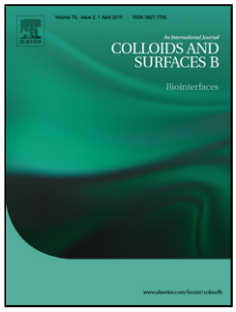


Title: Spironolactone loaded nanostructured lipid carrier gel for effective treatment of mild and moderate acne vulgaris: A randomized, double-blind, prospective trial

Author: Hamid Reza Kelidari Majid Saeedi Zohreh Hajheydari Jafar Akbari Katayoun Morteza-Semnani Javad Akhtari Hadi Valizadeh Kofi Asare-Addo Ali Nokhodchi

PII: S0927-7765(16)30367-8
DOI: http://dx.doi.org/doi:10.1016/j.colsurfb.2016.05.042
Reference: COLSUB 7891

To appear in: *Colloids and Surfaces B: Biointerfaces*

Received date: 5-2-2016
Revised date: 11-4-2016
Accepted date: 16-5-2016

Please cite this article as: Hamid Reza Kelidari, Majid Saeedi, Zohreh Hajheydari, Jafar Akbari, Katayoun Morteza-Semnani, Javad Akhtari, Hadi Valizadeh, Kofi Asare-Addo, Ali Nokhodchi, Spironolactone loaded nanostructured lipid carrier gel for effective treatment of mild and moderate acne vulgaris: A randomized, double-blind, prospective trial, Colloids and Surfaces B: Biointerfaces http://dx.doi.org/10.1016/j.colsurfb.2016.05.042

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]Department of Nanobiomedicine, School 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

Total number of words: 4455 (including the title and references)
Total number of tables: 2
Total number of figures: 5

*Corresponding authors: Ali Nokhodchi, PharmD, PHD, e-mail: a.nokhodchi@sussex.ac.uk; tel: +44 1273 872811 and Majid Saeedi, PharmD, PhD; e-mail: majsaeedi@gmail.com)

Graphical abstract

Highlights

- Spironolactone-SLN (SP-SLN) particles can be formulated in carbopol gel
- SP-SLN gel formulations showed non-Newtonian independent pseudoplastic behaviour
- Water content of the skin increased after using SP-SLN gels
- SP-SLN gels are more effective than Spironolactone alcoholic gels to treat acne

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.

Keywords: Acne, Nanostructured lipid carriers **(NLCs)**, Spironolactone, Topical, **Skin** condition

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 clinical 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 [8] 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 934P 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:

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** RPMI 1640 medium containing 2 mM l-glutamine supplemented with 10 % (v/v) heat-inactivated FBS (**fetal** bovine serum), 100 IU/mL penicillin and 100 mg/mL streptomycin (Gibco).

2.5. Treatment of cells with nanoparticles

The HFFF2 cells (1×10^4) were seeded in each well containing 100 µl of the RPMI medium supplemented with 10 % FBS in a 96-well plate. After 24 h of adhesion, a serial of doubling dilution of the SP-NLC was added to four replicate wells. Cell viability was assessed by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) reduction assay described below.

2.6. MTT reduction assay

After 1 and 2 days, 10 µl of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) (5 mg/ml stock solution) was added to each well and the plates were incubated at 37 °C for an additional 4h. The medium was discarded and the formazan blue, which formed in the cells, was dissolved using 200 µl dimethyl sulfoxide (DMSO). The absorbance was measured at 570 nm in a microplate reader (ELx800, BioTek Instruments, Inc., Winooski, VT). Cell viability data were expressed as the mean ± standard deviation (SD) of two independent experiments run in four replicates. The percentage of viability compared with the control wells

(the means optical density of untreated cells was set to 100 % viability) was calculated from the concentration–response curves by linear regression analysis.

The gels were kept in 4, 25 and 40 ˚C for physical stability evaluation (viscosity, syneresis, swelling and color change) for 2 weeks [18]. The selected formulation for clinical trial was prepared freshly and controlled microbiologically according to USP 30 (United States Pharmacopoeia).

2.7. Clinical study

In selecting volunteers for the clinical study, patients received a systemic or topical anti acne therapy 3 months before or during the study, pregnant patients, patients planning to become pregnant, lactating patients, patients with skin diseases that might interfere with the diagnosis or evaluation of their hyper pigmentation were excluded from the study. Seventy-six patients with mild to moderate acne, 8 years or older, defined as a score of 1–30 on the global acne grading system (GAGS) scale [17], who were not satisfied with their previous acne therapies participated in the double-blind clinical trial study after giving written informed consent. Permission was obtained from the Mazandaran University of **M**edical **S**ciences ethical committee before starting the study. At the first visit, a detailed questionnaire including data of demographic status, acne duration and medical history was completed for each patient. Thereafter, the patients were randomly assigned to one of the two treatment groups, namely group A (**where** SP-NLC gel was used) and group B (**where** SP-ALC gel was used). Both the physicians and patients were blinded to the type of treatment. Every morning and evening, patients washed their face with non-medicated soap, then thoroughly rinsed and dried it.

Over the 8-week course, each patient applied 2 tubes **(each tube contained 30 g gel with the patient instructed to apply about 1 g of the gel containing 10 mg SP for SP-NLC gel (group A) or 50 mg drug of the SP-ALC (group B) during the 8 weeks)** of the formulated gel and was asked to apply about 2 cm (knuckle) of the gel each morning and evening to the area and massage it for about 2 minutes. They were left on individual acne lesions for 2–3 h, after which they were washed off. Non-medicated cosmetics were permitted during the study. The patients were asked about adherence to the protocol and to report any side effects. During the trial the patients were prohibited from using any drugs or other skin care treatments for acne.

2.8. Clinical assessments

The patients were assessed for any changes in the facial lesion counts (non-inflammatory lesions: open and closed comedones; inflammatory lesions: papules, pustules, and nodules) at each clinic visit (0, 2, 4 and 8 Wk). At every session, the acne lesions were assessed based on their numbers, type and distribution. For the final assessment and to determine the efficacy of the treatment, the following two formulas were used:

Total Lesion Count (TLC) = comedones + papules+ pustules

Acne Severity Index (ASI) = papules + (2 pustules) + (comedones/4).

The hydratation, sebum, elasticity, melanin and redness of the skin were measured at each visiting time using the multi skin test instrument (MC 900; Enviro derm,

Gloucestershire, UK). The signs and symptoms were evaluated every 2 **weeks** from the baseline.

2.9. Statistical analysis

Statistical analysis was performed using SPSS for Windows (version 15; SPSS Inc., Chicago, IL, USA). Alterations in skin-related parameters were statistically examined using the Student's t-test. Differences were considered to be significant at $P < 0.05$.

3. Results

3.1. Characterization of the gels

The TEM micrographs of the SP-NLC gel (Figure 1) revealed that the SP-NLC particles **were** spherical in nature. The results **obtained from the zetasizer (Table 2S in supplementary materials)** showed smaller particle sizes and higher zeta potential for the SP-NLC gel (239.7±99.48 nm and -21.39±0.051 mV) compared to that of the SP-ALC gel formulation (8093.5±722.83 nm and 5.13±0.05 mV; $P<0.001$). SP-NLC particles showed lower polydispersity index of 0.286±0.051 compared to **the** SP-ALC gel which was 0.951±0.068. The diameters for both gels are an indication of good spreadability (between 5-6.5 cm) and also pH was found to be within acceptable limits (pH 4-6). Regarding the rheology, the gels exhibited non-Newtonian independent pseudoplastic and shear thinning **behaviour meaning** these systems have different viscosities at different shear rates (different rpm; $P>0.05$). For more detail**s** on these parameters please refer to supplementary data in Table 2S.

3.2. Cytotoxicity

The results of the cytotoxicity assays performed with the nanoparticles containing SP are presented in Figure 2. The results showed that SP-NLC was not toxic to the cell strain at 24 and 48h.

3.3. Demographic characteristics

A total of seventy six patients with mild to moderate acne were recruited in the present study. Among these patients, 36 (47.37 %) were in the group treated with SP-NLC gel (group A) and 40 (52.63 %) were in the group treated with SP-ALC gel (group B). In group A, thirty three (91.66 %) were female and three (8.34 %) were male; in group B, there were thirty seven (92.5 %) female and three (7.5 %) male participants. Among the 36 patients in group A, six (16.66 %) patients were excluded from the study for reasons cited as "personal reasons". Personal reasons were cited for four patients (10 %) in group B. There were also six (15 %) patients excluded for allergy reasons in group B. Hence, 30 patients in group A and 30 patients in group B were assessed for up to 8 weeks of treatment. The minimum and maximum age in group A were 11 and 38 years, respectively; and in group B these values were 8 and 34 years, respectively. There were no significant differences between patients' age in the two groups ($p = 0.232$). At baseline, no significant differences were noted between groups in terms of mean total lesion score ($P = 0.596$). This similarity was observed between groups in the mean inflammatory lesion score ($P = 0.988$) and the mean non-inflammatory score ($P = 0.811$) as well (Table 1).

3.4. Efficacy on Total Lesion Score (TLS)

The percentage reductions in the total lesion score are summarized in Figure 3(I). From the baseline to weeks 2, 4 and 8 the percentage reduction of total lesion scores in the group A compared to group B were not significant (P=0.596; P=987), however in both groups, the reduction in week 8 compared to the baseline was significantly higher (P < 0.004 and P = 0.074, respectively).

3.5. Efficacy on non-Inflammatory and inflammatory lesion scores

Decreases in non-inflammatory and inflammatory lesions over time are summarized in Figures 3(II) and 3(III), respectively. The mean number of non-inflammatory lesion significantly reduced (P <0.01) in both groups when week 8 **was** compared to the baseline. In group A, the mean number of non-inflammatory and inflammatory lesions decreased from 25.65 ± 8.12 to 13.45 ± 4.49 (**a** reduction rate **of** 47.6 %) and 8.08 ± 2.80 to 5.68 ± 1.94 (reduction rate 29.7 %), respectively. In group B, these values dropped from 29.26 ± 7.99 to 13.95 ± 5.22 (**a** reduction rate **of** 52.3 %) and 8.23 ± 2.66 to 6.36 ± 2.43 (**a** reduction rate **of** 22.7 %), respectively (P>0.05).

3.6. Efficacy on TLC and ASI

Figure 4(I) showed a statistically significant difference (week 8 compared to the baseline) in both groups A and B towards a decrease of the total lesion count (P<0.001). The TLC in group A was 37.165 ± 9.28 (baseline) and 19.63 ± 6.36 (Wk 8), (P = 0.003), and 32.60 ± 9.32 (baseline) and 18.33 ± 5.56 (Wk 8) for group B (P =0.022).

We report a representative case of a 15-year-old female patient, whose acne lesions remarkably improved after topical application of SP-NLC gel for 8 weeks (Figure 5).

3.7. Efficacy on skin condition

The data of patients in both groups' subjects for skin conditions are listed in Table 2. There were no differences in skin hydration, sebum, elasticity, melanin and redness between groups A and B during the study period (P>0.05). The skin elasticity, melanin and redness of both groups remained unchanged during the study period (P>0.05). Notably, skin hydration increased in weeks 4 and 8 in group A and the increase was more significant in week 8 (P =0.002). Also skin sebum decreased significantly (P =0.013) in the same time (Wk 8) in group B. There were no serious adverse experiences that were related to the treatment. Dryness and itching were the most frequent symptoms reported in group A (2.7 %) and B (15 %).

4. Discussion

4.1 Characterization of the gels

A reduction in the size of nanoparticles and PI in this study maybe as a result of the higher interfacial tension between nanoparticles and the aqueous phase which leads to **a** more homogenized nanoparticles in the aqueous phase. The phenomenon for zeta potential can be explained by the fact that oleic acid has negatively charged carboxylic groups. NLC revealed the highest zeta potential values, possibly due to the accumulation of oleic acid at the surface of the nanoparticles [20].

4.2. Clinical assessments

The purpose of this double-blind, randomized study was to compare the efficacy and tolerability of SP nanogel 1 % (SP-NLC) to an alcoholic SP gel 5 % (SP-ALC) formulation which had previously been reported to be beneficial in acne by Afzali et al, in the treatment of facial mild to moderate acne vulgaris [13]. In this present study we have demonstrated for the first time that daily application of the SP-NLC gel for up to 8 weeks has a therapeutic effect on mild to moderate acne vulgaris. Compared with the baseline, non-inflammatory lesions and TLC decreased significantly in groups A and B at week 8. In contrast, inflammatory lesion score and ASI did not change after 8 weeks in both groups. The dermatologic improvement of acne vulgaris in groups A and B maybe accompanied by a significant decrease in sebum content, which is over produced in acne [21]. The efficacy of 5 % topical SP cream acts as an anti-androgen in human sebaceous glands, competing with dihydrotestosterone (DHT) receptors and producing a decrease of labelled DHT [13]. Califano et al. reported that the patient's treatment with 5 % SP cream revealed complete regression of acne in 30 % and **an** improvement in 65 % of the patients [22]. Recently, the efficacy of a 5 % **SP** gel in the treatment of facial acne **was** studied in a randomized placebo-controlled trial by Afzali et al. [13]. They showed that TLC and ASI in a placebo and SP groups reduced significantly and emphasized that perhaps the alcoholic content of the gel caused this phenomenon to occur in both groups. This could be the reason here as well as for group B as around 65 % of non-inflammatory lesions reduced after 8 weeks treatment. But in group A however, SP-NLC may **have achieved** the

targeting to skin appendages, especially to the pilosebaceous units (hair follicles with their associated sebaceous glands). This is an alternative pathway for the dermal absorption of NLC, since it contributes significantly to the absorption of small molecules within the lag time after application. Walton et al. showed that when SP was applied topically in concentration of 3 and 5 % in humans there was no effect on sebum excretion. They suggested that, the lack of anti-androgen effect from topical SP cream maybe related to the vehicle which is used and has to be adequate to deliver the drugs to the target sebaceous glands [23]. Interest in pilosebaceous units is directed towards their utilization as reservoirs for localized therapy and also as a transport pathway for systemic drug delivery. Moreover, for some hair follicle related diseases such as acne, the hair follicle itself is the target site [24]. Some researchers hypothesize that lipid coating or lipophilic material properties may favour higher uptake into hair follicles, because the hair follicles are filled with sebum and provide relatively lipophilic environment [25]. Follicular deliveries play a key role *in-vivo* for the penetration of substances topically applied since the pilosebaceous unit is more permeable than corneocytes. Moreover, it has been demonstrated in reports that, colloidal particles larger than 10 μm can remain on the skin surface, those in the range of 3–10 μm accumulate in the follicle, when smaller than 3 μm, they can penetrate into follicles [26]. Furthermore, in this study the particle size of SP-NLC was 239 nm while SP-ALC was around 8 μm meaning SP-NLC may penetrate into **the** follicles **whilst the** SP-ALC may remain on the skin surface and that the alcoholic effect is what is paramount in the reduction of lesions. The lipid structures of NLC in contrast to an alcoholic formulation may affect the interaction with skin. Lipid-based carriers could attach themselves onto

the skin surface, making close contact with superficial junction of corneocyte clusters and channels between corneocyte islands. Finally it makes drug permeation easier, since the lipid cover could reduce corneocyte packing and widen the inter corneocytes gaps [27]. NLC's have occlusive properties, i.e. they can be used in order to increase the water content of the skin [28]. Occlusive compounds affect the skin hydration and penetration of compounds into the skin. The increase in skin moisture results in **group** A may be attributed to the retention of water content of the skin. Nanoparticles have been found to be 15-folds more occlusive than microparticles [27]. Wissing and Müller performed an *in-vivo* study investigating the skin hydration effect after repetitive application of an o/w cream containing lipid nanoparticles and a conventional o/w cream for 28 days. The lipid nanoparticles containing o/w cream increased the skin hydration significantly more than **the** conventional o/w cream [27].

5. Conclusion

The results of this randomized, double-blind trial demonstrated that the therapy of SP-NLC and SP-ALC were well tolerated and resulted in significantly greater improvement in mild to moderate acne vulgaris after 8 weeks treatment in comparison to the baseline. This therapy effectively treated non-inflammatory lesions, and showed high skin hydratation in the SP-NLC group (group A). However, the role of SP-NLC gel developed in the present investigation still requires clinical evaluation in a larger number of human subjects at different locations.

Acknowledgment

This work was supported by a grant from the research council of the Mazandaran University of Medical Sciences.

Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] J.S. Archer, R.J. Chang, Best. Pract. Res. Cl. Ob. 18 (2004) 737-754.

[2] K. Sato, D. Matsumoto, F. Iizuka, E. Aiba-Kojima, A. Watanabe-Ono, H. Suga, K. Inoue, K. Gonda, K. Yoshimura, Aesthet Surg J. 30 (2006) 689-694.

[3] H.-Y. Chiu, T.-F. Tsai, J Am Acad Dermatol. 65 (2011) 1048. e1041-1048. e1022.

[4] Y. Dong, W.K. Ng, S. Shen, S. Kim, R.B.H. Tan, Int. J. Pharm. 375 (2009) 84-88.

[5] M. Schäfer-Korting, W. Mehnert, H.-C. Korting, Adv. Drug. Deliv. Rev. 59 (2007) 427-443.

[6] M. Gupta, S.P. Vyas, Chem. Phys. Lipids 165 (2012) 454-461.

[7] R.H. Müller, R.D. Petersen, A. Hommoss, J. Pardeike, Adv. Drug. Deliv. Rev. 59 (2007) 522-530.

[8] J. Štecová, W. Mehnert, T. Blaschke, B. Kleuser, R. Sivaramakrishnan, C.C. Zouboulis, H. Seltmann, H.C. Korting, K.D. Kramer, M. Schäfer-Korting, Pharm. Res. 24 (2007) 991-1000.

[9] K.A. Shah, A.A. Date, M.D. Joshi, V.B. Patravale, Int. J. Pharm. 345 (2007) 163-171.

[10] J. Liu, W. Hu, H. Chen, Q. Ni, H. Xu, X. Yang, Int. J. Pharm. 328 (2007) 191-195.

[11] A.K. Jain, A. Jain, N.K. Garg, A. Agarwal, A. Jain, S.A. Jain, R.K. Tyagi, R.K. Jain, H. Agrawal, G.P. Agrawal, Colloids Surf. B: Biointerfaces 121 (2014) 222-229.

[12] J.C. Shaw, J. Am. Acad. Dermatol. 24 (1991) 236-243.

[13] B.M. Afzali, E. Yaghoobi, R. Yaghoobi, N. Bagherani, M.A. Dabbagh, J Dermatol Treat. 23 (2012) 21-25.

[14] R.N. Shamma, M.H. Aburahma, Int J Nanomedicine, 9 (2014) 5449.

[15] M. Joshi, V. Patravale, Int. J. Pharm. 346 (2008) 124-132.

[16] W. Liu, M. Hu, W. Liu, C. Xue, H. Xu, X. Yang, Int. J. Pharm., 364 (2008) 135-141.

[17] S. Bose, Y. Du, P. Takhistov, B. Michniak-Kohn, Int. J. Pharm. 441 (2013) 56-66.

[18] Z. Hajheydari, M. Saeedi, K. Morteza-Semnani, A. Soltani, J Dermatol Treat. 25 (2014) 123-129.

[19] A. Doshi, A. Zaheer, M.J. Stiller, Int J derm.36 (1997) 416-418.

[20] F. Marquele-Oliveira, D.C. de Almeida Santana, S.F. Taveira, D.M. Vermeulen, A.R. Moraes de Oliveira, R.S. da Silva, R.F.V. Lopez, J Pharm Biomed Anal. 53 (2010) 843-851.

[21] D.S. Berson, A.R. Shalita, J Am Acad Dermatol, 32 (1995) S31-S41.

[22] L. Califano, S. Cannavo, M. Siragusa, R. Girardi, Clin Ter. 135 (1990) 193-199.

[23] S. Walton, W. Cunliffe, P. Lookingbill, K. Keczkes, Br. J. Dermatol. 114 (1986) 261-264.

[24] Y. Zhai, G. Zhai, J. Con. Rel. 193 (2014) 90-99.

[25] U. Münster, C. NakamuraNachname, A. Haberland, K. Jores, W. Mehnert, S. Rummel, M. Schaller, H. Korting, C.C. Zouboulis, U. Blume-Peytavi, Die. Pharmazie. Int. J. Pharm.60 (2005) 8-12.

[26] H. Wosicka, K. Cal, J. Dermatol.Sci. 57 (2010) 83-89.

[27] J. Pardeike, A. Hommoss, R.H. Müller, Int. J. Pharm. 366 (2009) 170-184.

[28] S. Wissing, O. Kayser, R. Müller, Adv. Drug. Deliv. Rev. 56 (2004) 1257-1272.

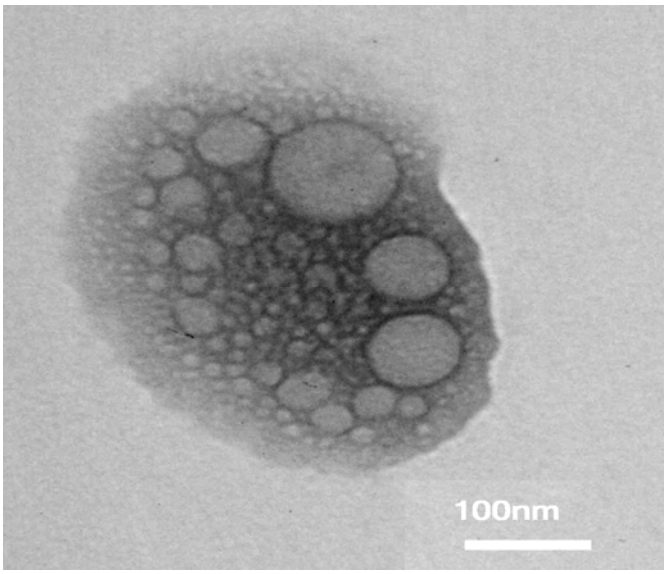


Figure 1. Transmission electron micrograph (TEM) of SP-NLC.

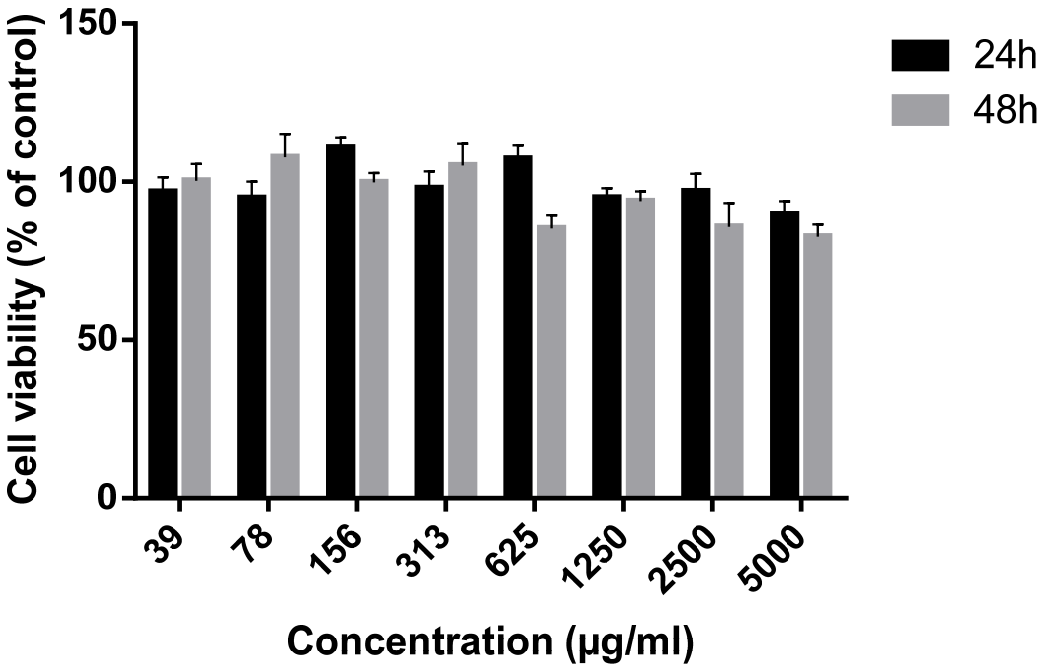


Figure 2. Cytotoxicity of SP-NLC in HFFF2 fibroblast. Data shown as mean ± standard deviation, n = 5. The nanoparticle concentration is presented in units of NLC concentration.

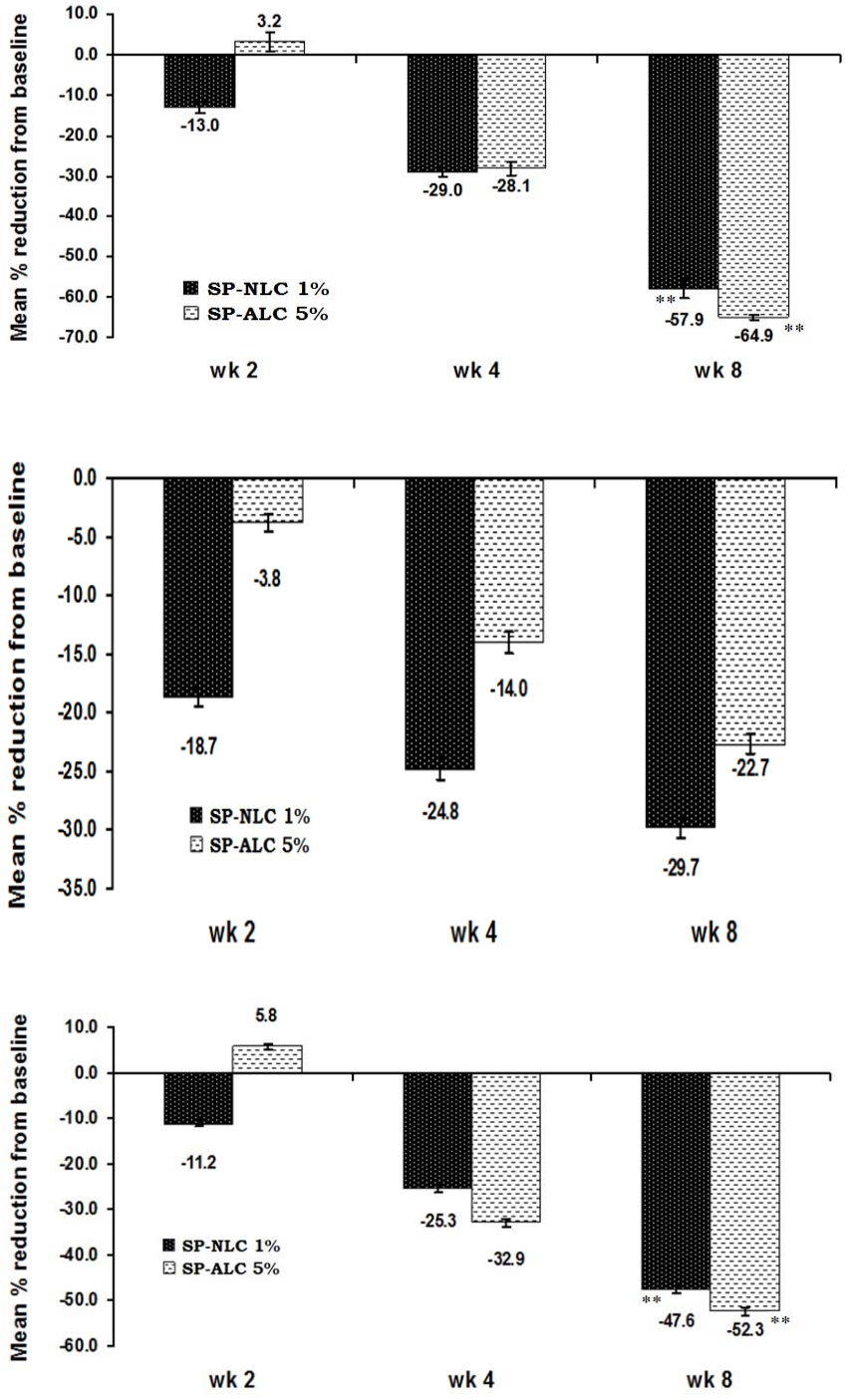
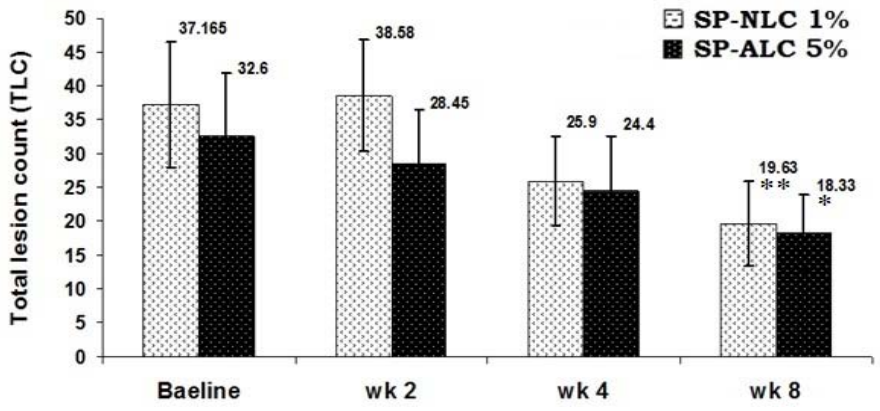
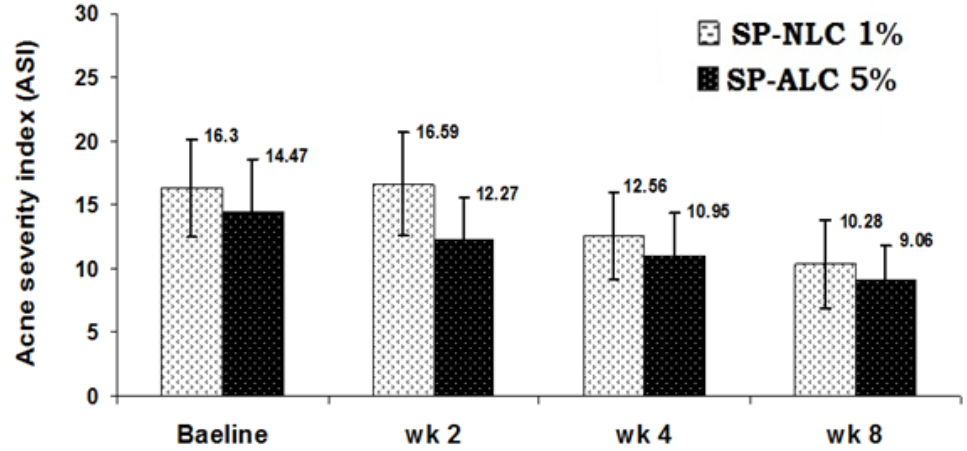


Figure 3. Mean percentage changes in: (I) total lesion scores from baseline; (II) inflammatory lesion scores from baseline and (III) non-inflammatory lesion scores from baseline (A = SP-NLC 1 % ; B = SP-ALC 5 %). **Values are expressed as mean ± standard deviation (n = 30).**



(I)



(II)

Figure 4. (I) Total Lesion count (TLC) from baseline and (II) acne severity index (ASI) from baseline (A = SP-NLC 1%; B = SP-ALC 5%). **Values are expressed as mean ± standard deviation (n = 30).**

Figure 5. Fifteen year old female patient at baseline (A) and after treatment with SP-NLC gel at week 8 (B)

Table 1. Baseline characteristics of the intent-to-treat population. **Values are expressed as mean ± standard deviation (n = 30).**

Demographic parameter	***SP-NLC 1 %***	***SP-ALC 5 %***	***p-value***
Mean age, y (±SD)	21.80 ± 5.97	20.86 ± 5.36	0.232
Sex, No. (%)			
Female	27 (% 87.5)	28 (% 93.3)	
Male	3 (% 12.5)	2 (% 6.67)	
Mean acne duration, m (±SD)	3.72 ± 2.67	3.54± 2.88	0.712
Patients who used medication, No.	36	40	
Patient who completed study, No.	30	30	
Reason for discontinuation			
Allergy to medication	0	6	
Personal reason	6	4	
Acne lesion count (mean±SD)			
Total	37.66 ±9.27	33.73 ±9.40	0.596
Non-inflammatory	29.26 ± 7.99	25.65 ± 8.12	0.988
Inflammatory	8.23 ± 2.66	8.08 ± 2.80	0.811
TLC (± SD)	37.16 ± 9.28	32.6 ± 9.32	0.564
ASI (± SD)	16.3 ± 3.85	14.47 ±4.14	0.121

TLC = Total Lesion Count; ASI = Acne Severity Index

Table 2. Changes of skin conditions from baseline to week 8 (%).**Values are expressed as mean ± standard deviation (n = 30).**

	BASELINE	WEEK 2	WEEK 4	WEEK 8
Hydratation (± SD)				
SP-NLC 1 %	37.44±8.85	40.46±10.09	41.25±12.80*	45.69±19.34**
SP-ALC 5 %	37.61±9.38	38.87±14.30	38.24±18.13	37.74±10.92
Sebum (± SD)				
SP-NLC 1 %	18.24±10.28	15.25±7.85	14.64±9.71	15.10±9.03
SP-ALC 5 %	21.93±12.66	16.78±10.09	15.64±8.49	13.87±7.15*
Elasticity (± SD)				
SP-NLC 1 %	73.92±7.61	69.35± 12.38	71.58±10.24	71.52±13.35
SP-ALC 5 %	71.20±11.89	68.69± 10.56	71.58±10.24	70.29±9.73
Melanin (± SD)				
SP-NLC 1 %	31.91±5.90	31.32± 6.45	30.33±5.89	30.25±6.16
SP-ALC 5 %	29.47±5.99	31.47±11.21	29.84±6.62	28.78±6.87
Redness (± SD)				
SP-NLC 1 %	37.26±6.09	37.03±5.52	36.15±6.05	35.86±5.95
SP-ALC 5 %	36.45±6.74	36.67± 5.70	36.01±5.73	35.89±6.11

* Values differ significantly ($P < 0.05$).
** Values differ significantly ($P < 0.001$).