International Journal of Pharmaceutics 516 (2017) 196–203

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Polymeric nanospheres for topical delivery of vitamin D3

Tannaz Ramezanli[a,b], Brian E. Kilfoyle[a,c], Zheng Zhang[c,d], Bozena B. Michniak-Kohn[a,b,*]

[a] Ernest Mario School of Pharmacy, Rutgers—The State University of New Jersey, 160 Frelinghuysen Road, Piscataway, NJ 08854, USA
[b] Center for Dermal Research, Rutgers—The State University of New Jersey, 145 Bevier Rd, Piscataway, NJ 08854, USA
[c] The New Jersey Center for Biomaterials, Rutgers—The State University of New Jersey, 145 Bevier Rd, Piscataway, NJ 08854, USA
[d] Mosaic Biosciences, 3415 Colorado Avenue, Boulder, CO 80309, USA

ARTICLE INFO

Article history:
Received 20 June 2016
Received in revised form 27 October 2016
Accepted 29 October 2016
Available online 31 October 2016

Keywords:
Vitamin D3
TyroSpheres
Nanoparticles
Skin permeation
Photostability

ABSTRACT

This study investigates the potential application of polymeric nanospheres (known as TyroSpheres) as a formulation carrier for topical delivery of cholecalciferol (*i.e.*, Vitamin D3, VD3) with the goal to improve the skin delivery and stability of VD3. High drug loading and binding efficiencies were obtained for VD3 when loaded in TyroSpheres. VD3 was released from TyroSpheres in a sustained manner and was delivered across the stratum corneum, which occurred independent of the initial drug loading. An *ex vivo* skin distribution study showed that TyroSphere formulations delivered 3–10 μg of active into the epidermis which was significantly higher than that delivered from Transcutol® (the control vehicle). In addition, an *in vitro* cytotoxicity assay using keratinocytes confirmed that VD3 encapsulation in the nanoparticles did not alter the drug activity. Photodegradation of VD3 followed zero-order kinetics. TyroSpheres were able to protect the active against hydrolysis and photodegradation, significantly enhancing the stability of VD3 in the topical formulation.

1. Introduction

Vitamin D3 (VD3) or cholecalciferol is a steroid hormone generated in the skin by UVB radiation of 7-dehydrocholesterol or it is obtained from dietary sources. The active form of cholecalciferol, 1,25-dihydroxycholecalciferol (calcitriol) plays an important role in the regulation of calcium homeostasis and mineralization of bones (Picciano, 2010). VD3 and its analogues have also been associated with other functions in the body. For example, they can influence keratinocyte differentiation and are therefore used in treatment of several skin disorders including psoriasis (Barker et al., 1999). Vitamin D3 and its analogues are also involved in the control of multiple intracellular pathways responsible for the melanin synthesis and melanocyte survival. They can control the activation, proliferation, and migration of melanocytes and therefore induce skin pigmentation, which is potentially useful in treatment of vitiligo (Birlea et al., 2009). Maxacalcitol, one of the active analogues of VD3, has proven to be effective in treatment of comedones (Hayhoe et al., 2010; Nieves et al., 2010) and there are a few studies reporting antineoplastic activity of calcitriol (Krishnan and Feldman, 2011). The anti-psoriatic properties of VD3 analogues are suggested from their effects in decreasing proliferation and promoting differentiation of keratinocytes, as well as immunomodulatory actions (Nagpal et al., 2005). Even though the exact mechanism behind these effects is not completely understood, it is well-known that VD3 anti-psoriatic effects are partially genomic and mediated via the vitamin D receptor (Lehmann, 2005). As far back as the 1930s, VD3 was used as an oral therapy for psoriasis but eventually fell out of favor due to hypercalcemic side effects. After the discovery of VD3 receptors on keratinocytes and fibroblasts, interest in topical therapy with VD3 analogues resurfaced (DiSepio et al., 1999). Human keratinocytes have an autonomous VD3 pathway and can convert VD3 to its hormonally active form, calcitriol (Lehmann, 2005).

Skin, despite its strong barrier properties provides several advantages as a route of delivery. Dermal drug delivery via topical application can localize a high concentration of active in the upper skin layers – which is the site of action for many drugs that treat dermatological disorders – and also minimize systemic exposure of the drug. Successful drug delivery depends on the physicochemical characteristics of the active and its carriers in the formulation. One of the approaches for formulating unstable and highly lipophilic compounds is by using particulate systems. Many studies have been focused on using nanoparticles for enhanced topical delivery of small and large molecules. A broad spectrum of particles (including liposomes, solid lipid nanoparticles (Jenning et al., 2000), polymeric

Abbreviations: SC, stratum corneum; VD3, vitamin D3; HPLC, High Performance Liquid Chromatography; DLS, dynamic light scattering.

* Corresponding author at: 145 Bevier Rd, Piscataway, NJ 08854, USA.
E-mail addresses: Michniak@dls.rutgers.edu, bomichniak@gmail.com (B.B. Michniak-Kohn).

http://dx.doi.org/10.1016/j.ijpharm.2016.10.072
0378-5173/

micelles (Kilfoyle et al., 2012), microemulsions (Bhatia et al., 2013), and liquid crystalline nanoparticles (Madheswaran et al., 2013; Angelova et al., 2011) have been studied for delivery of drugs and cosmetic actives to the skin. These delivery systems may be capable of providing enhanced solubility of the active, chemical and/or physical protection of the active, sustained and controlled release of the drug, and eventual enhancement of the biological absorption of the drug (Zhang et al., 2013).

A unique class of polymeric nanospheres, made of a family of biocompatible, amphiphilic tyrosine-derived ABA-triblock copolymers has been developed and characterized at The New Jersey Center for Biomaterials, Rutgers-The State University of New Jersey (Bourke and Kohn, 2003; Sheihet et al., 2007). The chemical structure of these copolymers is composed of hydrophobic B-block *i.e.*, oligomers of desaminotyrosyl-tyrosine ester (DTR) and diacid (Choueka et al., 1996) and hydrophilic poly(ethylene glycol) (PEG) A-blocks. These PEG-*b*-oligo(DTR-XA)-*b*-PEG triblock copolymers undergo self-assembly in an aqueous environment to form polymeric micelles referred to as TyroSpheres. They have very low critical aggregation concentration of 2.6×10^{-7} g/mL and are capable of encapsulating hydrophobic compounds (Sheihet et al., 2005). Previous studies have demonstrated that TyroSpheres are not cytotoxic and drug encapsulated within these nanoparticles does not lose its activity (Nardin et al., 2004). Evaluation of this family of polymers has shown that the triblock polymer PEG_{5K}-*b*-oligo(desaminotyrosyl-tyrosine octyl ester suberate)-b-PEG_{5K} (DTO-SA/5K) is optimal for the encapsulation of lipophilic drugs (see graphical abstract). The drug-loaded TyroSpheres showed reproducible hydrodynamic diameter of approximately 70 nm and low polydispersity index (0.17), making this polymer the lead candidate evaluated for nanosphere applications (Sheihet et al., 2005). TyroSpheres have been previously evaluated for topical delivery of lipophilic drug/dye molecules (Nile Red (Batheja et al., 2011 #159), paclitaxel (Kilfoyle et al., 2012), cyclosporine A (Goyal et al., 2015), and adapalene (Ramezanli et al., 2016)) to treat dermatological diseases such as psoriasis and acne. Additionally, theses nanocarriers were able to localize in the hair follicles and release the drug in the pilosebaceous unit (Ramezanli et al., 2016).

VD3 is very hydrophobic (log P = 9) and sensitive to many environmental factors (*e.g.* moisture, heat and light), which can induce isomerization or oxidation of its structure and adversely affecting its bioactivity (Ballard et al., 2007). Although the encapsulation of lipophilic compounds in nano-sized carriers has received significant attention in both the food and the pharmaceutical industry, few reports in literature are available for Vitamin D analogues (Guttoff et al., 2015; Almouazen et al., 2013). Preparation of some of these carrier systems involves high temperature (Delaurent et al., 1998) (which would induce inactivation of VD) or toxic solvents *e.g.* tetrachloride or petroleum ether (Shi and Tan, 2002). In this study, we propose to use TyroSpheres for the topical delivery of VD3. VD3-TyroSpheres were fabricated and characterized for their size, binding and loading efficiencies, stability, drug release and permeation to human skin. To the best of our knowledge there is no published study on topical delivery of VD3 using nanocarriers. This work aims to address the need of a suitable carrier system for enhancing dermal delivery of VD3 and improving its stability in the formulation.

2. Materials and methods

2.1. Materials

Suberic acid (SA), poly(ethylene glycol) monomethyl ether MW 5000 (PEG5K), Tween-80, Dimethylformamide (DMF), and Dulbecco's Phosphate Buffered Saline (PBS), Cholecalciferol, and diethylene glycol monoethyl (Transcutol®) were purchased from Sigma Aldrich (St. Louis, MO). Dulbecco's Modified Eagle Medium, high glucose (DMEM), trypsin (0.25% Trypsin-EDTA), Penicillin Streptomycin (10,000 U/mL), fetal bovine serum (FBS), and Dulbecco's Phosphate Buffered Saline without calcium chloride and magnesium chloride (DPBS) were purchased from Life Technologies (Grand Island, NY). HPLC grade water, acetonitrile, and methanol were obtained from Fisher Scientific (Pittsburgh, PA). HaCaT cells were received as a generous gift from Dr. Qing Ren (Department of Radiation Oncology at Thomas Jefferson University). AlamarBlue® Reagent was obtained from AbD Serotec (Raleigh, NC). PEG_{5K}-*b*-oligo(desaminotyrosyl-tyrosine octyl ester suberate)-*b*-PEG_{5K} (M_n = 22.9 kDa, Mw = 31.9 kDa, molecular weight distribution (MWD) = 1.39 obtained from gel permeation chromatography) was synthesized according to previously published and established procedures at the New Jersey Center for Biomaterials, Rutgers-The State University of New Jersey. Their chemical structure and purity were confirmed by ^{1}H NMR (Sheihet et al., 2005, 2007). All reagents were used as received.

2.2. Preparation of VD3 loaded-TyroSphere formulations

ABA triblock copolymers composed of hydrophilic A blocks of poly(ethylene glycol) and hydrophobic B blocks of desaminotyrosyl-tyrosine octyl ester and suberic acid were used for preparation of TyroSpheres. Drug loaded-TyroSphere dispersion was prepared and purified by methods described previously (Batheja et al., 2011; Sheihet et al., 2007). The final VD3-TyroSphere formulation was obtained by redispersing the pellet formed from ultracentrifugation and filtering through 0.22 μm PVDF syringe filters (Merck Millipore). The formulations were protected from light.

2.3. VD3- TyroSpheres characterization

2.3.1. VD3 High Performance Liquid Chromatography (HPLC) assay method

An Agilent 1100 high-performance liquid chromatography (HPLC) system (Agilent Technologies, USA) equipped with a UV/Vis detector and a C18 column (Waters XBridge 3.5 μm particle size, 4.6 × 50 mm) was used for chromatographic separations at 25 °C. A mixture of acetonitrile:water (isocratic A:B, 98:2) was applied as the mobile phase at a flow rate of 1 mL/min. The injection volume was 20 μL and the detection wavelength was set at 265 nm. Standard calibration curves were prepared at drug concentrations ranging from 0.1 to 100 μg/ml in both methanol and DMF. A full method validation was performed including variability, specificity, linearity, robustness, limit of detection, and limit of quantification.

2.3.2. VD3 solubility

The solubility of VD3 in PBS media with or without surfactant Tween 80 (0.1–10% w/w) was determined and compared to the VD3-TyroSphere formulation. Super-saturated solutions of VD3 were prepared by adding excess amount of drug to each medium. Samples were vortexed and placed in a shaker water bath (100 rpm) at 37 °C for 24 h. The samples were then centrifuged and filtered through 0.45 μm PVDF filters (Whatman, Clifton, NJ), lyophilized, and re-dissolved in methanol. Drug concentrations in each solution were determined by HPLC technique. The solutions were prepared and stored in amber vials to protect the active against photodegradation.

2.3.3. Encapsulation and loading efficiency

VD3 concentration in the final purified nano-dispersion was measured by extracting VD3 from lyophilized aliquots of the VD3-TyroSphere formulation in methanol, followed by filtrating and running on HPLC (as described above). Binding efficiency and loading efficiency were calculated from the weights of VD3-