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Nano-lipoidal carriers of tretinoin with enhanced percutaneous absorption, photostability, biocompatibility and anti-psoriatic activity

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ABSTRACT

Tretinoin (TRE) is a widely used retinoid for the topical treatment of acne, psoriasis, skin cancer and photoaging. Despite unmatchable efficacy, it is associated with several vexatious side effects like marked skin erythema, peeling and irritation, eventually leading to poor patient compliance. Its photo-instability and high lipophilicity also pose challenges in the development of a suitable topical product. The present study, therefore, aims to develop biocompatible lipid-based nanocarriers of TRE to improve its skin delivery, photostability, biocompatibility and pharmacodynamic efficacy. The TRE-loaded liposomes, ethosomes, solid lipid nanoparticles (SLNs) and nanostructured lipidic carriers (NLCs) were prepared and characterized for micromeritics, surface charge, percent drug efficiency and morphology. Bioadhesive hydrogels of the developed systems were also evaluated for rheological characterization, photostability, *ex vivo* skin permeation and retention employing porcine skin, and anti-psoriatic activity in mouse tail model. Nanoparticulate carriers (SLNs, NLCs) offered enhanced photostability, skin transport and anti-psoriatic activity *vis-à-vis* the vesicular carriers (liposomes, ethosomes) and the marketed product. However, all the developed nanocarriers were found to be more biocompatible and effective than the marketed product. These encouraging findings can guide in proper selection of topical carriers among diversity of such available carriers systems.

1. Introduction

Tretinoin, all-*trans* retinoic acid, is frequently used in topical form for the treatment of various dermatological problems such as acne, psoriasis, skin carcinoma and photoaging, and also for regulating growth and differentiation of epithelial cells, sebum production and collagen synthesis (Zouboulis, 2001; Ourique et al., 2011). Use of topical TRE is though reported for acne treatment since 1962, yet was approved by US-FDA in 1971. It is the single first generation retinoid which is available in the US market for topical acne treatment, whereas isotretinoin is also used in other parts of world, except in the US (Thielitz and Gollnick, 2008; Ourique et al., 2011). It influences a range of cellular processes, like cell surface alterations, cellular growth and differentiation and immune modulation. Several of the tissue effects are mediated by its interaction with specific cellular and nucleic acid receptors. The nuclear retinoic acid receptor family (RARs) consists of three main forms (RAR-α, RAR-β, RAR-γ) and is activated by TRE. By interaction with RARs, TRE executes its effect by one or more mechanism(s): (i) epidermal thickening due to initiation of epidermal proliferation; (ii) compaction of the stratum corneum; and (iii) biosynthesis and deposition of the glycosoaminoglycans (Griffiths et al., 1993; Mukherjee et al., 2006). Despite marked efficacy, it is not widely accepted by patients as around 88% of the patients receiving topical TRE report side effects like desquamation and erythema (Bulengo-Ransby et al., 1993). Other common problems with topical TRE are skin irritation, peeling and erythema, and physicochemical challenges like poor stability in air, light, and heat. It is so unstable that around 70% TRE is reported to be degraded after only 10 min of irradiation (Ourique et al., 2011). Additionally, its low aqueous solubility limits its incorporation in a suitable aqueous vehicle and compels the use of relatively toxic co-solvents such as ethanol and propylene glycol in the commercial products (Morrow et al., 2007; Ourique et al., 2011).

Colloidal drug delivery carriers have been successfully employed for the photo-protection, efficacy enhancement and targeting of a variety of drugs thereby augmenting the confidence level on such drug delivery carriers day-by-day (Katare et al., 2010; Shiva et al., 2012). Among a myriad of nano-colloidal carriers, each system has unique advantage(s) *vis-à-vis* the other. The

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phospholipid-based colloidal carriers, however, are reported to possess better drug delivery potential and offer more biocompatibility (Katare et al., 2010; Raza et al., 2013a,b). Only a couple of articles are traceable for comparing the drug delivery and efficacy enhancement attributes of various colloidal carriers, that too without phospholipids (Lin et al., 2013). Therefore, the present study aims to develop phospholipid-based particulate (SLNs and NLCs) and vesicular (liposomes and ethosomes) nano-colloidal carriers of TRE and to explore the impact of the carrier type on various critical attributes of drug, ranging from topical delivery, stability, biocompatibility and therapeutic efficacy.

2. Materials

Tretinoin (TRE; M/s Shalaks Pharmaceuticals, New Delhi, India), phosphatidylcholine (PL; Phospholipon 90 G; M/s Phospholipid GmbH, Nattermannallee, Germany) and Compritol 888 ATO GF3123 (M/s Gattefosse, Hauptstrasse, Germany; M/s Colorcon, Mumbai, India) were supplied *ex-gratis* by the respective companies. Isopropyl myristate (IPM; M/s Loba Chemie, Mumbai, India), butylated hydroxy toluene (BHT; M/s S.D. Fine Chemicals Ltd., Mumbai, India), cholesterol (CHOL; M/s Hi Media Lab. Ltd., Mumbai, India) and absolute alcohol (M/s Hong Yang Chemical Corporation, Xinxiang Nanlu, China) were procured from the respective sources. All the chemicals used in the presented study were of analytical grade, and were used as such without any further purification. Ultrapure water (Milli-Q® Integral system; M/s Merck Millipore, Billerica, USA) was employed throughout the study. Retino A™ cream (marketed tretinoin formulation; Treat-M; Batch No. Ko44; manufacturing date: January 2007; M/s Johnson & Johnson, Mumbai, India) was procured from local drug store.

3. Methods

Considering the photostability issues with TRE, all the formulation and characterization studies were performed in dark.

3.1. Preparation of the TRE-loaded nano-colloidal carriers

TRE-loaded liposomes were prepared by thin-film hydration technique (Raza et al., 2009). In a nutshell, TRE (5 mg), PL (400 mg), BHT (2.5 mg) and CHOL (40 mg) were dissolved in minimum volume of chloroform-methanol mixture (2:1 v/v) contained in a 100 mL round bottomed flask. The organic solvent was evaporated at 45 ± 1 °C and 80 rpm under reduced pressure using a rotary film evaporator (Buchi RE 121, Switzerland). After complete vapourization of the solvent, the flask was kept under vacuum for an overnight to remove remaining traces of the organic solvent. The obtained thin lipid film was hydrated at 45 ± 1 °C and 60 rpm with normal saline (20 mL) as the aqueous phase, using a rotary film evaporator. The obtained liposomal dispersion was kept at room temperature for 2 h for complete hydration of the PL.

Ethosomes were prepared by the cold method (Rakesh and Anoop, 2012). PL (400 mg), TRE (5 mg) and BHT (2.5 mg) were dissolved in 4 mL of ethanol to yield a clear yellow solution. This ethanolic solution was added in a streamlined fashion to normal saline (q.s. to 20 mL) continuously mechanically stirred at 900 rpm (RQ 122; M/s Remi Motors, Mumbai, India). Stirring was continued additionally for 5 min to form milky ethosomal dispersion.

The SLNs and NLCs were prepared by microemulsification technique (Raza et al., 2013b,c). For SLNs, TRE (5 mg) and BHT (2.5 mg) were dissolved in ethanol (0.8 g), while PL (200 mg) was dispersed in 10 mL of water along with Tween 80 (1.2 g). Compritol (200 mg) was melted at 70 °C, and mixed isothermally with the aqueous phase and drug solution to obtain a clear microemulsion. The hot microemulsion formed was poured into the remaining portion of cold water (q.s. to 10 mL), maintained at 4 °C. The resulting dispersion was stirred continuously at 3000 rpm for 20 min. The NLCs were prepared analogously with an extra component as IPM (60 mg), which was added to the alcoholic phase, and employing amount of Compritol as 140 mg.

3.1.1. Incorporation of nanocarriers in hydrogels

Incorporation of nanocarriers in the hydrocolloids is most widely employed technique for ease of application, adhesion and required drug penetration (Aggarwal and Goindi, 2012; Raza et al., 2013c). To make the nano-dispersions spreadable for topical application, these were further incorporated in 1.5% by weight of neutralized Carbopol® 934 hydrogel.

3.1.2. Ethical compliance

Taking cognizance that the research work adheres to the guidelines for the care and use of laboratory animals, all the animal investigations were performed as per the protocol approved by the Panjab University Animal Ethics Committee, duly approved for the purpose of control and supervision of experiments on animals by the Government of India (Ref. letter No. CAH/09/70; IAEC/156).

3.1.3. Statistical analysis

Multiple comparisons were made using one-way analysis of variance ANOVA followed by *post hoc* analysis using Student's *t*-test. Statistical significance was considered at $p < 0.05$.

3.2. Characterization of the developed nano-carriers

3.2.1. Micromeritics, surface charge and morphology

Particle size and zeta potential of the developed nano-carrier were determined using Delsa Nano particle analyzer (M/s Beckman Coulter, Inc., CA, USA) installed at Institute of Microbial Technology, Chandigarh. The mean value of three repeated measurements for each sample was reported as the final result. Transmission electron microscopy (TEM) was employed to determine the morphology of the nano-colloidal carriers. The developed carriers were negatively stained with aqueous solution of phosphotungstic acid (1% by mass), subsequently dried on a microscopic carbon-coated grid and viewed under microscope at suitable magnifications.

3.2.2. Percent entrapment efficiency (PDE)

In case of liposomes and ethosomes, mini-column centrifugation technique was used to removal the unentrapped drug (Raza et al., 2009). In brief, pre-saturated Sephadex G 50 columns were pre-saturated with plain (without drug) vesicular carriers. After pre-saturation of the columns, the drug-loaded vesicular carriers (0.2 mL) were placed in the column. Centrifugation was carried out at 3000 rpm (1565 × *g*) for 3 min to elute the drug-loaded vesicles. The eluted vesicles were collected and observed under microscope for the absence of un-entrapped drug crystal(s). The column was washed again with 0.2 mL of water and the eluate obtained was pooled with the previously collected eluate. Thereafter, a volume of 0.2 mL of the eluted vesicular dispersion was digested with methanol and analyzed for drug content employing a validated reverse phase high performance liquid chromatographic (RP-HPLC) method (Raza et al., 2013b). In brief, LC-2010CHT liquid chromatograph (Schimadzu, Japan) was run at 355 nm employing a reverse phase C-18 column of length 150 × 4.6 mm, 3 μ ODS1 Spherisorb (M/s Waters Corporation, Milford, USA). The mobile phase was composed of glacial acetic acid 1.96% v/v: methanol (27:73 v/v) and was delivered at a flow rate of 1.0 mL/min (Raza et al., 2013d).

In case of SLNs and NLCs, the dispersion was lyophilized and washed with methanol to remove the un-entrapped drug.