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Penetration enhancer containing vesicles as carriers for dermal delivery of tretinoin

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ABSTRACT

The ability of a recently developed novel class of liposomes to promote dermal delivery of tretinoin (TRA) was evaluated. New penetration enhancer-containing vesicles (PEVs) were prepared adding to conventional phosphatidylcholine vesicles (control liposomes) different hydrophilic penetration enhancers: Oramix® NS10 (OrNS10), Labrasol® (Lab), Transcutol® P (Trc), and propylene glycol (PG). Vesicles were characterized by morphology, size distribution, zeta potential, incorporation efficiency, stability, rheological behaviour, and deformability. Small, negatively charged, non-deformable, multilamellar vesicles were obtained. Rheological studies showed that PEVs had fluidity higher than conventional liposomes.

The influence of the obtained PEVs on (trans)dermal delivery of tretinoin was studied by *ex vivo* diffusion experiments through new born pig skin using formulations having the drug both inside and outside the vesicles, having TRA only inside, in comparison with non-incorporated drug dispersions of the same composition used to produce the studied vesicles. Main result of these experiments was an improved cutaneous drug accumulation and a reduced transdermal TRA delivery (except for PG-PEVs). TRA deposition provided by PEVs was higher for dialysed than for non-dialysed vesicles. Further, the accumulation increased in the order: control liposomes < PG-PEVs < Trc-PEVs ≤ Or-PEVs < Lab-PEVs. SEM analysis of the skin gave evidence of PEVs' ability to strongly interact with the intercellular lipids causing an enlargement of this region.

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1. Introduction

Liposomal formulations have been extensively studied to enhance efficiency of (trans)dermal drug delivery, since they offer many advantages over traditional topical dosage forms such as enhancement of drug effectiveness and bioavailability as well as reduction of side effects. However, the function of vesicles as transdermal or dermal delivery systems is controversial. Therefore, in the last two decades, as an alternative to liposomes and niosomes, innovative vesicular systems have been proposed (Paolino et al., 2008; Sinico and Fadda, 2009; Tavano et al., 2010). Several authors have investigated the ability of different molecules to modify vesicular bilayer properties, increasing their deformability and capability of delivering drugs to and through the skin. Although most studies have reported that high deformable liposomes are especially able to enhance transdermal drug delivery, this class of lipid vesicles has also been reported to improve *ex vivo* cutaneous drug deposition with a higher effectiveness than conventional vesicles (Cevc and Blume, 1992; van den Bergh, 1999; Touitou et al., 2000; Bouwstra and Honeywell-Nguyen, 2002; Paolino et al., 2005; Elsayed et al., 2006). Recently, different penetration enhancer (PE) molecules have been tested as "edge activator" in the formulation of new deformable vesicles by several authors (Verma, 2002; El Maghraby et al., 2004; Elsayed et al., 2007; Dragicevic-Curic et al., 2008; Manconi et al., 2009). In previous studies, we introduced the acronym PEVs to identify the penetration enhancer containing vesicles as carriers for dermal delivery of minoxidil and diclofenac (Manconi et al., 2009, 2011; Mura et al., 2009, 2011). These vesicles have shown to possess the advantage to combine liposome potential as carriers and PE ability to modify the order of stratum corneum (SC) packing (Mura et al., 2009, 2011), thus promoting skin delivery. Moreover, during last years the benefits of *trans*-retinoic acid (TRA) incorporation in liposomes and niosomes on its dermal delivery have also been extensively studied (Trapasso et al., 2009). *Trans*-retinoic acid or tretinoin (TRA) is a natural retinoid widely used in proliferative and inflammatory skin diseases, such

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as psoriasis, acne, and epithelial skin cancer. Unfortunately, this drug is very unstable in the presence of air, light and heat and, in addition, its topical application may cause irritation and peeling of the treated area (Manconi et al., 2006). Our previous works demonstrated that TRA-incorporated liposomes and niosomes are able to improve *ex vivo* drug localization in the superficial skin layers (Sinico et al., 2005; Manconi et al., 2006). Indeed, especially niosomes were shown to dramatically enhance the drug cutaneous retention, mostly if compared to the commercial formulation RetinA® and phosphatidylcholine liposomes. Furthermore, liposomes saturated with TRA demonstrated to be significantly more efficient in promoting drug accumulation than the corresponding non-saturated vesicles (Manconi et al., 2006).

Taking into account these results, with the aim of finding new formulations capable of improving TRA cutaneous retention, new PEVs were studied. In particular, in this work, PEVs were prepared using soy phosphatidylcholine (Phospholipon 50, P50) and one of four selected hydrophilic PEs: decylpolyglucoside (Oramix® NS10, OrNS10), caprylocaproyl macrogol 8-glyceride (Labrasol®, Lab), 2-(2-ethoxyethoxy)ethanol (Transcutol® P, Trc), and propylene glycol (PG). As a consequence of the results obtained in previous studies with Transcutol, in this work we evaluated only the influence of hydrophilic PEs. In fact, the further aim of this study is to compare Transcutol containing PEVs' properties to those of vesicles obtained with PEs with similar hydrophilic characteristic but different chemical structure. Therefore, all prepared formulations were obtained using the same PE concentration (i.e. 0.6%, w/v). Moreover, in an attempt to understand the PE capability of interacting with vesicle bilayer and affecting its properties, a rheological study was carried out. To shed some light on the possible mechanism by which these formulations act, *ex vivo* skin permeation and deposition behaviour of the PEVs was also studied using new born pig skin. To this purpose, formulations having the drug both inside and outside the vesicles (non-dialysed, ND), having TRA only inside (dialysed, D), and non-incorporated drug dispersions of the same composition used to produce the studied vesicles (MIX) were tested. Furthermore, to elucidate the effect and interaction between PEVs and skin, Scanning Electron Microscopy (SEM) was used to visualize the new born pig skin structure after the liposomal administration.

2. Materials and methods

2.1. Materials

Soy phosphatidylcholine (Phospholipon 50, P50) was kindly supplied by AVG S.r.l. (Garbagnate Milanese, Milan, Italy) and Lipoid GmbH (Ludwigshafen, Germany). Decylpolyglucoside (Oramix® NS10, OrNS10) was kindly donated by Seppic (Milan, Italy). 2-(2-Ethoxyethoxy)ethanol (Transcutol® P, Trc), caprylocaproyl macrogol 8-glyceride (Labrasol®, Lab) were a gift from Gattefossè (Saint Priest, France). Propylene glycol (PG), *trans*-retinoic acid (TRA), and all the other products were of analytical grade and were purchased from Sigma–Aldrich (Milan, Italy).

2.2. Vesicle preparation

Multilamellar liposomes (control) and PEVs were prepared according to the thin film hydration method but with a slight modification (hydration in two steps) that allowed us to obtain more homogeneous vesicular populations (polydispersity index < 0.4). P50 (1.2 g), TRA (22 mg) and PE (0.06 g), when appropriate, were dissolved in chloroform. The excess of drug (22 mg) was used to obtain saturated vesicles, which have shown to better promote drug accumulation in pig skin (Manconi et al., 2006). The lipid mixture was deposited as a thin film in a round-bottom flask by roto-evaporating (Rotavapor Büchi R110, Switzerland) the chloroform under vacuum, which was applied for 1 h to ensure total removal of solvent traces. Hydration of the film was performed in two steps: first, 5 ml of phosphate buffer solution (PBS, pH 7) was added to the flask and the concentrated dispersion was mechanically shaken for 1 h at room temperature. Then, a second 5 ml aliquot of PBS was added and the dispersion shaken for another hour. PE final concentration was 0.6% (w/v). All suspensions were prepared under yellow light and kept in the dark at all times. The presence of TRA crystals, which was used as an indicator of vesicle saturation with the drug, was checked using light microscopy. Each vesicle suspension was then purified from the non-incorporated drug by centrifugation (5000 rpm for 5 min), followed by exhaustive dialysis, avoiding any sample dilution and allowing a more precise dosage of tretinoin-loaded vesicles. Dispersions were loaded into dialysis tubing (Spectra/Por® membranes: 12–14 kDa MW cut-off, 3 nm pore size; Spectrum Laboratories Inc., USA) and dialysed against PBS at 4 ± 1 °C. Dialysis of each sample (2 ml) was carried out in 1000 ml of PBS that was changed 3 times during 24 h. Incorporation efficiency (*E*%), expressed as the percentage of the amount of TRA initially used, was determined by high performance liquid chromatography (HPLC) after disruption of vesicles with 0.025% non-ionic Triton X-100. TRA content was quantified at 350 nm using a chromatograph Alliance 2690 (Waters, Italy). The column was a Simmetry C18 (3.5 μm, 4.6 × 100 mm, Waters). The mobile phase was a mixture of acetonitrile, water and acetic acid (84.5:15:0.5, v/v), delivered at a flow rate of 1.2 ml/min.

2.3. Vesicle characterization

Vesicles were characterized by Transmission Electron Microscopy (TEM) for vesicle formation and morphology. A drop of the vesicular dispersion was applied to a carbon film-covered copper grid and stained with a 1% phosphotungstic acid. Then, samples were examined with a JEM-1010 (Jeol Europe, France) transmission electron microscope equipped with a digital camera MegaView III and Software "AnalySIS", at an accelerating voltage of 80 kV.

The average diameter and polydispersity index (P.I.) of the samples were determined by Photon Correlation Spectroscopy (PCS) using a Zetasizer nano-ZS (Malvern Instrument, UK). Samples were backscattered by a helium–neon laser (633 nm) at an angle of 173° and a constant temperature of 25 °C. The instrument systematically and automatically adapts to the sample by adjusting the intensity of the laser and the attenuator of the photomultiplier, thus ensuring reproducibility of the experimental measurement conditions. The P.I. was used as a measure of the width of the size distribution. P.I. less than 0.4 indicates a homogenous and monodisperse population. Zeta potential was estimated using the Zetasizer nano-ZS by means of the M3-PALS (Phase Analysis Light Scattering) technique, which measures the particle electrophoretic mobility in a thermostated cell. All the samples were analysed 24 h after their preparation.

A long-term stability study was performed by monitoring the vesicle average size, polydispersity, and surface charge over 90 days at 4 ± 1 °C. Tretinoin retention and chemical stability were also checked by measuring the amount of drug retained during storage, after dialysis, by HPLC.

2.4. Vesicle viscosity measurements

Just before the analyses, P50 dispersions were frozen and defrosted to break vesicle bilayer, in order to avoid their reconstitution prior to viscosity and shear rate measurements. The rheology study was carried out on a Bohlin Visco 88 rheometer (Bohlin Instruments, Cranbury, NJ) equipped with eight rotational speed

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