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Skin penetration and deposition of carboxyfluorescein and temoporfin from different lipid vesicular systems: In vitro study with finite and infinite dosage application

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ABSTRACT

The aim of the present research is to evaluate the influence of different lipid vesicular systems as well as the effect of application mode on skin penetration and deposition behaviors of carboxyfluorescein (hydrophilic model drug) and temoporfin (lipophilic model drug). All of the lipid vesicular systems, including conventional liposomes, invasomes and ethosomes, were prepared by film hydration method and characterized for particle size distribution, ζ-potential, vesicular shape and surface morphology, in vitro human skin penetration and skin deposition. Dynamic light scattering (DLS) and transmission electron microscopy (TEM) defined that all of lipid vesicles had almost spherical structures with low polydispersity (PDI < 0.2) and nanometric size range (*z*-average no more than 150 nm). In addition, all lipid vesicular systems exhibited a negative zeta potential. In vitro skin penetration and deposition experiments demonstrated that, in the case of CF with finite dose application (10 μl/cm^2) and infinite dose application (160 μl/cm^2), lipid vesicular systems, especially ethosomes and invasomes, compared with non-vesicular systems, can significantly improve the delivery of hydrophilic drug such as carboxyfluorescein into skin deep layers or across the skin. While in the case of mTHPC with finite and infinite dose application, most of drug accumulation was observed in the skin superficial layer for both lipid vesicular systems and non-vesicular systems. The results also revealed that the factors influencing the drug skin distribution concern the physicochemical characteristics of the drug, the choice of the vehicle formulation and the application mode applied.

1. Introduction

Skin covers a surface area from 1.5 to 2.0 m^2 and is regarded as the largest organ of the human body. From a pharmaceutical point of view, it offers advantages over other routes of administration, including avoidance of first-pass metabolism, smaller fluctuations in plasma drug levels for repeated dosing, good patient compliance (Brown et al., 2006). However, although skin delivery systems may have the described advantages, most drugs are not amenable to this mode of administration because of the barrier function of the skin. Anatomically, the skin consists of three distinct layers, including stratum corneum (SC) having a thickness of 10–20 μm, viable epidermis (50–100 μm), and dermis (1–2 mm). The most simplistic organizational description of SC is suggested as the classic "brick-and-mortar" assembly with the corneocytes as the bricks and the intercellular lipids as the mortar (Elias, 1983). It is the "brick and mortar" architecture and lipophilic nature of the SC, which primarily accounts for the barrier properties of the skin (Elias, 1983).

During the past decades, numerous techniques have been employed to overcome the barrier posed by the SC to improve transdermal drug delivery, one of which is the employment of lipid vesicular formulations as skin drug delivery systems to enhance drug transport across or into the skin barrier. Lipid vesicular systems such as conventional liposomes (Lasch and Wohlrab, 1986), transfersomes (Cevc, 1996; Cevc and Blume, 1992) and ethosomes (Touitou et al., 2000) offer a promising strategy for achieving the purpose of improved skin drug delivery. It should be pointed out that the great diversity of potential composition of these vesicles influences their physicochemical characteristics such as particle size, charge, thermodynamic phase and bilayer elasticity, which in turn have a significant effect on the interaction between vesicles and the skin and hence on the effectiveness of these vesicles as skin delivery systems.

Moreover, the SC is also known to exhibit selective permeability with respect to the type of diffusing molecules, meaning that the barrier nature of the skin imposes physicochemical limitations to the type of permeants that can traverse the skin, including

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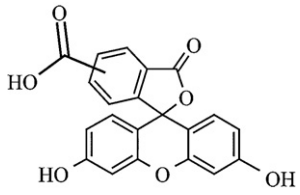

Fig. 1. Structure of 5(6)-carboxyfluorescein.

hydrophilicity, size, hydrogen-bonding ability and so on (Akomeah et al., 2007; Lian et al., 2008; Potts and Guy, 1992). On the other hand, drugs can be encapsulated and located at different positions in the lipid vesicular system according to their lipophilicity. Hydrophilic drugs can be entrapped into the internal aqueous compartment, whereas amphiphilic, lipophilic and charged hydrophilic drugs can be associated with the vesicle bilayer. For these reasons, two model drugs, including carboxyfluorescein (CF, Fig. 1, hydrophilic molecule, $\log P_{o/w} = -1.5$; Nicole et al., 1989) and temoporfin (mTHPC, Fig. 2, lipophilic one, $\log P_{o/w} = 9.24$ (unpublished experimental data from our department)), were selected to assess and compare the skin penetration enhancing effect of different lipid vesicular systems on them.

Furthermore, the enhancement effects and mechanisms of action of these lipid vesicular systems differ among the reports available. The compositions of these formulation change due to penetration into the skin or evaporation of volatile components. These changes depend on the amount of liposome applied and occlusion conditions. Many studies have employed non-occluded conditions, but the application amount was varied. The amounts of formulation have ranged from 10 μl/cm^2 (Dragicevic-Curic et al., 2008; Verma and Fahr, 2004) to 1.5 ml/cm^2 (Elsayed et al., 2007a). These differences may be related to variations in the observed effects of lipid vesicular systems, but there have been few reports aiming to clarify the effects of dose on lipid vesicular systems penetration.

In this study, the influence of different lipid vesicular systems (including conventional liposomes, invasomes and ethosomes) as well as the effect of application mode (including finite dose application and infinite dose application) on skin penetration and deposition behaviors of CF and mTHPC has been investigated. This should provide an insight into the mechanisms of action of different lipid vesicular systems for model drugs with different physicochemical properties when different application modes are used.

2. Materials and methods

2.1. Chemicals

Lipoid S 100 (Phosphatidylcholine from soybean lecithin) was a gift from Lipoid GmbH (Ludwigshafen, Germany). NAT 8539 (Soybean phosphatidylcholine (SPC) dissolved in ethanol containing: 77.3% phosphatidylcholine, 5% lysophosphatidylcholine, 3% cephaline and 1.1% phosphatidic acid of the dry residue) was purchased from Nattermann Phospholipid GmbH (Hermersberg, Germany). 5(6)-Carboxyfluorescein was purchased from Sigma–Aldrich, (Steinheim, Germany). Temoporfin (7,8-dihidro-5,10,15,20-tetrakis-(3-hydroxyphenyl) porphyrin, mTHPC) was a gift from biolitec AG, (Jena, Germany). Methanol (HPLC grade) was purchased from Carl Roth GmbH & Co. (Karlsruhe, Germany). DPPG (1,2-dipalmitoylsn-glycero-3-phosphatidylglycerol) was purchased from Genzyme Pharmaceuticals, Sygena Facility, (Liestal, Switzerland). Bovine albumin serum (BSA), Limonene, Citral and Cineole were purchased from Sigma–Aldrich, (Steinheim am Albuch, Germany). And all other solvents used in this study were of analytical grade and were purchased from Merck (Darmstadt, Germany).

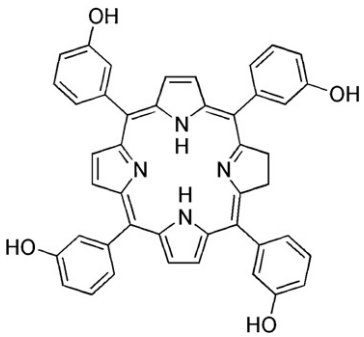

Fig. 2. Structure of temoporfin.

2.2. Preparation of lipid vesicles

All the lipid vesicles were prepared by a conventional rotary evaporation method (Bendas and Tadros, 2007; Ita et al., 2007). Briefly, the appropriate weights of lipid or lipids (and with mTHPC in the case of lipid vesicles containing mTHPC) (the compositions of different vesicular systems shown in Table 1) were dissolved in methanol/chloroform solution (1:2, v/v) in round bottom flask. Thin lipid films were obtained by removing the organic solvents under vacuum condition (500 mbar 10 min, 200 mbar 10 min, 100 mbar 10 min, 35 mbar 1 hr) at a temperature of 43 °C with a rotary evaporator (Rotavapor R-114, BÜCHI, Flawil, Switzerland). The resulted dry lipid films on the inside wall of round bottom flask were hydrated and dispersed with different hydration systems (and also containing CF in the case of lipid vesicles containing CF) corresponding to all formulations (Table 1) at room temperature. The obtained macroscopically homogenous solution was sonicated for totally 15 min in 3 cycles (5 min for each cycle and 5 min pause among these cycles) with a sonication ice-water bath. Then these suspensions were extruded through polycarbonate membrane (diameter: 19 mm, pore diameter: 100 nm, Armatis, Schriesheim, Germany) 21 times to produce liposomes of the desired size with the help of a Hamilton-Bonaduz extruder (GASTIGHT #1001, Bonaduz, Switzerland) (MacDonald et al., 1991).

2.3. Characterization of lipid vesicular systems

2.3.1. Particle size distribution and ζ-potential measurement

The particle size, polydispersity index (PDI) and ζ-potential of all the lipid vesicles were determined by photon correlation spectroscopy (Zetasizer Nano series, Nano-ZS, Malvern Instruments Ltd., Worcestershire, UK). Before the size and ζ-potential measurements, different liposomes samples were diluted 100-times with PBS (pH 7.4, 10 mM), which were also filtered throughpolycarbonate membrane (Minisart® syringe end filters 0.2 μm, Sartorius AG, Goettingen, Germany). Measurements were made at 25 °C with a fixed angle of 137°. Sizes quoted are the z-average mean for the liposomal hydrodynamic diameter (Nanda and Khan Ghilzai, 2006). Calculation of ζ-potential (mV) was done by the instrument from electrophoretic mobility (Fang et al., 2008).

2.3.2. Visualization by cryo-transmission electron microscopy

The different lipid vesicles (CL, INS and ETS) were visualized by cryo-electron microscopy and their shape and lamellarity was investigated.5 μl of test formulation were placed on a perforated copper grid (Quantifoil R 1.2/1.3) and excess liquid was blotted automatically for 2 s between two pieces of filter-paper-strips. Shortly after that, the samples were frozen by rapidly plunged in liquid ethane (cooled to −170 to −180 °C) in a cryo-box (Carl Zeiss NTS GmbH, Germany). Excess ethane was removed with a piece of