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Comparison of liposomal drug formulations for transdermal iontophoretic drug delivery

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ABSTRACT

This study was aimed to evaluate the *in vitro* transdermal direct/pulsed current iontophoretic delivery of an amphiphilic model compound from various lipid vesicle-encapsulated formulations compared to free-drug formulation. Conventional, pegylated, ultradeformable liposomes (transfersomes) and ethosomes loaded with a negatively charged drug diclofenac sodium (DS) were prepared and characterized. All the liposomes possessed an average size of ≈ 100–150 nm and negative zeta potential. No changes in colloidal stability were detected after 8 h incubation of any vesicle formulation under constant or pulsed iontophoretic current. DS was released from all the liposome formulations with a similar, limited rate (≈ 50% in 24 h), leading therefore to significantly lower transdermal fluxes across full-thickness porcine skin compared to the respective free drug formulation. From the tested lipid vesicle formulations, the transfersomes resulted in the highest passive flux and the ethosomes in the highest iontophoretic flux under direct constant current treatment. Higher negative surface charge of the vesicle led to better transport efficiency due to the higher mobility of the drug carrier under electric field. Pulsed current iontophoresis had no advantage over constant current treatment in combination with any type of lipid vesicular nanocarriers, in contrast to what has been described earlier with drug-loaded polymeric nanocarriers.

1. Introduction

Transdermal delivery of drugs across the skin to the systemic circulation provides a convenient administration route for a variety of clinical indications (Pastore et al., 2015). In addition to avoiding the hepatic first-pass effect and chemical degradation of drugs in the gastrointestinal tract, patient compliance can be improved by reducing the frequency of dosing due to the continuous drug input. Despite of being an attractive alternative to oral and parenteral administration, however, transdermal and topical delivery of therapeutics has been clinically realized only for a handful of drugs, owing to the formidable barrier properties of *stratum corneum*, the outermost layer of skin. Therefore, only a limited number of molecules with appropriate balance of hydro − /lipophilicity, small size, no charge, and relatively high potency are able to pass this layer passively in therapeutic amounts (Kalia et al., 2004). In order the expand the range of molecules being able to overcome such resistance, strategies have been developed to improve the transport across or into the skin by enhancing the permeability properties of the *stratum corneum* or providing a driving force acting directly on the drug (Herwadkar and Banga, 2012; Parhi et al., 2012).

One such technique, iontophoresis, involves an application of mild electric current to deliver ionized or polar molecules across biological membranes (Hirvonen, 2005). The drug dose delivered by iontophoresis is directly proportional to the amount of charge passed through the skin and can be therefore controlled by the electric input of the iontophoretic system: current density, type of the current and the application time of the iontophoretic treatment. The efficiency of drug transport by iontophoresis can therefore be described by a transport number (t_d) that reflects the proportion of the current carried by the drug, as compared to other migrating species in formulation, and is determined by its mobility (μ_d), charge (z_d) and concentration (c_d) (Phipps and Gyory, 1992):

$$t_d = \frac{\mu_d \cdot z_d \cdot c_d}{\sum_i \mu_i \cdot z_i \cdot c_i} \quad (1)$$

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Iontophoresis is usually carried out by a continuous direct current (DC) that is typically considered to be the most efficient current type in transdermal delivery. However, it has been suggested that the long-term use of DC may have the problem of creating a polarizing current that will decrease the efficiency of DC applied (Lawler et al., 1960). This could be overcome by a current delivered in a periodic manner (pulsed current; PC) that allows skin to depolarize and return to its initial state before the onset of next pulse (Zakzewski et al., 1992). PC is also considered to be less damaging to skin and cause less patient discomfort (Clemessy et al., 1994). Furthermore, compared to conventional DC, PC has been more effective in promoting transdermal transport of large drug-like peptides or proteins (Chien et al., 1989; Knoblauch and Moll, 1993; Malinovskaja et al., 2014; Raiman et al., 2004; Singh et al., 1998), or when the compound of interest has been loaded into polymeric nanoparticles prior administration (Malinovskaja-Gomez et al., 2016).

Alternatively, in the search of improved dermal or transdermal delivery, attempts have been made to design new nanosized carrier systems, such as liposomes, micelles, nanoparticles, nanoemulsions and dendrimers, in order to ensure adequate penetration into or across the skin (Cevc and Vierl, 2010). Among those, liposomal carriers have shown to be promising drug delivery systems to transport therapeutics mainly to the different layers of skin, and by special vesicular systems (*e.g.* niosomes, ethosomes, transfersomes *etc.*) also for systemic drug delivery purposes (Pierre and Dos Santos Miranda Costa, 2011). Potential advantages of liposome use include enhanced drug delivery, solubilization of poorly soluble drugs, drug protection against proteolytic degradation, local skin depot for sustained release, reduction of side-effects and incompatibilities, or formation of rate-limiting barrier for systemic absorption (Weiner et al., 1994). There are many reports on the separate use of liposomes and iontophoresis for skin penetration enhancement, however, the combined use of both approaches has gained little attention (Essa et al., 2002a, 2002b, 2004; Fang et al., 1999; Han et al., 2004; Kajimoto et al., 2011; Kigasawa et al., 2012; Vutla et al., 1996). Combining transdermal iontophoresis with liposome-encapsulated formulations could offer some additional benefits, including improvement of drug delivery by liposome membrane/surface charge modifications and more predictable and controlled drug transport, resulting in drug fluxes less dependent on skin variables.

The model drug used in this study was diclofenac sodium (DS), the most widely prescribed non-steroidal anti-inflammatory drug (NSAID) worldwide for the management of acute conditions of inflammation and pain, musculoskeletal disorders, arthritis and dysmenorrhea (Altman et al., 2015). Although widely used by oral administration, alternative delivery approaches would desirable due to the gastrointestinal side effects, such as gastric ulcers and bleeding, extensive first pass metabolism, and short biological half-life. Therefore, topical DS preparations have been developed with the aim of treating local pain and inflammation while limiting systemic exposure and potentially minimizing the risk of side effects associated with the treatment of oral NSAIDs. The physicochemical parameters (small molecular weight, lipophilic nature of the DS form while its salts are water soluble at neutral pH) makes it an excellent candidate drug to be used for transdermal delivery. Although effective passive delivery of DS across the skin has been demonstrated, the iontophoretic administration of this drug should be investigated as means to improve both the rate and extent of drug delivery, as well to decrease the variability of transdermal fluxes.

The objective of this study was to test drug delivery systems that combine drug-loaded lipid vesicles and iontophoresis for the controlled transdermal delivery of small molecular weight hydrophilic model compound. In more detail, we aimed: (Altman et al., 2015) to develop a range of different DS-loaded liposome formulations suitable for transdermal iontophoretic administration, with regards to drug loading, colloidal properties, electrochemical stability and drug release kinetics, (Bahia et al., 2010) to study the effect of the surface charge of liposomal carrier, or (Caddeo et al., 2013) liposome type (conventional *vs.* special vesicular systems) on the permeation of loaded model drug under iontophoretic delivery, and (Cagdas et al., 2011) to determine the suitable iontophoretic current (constant *vs.* pulsed) type to be combined with the DS loaded vesicles.

2. Materials and methods

2.1. Chemicals

Soya phosphatidylcholine (Emulmetik 930; phosphatidylcholine content 95%) was obtained from Lucas Meyer Cosmetics (Champlan, France), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy (polyethylene glycol)-2000] ammonium salt from Avanti Polar Lipids (Alabaster, AL, US), and cholesterol, Tween-80 and DS from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were at least of analytical grade. Deionized water ($\geq 18.2\ M\Omega/cm$ of resistance; Millipore, Molsheim, France) was used to prepare all the solutions.

2.2. Preparation of the liposomes

The conventional liposomes, the pegylated liposomes and the transfersomes were prepared by the classic cast film method. Briefly, phosphatidylcholine (PC), cholesterol (Chol), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-methoxy-polyethylene glycol-2000 ammonium salt (DSPE-PEG2000) and Tween-80 were dissolved in absolute ethanol in a clean, dry, round-bottom flask. The organic solvent was removed by evaporation for 20 min at 45 °C to obtain homogenous thin lipid film on the inner surface of the flask. The deposited film was then hydrated with 2.5 mg/ml DS solution in 10 mM Hepes buffered saline pH = 7.4 for 1 h at 45 °C. The resulting vesicles were extruded 11 times through a 100 nm polycarbonate membrane (Avanti Polar Lipids, Alabaster, AL, US) at 45 °C.

In order to prepare the ethosomes, the lipids and DS were dissolved in absolute ethanol. This mixture was heated to 30 °C ± 1 °C in a water bath. Buffer (10 mM Hepes buffered saline pH = 7.4), also heated to 30 °C ± 1 °C, was added slowly as a fine stream to lipid mixture with constant stirring at 700 rpm in a closed vessel. Mixing was continued for an additional 5 min, while maintaining the system at 30 °C ± 1 °C. The size of the vesicles was reduced by sonication for 15 s at 15 W, followed by extrusion for 11 times through the 100 nm polycarbonate membrane at ambient temperature.

2.3. Characterization of the liposomes

The colloidal characteristics (hydrodynamic diameter, polydispersity index, zeta potential) of the DS loaded liposomes were determined by Malvern Zetasizer Nano (Malvern Instruments, Malvern, UK). The liposome-encapsulated DS was separated from the unentrapped drug by ultracentrifugation at 30000 rpm for 6 h at 4 °C. The liposomes were lysed with absolute ethanol and the released DS was quantified by HPLC. The percent of encapsulation efficiency (EE%) was then calculated according to the following equation:

$$EE\% = \frac{\text{amount of drug in liposomes}}{\text{total amount of drug}} \times 100 \quad (2)$$

The concentration of phospholipids in each formulation was quantified using the phosphate assay method (Rouser et al., 1970). The stability of the liposomes under 100% constant direct current (DC) and 75% on/25% off pulsed current (PC; cathodal, current density 0.5 mA/cm^2, frequency of pulsing 500 Hz) profiles for 8 h was evaluated at 37 °C in small glass vials as a change in hydrodynamic diameter and polydispersity index (PDI).