Available online at www.sciencedirect.com

SCIENCE DIRECT®

International Journal of Pharmaceutics 298 (2005) 1–12

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Effect of cholesterol and ethanol on dermal delivery from DPPC liposomes

J.M. López-Pinto, M.L. González-Rodríguez, A.M. Rabasco *

Department of Pharmaceutical Technology, University of Seville, C/ Prof. García González 2, 41012 Seville, Spain

Received 24 March 2004; received in revised form 21 January 2005; accepted 9 February 2005
Available online 17 May 2005

Abstract

The main objective of the present work was to compare the dermal delivery of minoxidil (Mx), a lipophilic drug from ethosomes versus classic liposomes, containing different cholesterol (CHOL) concentrations. All the systems were characterized for shape, lamellarity, particle size and entrapment efficiency percentage (EE), by transmission electron microscopy (TEM), confocal laser scanning microscopy (CLSM), laser diffraction and ultracentrifugation or dialysis methods, respectively. Multilamellar vesicles (MLVs) were obtained and one to six lamellae were visualized by CLSM. The presence of ethanol in the formulations affects the particle size in terms of reducing this parameter. In addition, it was possible to appreciate the influence of CHOL on the vesicle size, because it was increased, as CHOL concentration was higher. When the EE was determined by two different methods (ultracentrifugation and dialysis methods), a clear losing of entrapped drug by the ultracentrifugation method was observed, because the strong energy transmitted to the samples disrupted vesicles.

Vesicles were non-occlusively applied on rat skin and the permeation pattern of the different systems, depth into the skin and the main permeation pathway were studied by using β-carotene as a fluorescent probe. CLSM studies showed that ethosomal systems were much more efficient at delivering the fluorescent substance into the skin in terms of quantity and depth, than either liposomes or hydroalcoholic solutions.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Liposome; Ethosome; Lamellarity; Confocal laser scanning microscopy; Franz cell; Minoxidil

1. Introduction

For the last 2 decades, topical delivery of drugs from liposomal formulations has evoked a considerable interest. Many reports on enhancing percutaneous delivery focus on the use of liposomes, because they can aid the transport of hydrophilic and lipophilic compounds (Coderch et al., 1996). However, it is generally agreed that classic liposomes are of little or no value as carriers for transdermal drug delivery, because they do not deeply penetrate the skin, but rather remain confined to the upper layer of the stratum corneum. Only specially

* Corresponding author. Tel.: +34 954 556724;
fax: +34 954 556726.
E-mail address: amra@us.es (A.M. Rabasco).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.02.021

designed vesicles were shown to be able to allow transdermal delivery, such as transfersomes (Cevc et al., 1998, 2002).

Ethanol is known as an efficient permeation enhancer. This solvent is commonly believed to act by affecting the intercellular region of the stratum corneum (Magnusson et al., 1997), although other mechanisms have been reported, such as coadjuvant to diminish the melting point of drugs, such as lidocaine, achieving a higher concentration in the oil phase of an emulsion and prolonging the anaesthetic latency time (Kang et al., 2001). Its inclusion in liposomes to form ethosomes has already been investigated by other authors (Kirjavainen et al., 1999; Dayan and Touitou, 2000; Godin and Touitou, 2004).

In this study, Mx was selected as a model lipophilic drug. It has potential applications in the case of androgenetic alopecia (Olsen et al., 2002). However, the appearance of some complaints in the patients with this treatment (pruritus and scaling of the scalp) as a consequence of irritant contact dermatitis, allergic contact dermatitis or an exacerbation of seborrheic dermatitis, has been reported (Friedman et al., 2002).

In this paper, vesicle systems (liposomes and ethosomes) were prepared. Ethosomal formulations included ethanol in relatively high concentrations, incorporating it in the aqueous phase. Mx was dissolved in the hydroethanolic solution and was included in the aqueous compartment of vesicles.

This paper focuses on the preparation and characterization of ethosomal formulations, for Mx transdermal delivery. So, the effect of ethanol and CHOL on the permeation of two lipophilic substances (Mx and carotene) through rat skin was evaluated.

2. Materials and methods

2.1. Materials

High-purity α-dipalmitoylphosphatidylcholine (α-DPPC) and CHOL were purchased from Sigma (Barcelona, Spain). The lipid purity was higher than 99%, and it was used without further purification. Mx was purchased from Genox Farma (Barcelona, Spain) and was used as a model drug because of its poor water solubility. β-carotene (βC) was used as a fluorescent probe, obtained from Faisa (Seville, Spain).

Chloroform and ethanol were received from Panreac Chemistry (Barcelona, Spain). All other chemicals were at least reagent grade and used as received.

2.2. Mx solubility measurements

The solubility was determined in water at 25 °C. Briefly, an excess of Mx was added to 2 mL of solvent; the suspensions were vigorously shaken for 7 days (*Selecta Rotaterm*) and then left to equilibrate. Samples were ultracentrifuged (*PACISA FP-S10*), at 40,000 rpm for 1 h, the supernatant was filtered (*Millipore* 0.45 μm) and diluted. The Mx concentration was determined by HPLC as further described.

2.3. HPLC assay for Mx quantification

Mx was quantified by an HPLC method proposed by other authors (Touitou et al., 2000). It was used a Hitachi system manager D-7000, equipped with a Rheodyne injector 77251, isocratic pump L-7100 and detector L-7455. Separation was carried out on Merck LiChrospher® 100 RP-18 column with a particle size of 5 μm, 12.5 cm × 4 mm and kept at room temperature. The mobile phase was methanol:water:glacial acetic acid (7:3:0.1) pH 3.0 and 3 g/L of sodium docusate. Mx was detected at 254 nm, and the flux rate was 1 mL/min.

2.4. Preparation of liposomes

Multilamellar liposomes were prepared by the hydration method. In a typical procedure, 60 mg (81.7 μmol) DPPC, 0–40 mg (0–103.44 μmol) CHOL and 40 mg (191.16 μmol) Mx or fluorescent probe (5 mg) were dissolved in a small amount of methanol:chloroform (1:3) mixture. The solution was placed in a rotary evaporator (*Rotavapor R 200/205, Buchi*) at 55 °C until a thin lipid film on the wall of a round-bottomed flask, was obtained. The resulting lipid film was kept under vacuum in order to eliminate the traces of organic solvent. This lipid film was then hydrated with 4 mL of the appropriate aqueous solution, by mixing in a vortexing device, consisting in 1 min of vortex treatment and 5 min in a thermostatized bath at 55 °C, value that is above the phase-transition temperature (T_c) of DPPC (41 °C) according to Lasic et al. (1998). All the samples were sonicated for 10 min by using an ultrasound bath (*Transonic 460 H, Singen*),

Download English Version:

https://daneshyari.com/en/article/9918731

Download Persian Version:

https://daneshyari.com/article/9918731

Daneshyari.com