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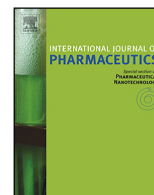

Pharmaceutical nanotechnology

Effect of diclofenac and glycol intercalation on structural assembly of phospholipid lamellar vesicles

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ABSTRACT

The aim of the current study was to improve the knowledge of drug–glycol–phospholipid-interactions and their effects in lamellar vesicle suitability as drug delivery systems. Liposomes were prepared using hydrogenated soy phosphatidylcholine (P90H, 60 mg/ml) and diclofenac sodium salt at two concentrations (5–10 mg/ml). To obtain innovative vesicles two permeation enhancers with glycol group, diethyleneglycol monoethyl ether and propylene glycol, were added to the water phase at different ratios (5%, 10%, and 20%).

Vesicle organization was deeply investigated by physico-chemical characterization, including differential scanning calorimetry and small-angle diffraction signal analysis while macroscopic structure behavior was evaluated by rheological studies. Results evidenced that the presence of the penetration enhancer and diclofenac sodium salt led to structural rearrangements within and among vesicles forming a tridimensional and complex architecture in which vesicles were closely packed and interconnected. This new design allowed a change in the physical state of dispersions that became highly viscous liquid or soft-solid-like, thus forming an ideal system for topical application able of both adhering to the skin and delivering the drug.

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1. Introduction

Several studies have been carried out to explore the interactions of drug or other components with lamellar vesicles and their effects on the bilayer structure (Kranenburg et al., 2004; Pignatello et al., 2006; Rosser et al., 1999). Any small molecules might cause bilayer packing alteration and among these chemicals, aliphatic alcohols are the best known and studied (Adachi et al., 1995; Kranenburg et al., 2004; Löbbecke and Cevc, 1995). In particular, a large number of studies have been focused on ethanol effects in phospholipid vesicles (Celia et al., 2009; Touitou et al., 2000). Results suggested that ethanol plays a significant role in the arrangements of phospholipids on the bilayer, changing the acyl chain orientation inside the lamellar structure and usually forming an interdigitated structure (Adachi et al., 1995; Komatsu and Okada, 1995; Simon and McIntosh, 1984). Thanks to this, ethanol forms soft and fluid lamellar vesicles able to improve skin delivery of several drugs. Alternatively to ethanol, others water miscible cosolvents such as isopropyl alcohol and propylene glycol, have been used as additive of vesicle bilayer to make innovative phospholipid vesicles able to facilitate drug delivery, especially skin delivery (Caddeo et al., 2012; Chessa et al., 2011; Elmoslemany et al., 2012; Elsayed et al., 2007; Manconi et al., 2011, 2012). However, the interactions involved among drug, water, cosolvent and phospholipid and the possible rearrangements that take place into bilayer were not deeply investigated. We recently reported the effect of diethylene glycol monoethyl ether (Transcutol) and propylene glycol on self-assembling and rheological properties of phosphatidylcholine lamellar vesicles and we found that 10–20% of glycols allowed the formation of vesicles with the more suitable and stable features (Manconi et al., 2009).

Here, the interactions between hydrogenated phosphatidylcholine bilayer vesicles and the two glycols, differing in their structure, have been extensively studied. In particular, the effective glycol ability to perturb phospholipid vesicle bilayer and the simultaneous effect of diclofenac sodium salt, were evaluated. This potent antinflammatory drug is commercially available in semisolid preparations for topical application (Escribano et al.,

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2003). In this work it was used as model drug able to perturb phospholipid bilayer structure, thanks to its amphiphilic properties (López et al., 2002; Manconi et al., 2011, 2009; Schreier et al., 2000).

The aim of the current study was to improve the knowledge of drug–glycol–phospholipid-interactions and their effects in lamellar vesicle aptitude as topical carrier. The simultaneous addition of diclofenac sodium salt and a hydrophilic glycol (Transcutol or propylene glycol) to phospholipid dispersion led to the formation of a vesicle structure with a complex intervesicle architecture. These important structural rearrangements were disclosed by a change on physical state from liquid to high viscous liquid or soft-solid like. The effects, at molecular level, have been monitored by combining the information obtained by differential scanning calorimetry (DSC), small- and wide-angle diffraction signals (SAXS) and rheology studies. These techniques permitted to build a detailed picture of intra- and inter-vesicle assembly.

2. Materials and methods

2.1. Materials

Hydrogenated soy phosphatidylcholine (Phospholipon® 90H, P90H) was kindly supplied by AVG S.r.l. (Milan, Italy) and Lipoid GmbH (Ludwigshafen, Germany). Diethylene glycol monoethyl ether (Transcutol® P, Trc) was kindly provided by Gattefossè (Saint Priest, France). Phosphate buffer solution (PBS, pH 7) was purchased from Carlo Erba Reagents (Milan, Italy). Diclofenac sodium salt (DCF_{Na}), cholesterol (Chol), and propylene glycol (PG) were purchased from Sigma–Aldrich (Milan, Italy).

2.2. Vesicle preparation

Liposomes were prepared by using P90H (60 mg/ml), Chol (2 mg/ml), DCF_{Na} (5 and 10 mg/ml) and PBS. PEVs were obtained using the same lipid phase (P90H, Chol), DCF_{Na} (5 and 10 mg/ml) and a mixture of Transcutol/PBS or propylene glycol/PBS (5, 10, 20%, v/v) as water phase (Table 1). All components were weighted in a glass flask, hydrated with the water phase, and finally sonicated (5 s on and 2 s off, 30 cycles; 14 μm of probe amplitude) with a high intensity ultrasonic disintegrator (Soniprep 150, MSE Crowley, London, United Kingdom) (Madrigal-Carballo et al., 2008; Manconi et al., 2003). Each formulation was purified from the non-encapsulated drug by dialysis. Samples were loaded into dialysis tubing (Spectra/Por® membranes: 12–14 kDa MW cut-off, 3 nm pore size; Spectrum Laboratories Inc., DG Breda, The Netherlands) and dialysed against PBS (liposomes) or appropriate glycol/PBS (PEVs) mixtures for 2 h at 5 °C, to allow the dissolution and removal of the non-encapsulated drug and non-aggregated phospholipids.

2.3. Vesicle characterization

Vesicle formation and morphology were assessed by cryo transmission electron microscopy (cryo-TEM) analysis. A thin aqueous film was formed by placing a 5 μl sample drop on a glow-discharged holey carbon grid and then blotting the grid against filter paper. The resulting thin films spanning the grid holes were vitrified by plunging the grid (kept at 100% humidity and room temperature) into ethane, which was maintained at its melting point with liquid nitrogen, using a Vitrobot (FEI Company, Eindhoven, The Netherlands). The vitreous films were transferred to a Tecnai F20 TEM (FEI Company) using a Gatan cryotransfer (Gatan, Pleasanton, CA), and the samples were observed in a low dose mode. Images were acquired at 200 kV at a temperature between 170 and 175 °C, using low-dose imaging conditions not exceeding 20 $e^-/Å^2$, with a CCD Eagle camera (FEI Company).

The average diameter and polydispersity index (PI), as a measure of the width of the size distribution of the samples, were determined by Photon Correlation Spectroscopy using a Zetasizer nano-ZS (Malvern Instruments, Worcestershire United Kingdom). Samples were backscattered by a helium–neon laser (633 nm) at an angle of 173° and a constant temperature of 25 °C. Zeta potential was estimated using the Zetasizer nano-ZS by means of the M3-PALS (Mixed Mode Measurement-Phase Analysis Light Scattering) technique, which measures the particle electrophoretic mobility in a thermostated cell. Each sample (10 μl) was diluted with 10 ml of PBS or the appropriate glycol/PBS mixture before analysis.

Drug entrapment efficiency (EE%), expressed as the percentage of the drug amount after dialysis versus that initially used, was determined by high performance liquid chromatography (HPLC) after disruption of vesicles with methanol (1:1000 dilution). Diclofenac sodium content was quantified at 227 nm using a chromatograph Alliance 2690 (Waters, Milan, Italy) equipped with a column Symmetry C18 (3.5 lm, 4.6 100 mm, Waters). The mobile phase was a mixture of water/acetonitrile (30/70, v/v), delivered at a flow rate of 0.5 ml/min (Manconi et al., 2011).

The lipid content of dispersions was determined by the Stewart (1980) assay. Briefly, an aliquot of liposomes or PEV dispersions was added to a biphasic mixture of aqueous ammonium ferrithiocyanate solution (0.1 N) and chloroform. The concentration of P90H was obtained by measuring absorbance at 485 nm of the organic

Table 1
Acronyms, composition and physical state of liposomes and PEVs. Vesicles appeared as liquid, high viscous liquid (HV liquid) or soft solid.

Sample	P90H (mg/ml)	Chol (mg/ml)	DCF_{Na} (mg/ml)	Trc (%v/v)	PG (%v/v)	PBS (%v/v)	Physical state
Empty liposomes	60	2	0	0	0	100	Liquid
5DCF liposomes	60	2	5	0	0	100	Liquid
5DCF 5Trc-PEVs	60	2	5	5	0	95	Liquid
5DCF 10Trc-PEVs	60	2	5	10	0	90	HV liquid
5DCF 20Trc-PEVs	60	2	5	20	0	80	Soft-solid
5DCF 5PG-PEVs	60	2	5	0	5	95	Liquid
5DCF 10PG-PEVs	60	2	5	0	10	90	HV liquid
5DCF 20PG-PEVs	60	2	5	0	20	80	Soft-solid
10DCF liposomes	60	2	10	0	0	100	Liquid
10DCF 5Trc-PEVs	60	2	10	5	0	95	HV liquid
10DCF 10Trc-PEVs	60	2	10	10	0	90	Soft-solid
10DCF 20Trc-PEVs	60	2	10	20	0	80	Soft-solid
10DCF 5PG-PEVs	60	2	10	0	5	95	HV liquid
10DCF 10PG-PEVs	60	2	10	0	10	90	HV liquid
10DCF 20PG-PEVs	60	2	10	0	20	80	Soft-solid

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