Journal of Controlled Release 242 (2016) 50–63

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Tailored dendritic core-multishell nanocarriers for efficient dermal drug delivery: A systematic top-down approach from synthesis to preclinical testing

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ARTICLE INFO

Article history:
Received 30 April 2016
Received in revised form 16 June 2016
Accepted 22 June 2016
Available online 24 June 2016

Keywords:
Dendritic core-multishell nanocarriers
Biocompatibility
Dexamethasone
Inflammatory skin disease
Dermal drug delivery
Skin model

ABSTRACT

Drug loaded dendritic core-multishell (CMS) nanocarriers are of especial interest for the treatment of skin diseases, owing to their striking dermal delivery efficiencies following topical applications. CMS nanocarriers are composed of a polyglycerol core, connected by amide-bonds to an inner alkyl shell and an outer methoxy poly(-ethylene glycol) shell. Since topically applied nanocarriers are subjected to biodegradation, the application of conventional amide-based CMS nanocarriers (10-A-18-350) has been limited by the potential production of toxic polyglycerol amines. To circumvent this issue, three tailored ester-based CMS nanocarriers (10-E-12-350, 10-E-15-350, 10-E-18-350) of varying inner alkyl chain length were synthesized and comprehensively characterized in terms of particle size, drug loading, biodegradation and dermal drug delivery efficiency. Dexamethasone (DXM), a potent drug widely used for the treatment of inflammatory skin diseases, was chosen as a therapeutically relevant test compound for the present study. Ester- and amide-based CMS nanocarriers delivered DXM more efficiently into human skin than a commercially available DXM cream. Subsequent *in vitro* and *in vivo* toxicity studies identified CMS (10-E-15-350) as the most biocompatible carrier system. The anti-inflammatory potency of DXM-loaded CMS (10-E-15-350) nanocarriers was assessed in TNFα supplemented skin models, where a significant reduction of the pro-inflammatory cytokine IL-8 was seen, with markedly greater efficacy than commercial DXM cream.

In summary, we report the rational design and characterization of tailored, biodegradable, ester-based CMS nanocarriers, and their subsequent stepwise screening for biocompatibility, dermal delivery efficiency and therapeutic efficacy in a top-down approach yielding the best carrier system for topical applications.

1. Introduction

The excellent barrier properties of human skin restrict or prevent the dermal delivery of most drugs. Outside of direct chemical modification to the compound in question, one of the main strategies to overcome this natural barrier is the use of drug delivery systems [1,2]. Though numerous systems have been designed to this end, their reported success is limited due particle instability or problematic safety profiles [3]. In recent years, polymeric nanoparticles composed of biocompatible and biodegradable polymers have received increasing attention as a modern approach for dermal drug delivery. Thanks to their synthetic nature, one is able to precisely tailor polymeric particles with efficient, site specific and environmentally controlled drug release, which alongside the use of biocompatible polymers, can greatly improve the safety profiles of such therapies [4–8].

Dendritic core-multishell (CMS) nanocarriers have gained particular interest in the field of dermal drug delivery. They are composed of a hyperbranched polyglycerol (hPG) amine core, conjugated to a surrounding lipophilic inner shell, followed by a hydrophilic outer shell [9]. The loading of lipophilic or hydrophilic substances onto CMS nanocarriers significantly increases their delivery to viable skin layers [10–13] and skin appendages [14]. Whilst biomacromolecules cannot be loaded [15], peptide loaded CMS nanocarriers have also demonstrated enhanced skin penetration [16].

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http://dx.doi.org/10.1016/j.jconrel.2016.06.030
0168-3659/

Unlike larger, rigid nanoparticles [17], CMS nanocarriers are able to enter the viable epidermis following prolonged topical exposure or in barrier-impaired skin [12]. Whilst it is probable that penetrance of nanoparticles into the viable epidermis changes considerably when applied to inflamed or lesional skin, surprisingly few studies have investigated the use of drug loaded nanoparticles in the skin diseases for which they are intended. Reasons for this apparent lack include the limited availability of diseased human skin for experimental purposes and the popularity of animal models in the assessment of nanoparticle mediated anti-inflammatory effects [18]. Rodent skin however demonstrates major anatomical and physiological differences relative to human skin, something that skin models aim to overcome [19–21]. Reconstructed human skin has been used to model atopic dermatitis [22], ichthyosis vulgaris [23] and non-melanoma skin cancer *in vitro* [24], and to assess nanoparticle-mediated delivery [12] and therapeutic efficacies [25,26].

Besides enhancing topical bioavailability, the main challenge in the cutaneous therapy of inflammatory skin diseases is to reduce the dose and frequency of drug administration for existing therapies [27]. Treatment of chronic inflammatory skin diseases such as atopic dermatitis and psoriasis involve the use of corticosteroids, which induce strong anti-inflammatory, immunosuppressive and anti-proliferative effects. Topical administrations however, lead to local side effects including rosacea and skin infections. Most importantly, the risk of irreversible skin atrophy limits the clinical use of glucocorticoids in chronic and recurring skin diseases [28]. Hence over the years, research has focused on strategies to optimize the potency of corticosteroids while minimizing adverse effects caused by drug absorption across the skin. To this end, liposomes [29], lipid nanoparticles [30], and polymeric nanoparticles [31,32] have been proposed as potential drug carrier systems that could improve the retention of encapsulated corticosteroids within the skin, such as demonstrated in solid lipid nanoparticles [30,33].

In this context, the aim of our study was to synthesize new biodegradable ester-based CMS nanocarriers loaded with the corticosteroid dexamethasone (DXM), and to assess their delivery efficiencies, biocompatibility and anti-inflammatory effects in a model of inflammatory skin disease. Here, the amide linker between the polyglycerol core and inner alkyl shell found in amide-based CMS nanocarriers has been replaced by an ester bond. This is intended to facilitate the biodegradation of the nanocarrier whilst avoiding the formation of toxic polyglycerol amines, as seen upon degradation of amide-based CMS nanocarriers [34,35]. Hence, this rational study describing a top-down approach, started with the synthesis of three nanocarrier candidates followed by systematic *in vitro* an *in vivo* toxicological screening, to identify the best carrier system for further use. Three novel ester-based CMS nanocarriers (10-E-12-350, 10-E-15-350, 10-E-18-350) possessing inner-shell alkyl chains of varying length (C12, C15 and C18) were synthesized and fully characterized in terms of particle properties and biodegradability. To identify the most suitable nanocarriers of these, *in vitro* and *in vivo* toxicological profiles and delivery efficiencies were determined for each particle, with comparison to the conventional amide-based CMS nanocarriers (10-A-18-350). Finally, an inflammatory skin model was established to assess the anti-inflammatory effects of DXM loaded CMS nanocarriers with comparison to commercially available DXM cream.

2. Material and methods

2.1. Reagents and chemicals

Anhydrous methylene chloride and pyridine were purchased from Merck (Darmstadt, Germany), methoxy poly(ethylene glycol) (M_n = 350 g/mol, 1 equiv.; $mPEG_{350}$) from Acros Organics (Geel, Belgium) and pentadecanedioic acid from ToniChemPharma (Huizhou, China). hPG (M_n = 10,000 g/mol) was prepared analogous to the published method [36]. Analytical grade solvents and all other chemicals were purchased from Sigma Aldrich (Taufkirchen, Germany). All chemicals and reagents were used without further processing.

2.2. Preparation of ester-based CMS nanocarriers

2.2.1. Synthesis of shell components: 1-methoxy-poly(ethylene glycol)yl dodecanedioate ($mPEG_{350}$-C12) and 1-methoxy-poly(ethylene glycol)yl pentadecanedioate ($mPEG_{350}$-C15)

In a three-neck flask equipped with a gas inlet, thermometer with quick fit and septum, mixtures of $mPEG_{350}$ (0.3 mol, 1 equiv.) with dodecanedioic acid (C12, 0.9 mol, 3 equiv.) or pentadecanedioic acid (C15, 0.9 mol, 3 equiv.) were stirred under high vacuum and heated to 120 °C. The mixture was kept at this temperature for at least 1.5 h until a clear melt was obtained. The temperature was then raised to 180 °C and stirred for an additional 4.5 h. The reaction mixture was kept under vacuum and allowed to cool to 120 °C. While still a melt, the hot reaction mixture was transferred to a beaker and cooled to room temperature. The still warm waxy solid was chopped, 2000 ml of methylene chloride added, and the resulting mixture vigorously stirred until a homogeneous suspension was formed. The suspension was filtered and the filtrate concentrated to a final volume of 600 ml by rotary evaporation. The solution was kept a 5 °C for 18 h. Precipitated excess of the diacids was removed by filtration. The remaining filtrate was concentrated by rotary evaporation and subsequently dried under high vacuum to yield a colorless wax (69%) of $mPEG_{350}$-C12 or a pale yellow wax (68%) of $mPEG_{350}$-C15.

$mPEG_{350}$-C12. ^{1}H-NMR (500 MHz, MeOH-d_4, TMS): δ (ppm) = 4.22–4.18 (m, 2H, —C*H*$_2$—OCO—), 3.75–3.50 (m, 30H, mPEG backbone), 3.36 (s, 3H, —*O*—C*H*$_3$), 2.33 (t, 2H, *J* = 7.4 Hz, ROOC—*CH*$_2$—CH$_2$—), 2.28 (t, 2H, *J* = 7.4 Hz, HOOC—*CH*$_2$—CH$_2$—), 1.65–1.55 (m, 4H, —CO—CH$_2$—*CH*$_2$—), 1.37–1.20 (m, 12H, —CH$_2$—(*CH*$_2$)$_6$—CH$_2$—).

^{13}C-NMR (125 MHz, MeOH-d_4, TMS): δ (ppm) = 177.4, 175.2, 72.9, 71.7–71.3, 70.3, 70.1, 64.5, 64.3, 59.1, 59.1, 34.9, 34.9, 30.7, −30.1, 26.0, 26.0.

IR (cm^{-1}): 2923, 2856, 1732, 1456, 1349, 1247, 1099, 1040, 946, 849, 724.

GPC: M_n = 680 g/mol, M_w = 750 g/mol, D = 1.10.

$mPEG_{350}$-C15. ^{1}H-NMR (500 MHz, MeOH-d_4, TMS): δ (ppm) = 4.22–4.18 (m, 2H, —C*H*$_2$—OCO—), 3.75–3.50 (m, 30H, mPEG backbone), 3.36 (s, 3H, —O—C*H*$_3$), 2.33 (t, 2H, *J* = 7.4 Hz, ROOC—*CH*$_2$—CH$_2$—), 2.28 (t, 2H, *J* = 7.4 Hz, HOOC—C*H*$_2$—CH$_2$—), 1.65–1.55 (m, 4H, —CO—CH$_2$—*CH*$_2$—), 1.37–1.20 (m, 18H, —CH$_2$—(*CH*$_2$)$_9$—CH$_2$—).

^{13}C-NMR (125 MHz, MeOH-d_4, TMS): δ (ppm) = 177.5, 175.3, 72.9, 71.6–71.5, 71.3, 70.1, 64.5, 59.1, 34.9, 34.9, 30.7, −30.1, 26.0, 26.0.

IR (cm^{-1}): 2922, 2854, 1733, 1456, 1349, 1248, 1099, 1040, 946, 850, 723.

GPC: M_n = 680 g/mol, M_w = 735 g/mol, D = 1.08.

2.2.2. Synthesis of the shell component: 1-methoxy-poly(ethylene glycol)yl octadecanedioate ($mPEG_{350}$-C18)

Similar to a published method [9], 14.94 g (42.7 mmol) $mPEG_{350}$ (dried overnight at 70 °C under low pressure, <5 x 10^{-2} mbar) and 47.74 g (15.2 mmol) 1,18-octadecanedioic acid were added without solvent into a Schlenk-flask. The reaction mixture was heated up to 185 °C and stirred vigorously for 3 h under vacuum (5 x 10^{-2} mbar). After 3 h, the mixture was allowed to cool down to 140 °C and a reflux condenser was installed. 300 ml toluene was added and the mixture was slowly cooled to 0 °C. The resulting suspension was filtrated and the residue was washed with 300 ml of cold toluene. The filtrate and washings were combined and concentrated by rotary evaporation and the remaining solvent was removed under low pressure (<5 x 10^{-2} mbar) at 50 °C. 5 g of the crude product (19.8 g, 72% yield) were purified *via* HPLC (Gemini Phase, Phenomenex C18, 5 μm, 110 Å,