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Research Paper

Size dependent skin penetration of nanoparticles in murine and porcine dermatitis models

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ABSTRACT

A major limitation in the current topical treatment of inflammatory skin diseases is the inability to selectively deliver the drug to the inflammation site. Recently, smart drug delivery systems such as nanocarriers are being investigated to enhance the selective deposition of anti-inflammatory drugs in inflamed areas of the skin to achieve higher therapeutic efficacy with minimal side effects. Of such systems, polymeric nanoparticles are considered very efficient carriers for the topical drug delivery.

In the current work, poly(L-lactide-co-glycolide) nanoparticles of nominal sizes of 70 nm (NP70) and 300 nm (NP300) were studied for their intra-epidermal distribution in murine and pig atopic dermatitis models over time against the respective healthy controls. Confocal laser scanning microscopical examination of skin biopsies was utilized for the qualitative and semi-quantitative analyses of nanoparticles skin deposition and penetration depth.

While no skin penetration was found for any of the particles in healthy skin, the accumulation of NP70 was significantly higher than NP300 in inflamed skin (15-fold in mice, 5-fold in pigs). Penetration depth of NP70 decreased over time in mice from 55 ± 3 μm to 20 ± 2 μm and similar tendencies were observed for the other formulations. In inflamed pig skin, a similar trend was found for the penetration depth (NP70: 46 ± 12 μm versus NP300: 23 ± 3 μm); however, the NP amount remained constant for the whole analyzed period.

Their ability to penetrate specifically into inflamed skin combined with minimal effects on healthy skin underlines small polymeric nanoparticles' potential as selective drug carriers in future treatment of chronic inflammatory skin diseases such as atopic dermatitis.

1. Introduction

Although topical drug administration to the inflamed skin is one of the most common treatment modalities of dermatitis, efforts toward higher specificity and enhanced local delivery to the inflammation site are surprisingly limited. The conventional topical formulations used to treat inflammatory skin diseases locally bare the problem of drug delivery into the skin without providing specific penetration toward the inflamed tissues.

In particular, atopic dermatitis requires increasing efforts for a better therapy with a prevalence that reaches 20% in children and over 3% of adults and since a long-term therapy distinctly alters the patient quality of life [1].

Recently, different approaches have been tested in order to increase the therapeutic specificity [2,3]. Initial studies revealed the beneficial use of nanoparticles for an anti-inflammatory therapy [4,5]. In particular, the ability to penetrate more profoundly into the skin and forming a drug reservoir that retains the drug locally at the site of action represents a promising strategy for the treatment of chronic inflammatory skin diseases [4].

These studies provided insights into the effect of particle size and surface properties [4,5] as well as the general superiority of polymeric matrix material for the local delivery compared to lipid carriers [6–8]. However, an ambiguity discussed in the context of these studies was, despite the use of mice in several models with atopic dermatitis like features over the last years [9–14], the significant differences in morphology compared with human skin [15].

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Table 1
Physicochemical characterization of particles for in vivo experimentation (results are shown as mean ± SD, $n = 3$).

Experiments	Size (nm)	PDI ± SD	Zeta potential (mV)
Mouse	72 ± 0.4	0.12 ± 0.01	−0.3 ± 0.1
Mouse	281 ± 0.3	0.29 ± 0.10	−11.5 ± 0.2
Pig	66 ± 0.3	0.14 ± 0.01	−0.8 ± 0.2
Pig	355 ± 0.8	0.32 ± 0.14	−11.9 ± 0.3

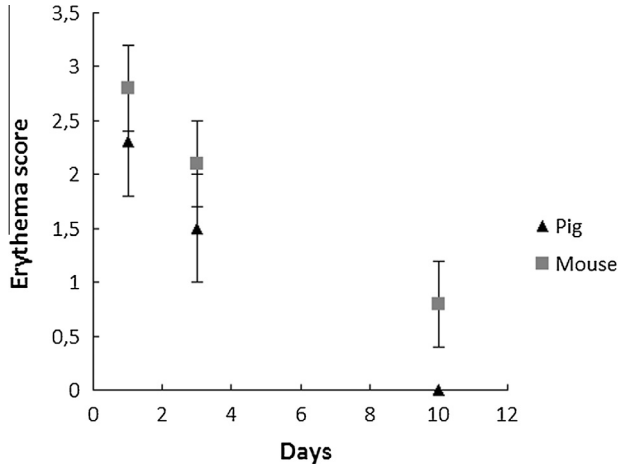

Fig. 1. Erythema score in pig and mouse experimental oxazolone-induced dermatitis.

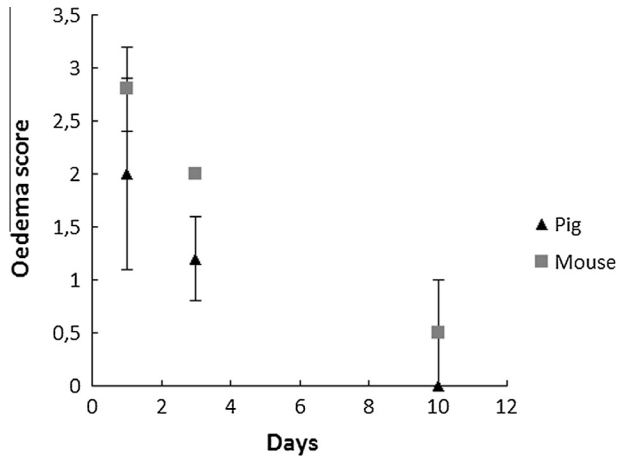

Fig. 2. Edema score in pig and mouse experimental oxazolone-induced dermatitis.

Beside other structural features, rodent skin generally shows higher permeation rates compared with human skin [16].

A very interesting alternative is the porcine oxazolone-induced allergic dermatitis model [17]. Pig skin has seemingly the advantage of being currently the best model to mimic the human skin due to its very similar skin morphology and physiology [15,18,19]. The skin of both man and pig is characterized by a spare hair coat, a thick epidermis that has a well-differentiated undersculpture, a dermis that has a well-differentiated papillary body and a large content of elastic tissue [20]. Besides, the immune system of the pig notably Langerhans cells, is close to that what is observed in humans [21–23].

In this study we focus on the in vivo penetration behavior of polymeric particles into oxazolone induced inflamed skin. Beside the currently used mouse ear model, we have run a comparative study in a newly developed pig skin dermatitis model in order to detect potential species-related difference in nanoparticle skin penetration and also to elucidate the relevance of earlier results from murine models. Experiments were performed with poly(L-lactide-*co*-glycolide) (PLGA) nanoparticles where PLGA was covalently bound to fluoresceinamine to ensure the accurate detection of the nanocarriers in the skin samples [24]. Confocal laser scanning microscopy (CLSM) was employed to detect particle distribution in the skin samples and free fluorescein in solution was run in parallel as a control.

2. Materials and methods

2.1. Materials

Resomer® RG502H, poly(L-lactide-*co*-glycolide) was provided by Evonik AG (Ingelheim, Germany). 5-Fluoresceinamine [14], Fluorescein sodium salt, 1-ethyl-3-(3-Dimethylaminopropyl)-carbodii mide hydrochloride (DMAP), Polyvinyl Alcohol (PVA), and Oxazolone (4-ethoxymethylene-2-phenyl-oxazol-5-one) were purchased from Sigma–Aldrich (Saint-Quentin Fallavier, France). Cryomatrix™ gel was provided by Thermo Scientific (Villebon sur Yvette, France). All other chemicals were of analytical grade and were obtained from Sigma–Aldrich (Saint-Quentin Fallavier, France).

2.2. Preparation of fluorescein sodium solution and fluoresceinamine bound PLGA (FA-PLGA)

Fluorescein sodium solution at 0.0050 mg ml^{-1} was prepared by dissolution of the dye in water. Fluoresceinamine grafted to PLGA (FA-PLGA) was prepared according to the method described by Horisawa et al. [25]. Briefly, PLGA (3 g) and FA (0.058 g) were dissolved in a solution of DMAP into acetonitrile at 0.0013 g ml^{-1}. The mixture was incubated at room temperature for 24 h under light protection and stirring. FA-PLGA was obtained by precipitation with the addition of purified water and was separated by centrifugation. In order to remove the reagents in excess, the polymer was successively dissolved in acetone and precipitated in ethanol until no fluorescence was detected in the precipitation medium. Purified polymer was finally lyophilized at −52 °C for 24 h. Infrared spectroscopy analyses were used to confirm the coupling of FA-PLGA. Fluorescence from FA-PLGA was quantified in dimethyl sulfoxide against a calibration curve of free fluoresceinamine. The number of bound FA was calculated against the number of free carboxy groups obtained by the acid value of pure PLGA which led to a labeling efficiency of 93 ± 3%. All experiments were performed with the same batch of FA-PLGA.

2.3. Preparation and physicochemical characterization of polymeric particles

FA-PLGA particles were prepared by dissolving 100 mg of FA-PLGA in 3 ml of ethyl acetate under magnetic stirring. The whole organic phase was then emulsified by sonication (Bandelin Sonoplus, Berlin, Germany) during 4 min in 10 ml of 0.1 (NP300) or 5% (NP70) PVA aqueous solution in an ice bath. The organic solvent was removed under reduced pressure.

Nanoparticle size, polydispersity index (PDI) and zeta potential analyses were carried out at 25 °C by dynamic light scattering (Zetasizer Nano, Malvern, UK). All measurements were performed in triplicate.