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Research paper

Innovative drug vehicle for local treatment of inflammatory skin diseases: *Ex vivo* and *in vivo* screening of five topical formulations containing poly(lactic acid) (PLA) nanoparticles

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ABSTRACT

One of the main goals in the galenic development of innovative topical treatment options for inflammatory skin diseases such as psoriasis and atopic dermatitis is to selectively deliver the drug at the inflammation site. Recent studies have highlighted the beneficial use of polymeric nanoparticles for anti-inflammatory therapy and topical anti-inflammatory drug delivery due to their ability to form a drug reservoir retaining the drug locally at the site of action. Our approach consisted in designing innovative topical semi-solid formulations of poly(lactic acid) (PLA) nanoparticles as anti-inflammatory drug vehicles for local treatment of inflammatory skin diseases. In the course of this work, five topical formulations containing fluorescent PLA nanoparticles were initially developed, and then screened depending on their physico-chemical properties, toxicity and delivery efficacy. The penetration and permeation of a fluorophore vectorized by PLA nanoparticles into healthy and inflammatory skin were assessed using an alternative device to classical Franz cells: VitroPharma. All these investigations led to the selection of two satisfactory formulations out of five initial candidates.

1. Introduction

Inflammatory skin diseases such as atopic dermatitis (AD) and psoriasis are highly prevalent cutaneous disorders that can deeply affect patient's quality of life [1]. Depending on the severity of the disease, various treatments are applied.

With increasing discoveries in the immuno-pathogenesis of these pathologies, the development of targeted therapies (TNF-alpha inhibitors, anti-IL17, anti-IL12/23, anti-IL4/13, anti-IL5, JAK inhibitors) has gained ground in the past decade [2,3]. Those treatments are usually prescribed for patients with severe forms of cutaneous inflammatory disorders. Though very efficient, those molecules are considered as immunosuppressive treatments and are associated with an increased risk of side effects such as infectious diseases, sometimes life threatening [2].

For patients with mild to moderate forms of cutaneous inflammation, topical therapies are most frequently used. The development of innovative topical therapies for the treatment of cutaneous inflammatory disorders has been neglected over the past decades, and corticosteroids and vitamin D3 analogs are still the gold standard of those therapies [4,5] while they may be associated with low efficacy, poor tolerability or important cutaneous side effects due to prolonged use [6]. However, the severity of

Abbreviations: PLA, poly(lactic acid); AD, atopic dermatitis; JAK, janus kinase; PBS, phosphate-buffered saline; BSA, bovine serum albumin; PFA, paraformaldehyde; PDI, polydispersity index; NP, nanoparticle; $_{fluo}$NP, fluorescent nanoparticle; rpm, *rotation* per minute; EE, entrapment efficiency; RT, room temperature; SEM, scanning electron microscopy; HES, hematoxylin/eosin/safran; BCA, bicinchoninic acid assay; ELISA, Enzyme-Linked ImmunoSorbent Assay; IMQ, imiquimod; OECD, Organisation for Economic Co-operation and Development; SD, standard deviation; ANOVA, analysis of variance; SCORAD, severity scoring of atopic dermatitis; PASI, psoriasis area severity index; TMA, trimellitic anhydride.

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the disease does not justify a systemic delivery of one of the treatments described above, for which the balance of risk and benefice would be detrimental to the patient. On the other hand, local administration of previously described new immune-targeted therapies may be at least as effective as corticosteroids while limiting their local and systemic side-effects, providing that they are properly delivered. For all these reasons, there is a need to develop new and more effective formulations for topical delivery of innovative agents, both designed for a short and/or long term use, with lesser side effects than corticosteroids.

Recent studies have highlighted the beneficial use of polymeric nanoparticles for anti-inflammatory therapy [7,8] and topical anti-inflammatory drug delivery [9] due to their ability to form a drug reservoir retaining the drug locally at the site of action. It has also been shown that polymeric materials have a higher potential for local delivery to the skin than lipid carriers [10–12].

However, aqueous suspensions of polymeric nanoparticles cannot be easily applied on skin for *in vivo* studies and the therapeutic drug vectorized by those particles tends to run-off. Hence, there is a need to develop specific delivery systems allowing topical administration of polymeric nanoparticles. Numerous innovative approaches have been developed over the past few years, such as microneedles [13], iontophoresis [14,15], sprays and patches [16]. For now, harsh manufacturing process of microneedles impacts on the particles that should be delivered and on the non-standardized doses [17]. Regarding iontophoresis, this technique is not easily transferable for a daily use by patients.

Our approach consisted in designing innovative topical semi-solid formulations of poly(lactic acid) (PLA) nanoparticles as anti-inflammatory drug vehicles for local treatment of inflammatory skin diseases. PLA nanoparticles are biodegradable and biocompatible nanovectors with a strong safety background for human use. They also have been shown a preferential accumulation in hair follicles following topical application, due to their lipophilicity, thus reducing the trans-epidermal pathway [18,19]. Furthermore, drug encapsulation into PLA nanoparticles can result in a sustained and controlled drug release in the epidermis, as shown by Luengo et al. [20]. Thereby, they have an interesting profile for new dermatotherapies [18,21].

Five topical formulations containing PLA nanoparticles were initially developed. These formulations were screened depending on their physico-chemical properties, toxicity and delivery efficacy. In order to assess this last point, we used the well-known mouse model of Imiquimod-induced psoriasis developed by van der Fits et al. [22] as inflammatory skin model, in comparison with healthy mouse skin. Penetration and permeation of a fluorophore, encapsulated in the PLA nanoparticles, into healthy and inflammatory skin were assessed using an alternative device to classical Franz cells: VitroPharma [23]. All these investigations led to the selection of two satisfactory formulations out of five initial candidates.

2. Materials and methods

2.1. Materials

Viscarin® GP 209F and Avicel® RC-591 were purchased from FMC BioPolymer (USA), Sepineo™ P 600 was procured from SEPPIC (France), Pentravan® was purchased from Fagron (USA), and Lutrol® F 127 was purchased from BASF (Germany). CellTrace BODIPY® TR Methyl Ester was purchased from Molecular Probes (USA). Acetonitrile, physiological serum, acetone, ethanol, paraformaldehyde, phosphate-buffered saline (PBS), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich, USA. 16% formaldehyde solution (PFA) was purchased from ThermoScientific (USA). Hematoxylin, eosin and safran stains were procured from the platform PrImaTiss (Lyon).

2.2. Animals

Male BALB/cByJ mice between 7 and 8 weeks old were purchased from Charles River Laboratories and housed in Mice experimental biology facility (ENS de Lyon, France). All animals were handled according to the institutional guidelines. All studies and procedures were approved by the Comité Rhône-Alpes d'Ethique pour l'Experimentation Animale (Lyon, France).

2.3. PLA nanoparticles synthesis

Poly(D,L-lactide) (PLA) of Mn = 32,000 g mol^{-1} (polydispersity index, PDI = 2.08) was produced in the laboratory following the patent FR2745005A1 of Phusis (Grenoble, France).

PLA nanoparticles (NPs) were synthesized as previously described in Lamalle-Bernard et al. [24] using the solvent diffusion method initially described by Fessi et al. [25], without the use of any surfactant. Briefly, the anionic polymer was dissolved in acetone at a concentration of 2% w/v, and this solution was added dropwise to an aqueous phase under moderate magnetic stirring. The solvents were then removed under reduced pressure using a Rotavapor® R-II (Buchi, France). The final PLA concentration was precisely determined by weighing the wet and dried materials. Fluorescent nanoparticles were obtained as above, dissolving the hydrophobic fluorescent dye (CellTrace BODIPY®) at a concentration of 0.04% w/w dye: PLA in acetone with PLA prior to nanoprecipitation, leading to a final concentration of fluorophore of 14.6 μg/mL after solvent evaporation. PLA NPs suspensions were carefully protected from light throughout the experimental procedure.

2.4. Characterization of PLA nanoparticles

2.4.1. Physico-chemical characterization

Hydrodynamic diameter and polydispersity index of blank (non-fluorescent) and fluorescent PLA nanoparticles were determined after their dilution in a 1 mmol L^{-1} NaCl solution by Dynamic Light Scattering at 25 °C at an angle of 173°, using a Zetasizer Nano ZS Plus (Malvern Instrument, UK). Each value provided is the average of four series of measurements. In the same dilution conditions, Zeta potential was converted from electrophoretic mobility according to Smoluchowski's equation, using a Zetasizer Nano ZS Plus.

2.4.2. Fluorescent characteristics of PLA $_{fluo}$NPs

Maximum of excitation and emission wavelengths of fluorescent NPs was also determined from excitation and emission spectra measured with a Tecan infinite M1000 fluorescence spectrophotometer (Tecan, Swiss). To determine the dye entrapment efficiency (EE) within PLA particles, 250 μL of PLA $_{fluo}$NPs was centrifuged during 30 min at 14,000 rpm (Eppendorf centrifuge 5418). Supernatant containing the non-encapsulated dye was then collected, and the pellet of $_{fluo}$NPs was washed 2 times with water before its dissolution in 250 μL of acetonitrile. The fluorescent dye was quantified in the fluoNPs suspension (free + encapsulated fractions) and in the pellet of $_{fluo}$NPs (encapsulated fraction), after solubilization of PLA NPs in acetonitrile, by spectrofluorimetry. Bodipy was detected at an excitation/emission fluorescence wavelength of 585/620 nm in a linear range from 0.5 ng/mL to 5 μg/mL with a correlation coefficient (R^2) $\geqslant$ 0.99. Fluorescence measurement on supernatants (non-encapsulated fraction) was measured as a control. The entrapment efficiency (EE) was calculated according to the following equation: