European Journal of Pharmaceutics and Biopharmaceutics 79 (2011) 241–249

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Improved efficacy in the treatment of contact dermatitis in rats by a dermatological nanomedicine containing clobetasol propionate

M.C. Fontana [a,c,1], J.F.P. Rezer [b,1], K. Coradini [a], D.B.R. Leal [c], R.C.R. Beck [a,*]

[a] Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil
[b] Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil
[c] Programa de Pós-Graduação em Ciências Farmacêuticas, Centro de Ciências da Saúde, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil

ARTICLE INFO

Article history:
Received 18 February 2011
Accepted in revised form 4 May 2011
Available online 14 May 2011

Keywords:
Clobetasol propionate
Contact dermatitis
Hydrogels
In vitro release
Nanocapsules
NTPDase activity

ABSTRACT

We developed a dermatological nanomedicine containing clobetasol propionate-loaded nanocapsules and evaluated its efficacy in a model of contact dermatitis after topical administration in rats. Hydrogels containing clobetasol propionate-loaded lipid-core nanocapsules or nanoemulsion (HG-CP-NC and HG-CP-NE, respectively) were prepared to evaluate the influence of the polymeric wall. They presented adequate pH values (5.50–6.50) and drug content (0.5 mg g^{-1}) and their rheograms exhibited a non-Newtonian pseudoplastic behavior. The best *in vitro* drug release control was obtained for HG-CP-NC (1.03 ± 0.11 μg cm^{-2} h) compared to the HG-CP-NE (1.65 ± 0.19 μg cm^{-2} h) and the hydrogels containing nonencapsulated drug (HG-CP) (2.79 ± 0.22 μg cm^{-2} h). A significant increase in NTPDase activity was observed in lymphocytes for the group treated with 0.05% HG-CP-NC every other day compared to the group treated with 0.05% HG-CP every day using the *in vivo* model of contact dermatitis. The nanoencapsulation of clobetasol in nanocapsules led to a better control of the drug release from the semisolid nanomedicine and provided better *in vivo* dermatological efficacy.

1. Introduction

Topical corticosteroids have been widely used to treat skin diseases. Their clinical effectiveness in the treatment of psoriasis and atopic dermatitis is related to their vasoconstrictive, anti-inflammatory, immunosuppressive, and antiproliferative effects [1]. However, the use of topical glucocorticoids, after systemic and topical administration, is limited due to their adverse effects, such as skin atrophy, steroid acne, hypopigmentation, and allergic contact dermatitis [2,3]. Currently, researches have been focusing mainly on the development of strategies to improve the benefit-risk ratio of glucocorticosteroids [4–6]. With a view to decreasing the adverse effects, nanotechnology collaborates through the reduced particle size of its nanosystems, improving the absorption and therapeutic concentration of the drug in the target tissue, allowing reproducible and long-term release of the drug at the target site, reducing the frequency of drug administration, and improving its pharmacokinetics [7]. Clobetasol propionate (CP) is a super high-potency dihalogenated corticosteroid used for the treatment of skin disorders such as atopic dermatitis and psoriasis [8,9].

Polymeric nanocapsules are vesicular nanocarrier systems in which an oily phase is confined in a cavity surrounded by a thin polymeric membrane [10,11]. The preparation of semisolid formulations containing polymeric nanostructured systems for cutaneous application has been studied recently, aiming to control the release of some active substances, to improve their photostability [6,12–16], and to promote the drug penetration in the stratum corneum [15]. Moreover, the small particle size of the nanocarriers ensures close contact with the stratum corneum [17].

Milão et al. [12] showed that hydrophilic gels (Carbomer 940®) containing diclofenac-loaded nanocapsules present non-Newtonian behavior and plastic properties. Intact nanostructures were observed in gels by freeze-fracture electron microscopy after 3 months of storage at room temperature. Jiménez et al. [13] demonstrated that the skin accumulation of octyl methoxycinnamate after the application of an oil-in-water emulsion containing its free form was significantly lower in comparison with the formulation containing octyl methoxycinnamate-loaded nanocapsules. The inclusion of octyl methoxycinnamate-loaded nanocapsules in sunscreen formulations decreases its accumulation in the skin, since the *in vitro* octyl methoxycinnamate release mechanism is governed by its high lipophilicity and by the hydrophobicity and crystallinity of the polymeric material.

A comparison of Carbomer 940® hydrogels containing different nimesulide-loaded nanoparticles (nanocapsules, nanospheres, and nanoemulsion) was reported by Alves et al. [14]. All formulations

* Corresponding author. Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Av. Ipiranga, 2752, CEP 90610-000, Porto Alegre, RS, Brazil. Tel.: +55 51 3308 5951, fax: +55 55 3308 5090.
E-mail address: ruy.beck@ufrgs.br (R.C.R. Beck).
[1] These authors contributed equally to this work.

0939-6411/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2011.05.002

presented pseudoplastic characteristics according to the Ostwald model and no thixotropic phenomenon was detected, regardless of the type of nanoparticles. In the following study, the penetration and distribution of nimesulide in human skin after the topical application of these formulations was investigated using the tape stripping technique and Franz-type diffusion cells [15]. Hydrogels containing nimesulide-loaded nanocapsules presented a higher penetration of nimesulide to the deeper skin layers in comparison with the formulations containing nimesulide-loaded nanospheres or nanoemulsion.

The efficiency of hair follicles to act as a drug reservoir after the topical application of a formulation containing nanoparticles was demonstrated by Ladermann et al. [18]. In this study, the authors observed deeper penetration of a dye into hair follicles after the application (with massage) of a formulation containing dye-loaded nanoparticles in comparison with one containing the dye in non-particle form. The differential stripping method revealed that nanoparticles were stored in the hair follicles for up to 10 days, while the nonparticle form could be detected only up to 4 days. In addition, Paese et al. [16] showed that the presence of polymeric nanocapsules in hydrogels did not produce contact sensitization in mice, demonstrating their low potential to cause contact allergic reactions.

Studies have also been conducted to develop semisolids containing CP-loaded nanoparticles. In this regard, Rao and Murthy [19] reported a lower absorption of the drug into the blood stream after topical application of HPMC gels containing CP-loaded liposomes compared to the same formulation containing the free drug. This result was attributed to higher drug accumulation in the skin, considering the results of *in vitro* studies on CP diffusion across rat skin membranes. Furthermore, Capó et al. [4] showed that the liposomal CP formulation presented a potency of 2.35 times higher compared to the formulation containing the free form. According to the authors, this increase could help to reduce the dosage of the drug, leading to a decrease in its adverse effects.

Kalariya et al. [5] demonstrated a lower mean flux value of CP from a cream containing drug-loaded solid lipid nanoparticles compared to a marketed cream containing the free drug. In addition, the nanostructured cream presented a better therapeutic response (1.9-fold higher for inflammation and 1.2-fold for itching) than the marketed formulation. Moreover, Senyigit et al. [20] showed that the incorporation of CP into lecithin/chitosan nanoparticles induced an accumulation of CP especially in the epidermis without any significant permeation across pig ear skin. Dilution of CP-loaded nanoparticles with chitosan gel (1:9) produced similar enhanced retention of CP in the epidermis and dermis compared to the commercial cream, even though the former contained ten times less CP. This is a remarkable finding in terms of reducing the side effects of CP.

Contact dermatitis is a frequent inflammatory skin disease that can be induced by exposure to low molecular weight chemicals, with both proinflammatory and antigenic properties [21]. This disease occurs in a delayed hypersensitivity reaction, mediated by cells through a mechanism that sensitizes the immune T lymphocyte to an antigen protein or a hapten linked to a protein [21]. Notable among the mediators able to modulate the actions of lymphocytes are adenine nucleosides and nucleotides, in particular, extracellular ATP, which is able to regulate the cell–cell interactions and is important in the processes of cell activation, differentiation, development, proliferation, and death, as well as effector lymphocyte response [22].

Extracellular nucleotides are messengers that modulate the exocrine and endocrine systems, the vasculature and hemostatic mechanisms, and musculoskeletal, immune and inflammatory cells [23]. These nucleotides represent an important means of modulating the activity of lymphocytes. The presence of an enzymatic mechanism is essential to maintaining the concentration of nucleotides in the extracellular space constant [22]. Adenine nucleotides (ATP, ADP, and AMP) and their nucleoside derivative, adenosine, are important signaling molecules that mediate diverse biological and pathological processes [24]. NTPDase (ecto-nucleoside triphosphate diphosphohydrolase; CD39) is an integral membrane protein that metabolizes extracellular ATP and ADP to AMP [25]. Kansas et al. [26] described the presence of CD39 on the surface of lymphocytes as well as endothelial cells. This enzyme has also been reported to be present on other leukocytes [27,28] as well as neoplastic cells in a number of hematologic malignancies [29–31]. CD39 has been demonstrated to have other effects in leukocytes including modulation of cytokine expression and the inflammatory response, as well as cell–cell adhesion [26,28,32]. It also affects the cell proliferation and apoptosis via its modulation of ATP levels in the pericellular fluid milieu [33]. The activity of NTPDase has been recognized as an activation marker necessary for effector lymphocyte function and participates in the processes of antigen recognition [22].

Recently, we reported the development of CP-loaded nanocarriers (nanocapsules, nanospheres, and nanoemulsion) as alternatives to topical administration of the drug [34]. All drug-loaded nanocarriers showed a controlled drug release and protection of the drug against UVA radiation. The polymer, poly(ε-caprolactone) (PCL), and the oil demonstrated an important influence on the drug release profile. Furthermore, PCL nanocapsules provided a better control over the drug released compared to other polyesters [35].

Taking all these considerations into account, the aim of this study was to develop a dermatological nanomedicine (hydrogel) containing CP-loaded polymeric nanocapsules and to evaluate its efficacy in the treatment of contact dermatitis, in order to obtain a new alternative for the treatment of skin disorders. Hydrogels containing CP-loaded nanoemulsions were also prepared in order to evaluate the effect of the polymeric layer on their physicochemical and rheological properties. Our main hypothesis was that by controlling the clobetasol propionate release from hydrogels through its association with nanocarriers, it may be possible to reduce the total dose of drug to be administered in a cutaneous disorder-like contact dermatitis. The *in vivo* protocol was based on the induction of contact dermatitis in rats using a dispersion of nickel sulfate in solid Vaseline at 5%.

2. Materials and methods

2.1. Materials

The CP used in this study was donated by Neo Quimica (Goiás, Brazil). The PCL and sorbitan monostearate were acquired from Sigma–Aldrich (São Paulo, Brazil). The caprylic/capric triglyceride mixture and imidazolidinyl urea were supplied by Brasquim (Porto Alegre, Brazil) and Alpha Quimica (São Paulo, Brazil), respectively, and polysorbate 80 and polyethylene glycol 400 by Henrifarma (São Paulo, Brazil). Acetate cellulose membranes (0.45 μm pore size) were acquired from Millipore (São Paulo, Brazil). Carbomer Ultrez® 10 NF and triethanolamine were acquired from DEG (São Paulo, Brazil). Clobetasol propionate commercial gel (Clob-X 0.05%, batch 2880038, Galderma) was purchased locally. HPLC grade methanol was acquired from Tedia (São Paulo, Brazil). Ethanol and acetone were acquired from Impex (São Paulo, Brazil). Nucleotides, sodium azide, HEPES, and Trizma base were purchased from Sigma Aldrich (St. Louis, MO, USA). Ficoll-Hypaque (Lymphoprep™) was purchased from Nycomed Pharma (Oslo, Norway), and all other reagents used in the experiments were of analytical grade and of the highest purity. All chemicals and solvents were used as received.