer (Thermo Scientific, USA). A cone-plate sensor was used with a cone angle of 2°, cone diameter of 35 mm and 0.105 mm gap at the cone tip. Temperature and shear rate were set to 20 °C and 4.3 s^{-1}, respectively. About 500 μL of hydrogel was used for each measurement.

2.8. pH measurement

pH was measured using a Metrohm 827 pH lab (Switzerland). Prior to measurements the instrument was calibrated using standard solutions (Metrohm buffer solutions pH 4 and 7). Measurements were performed at room temperature (RT) under continuous stirring.

2.9. Tissue extraction

Tacrolimus extraction from skin tissue was performed as previously described [14]. For each sample, a 0.28 cm^2 skin surface was sampled using a Sklar® Biopsy punch. The two groups of TAC ointment *versus* TAC hydrogel treated animals had similar skin thickness, close to normal skin, on the day of sample collection. Each sample was carefully washed with ethanol, in order to ensure the removal of the residual formulation from the skin surface. Skin samples were dried with a cotton swab. Identical cleaning procedure was applied for each sample. The skin was subsequently cut into small pieces, and TAC deposited within the skin was extracted by soaking the pieces in 0.56 mL of acetonitrile for 4 h at RT under continuous stirring. The extraction samples were centrifuged at 8000 ×*g* for 15 min prior to UHPLC-MS/MS analysis.