Colloids and Surfaces B: Biointerfaces 121 (2014) 222–229

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Adapalene loaded solid lipid nanoparticles gel: An effective approach for acne treatment

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ARTICLE INFO

Article history:
Received 12 January 2014
Received in revised form 27 May 2014
Accepted 29 May 2014
Available online 6 June 2014

Keywords:
Adapalene
Solid lipid nanoparticles (SLNs)
Acne
Epidermal targeting
Rheology
Topical delivery

ABSTRACT

Salient features such as controlled release, target ability, potential of penetration, improved physical stability, low cost compared to phospholipids, and ease of scaling-up makes solid lipid nanoparticles (SLNs) a viable alternative to liposomes for effective drug delivery. Adapalene (ADA) is a second generation retinoid effective in treating various dermatologic disorders such as *Acne vulgaris* with a few noticeable dose-mediated side effects. The present study was aimed at developing and characterizing ADA loaded SLNs for effective topical delivery. The formulated SLN system was characterized for particle size, poly dispersity index, entrapment efficiency and drug release properties. The resultant formulation (ADA loaded SLNs incorporated into carbopol hydrogel) was evaluated for *in vitro* drug release, skin permeation and bio-distribution, rheological behaviour, and texture profile analysis. The SLNs based ADA gel has shown its potential in targeting skin epidermal layer, and reducing systemic penetration. The developed system can avoid systemic uptake of ADA in skin layers, and can localize drug in skin epidermis as confirmed by rat skin model. Our results advocate potential of SLNs as a novel carrier for topical delivery of ADA in topical therapeutic approaches. This study open new avenues for drug delivery which better meets the need of anti-acne research.

1. Introduction

Acne vulgaris (AV) is the most common dermatological disorder rarely posing a serious threat, but affecting overall performance millions of individuals [1]. AV is usually associated with inflammation of pilosebaceous units caused by the gram-positive organism, *Propionibacterium acnes* on mainly face skin, neck, chest and upper back [2,3]. The microenvironment of sebaceous follicles undergoes selective changes that leads plugging of pilosebaceous follicles and development of micro-comedo resulting in to acne lesions, including non-inflammatory as well as inflammatory nodules [4]. There are effective treatments available such as topical and oral antibiotics, topical and oral retinoids. The retinitis is one of the regularly prescribed classes of medicine. The topical treatment is the first choice in mild and moderate acne, whereas systemic therapy is applied to treat severe and moderate cases [5]. The topical treatment of mild to moderate acne with all trans retinoic acid (RA) has been effective in acne therapeutic [6].

Retinoids, natural or synthetic derivatives of vitamin A, due to their ability to modify abnormal follicular keratinization are highly effective in *Acne vulgaris* therapeutics [4]. The topical application of RA follows high incidences of skin irritation, photosensitivity, and low patient compliance. The systemic therapy with antibiotics has its own disadvantages such as nausea, vomiting, and contraceptive failure in pregnant women [7]. The administration of a drug *via* topical route is a better option than systemic route using novel drug delivery systems, and present potential to reduce side effects without having an effect on drug efficacy [8].

Solid lipid nanoparticles (SLNs) as novel nano-particulate carrier systems have drawn considerable attention due to improved delivery and stability of drugs. SLNs consist of biocompatible lipid core and an amphiphilic surfactant at the outer shell [9]. They have

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http://dx.doi.org/10.1016/j.colsurfb.2014.05.041
0927-7765/

shown advantages over fat emulsions, polymeric nanoparticles and liposomes. They circumvent limitation observed with other carriers, and safeguarding drug to a greater extent against chemical degradation compared to that seen with liposomes [10–12]. Moreover, these systems may be industrialized because of their virtue of no or minimal requirement of organic solvents [13].

Adapalene (ADA), 6-[3-(1-adamantyl)-4-methoxy-phenyl] naphthalene-2-carboxylic acid, is a topical anti-acne agent with a few clinical effects similar to tretinoin as well as iso-tretinoin. However, ADA have shown better acceptability than retinoids [14], and thus considered an appropriate first-line therapy for all cases of acne with a few exceptions [15]. The role of ADA played in reducing sebum production by sebaceous glands remains to be demonstrated [16]. One day treatment therapy by ADA reverses uncharacteristic follicular conditions caused by the comedone formation and cutaneous inflammatory reactions involved in pathogenesis of acne [14,17]. It has been previously reported that ADA selectively binds to RAR subtypes b and g [17] and forms ADA-RAR complex. The retinoids perform their biological functions by an interacting with specific nuclear retinoic acid (RAR) and retinoid X (RXR) receptors [18]. Eventually, ADA-RAR complex binds to RXR, and ADA/RAR/RXR mediating regulation of transcription [17].

The current study was designed to develop and explore delivery potential of SLNs based hydrogel for targeted and sustained ADA delivery to affected sites. The SLN loaded ADA system was formulated and characterized for their size, entrapment efficiency and surface charge distribution. The characterized SLNs were incorporated into 1% Carbopol® 934 gel and formulations were investigated for *in vitro* drug release, stability study, and *in vitro* permeation and biodistribution into different layers of skin. In brief, our results validate the suitability of the delivery vehicle and set platform to establishing an effective treatment for acne.

2. Materials and methods

2.1. Materials

The ADA was a generous gift from Glenmark pharmaceuticals Ltd. (Nasik, India). Hydrogenated soya phosphatidylcholine (HSPC) was a kind gift from Lipoid, Ludwigshafen, Germany. Tristearin, Triton X-100 was procured from Sigma Aldrich (Germany). Cellulose dialysis bag (MWCO 10 kDa) and G-50 Sephadex were purchased from Himedia (Mumbai, India). Nylon membrane filter (0.22 and 0.45 μm) was acquired from Pall Gelman Sciences (USA). The deionised and filtered water was used all over the study.

2.2. Fabrication of ADA loaded solid lipid nanoparticles (SLNs-A)

The SLN was prepared by the solvent injection method as reported by elsewhere [19] with slight modifications. In brief, the tristearin (1%, w/v), soya lecithin (PC; 0.3%, w/v) and ADA (0.1%, w/v) were dissolved in 10 ml acetone and ethanol mixture (1:1, v/v), while temperature was maintained 70 °C on a water bath with continuous stirring. The heated lipid phase was added into aqueous phase (with 0.2% (w/v) Tween 80) drop by drop using a syringe at a constant flow rate of 5 ml/min at said temperature with stirring. The dispersion was stirred by mechanical stirrer (Remi Instrument, Mumbai, India) at 4000 rpm for 1 h followed by sonication for 1 min by using probe sonicator (Lark innovative technology, Chennai) to generate nano-size suspension. The unentrapped or free drug was removed by cellulose dialysis bag (MWCO 10 kDa) and resulting dispersion was filtered through membrane filter (0.45 μm) to remove excess lipid. The suspension was subjected to FTIR spectroscopic studies by KBr pellet method after adsorption of small amount of suspension on KBr pellet using an IR spectroscope (Perkin-Elmer, USA). The separated SLNs-A suspension was lyophilized (VirTis AdVantage) and stored.

2.3. Drug content determination

The SLNs-dispersion was filled into the cellulose dialysis bag (MWCO 10 kDa), and was extensively dialyzed with magnetic stirring (50 rpm) against double distilled water (DDW) under sink conditions for 10 min to remove un-entrapped drug from formulation. The samples were collected in HPLC vials and diluted with the solvent (methanol and dimethyl formamide). The ADA was estimated by HPLC method as reported earlier [20] with minor modifications. Briefly, HPLC analysis was isocratically performed using Merck RP-8 column (250 mm × 4.6 mm i.d., particle size 5 μm) and acetonitrile–water (65:35, v/v; the pH was adjusted to 2.5 with ortho-phosphoric acid) as the mobile phase (flow rate, 1.3 ml/min) and previously degassed by bath sonicator for 15 min. The injectable volume was 20 μL for all solutions, and detection wavelength was set at 321 nm [20]. The entrapment efficiency (EE) was calculated according to the following equation:

$$\text{Entrapment Efficiency}\,(\%) = \frac{\text{Total amount of drug added} - \text{Amount of drug in collected sample}}{\text{Total amount of drug added}} \times 100$$

2.4. Fabrication of SLNs-A gel

SLNs-A dispersion was incorporated into concentrated Carbopol® 934 gel base so that the final concentration of Carbopol® 934 remained 1% (w/v) and gel was assented to hydrate for 24 h. The resulting mixture was stirred for 3–5 h at room temperature with magnetic stirrer followed by neutralization with Tri-ethanolamine to obtain an adequate semisolid carbopol gel matrix at pH 6.0. The carbopol gel was appropriately viscous when neutralized to adjust pH 6.0 [21].

2.5. Characterization of SLNs-A and SLNs-A gel

2.5.1. Particle size

The average particle size and polydispersity index of SLNs-A were determined by photon correlation spectroscopy using zetasizer (PCS, Nano ZS90 zetasizer, Malvern Instruments Corp, UK). The sample was diluted with filtered deionized water in polystyrene cuvettes and was observed at a fixed angle of 90° at 25 ± 0.1 °C.

2.5.2. Zeta potential

The zeta potential of the SLNs-A was determined in folded capillary cells by laser Doppler anemometry using Malvern zetasizer which is also called as Doppler-Electrophoretic Light Scatter Analyzer. The zeta potential was measured on samples well-dispersed in deionised water at temperature, 25 ± 0.1 °C and electric field, 15.24 V/cm.

2.5.3. Transmission electron microscopy (TEM)

The SLNs-A was characterized for size and morphology by TEM using a Philips CM 10 electron microscope with an accelerating voltage of 3 kV (Morgani, 268D; Holland). A drop of sample was placed on a carbon coated copper grid to leave a thin film on the grid. Sample was negatively stained with 1% phosphotungustic acid