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Research paper

Preparation and characterization of dutasteride-loaded nanostructured lipid carriers coated with stearic acid-chitosan oligomer for topical delivery

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ABSTRACT

Dutasteride, used for treating benign prostate hyperplasia (BPH), promotes hair growth. To enhance delivery to the hair follicles and reduce systemic effects, in this study dutasteride has been formulated for topical application, in a nanostructured lipid carrier (NLC) coated with chitosan oligomer-stearic acid (CSO-SA). CSO-SA has been successfully synthesized, as confirmed using ^{1}H NMR and FTIR. Formulation of dutasteride-loaded nanostructured lipid carriers (DST-NLCs) was optimized using a 2^3 full factorial design. This formulation was coated with different concentrations of stearic acid-chitosan solution. Coating DST-NLCs with 5% SA-CSO increased mean size from 187.6 ± 7.0 nm to 220.1 ± 11.9 nm, and modified surface charge, with zeta potentials being −18.3 ± 0.9 mV and +25.8 ± 1.1 mV for uncoated and coated DST-NLCs respectively. Transmission electron microscopy showed all formulations comprised approximately spherical particles. DST-NLCs, coated and uncoated with CSO-SA, exhibited particle size stability over 60 days, when stored at 4–8 °C. However, NLCs coated with CSO (without conjugation) showed aggregation when stored at 4–8 °C after 30 days. The measured particle size for all formulations stored at 25 °C suggested aggregation, which was greatest for DST-NLCs coated with 10% CSO-SA and 5% CSO. All nanoparticle formulations exhibited rapid release in an *in vitro* release study, with uncoated NLCs exhibiting the fastest release rate. Using a Franz diffusion cell, no dutasteride permeated through pig ear skin after 48 h, such that it was not detected in the receptor chamber for all samples. The amount of dutasteride in the skin was significantly different ($p < 0.05$) for DST-NLCs (6.09 ± 1.09 μg/cm^2) without coating and those coated with 5% CSO-SA (2.82 ± 0.40 μg/cm^2), 10% CSO-SA (2.70 ± 0.35 μg/cm^2) and CSO (2.11 ± 0.64 μg/cm$^{2)}$. There was a significant difference ($p < 0.05$) in the cytotoxicity (IC_{50}) between dutasteride alone and in the nanoparticles. DST-NLCs coated and uncoated with CSO-SA increased the maximum non-toxic concentration by 20-fold compared to dutasteride alone. These studies indicate that a stearic acid-chitosan conjugate was successfully prepared, and modified the surface charge of DST-NLCs from negative to positive. These stable, less cytotoxic, positively-charged dutasteride-loaded nanostructured lipid carriers, with stearic acid-chitosan oligomer conjugate, are appropriate for topical delivery and have potential for promotion of hair growth.

1. Introduction

Androgenic alopecia (AGA, male-pattern baldness) affects almost 50% of men during their lives [1]. Androgen is one of the prerequisites for male-pattern baldness [2]. Normally, patients with androgenic alopecia have higher levels of dihydrotestosterone (DHT) and 5-α reductase enzyme activity on their balding scalp area than those with a non-balding scalp area [3]. In hair-loss patients, testosterone is converted to DHT which results in miniaturization of the hair follicle and hair shedding [4]. There are two types of enzymes that contribute to androgenic alopecia: type I 5α-reductase enzyme is present in the skin, including scalp, and type II is in the hair follicles and prostate [5]. Dutasteride (a type I and type II 5α-reductase inhibitor) is approved by the US Food and Drug Administration (FDA) for treating benign prostate hyperplasia (BPH), and has been studied for hair growth efficacy, with an

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oral dose of 0.5 mg daily [6]. Dutasteride taken orally will reduce DHT levels throughout the body. In order to reduce the systemic effects of lowered DHT levels, such as diminished sexual desire, increased depression and ejaculation disorder [7,8], topical administration of dutasteride for treating AGA would be an appropriate drug-delivery strategy. Little research has been conducted using dutasteride for topical application. Ansari et al. [9] prepared nanoemulsions using different ratios of oleic acid and eucalyptus oil for delivery to the skin, in the size range of approximately 20–210 nm; no information on surface charge, entrapment efficiency or drug loading was provided. Madheswaran et al. [10] used monoolein to produce liquid crystalline nanoparticles of finasteride and dutasteride, surface-modified with chitosan (low molecular weight) to give a positive charge. The mean particle size was 239–259 nm, with zeta potential +19.8 to +48.5 mV. The surface-modified nanoparticles enhanced transdermal delivery of the 5-α reductase inhibitors, increasing permeation of finasteride and dutasteride. No stability studies were undertaken. Unlike most previous studies of dutasteride employing oral administration, this work aims to achieve local delivery of dutasteride, by formulation in a nanostructured lipid carrier (NLC) system for topical delivery to the skin/hair follicles. Previous studies have determined the transfollicular delivery of materials, including drugs using nanoparticles. Lademann et al. [11] found that dye-containing nanoparticles with a mean size of 320 nm penetrated deeper into the hair follicles, when massage was applied, than the dye similarly applied in non-nanoparticulate form. Blume-Peytavi et al. [12] compared the transfollicular and percutaneous delivery of a minoxidil foam. Minoxidil was detected in the blood faster when the hair follicle orifices were opened compared to the blocked hair follicle orifices, demonstrating more rapid drug delivery and transport via the follicular route. Patzelt et al. [13] reported that the penetration depth of particles into the hair follicles was dependent on their size; medium sized particles (643 and 646 nm) penetrated deepest into porcine hair follicles, whereas smaller (122 nm, 230 nm, 300 nm and 470 nm) and larger particles (860 nm, 920 nm and 100 nm), penetrated the follicle, but to shorter depths.

Nanostructured lipid carriers (NLCs) were introduced by Muller et al. [14] subsequent to solid lipid nanoparticles (SLNs), to overcome some of the limitations associated with SLNs. NLCs can increase loading capacity, physical and chemical long-term stability and prolong release of incorporated molecules. They are also suitable for topical formulations [15]. Different types of solid lipid can be used in the production of SLNs and NLCs. The term solid lipid includes fatty acids (e.g. myristic, stearic and palmitic acid), triglyceride (e.g. tripalmitin and tristearin), diglyceride (e.g. glyceryl behenate), monoglyceride (e.g. glyceryl monostearate), steroids (e.g. cholesterol) and waxes (e.g. cetyl palmitate and beeswax). Different types of solid lipid have different degrees of crystallization that may impact drug entrapment and loading, size and charge, and also efficacy. The lipid particle matrix is solid at both ambient and body temperatures [16]. In this study, stearic acid has been chosen as a solid lipid due to its potential for promoting hair growth [17]. Previous research [18] has demonstrated that free fatty acids (α-linolenic, linoleic, palmitic, elaidic, oleic and stearic acid) are potent 5α-reductase inhibitors and promote hair regrowth. Inclusion of stearic acid in the formulation, might have an additional effect with dutasteride for the promotion of hair growth. Due to the properties of dutasteride, which is poorly water soluble (0.038 ng/mL; Log P = 5.09) [19], NLCs would seem useful as a potential carrier. NLCs composed of biocompatible and biodegradable lipids have low toxicity and cytotoxicity, are suitable for topical application and have an occlusive effect on the skin, increasing skin hydration [20]. Mittal et al. [21] reported that NLCs with a size of approximately 300 nm were found in the transfollicular region of the skin. Hamishehkar et al. [22] found higher skin deposition of flutamide-loaded SLNs (volume mean diameter approximately 200 nm) compared to flutamide hydroalcoholic. This higher accumulation of flutamide-SLNs in the hair follicles suggested a greater growth of new hair follicles based on the histological assessment.

Chitosan, a naturally occurring polysaccharide, contains free amino groups, is cationic in neutral or basic pH conditions, and is commonly used for drug delivery applications, particularly where a positive charge is advantageous. Hair is negatively charged [23]; consequently, introducing a positive charge to nanoparticles may enhance targeting. It is also reported that due to the bioadhesive properties of chitosan, it may increase retention of the drug/-carrier in the targeted area [24]. Taking this into account, DST-NLCs coated with a positively-charged, chitosan-based polymer have been investigated here for topical/transfollicular delivery.

In the present study, stearic acid was selected for use as a lipophilic ingredient and synthesized with chitosan to enhance the lipophilicity of the aqueous-soluble chitosan. The carboxylic group of stearic acid was reacted with the amine group from chitosan with addition of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl), creating the hydrophobic drug-delivery moiety.

Therefore, it is suggested that when dutasteride is incorporated in the nanostructured lipid carrier (NLC), the drug will be entrapped in the hydrophobic region where surfactants are added to stabilize it. A solution of stearic acid-chitosan (CSO-SA) having a positive charge will coat the nanoparticles.

The aim of this study is then to design and formulate a stable and less cytotoxic formulation of DST-NLCs coated with CSO-SA for the promotion of hair growth. This study anticipates that surface modification of DST-NLCs with CSO-SA would produce a slow release of the drugs in the skin/hair follicles with potential to reduce systemic effects.

2. Materials and methods

2.1. Materials

Stearic acid was purchased from Tokyo Chemical Industry (UK). Chitosan oligomer (CSO), Mwt < 3000 Da and dutasteride (purity > 98.0%) were obtained from Carbosynth (UK). Ethanol (96% v/v analytical grade), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl), ninhydrin, Sephadex G-50 and acetic acid-d_4 (99.9 atom %D) were obtained from Sigma-Aldrich (UK). Deuterium oxide (99.9 atom %D) was purchased from Cambridge Isotope Lab. Inc. (USA), Phosal® 53 MCT and Lutrol® micro 68 were supplied by Lipoid GmbH (Germany) and BASF Group (Ludwigshafen, Germany), respectively. Acetone, water (HPLC grade) and acetic acid glacial (analytical reagent) were purchased from Fisher Scientific (United Kingdom). Deionized water was prepared in-house (PURELAB, ELGA, UK).

2.2. Synthesis of stearic acid-chitosan (CSO-SA)

Chitosan oligomer (CSO) was conjugated by the reaction of the carboxyl groups of stearic acid in the presence of EDC.HCl with the free amino groups of chitosan oligomer (Fig. 1), using an established method with minor modifications [25,26]. CSO (1 g) was dissolved in 120 mL of deionized water with magnetic stirring for 2 h at 80 °C. Meanwhile, 0.5 g stearic acid (SA) was dissolved in ethanol (80 mL) and heated at 60 °C, with stirring. SA solutions were activated by EDC.HCl (1:5 M ratio) and stirred for 2 h at 60 °C. The solutions of SA were then added using a syringe with needle (BD Micro Lance™ 3, Spain) into the CSO solution with continuous stirring for 6 h.