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Original Research

The efficacy of isotretinoin-loaded solid lipid nanoparticles in comparison to Isotrex® on acne treatment

Pouran Layegh[1], Navid Mosallaei[2], Danial Bagheri[2], Mahmoud Reza Jaafari[3], Shiva Golmohammadzadeh[3*]

[1]Research Center for Cutaneous Leishmaniasis, Department of Dermatology, Mashhad University of Medical Sciences, Mashhad, Iran
[2]School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Nanotechnology Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

Abstract

Objective(s): Topical retinoids are considered as the first line therapy in the treatment of acne vulgaris, but they are associated with cutaneous irritation.
Materials and Methods: In this study, isotretinoin-loaded solid lipid nanoparticles (IT-SLN) were prepared to treat the mild to moderate acne. Also using IT-SLN would minimize IT adverse effects in comparison to commercial product, Isotrex®. This study was conducted to prepare and characterize IT-SLN and assessing the efficiency of IT-SLN comparing to Isotrex® acne.
IT-SLN was prepared using hot high pressure homogenization method. IT-SLN contained 0.05% IT in 5% of lipid phase (Glyceryl monostearate- GMS) and tween 80 (2.5 % w/v) was used as surfactant in the aqueous phase. IT-SLN was characterized by particle size analyzing, differential scanning calorimetry and transmission electron microscopy. Encapsulation efficacy was also obtained using spectrophotometry. The efficacy of IT-SLN was evaluated in a randomized, single-blind, parallel-group study and compared with Isotrex®. Forty patients encountered in the study and divided in two groups. Treatment regimen was once-nightly topical administration accompanied with topical administration of clindamycin 2% solution twice a day for 8 weeks.
Results: The particle size of IT-SLN was around 60 nm with PDI of 0.4 and zeta potential was about -40 mV. Encapsulation efficacy of IT in SLN in crystalline form was 84±0.21%. IT-SLN produced significantly better treatment than Isotrex® in both non-inflammatory and inflammatory lesions according to its recovery percent after 8 weeks. Also IT-SLN gained better global assessment scores.
Conclusion: Our results showed that IT-SLN had higher efficacy than Isotrex® to clear non-inflammatory and inflammatory lesions.

Keywords: Acne, Clinical trial, Isotretinoin, Solid lipid nanoparticles (SLN)

*_**Corresponding author:**_ *Shiva Golmohammadzadeh, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.*
Tel: +98511-8823255, Email: GolmohamadzadehSh@mums.ac.ir

Introduction

Isotretinoin, as a derivative of retinoic acid (13-cis-retinoic acid), is included in the first generation of retinoids (1). It has been commonly used for the treatment of severe acne and the other dermatological diseases like neoplastic diseases (2).

In recent years, dermatologists have used Isotretinoin to treat patients with less severe forms of acne which have the risk of scarring or excessive psychological distress. This regimen of therapy was preferred when; acne has demonstrated resistance to other conventional systemic treatments such as oral antibiotics (3). IT is also commonly used for treatment of some acne variants, such as acne conglobata, acne fulminans (in combination with corticosteroids) and acne inversa, as well as for acne with gram-negative folliculitis (4). IT adverse effects which may occur after oral administrations consist of mucosal dryness, visual disturbance, and skeletal hyperostosis with musculoskeletal symptoms. Elevation of serum triglycerides, blood glucose and hepatic enzymes are reported as well and should be closely monitored. One of the most important contraindications for IT is pregnancy (X) (5, 6, 7). Topical retinoids are highly effective in the treatment of both comedonal and inflammatory lesions of acne and are a vital part of almost any acne regimen. Topical IT should be primarily prescribed in the case of a relapse as a conventional treatment as well (1, 7). Hence a topical formulation of IT seems to be worthy. Topical preparations of IT gel and cream can be found on the market. Unfortunately, they showed systemic absorption, and skin irritation. Therefore, it is valuable to improve the skin uptake and acne treatment efficacy, while reducing irritation and systemic adverse effects of IT (6, 8, 9, 10).

There are 4 major pathogenic steps in the production of acne: (1) increased sebum production; (2) abnormal keratinization of the follicular infundibulum; (3) Propionibacterium acnes-mediated responses; and (4) inflamemation. Topical retinoids show their efficacy in the treatment of acne through these pathways. Of course retinoids have no direct effect on decreasing sebum production but the other three factors are closely linked and modulation of one will often have a direct or indirect effect on another (1, 11).

Adverse effects that patients usually complain about after topical administration of IT are erythema, dryness, peeling and skin photosensitivity. These adverse effects cause patient incompliance and the medication may be refused (1).

In addition, IT is a photosensitive molecule which degrades while it is exposed to UV light. Thus, it was necessary to improve photostability, skin uptake and reduce systemic absorption of IT using a carrier which we showed in our previous study (12).

Nanoparticulate carriers which are developed not only enhance percutaneous absorption but also target the active pharmaceutical ingredient to the skin or its substructure. Thus these systems like liposomes, nano-emulsions, niosomes and solid lipid nanoparticles (SLN) are potential for an improved benefit/risk ratio in topical drug therapy (10).

SLN has been introduced as a novel drug delivery system for pharmaceutical drugs in various application routes of intravenous, intramuscular, oral and topical (13). SLN production by the aim of topical usage should be made up of lipids that remain in solid state at skin (32 °C) and room temperature. Its particle surface is covered by a surfactant which stabilizes the dispersion (10). As a skin drug delivery system, they are promising carriers for cosmetic active ingredients due to their numerous advantages over existing conventional formulations. SLN merits can be introduced as: (1) Protecting labile compounds against chemical degradation (14). (2) Producing controlled release of the active ingredients. This property depends on the way that active ingredient encapsulation. SLN with a drug-enriched shell shows burst release characteristics whereas SLN with a drug-enriched core leads to sustained release (15, 16). (3) SLN has occlusive properties, i.e. they can be used in order to increase the water

content of the skin (17, 18). (4) SLN shows a UV-blocking potential and they can act as physical sunscreens on their own by scattering sunlight (19, 20). (5) They have no biotoxicity of excipients. (6) They are produced in avoidance of using organic solvent and (7) ease of large scale production (21). Therefore, SLN has proven its ability as an effective skin drug delivery system and is used for many drugs like topical gluco-corticoide, tretinoin, antimycotics (22, 23, 24, 25).

In the current study we prepared and characterized SLN containing IT and evaluated its efficacy in patients suffering from mild to moderate acne. It is considered that in patients with mild-to-moderate mixed inflammatory and comedonal acne, a combination of a topical antimicrobial, either benzoyl peroxide or antibiotic, is optimal. Since it is well established that combination therapy works faster and more effective than monotherapy, we prepared and added topical solution of clindamycin 2% to the regimen (11). We compared the efficacy of our new nanoparticulate formulation with Isotrex® while they were used beside clindamycin 2% topical solution.

Materials and Methods

Materials

Isotretinoin (IT, Across, America), glyceryl monostearate (GMS, Gattefossé, France) and Tween 80 (Sigma, Germany) were used as received. Isotrex® 0.05% was manufactured in Stiefel, Ireland. Clindamycin phosphate powder was purchased from Sepidaj, Iran. Ethanol (Noor-e-Zakaria Razi, Iran) and double distilled water were used in experiments. Isopropyl alcohol, HCl and propylene glycol were obtained from Iranian companies.

Preparation of IT-SLN

The best formulation of IT-SLN was invest-tigated in our previous study. Briefly, lipid phase contained GMS (500 mg; 5% w/v) melted at 80 °C in a pear-shaped vessel. IT (5 mg; 0.05% w/v of the formu-lation) dissolved in ethanol and poured on GMS. Ethanol was removed using rotary evaporator under vacuum condition. Then the isothermal aqueous phase containing Tween 80 (250 mg; 2.5 % w/v) was added to the melted lipid and homogenized using T 25 Ultra Turrax (Janke und Kunkel GmbH and Co KG Staufen, Germany) for 1 min at 20000 rpm and the pre-emulsion was produced. This pre-emulsion was processed at 1000 bar, at 80 °C, for 5 cycles using a high pressure homogenizer (EmulsiFlex-C5®, Avestin Inc., Canada) and IT-SLN suspension was obtained. Homo-genized samples were then cooled to 4 °C and stored for 24 h before performing further characterizations (8, 12, 14).

Particle size and zeta potential

Dynamic light scattering (ZetaSizer Nano-ZS; Malvern Instruments Ltd., United Kingdom) was used to evaluate the mean particle size (Z-average (nm)), polydispersity index (PDI) and zeta potential (mV) of SLN dispersions. Samples were prepared by adding 990 µl deionized water to 10 µl SLN suspension. The mean diameters and zeta potential values during three months were calculated from the measurements performed at least in triplicates (8, 12).

Transmission electron microscopy (TEM)

TEM (CEM 902A; Zeiss, Germany) was performed to characterize the morphology of IT-SLN. IT-SLN suspension was diluted 50 times with double distilled water and placed on a carbon-coated copper grid and after 30 seconds, the excess water was wiped off with paper filter. Twenty µl of uranyl acetate 2 % in water was spread on SLN and after 30 seconds wiped off with paper filter. The grid was dried at room temperature and then observed through TEM (8, 12).

Differential scanning calorimetry (DSC)

DSC was performed to investigate the melting and recrystallization behavior of crystalline materials after solidification. DSC scans of IT, empty and IT-SLN was carried out in a Mettler DSC 821e (Mettler Toledo,

Germany). Approximately, 5 mg of samples were filled in aluminum oxide pans, sealed and analyzed. An empty aluminum pan served as reference. DSC was done at 25-250 °C temperature range by rate of 5°C/min under N_2 flow and the melting point of SLN dispersions was compared to the bulk lipid (14, 26).

Entrapment efficiency (EE)

Calibration curve was resulted using UV-spectroscopy technique. IT solutions in acidic isopropyl alcohol (HCl 10^{-5} N in isopropyl alcohol) were prepared at concentrations of 0.25, 0.5, 1, 2, 3, 4 and 5 mg/ml and its absorbance was detected at 349 nm wavelength. Calibration curve was drawn in triplicate, inter- and intra-daily during three days. Acidic isopropyl alcohol was served as blank.

Encapsulation efficiency percent was determined indirectly by measuring the concentration of unentrapped IT (12, 27). A known dilution of the SLN dispersion was prepared and 500 µl was transferred to the upper chamber of centrifuge tubes fitted with an ultrafilter (Amicon Ultra-15, PLHK Ultracel-PL Membrane, 100 kDa, Millipore). Amicon tubes were centrifuged at 10000 rpm for 30 min. The filtrate was analyzed for unencapsulated IT at 349 nm using a validated UV-spectrophotometric method after suitable dilution and the entrapment efficacy was measured (27, 28).

Preparation of clindamycin solution 2%

To prepare 100 ml Clindamycin solution, its phosphate salt was dissolved in 27 ml double distilled water and added to 10 ml propylene glycol and mixed gently. This was then reached to the volume of 100 ml by ethanol 96 °.

Evaluation of IT-SLN and Isotrex® efficacy on acne treatment in human

The efficacy of IT-SLN (0.05%) was evaluated in a randomized, single-blind, parallel-group study. The design included a screening and a baseline evaluation followed by randomization to the treatment with either IT-SLN, or commercial IT gel (Isotrex® 0.05%) at a once-nightly topical adminis-tration for 8 weeks. The regimen was accompanied by topical administration of clindamycin 2% solution twice a day.

At weeks 0 (baseline), 4 and 8, numbers of non-inflammatory (open and closed comedones) and inflammatory (papules and pustules) lesions were counted for each patient. Clinically significant improvement was defined as a between-group difference of ≥15% reduction in the percentage of total lesion count (sum of non-inflammatory and inflammatory lesions) from baseline to the end of the study. At the baseline, photographs were taken. Pictures were used to compare patients' assessments of global response.

Each patient's global response to the treatment was assessed by a physician by counting the lesions at the first, fourth and eighth week of the treatment using a 7-point scale. The scale was as follows: 0 = completely cleared; 1= almost cleared (~90% improvement- very significant clearance in disease, with only traces of disease remaining); 2 = marked response (~75% improvement- significant improvement, with some disease remaining); 3 = moderate response (~50% improvement- intermediate between slight and marked response); 4 = slight response (~25% improvement- some improvement but significant disease remains); 5 = condition unchanged; and 6 = condition worsened. Treatment success was defined as a global assessment score of at least 3 points (≥50% global improvement) at the end of study (29).

Before enrollment, written informed consent was obtained from each patient or from the patient's parent or guardian if the patient was under the legal age of consent. The ethical committee code was 511/0363 (30). A dermatologist evaluated the result of treatment regimens and any side effects in weekly visits.

Study population

Patients for this study were recruited from dermatology department of Qaem Hospital, Mashhad University of Medical Sciences (MUMS). A total of 40 patients were scre-

ened, enrolled and randomized to the treatment.

Inclusion and exclusion criteria

Male and female patients aged 13-30 years with mild to moderate facial acne vulgaris who were candidate of treatment with topical agents were enrolled. The following washout periods were required: 2 weeks for topical acne medications, 4 weeks for oral antibiotics, 12 weeks for hormone therapy (unless treatment had been used for >12 consecutive weeks immediately before study entry and was expected to continue throughout the study), and 6 months for oral retinoids.

Patients were excluded if they had participated in any other study in the previous 30 days; had uncontrolled systemic disease; had any other skin condition that might interfere with assessment of the study medication like eczema; or were expected to undergo surgery, hospitalization, or excessive or prolonged exposure to the ultraviolet light (eg, sunlight, tanning bed) during the study. Volunteers were suggested to use the same oil-free sunscreen. Female patients who were pregnant, breastfeeding or planning to become pregnant were excluded (29). Recovery percent (treatment efficacy) was calculated using the following formula:

$$\text{Recovery}(\%) = (\text{lesion count at the baseline} - \text{lesion count after the medication}) / (\text{lesion count at the baseline}) \times 100$$

Statistical analysis

All the experiments of each preparation were repeated three times and data were expressed as Mean ± SD. Analysis of variance (ANOVA) was used to evaluate the statistical significance of differences among groups. Statistical data were analyzed using nonparametric techniques with a Tukey-Kramer test. Results with $P < 0.05$ were considered as statistically significant.

Results

Preparation of IT loaded SLN (IT-SLN)

IT was dispersed in the pre-emulsion homogenously, since we had dissolved it in ethanol and it was homogenized using a high speed stirrer (Ultra Turrax). Also we used the advantageous role of a hot water bath, in order to keep the pre-emulsion temperature over the melting point of lipids while it was forming. Hot high pressure homogenization method was performed to prepare our final product. The viscosity was enough to be stable and applicable on the skin without any excess excipient. The product had a milky color and good viscosity to apply.

Particle size, polydispersity and zeta potential of IT-SLN

Table 1 shows that particle size of IT-SLN was around 60 nm according to the number and PDI was 0.4. The results of particle size analyzing also showed an increase while IT was added to the formulation.

This formulation was stable in view of particle size and zeta potential for 3 months, since no statistical significant change was occurred during the storage at 4 °C. Zeta potential was about -40 mV which can produce particle repulsion and would inhibit aggregations.

Encapsulation efficacy of IT-SLN

The maximum light absorbance of IT was observed at the wavelength of 349 nm using spectrophotometry technique. The resulted calibration curve equation of mentioned concentrations was $A = 0.2195\ C - 0.0171$ and $R^2 = 0.99$ (A and C are absorbance and concentration (mg/ml), respectively).

Encap-sulation efficacy of IT-SLN was 84±0.21 % (n=3). As a reason of high encapsulation efficacy, un-encapsulated IT was not washed out in the following clinical experiments.

Thermal analysis of IT-SLN

Figure 1 investigates crystalline behavior of IT-SLN through DSC. It can be seen that GMS formed a basic structure in which IT entrapped in a crystalline state. GMS wide domain and IT sharp peak appeared around 50 and 177 °C, respectively.

Table 1. Z-average (nm), particle size by number (nm), PDI and zeta potential (mV) of IT-SLN prepared by high pressure homogenization technique. Data are shown in Mean±SD, n=3.

Formulation	Z-average (nm)	Size by number (nm)	PDI	Zeta potential (mV)
Blank SLN	191.9±6.4	50.4±2.1	0.42±0.02	-31.2
IT-SLN after 24 h	231.2±8.7	61.1±1.4	0.46±0.01	-37.1
IT-SLN after 1 month	239.2±8.	62.4±2.8.	0.47±0.07	-38.5
IT-SLN after 2 months	249.4±5.3	62.3±3.6	0.47±0.07	-39.8
IT-SLN after 3 months	252.1±2.7	62.9±7.7	0.47±0.08	-42.7

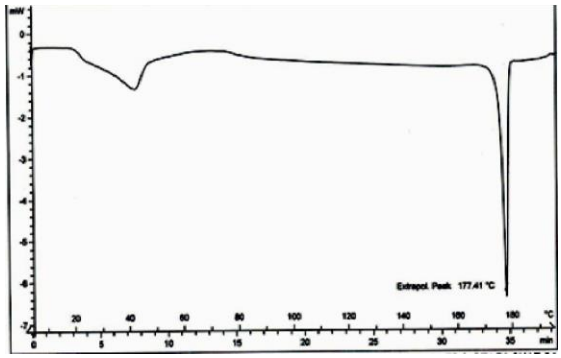

Figure 1. DSC thermogram of IT-SLN prepared by 5 cycles of high pressure homogenization technique, exo-up. Five mg of sample was used in each run.

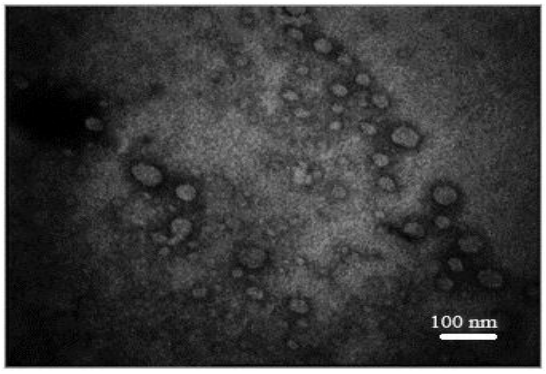

Figure 2. Transmission electron microscopy (TEM) image of IT-SLN prepared using high pressure homogenization.

Morphology of IT-SLN

The TEM imaging of IT-SLN can be observed in Figure 2. Particle sizes that resulted in this method were in accordance with particle size analyzing data (Table 1). The picture exhibited that SLN prepared in this study were almost uniform and had a spherical shape.

IT-SLN vs. Isotrex® efficacy in human acne treatment

Ten patients were omitted from the study for the following reasons: lack of on-time visits(three), wrong phone numbers for follow-up (two), wrong administration (two) and sensitivity to the treatment (three). Finally thirty patients remained, fifteen in each group.According to Figure 3A, it was resulted that recovery percent in non-inflammatory lesions after 4 weeks was 41.49±6.24% and 32.25±6.61% in IT-SLN and Isotrex® group, respectively. After 8 weeks, this amount was increased to 77.05±4.79% and 53.69±7.34%, respectively. So IT-SLN showed significantly higher recovery percent than Isotrex® in treatment of non-inflammatory lesions ($P<0.05$ and $P<0.01$ after 4 and 8 weeks, respectively).

Figure 3B shows the recovery percent in inflammatory lesions. The difference was not significant between IT-SLN and Isotrex® after 4 weeks.

However after 8 weeks IT-SLN showed significantly higher recovery percent (83.59±3.79%) than Isotrex® (61.15±5.34 %) with p-value of <0.01.Figure 4 shows the reduction of papulopustular lesions after the treatment with introduced regimen of topical therapy containing IT-SLN and clindamycin 2%.

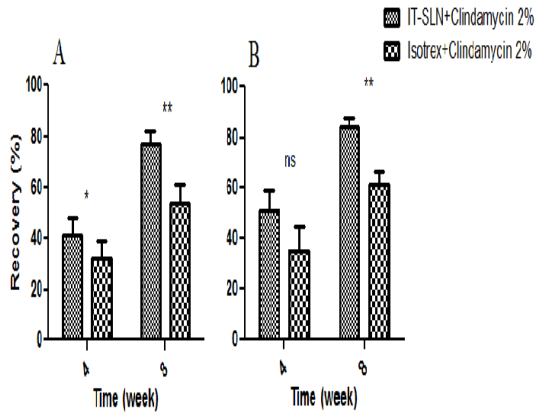

Figure 3. Recovery percent in non-inflammatory (A) and inflammatory (B) lesions of acne after 4 and 8 weeks of the treatment. (Mean±SD, n=15, ns: not significant, * P<0.05, ** P<0.01).

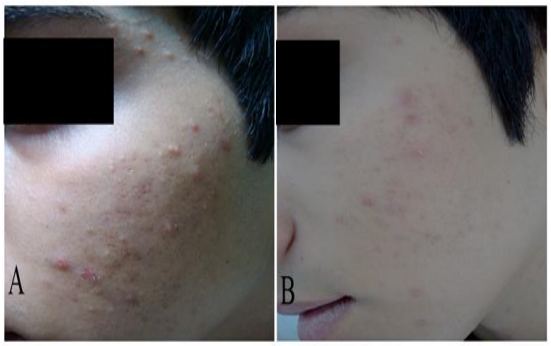

Figure 4. 17-year-old male patient with papulopostular acne. (A) before treatment (baseline) and again (B) after 8 weeks of treatment with IT-SLN and Clindamycyn 2% solution.

Figure 5-A demonstrates that all inflammatory lesions on the face was removed in about 30% of patients who administered our new formulation for 8 weeks.

It is resulted that about 70% of those who used IT-SLN got the global score of 0 and 1. Through a wider view to the treatment of non-inflammatory and inflammatory acne, it is concluded that 54 and 46% of the patients who consumed IT-SLN were scored 1 and 2, respectively (figure 5B). But in the opposite pole, 26 and 66% of those who consumed Isotrex® got the global scores of 2 and 3 in respect (Figure 5B). These results supports higher efficacy of IT-SLN than Isotrex® in treatment of acne. It was also meaningful that none of the patients in IT-SLN group complained about any skin irritancy while three

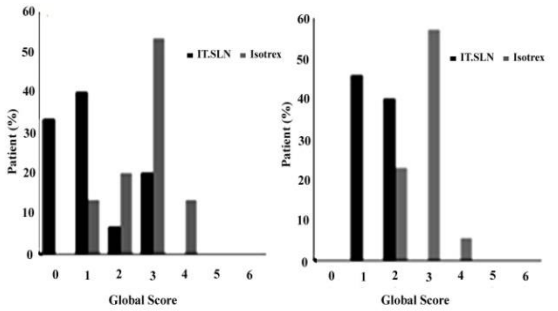

Figure 5. Global assessments at the end of treatment evaluation (8 weeks) for (A) inflammatory and (B) total lesions. 0 = completely cleared; 1 = almost cleared; 2 = marked response; 4 = slight response; 5 = condition unchanged; and 6 = condition worsened.

patients in Isotrex® group were omitted because of low compliance to the local adverse effects.

Discussion

Isotretinoin, as a derivative of retinoic acid is used in the treatment of different types of acne (2, 3, 4).

Topical IT is preferred because of omitting systemic adverse effects which can be seen in oral administration. These systemic adverse reactions consist of mucosal dryness, visual disturbance, skeletal hyperostosis with musculoskeletal symptoms, elevation of serum triglycerides, blood glucose and hepatic enzymes (5, 6, 7). Solid lipid nanoparticles (SLN) is a novel drug delivery system in various application routes of intravenous, intramuscular, oral and topical (13).

In this study, we prepared and characterized SLN containing IT and evaluated its efficacy in patients suffering from mild to moderate acne. In our previous and other researchers studies, it is well established that using SLN as topical drug delivery has advantages of reducing systemic side effects, protecting active pharmaceutical ingredient from hydrolysis, oxidation or photo degradation, better skin penetration, occlusive property, reducing IT induced skin irritation and ease of commercialization (10, 12, 21, 31).

The reason that we used 0.05% IT in formulations was that no higher efficacy was seen in stronger formulations (0.1%)

(32). Also the potency of the commercial product that we wanted to compare the formulation with was 0.05%, so in this way the judgment is more precise. IT-SLN showed suitable stability during three months of storage at 4 °C. This proper physical stability might have been resulted from the slow transition of lipid into SLN, small particle size, high zeta potential which produces particle repulsion, the effect of Tween 80 as surface stabilizer and the presence of GMS as a self emulsifier (8, 12, 33).

The small particle size would introduce proper occlusive factor, however, the occlusion factor of lipid nanoparticles relates to various factors but at identical lipid content, reducing the particle size leads to an increase in particle number and the film which forms on the skin will become denser and therefore the occlusion factor increases (12, 34). TEM image supports particle size of around 60 nm and proved spherical particle shape. Spherical shape of nanoparticles has some benefits like longest pathway of diffusion which can result in controlled release and higher protection of active ingredient (35).

It was detected that encapsulation efficacy of 84% was achieved. The presence of IT sharp peak at 177 °C in DSC experiment shows that IT was encapsulated in SLN lattice in a crystalline form.

Physical statues of IT-SLN suspension, showed good stability on the human skin after topical administration however it can also be used in a gel basis in future studies. Its minimum ratio of lipid content (5%) inhibited the oily view of the skin. Low lipid content is also suitable in acne therapy.

All of the preparations were stored at 4 °C to reduce any probable degradation of IT (8). We used a combination therapy (IT containing product + Clindamycin 2% topical solution) to ensure the faster and more efficient treatment (11).

This new formulation introduced higher efficacy in both non-inflammatory and inflammatory lesions in comparison to Isotrex® (Figure 4). However its effective response to the inflammatory lesions needs more time since the difference with the commercial product was not significant after first 4 weeks. This better improvement must be due to the better permeability of IT in SLN formulation to the skin and is due to the result of nano sized particle and high encapsulation efficacy. On the other side, SLN protects IT from UV degradation and increases its stability on the skin. In this point of view, it would be obvious to have higher drug amount, higher drug penetration and higher efficacy (12). IT-SLN produced higher efficacy than Isotrex® in inflammatory lesions and cleared them in almost 75% of patients after 8 weeks (Figure 5A).

Of course, further studies are also necessary to determine the ideal treatment length, especially in relation to varying dosage schedules. However, it should not be neglected that IT-SLN, even in this short time of study, produced better response comparing to Isotrex® in both non-inflammatory and inflammatory lesions.

In conclusion, our new formulation introduced better results when it is compared to Isotrex® because of its better global scores as seen in Figure 5B.

It was meaningful that no patient in IT-SLN group suffered from this formulation, while irritation caused cessation in three cases who administered Isotrex®. Triglycerides that were used in SLN structure are one of the physiologic lipids and this produces emollient property which can reduce IT irritancy.

Also IT is irritant itself when it is used on the skin and here SLN structure encapsulates IT and inhibits its direct contact to the skin (34). Besides, patients reported that they had good compliance to IT-SLN. These data are in accordance with our previous findings that proved low irritancy of IT-SLN comparing to Isotrex® (12).

Conclusion

Pharmaceutical results showed that IT-SLN was stable for three months in particle size and physical view while it is stored at 4 °C. No gelling or particle aggregation was occurred. In clinical experiment it was observed that IT-SLN had higher efficacy than Isotrex® to clear non-inflammatory and inflammatory lesions and finally showed better improvement rate. We propose it is now time to expand our study and gain more results by increasing the number of patients and length of treatment.

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