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Spironolactone loaded nanostructured lipid carrier gel for effective treatment of mild and moderate acne vulgaris: A randomized, double-blind, prospective trial

Hamid Reza Kelidari[a], Majid Saeedi[b,**], Zohreh Hajheydari[c], Jafar Akbari[b], Katayoun Morteza-Semnani[b], Javad Akhtari[d], Hadi Valizadeh[e], Kofi Asare-Addo[f], Ali Nokhodchi[e,g,*]

[a] Pharmaceutical Sciences Research Center, Mazandaran University of Medical Sciences, Sari, Iran
[b] Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran
[c] Department of Dermatology, Boo Ali Sina (Avicenna) Hospital, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[d] Immunogenetics Research Center, Department of Physiology and Pharmacology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[e] Drug Applied Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
[f] Pharmacy Department, University of Huddersfield, Huddersfield, HD1 3DH, UK
[g] Pharmaceutics Research Laboratory, School of Life Sciences, University of Sussex, Brighton BN1 9QJ, UK

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ABSTRACT

Spironolactone (SP) known as an anti-androgen drug, has been proven to be effective in treatment of acne. The quest to minimize the unnecessary systemic side effects associated with the oral drug administration of spironolactone, has led to a growing interest of loading SP on lipid nanoparticles to deliver the drug in a topical formulation. The aim of the current investigation was to prepare and compare the performance of SP loaded nanostructured lipid carrier (SP-NLC) and SP alcoholic gels (SP-ALC) on two groups of respective patient populations, group A and group B in the treatment of mild to moderate acne vulgaris. The results showed that SP-NLCs were spherical in shape with an average diameter of ~240 nm. The polydispersity index (PI) and zeta potential of these nanoparticles were 0.286 and −21.4 respectively. The gels showed non-Newtonian independent pseudoplastic and shear thinning behavior. The SP-NLCs was not toxic to fibroblast cell strains at the 24 and 48 h periods. Results showed that the mean number of total lesions (37.66 ± 9.27) and non-inflammatory lesions (29.26 ± 7.99) in group A significantly decreased to 20.31 ± 6.58 ($p < 0.05$) and to 13.95 ± 5.22 ($p < 0.05$) respectively. A similar pattern was observed for group B where the mean number of total lesions and non-inflammatory lesions reduced from 33.73 ± 9.40 to 19.13 ± 5.53 ($p < 0.05$) and from 25.65 ± 8.12 to 13.45 ± 4.48 ($p < 0.05$) respectively. The total lesion count (TLC) was significantly decreased from 37.16 ± 9.28 to 19.63 ± 6.36 (for group A; $p < 0.071$) and 32.60 ± 9.32 to 18.33 ± 5.55 (for group B; $p < 0.05$) respectively. After treatment with SP-NLC for 8 weeks, the water content of the skin significantly ($p < 0.05$) increased from 37.44 ± 8.85 to 45.69 ± 19.34 instrumental units. Therefore, the SP-NLC gel may help in controlling acne vulgaris with skin care benefits.

1. Introduction

Acne vulgaris is a disease of the pilosebaceous glands that usually occurs in adolescence following a sharp increase in androgen [1]. Spironolactone (SP) is classified as a BCS class II drug (high permeability and poor solubility). The BCS is a tool which is used to differentiate the drugs on the basis of their solubility and permeability as follows: Class I covers drugs with high permeability and solubility; Class II covers drugs with high permeability but poor solubility; Class III drugs have low permeability but high solubility and Class IV drugs have low permeability and solubility. As an anti-androgen drug, it has proved effective in reducing the sebum secretion rate in various clinical reports [2,3]. However, following oral administration, SP is poorly absorbed from the gastrointestinal (GI) tract and observed endocrine side effects restricted its clin-

* Corresponding author at: Pharmaceutics Research Laboratory, School of Life Sciences, University of Sussex, Brighton.
** Corresponding author at: Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.
E-mail addresses: majsaeedi@gmail.com (M. Saeedi), a.nokhodchi@sussex.ac.uk (A. Nokhodchi).

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ical application due to its variable oral bioavailability [4]. It has been shown that the topical delivery of SP can allow high drug levels at the site of action which in turn can lessen the systemic side effects and also improve patient compliance [5,6]. Nanostructured lipid carriers (NLCs) represent a relatively new type of colloidal drug delivery system that consists of solid-lipid and liquid-lipid, and offers the advantage of improved drug loading capacity and release properties. These nanoparticles contain non-irritative and non-toxic lipids and as such well suited for use on inflamed and damaged skin [7]. The small particle size of these nanoparticles ensures close contact with the stratum corneum and also increases the amount of encapsulated compounds penetrating into the skin due to the formation of an intact film on the skin surface upon drying. This lipid nanocarrier have been used to improve the skin/dermal uptake of several drugs such as cyproterone acetate [8], tretinoin [9], isotretinoin [10] and adapalene [11] which supports the notion that these nanocarriers can be employed for the topical delivery of SP. Clinical studies with alcoholic topical formulation of SP have been previously reported and the results demonstrate beneficial effects in patients with acne without any systemic hormonal changes [3,12,13]. Shamma and Aburahma used spironolactone loaded NLC for follicular targeting of drug molecules for the management of alopecia and they successfully showed the presence of SP in the scalp hair follicles and decreasing androgen production within sebaceous glands and blocking the androgen receptor in dermal papillae [14]. The efficacy of SP gel 5% in the treatment of facial acne also showed that total lesion count and acne severity index reduced significantly however, efficacy on non-inflammatory lesion (comedones) was more effective than on inflammatory ones (papules and pustules) because of poor ability of SP penetration to specific micro environmental conditions in the inflammatory acne lesions [13]. The aim of this current research was to develop novel NLC formulations in gel containing SP with a suitable rheology (spreadability), pH and better efficacy and tolerability (SP-NLC; 1%) versus alcoholic SP gel (SP-ALC; 5%) in the treatment of facial mild to moderate acne vulgaris.

2. Material and methods

2.1. Materials

Spironolactone (SP) was supplied by Behdashtkar Co. (Tehran, Iran). Stearic acid (SA), Oleic acid (OA), Tween 80, Span 80, hydroxyethyl cellulose, propylene glycol, Methyl paraben and triethanolamine were purchased from Merck Co. (Germany). Carbopol 934 P was obtained from BF Goodrich (Cleveland, Ohio, USA). Carbopol gels are approved for pharmaceutical use in several different administration routes. The cutaneous use of these gels is advantageous as they possess good rheological properties resulting in long residue times at the site of administration [15,16], therefore Carbopol was selected as the gelling agent in the present study. Deionized water was purified using a Milli-Q system (Millipore, Direct-Q). Dimethyl sulfoxide (DMSO, solvent) was purchased from Merck and Tetrazolium salt (MTT) was supplied from Sigma-Aldrich. Human Caucasian foetal foreskin fibroblast (HFFF2) was purchased from the National Cell Bank of Iran (Pasteur Institute, Tehran, Iran).

2.2. Preparation of the formulations

SP loaded NLC was prepared using the probe-ultrasonication method as described elsewhere [17]. Briefly, solid lipid (stearic acid 2.8 g) in combination with liquid lipid (oleic acid 1.2 g), lipophilic surfactant (Span 80 2.5 g) and SP (1 g), were melted at 85 °C using a hot plate. The hot lipid phase produced was dispersed in a 1/3 (28.77 g) of the aqueous solution of hydrophilic surfactant (Tween 80) prepared by weighing out 1.67 g Tween 80 heated at the same temperature and sonicated by using a probe sonicator (Bandelin sonopuls, Berlin, Germany) for 5 min (Model HD 3200, Prob TT25, 50% power and 19.82 KJ) to form a coarse pre-emulsion. At the end of the sonication, the mixture was dispersed into the remaining 2/3 of the hydrophilic surfactant solution (containing 3.33 g Tween 80) maintained in an ice bath. The final mixture was sonicated again for 10 min (50% power and 51.77 KJ) whilst still immersed in the ice-bath. This cooling step promoted the formation of the lipid nanoparticles. SP-NLC dispersion was incorporated into 1% w/v Carbopol gel containing 0.2 g methyl paraben as preservative. The obtained gel was allowed to hydrate for 24 h. The resulting mixture was then stirred followed by neutralization with tri-ethanolamine (approximately 10 drops) to obtain an adequate semisolid carbopol gel matrix (for a full composition of each formulation refer to Table 1S in the Supplementary material).

Alcoholic SP (SP-ALC) was composed of SP, hydroxyethyl cellulose, propylene glycol and methyl paraben in a base of deionized water. Briefly, 5 g of SP was dissolved in hydroxyethyl cellulose (5 g) and propylene glycol (10 g) for 10 min under stirring (300 rpm). 78.8 g of water was then added to the solution under stirring (300 rpm) for an additional 5 min stirring. This was followed by the addition of the methyl paraben (0.2 g) and carbopol (1 g). This solution was then stirred for a further 10 min. The final solution was then neutralized by triethanolamine (about 10 drops) under stirring condition (500 rpm) to obtain the SP-ALC gel.

2.3. Characterization of the gels

In order to determine the shape of SP-NLC, the transmission electron microscopy (TEM, CM 30, Phillips, Netherlands) was utilized. Briefly, the SLN samples were first diluted two times with distilled water. One drop of the diluted sample was placed on a 200-mesh carbon-coated copper grid, stained with 2% phosphotungstic acid solution and dried at room temperature. Representative images of each sample were reported.

Photon correlation spectroscopy (PCS) with a Malvern zetasizer ZS (Malvern Instruments, UK) was used to determine the particle size, profile the size distribution (polydispersity index, PI) and zeta potential of the nanoparticles. Briefly, the Zeta potential and the poly dispersity index (PDI) of the nanoparticle formulations were determined using the Zetasizer (Nano ZA, Malvern Instruments, UK). In this method the sample was measured at 25 °C with an angle detection of 90°. The concentration of the samples for analysis on the Zeta Sizer was 20–400 kilo counts per second (KCPS) and the intensity of diffraction was 100000 counts per second.

For spreadability 500 mg of the formulated gel was placed within a circle of 1 cm diameter pre-marked on a flat glass plate over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for 5 min. The increase in the diameter due to spreading of the formulated gel was noted. Finally, 1 g of the formulated gel was dissolved in 100 ml of distilled water and stored for 2 h. The pH of aqueous dispersion of the formulated gel was determined using Jenway Digital pH meter Model 3510, standardized using pH 4.0 and 7.0 standard buffers before use. The rheology of the SP-NLC and SP-ALC gels was obtained with a Brookfield Viscometer (Model DV-II+, Brookfield Engineering Laboratories, Inc., USA) using spindle TD. Viscosity was measured by increasing the shear rate from 0.5 rpm to 100 rpm at 25 ± 1 °C.

2.4. Cytotoxicity assays

2.4.1. Cell lines and culture

Human Caucasian foetal foreskin fibroblast (HFFF2) was cultured at 37 °C in a 5% CO_2/95% air humidified atmosphere in a