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Development of novel diolein–niosomes for cutaneous delivery of tretinoin: Influence of formulation and *in vitro* assessment

Maria Letizia Manca [a], Maria Manconi [a], Amparo Nacher [b], Claudia Carbone [c], Donatella Valenti [a], Anna Maria Maccioni [a], Chiara Sinico [a], Anna Maria Fadda [a,*]

[a] Department of Life and Environmental Sciences, CNBS, University of Cagliari, Cagliari, Italy
[b] Department of Pharmacy and Pharmaceutical Technology, University of Valencia, Burjassot, Valencia 46100, Spain
[c] Laboratory of Drug Delivery Technology, Department of Drug Sciences, University of Catania, Italy

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ABSTRACT

This work describes innovative niosomes, composed of diolein alone or in association with the hydrophilic penetration enhancer Labrasol®, as carriers for cutaneous drug delivery. The model drug was tretinoin and conventional, and Labrasol® containing liposomes was used as controls to evaluate the influence of vesicle composition and the role of Labrasol® on vesicle physico-chemical properties and performance as skin delivery system.

Vesicles, prepared by the thin film hydration technique, were characterized in terms of size distribution, morphology, zeta potential, structure, incorporation efficiency, and rheological properties. The influence of carrier composition on tretinoin delivery to human skin was evaluated by *in vitro* percutaneous experiments, while formulation distribution on human skin and cellular uptake in human keratinocytes were studied using confocal laser scanning microscopy.

Result showed that tretinoin loaded diolein–niosomes formed unilamellar vesicles very similar in physico-chemical properties to liposomes. The role of Labrasol® was similar in niosomes and liposomes. Its addition affected vesicle structure and size, by formation of an interdigitate bilayer with higher curvature and larger vesicle size, and rheological properties. Indeed, the presence of Labrasol® allowed both niosomes and liposomes to shift from Newtonian to pseudo-plastic behavior.

Confocal laser microscopy highlighted an important contemporaneous deposition of hydrophilic and lipophilic vesicle components in stratum corneum and a high vesicle affinity for skin appendages when Labrasol® was added to the diolein–niosomes. Moreover, all samples were internalized in human keratinocytes *in vitro*.

1. Introduction

Design of new vehicles suitable for dermal delivery represents an important goal of pharmaceutical research, since, therapy of dermatological disorders can benefit from topical application of drugs. Several nanocarriers have been largely studied as drug delivery systems (DDS) for improving treatment of skin pathologies. Among these, vesicles, such as liposomes and niosomes provide an alternative for improved drug local efficacy, although, their function as skin DDS is controversial with variable effects being reported in relation to the type of vesicles and their composition. Therefore, in the attempt to improve drug delivery into and through the skin, over the last two decades new classes of lipid vesicles have been developed. These include transfersomes, ethosomes, glycerosomes, and the so called penetration enhancer-containing vesicles (PEVs), *i.e.*, phospholipid vesicles containing in their composition a penetration enhancer (PE) (Ainbinder et al., 2010; Cevc et al., 1998; Chessa et al., 2011; Manca et al., 2013b; Manconi et al., 2012, 2011; Mura et al., 2011, 2009; Sinico and Fadda, 2009). Several PEs were tested as secondary components of phospholipid vesicles and their effect on skin delivery of different drugs was evaluated. Among others, the addition of the well known hydrophilic PE Labrasol® (a mixture of hydrophilic surfactants containing caprylocaproyl 104 macrogol 8-glyceride) has shown promising properties in increasing skin deposition of minoxidil, quercetin, and tretinoin (Chessa et al., 2011; Manconi et al., 2011; Mura et al., 2009). Results of *ex vivo* penetration and permeation studies have shown that the presence of such a PE

* Corresponding author at: Department of Life and Environmental Sciences, CNBS, University of Cagliari, Via Ospedale 72, 09124, Cagliari, Italy.
Tel.: +39 706758716; fax: +39 706758710.
E-mail address: mfadda@unica.it (A.M. Fadda).

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molecule in the composition of PEVs was especially crucial for improving dermal delivery of a lipophilic drug, such as tretinoin (Manconi et al., 2011).

In the last years, research concerning lipid vesicles suitable to allow drugs to overcome the skin barrier has been particularly focused on phospholipid systems. However, the advantages offered by niosomes (*i.e.*, lower costs, higher stability, and great availability of surfactant classes) have led researchers to also investigate these non-ionic surfactant vesicles (NSVs) as an alternative.

Q2 Since the first reports from L'Oreal laboratories in the seventies (Vanlerberghe and Handjani-Vila, 1975; Handjani-Vila et al., 1979), much research has been carried out into the vesicle forming ability of an ever increasing number of amphiphilic lipids, showing different chemical structures with different hydrophilic and hydrophobic moiety (Carafa et al., 1998; Gopinath et al., 2004, 2002; Manconi et al., 2002; Muzzalupo et al., 2007; Paolino et al., 2008; Sinico and Fadda, 2009; Uchegbu and Florence, 1995; Uchegbu and Vyas, 1998).

Combining these results and aiming at designing a suitable formulation for skin delivery, new NSVs were obtained using a surfactant mixture mainly containing polyglyceryl-3 dioleate (diolein, Plurol® Oleique CC), which, similarly to phospholipids, possesses a hydrophilic head and two acyl chains and can be used as a cheap alternative to form lamellar vesicles. Dioleins are biodegradable, non-toxic, biocompatible excipients, usually used in pharmaceutical formulations as penetration enhancers for their ability to temporarily and reversibly disrupt the ordered lamellar structure of stratum corneum (SC) (Steluti et al., 2005). Moreover, similarly to PEVs, diolein vesicles were also prepared by adding in their composition the hydrophilic Labrasol® to verify the effect of this PE on the niosome properties.

In this work, tretinoin (TRA) was chosen as the model drug because it is topically used in the treatment of proliferative and inflammatory skin diseases, such as psoriasis, acne, and epithelial skin cancer. However, its topical use is limited by several drawbacks, such as low aqueous solubility and high instability in the presence of air, light, and heat. Moreover, its topical application may causes irritation and peeling of the treated area (Manconi et al., 2006). Local administration of this drug into appropriate delivery systems would permit to circumvent these problems, and appears to be a promising approach to increase drug bioavailability in the skin. Above all, liposomes and niosomes seem to be ideal candidates to this purpose as their capability of improving skin accumulation of different drugs has already been demonstrated (Sinico et al., 2005; Tavano et al., 2010a,b). Literature has shown that TRA-incorporated liposomes and niosomes are able to reduce drug photodegradation and improve *ex vivo* drug localization in the superficial skin layers (Manconi et al., 2006; Marianecci et al., 2014; Sinico et al., 2005; Trapasso et al., 2009).

Therefore, the first aim of this work was to test the diolein ability to form tretinoin loading vesicles and to evaluate their physico-chemical features and skin delivery properties in comparison with those of liposomes. In addition, a further aim was to study the influence of Labrasol® association to the diolein–niosomes on their properties as well as on their ability to deliver TRA into the human skin and keratinocytes. Indeed, to the best of our knowledge, Labrasol® has never been used before in association with non-ionic surfactants in the formulation of NSVs, and therefore, the assessment of its influence on the vesicle properties, in comparison with phospholipid vesicles, seems to be of value.

Hence, obtained diolein vesicle features and tretinoin carrier ability were evaluated and compared with those of phospholipid liposomes. In addition, the hydrophilic PE Labrasol® was added to both liposomes and niosomes to give Lab-PEVs or the new PE-containing niosomes (Lab-NSVs).

Moreover, an in depth and detailed study was performed to evaluate the influence of vesicle composition on their structure and properties. Therefore, all prepared vesicles were characterized in terms of structure, size distribution, surface charge, stability, incorporation efficiency (*E%*), and rheological behavior. In particular, effective vesicle formation and structure were confirmed by transmission electron microscopy (TEM) and small and wide-angle X-ray scattering (SWAXS). The influence of the carrier composition on tretinoin delivery to human skin was evaluated *in vitro* by percutaneous experiments and confocal laser scanning microscopy (CLSM) investigations. Finally, human epidermal keratinocyte cells were used as an appropriate *in vitro* model to evaluate cellular uptake of TRA incorporating vesicles by CSLM observations.

2. Experimental methods

2.1. Materials

Phospholipon® 50 (P50) a mixture of soy lipids (45% of phosphatidylcholine, 10–18% of phosphatidylethanolamine, 37% of various polar lipids, and 3% of triglycerides) and 1,2-dioleolyl-sn-glycero-3-phosphoethanolamine-*N*-lissamine-sulfo-rhodamineB (Rho-PE) were purchased by AVG Srl (Garbagnate Milanese, Milan, Italy), local distributor of Lipoid GmbH (Ludwigshafen, Germany). Labrasol® (Lab 30% of mono-, di-, and tri-glycerides of C8 and C10 fatty acids, 50% of mono- and di-esters of PEG, 20% of free PEG 400; HLB 14) and Plurol® Oleique CC (PO, polyglyceryl-3 dioleate, HLB 6) were kindly provided by Gattefossé (Saint Priest, France). Phosphate buffer (PBS, pH 7) was purchased from carlo erba reagents (Rodano, Italy). *trans*-Retinoic acid (TRA), 5(6)-carboxy-fluorescein (CF), Hoechst 33,258, and all other products were of analytical grade and were purchased from Sigma–Aldrich (Milan, Italy). Tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and other cell medium and reactive were purchased by Life Technologies Europe, (Monza, Italy).

2.2. Vesicle preparation

Multilamellar vesicles (MLVs) were prepared according to the thin film hydration method. Vesicle components, P50 (120 mg/ml) or Plurol Oleique (100 mg/ml), Labrasol® (100 mg/ml) when appropriate, and TRA (3.0 mg/ml) were dissolved in chloroform and lipid mixture was deposited as a thin film in a round bottom flask by rotoevaporating the solvent under vacuum (Rotavapor Buchi R110, Flawil, Switzerland). The thin lipid film was then hydrated using PBS under mechanical stirring at room temperature for two hours. MLV dispersions were sonicated for 60 s (3 s on and 3 s off) using a Soniprep 150 ultrasonic disintegrator (MSE Crowley, London, UK). Sample compositions are reported in Table 1.

Table 1
Composition of TRA loaded liposomes and niosomes.

	TRA (mg/ml)	P50 (mg/ml)	PO (mg/ml)	Chol (mg/ml)	Lab (mg/ml)
Liposomes	3.0	120	–	–	–
Lab-PEVs	3.0	120	–	–	100
Diolein-NSVs	3.0	–	100	20	–
Lab-NSVs	3.0	–	100	–	100

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