Decrease of Liposomal Size and Retarding Effect on Fluconazole Skin Permeation by Lysine Derivatives

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Received 13 December 2010; revised 13 January 2011; accepted 22 January 2011

Published online 11 February 2011 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.22513

ABSTRACT: Liposomes are ideal dermal drug delivery systems because of their ability to alter the biodistribution profile of incorporated drugs. In a novel approach to optimize the liposomal microstructure, lysine derivatives were employed. The effect of the oligopeptides Lys-5 and Lys-7 on the structure as well as on the skin permeation of the antimycotic drug fluconazole in 1,2-dipalmitoyl-sn-glycero-3-phosphocholine vesicles was studied using a variety of techniques. It was demonstrated by addition of the shift reagent praseodymium(III)chloride and subsequent ^{31}P NMR measurements that the liposomes produced consisted mainly of unilamellar vesicles. This was confirmed by cryo-transmission electron microscopy. The addition of Lys-5 and Lys-7 induced a structural change resulting in a decrease in particle size between 10% and 40% and a retarding effect on fluconazole skin permeation. © 2011 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:2911–2919, 2011

Keywords: liposomes; ^{1}H NMR; ^{19}F NMR; ^{31}P NMR; calorimetry (DSC); stability; skin; lysine derivatives

INTRODUCTION

Dermal application is desired for a variety of drugs due to well-known advantages of this way of administration. Colloidal drug delivery systems, especially liposomes, have been known as efficient vehicles for drug delivery on skin for a long time.[1] Although the penetration mechanism is still not completely understood, liposomes can be used for localized or transdermal administration. However, their long-term stability is rather poor. In this work, we chose an entirely new approach to resolve this limitation: The incorporation of novel lysine oligomers. Arginine- and lysine-rich peptides have previously been reported to interact strongly with lipid bilayers and to modify the vehicles' mobility in the human body.[2,3] In this context, the amino residues of arginine and lysine seem to play a major role. So far, mostly peptides with lysine- and arginine- or only-arginine groups were designed and investigated.[4,5] In the present work, novel lysine derivatives consisting of five to seven amino acid residues (Lys-5 and Lys-7) were synthesized and incorporated into 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) liposomes. Furthermore, the active compound fluconazole was chosen for encapsulation in the vesicles because of its possible application in topical antifungal therapy. Moreover, the presence of two fluorine atoms in the fluconazole molecule is advantageous as ^{19}F NMR is feasible for analysis.

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Journal of Pharmaceutical Sciences, Vol. 100, 2911–2919 (2011)

In short, the aims of the present study were as follows:

(i) to investigate the influence of Lys-5 and Lys-7 on blank and fluconazole-loaded liposomes in terms of physicochemical long-term stability parameters such as particle size and polydispersity index (PDI),
(ii) to elucidate the changes in microstructure of the liposomes induced by the lysine derivatives using NMR, cryo-transmission electron microscopy (cryo-TEM) and micro differential scanning calorimetry (micro-DSC), and
(iii) to compare the fluconazole skin permeation from liposomes with and without lysine oligomers.

MATERIALS AND METHODS

Materials

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine was obtained from Lipoid (Steinhausen, Switzerland). The product consists of synthetic Lipoid PC 16:0/16:0. The content of phosphatidylcholine was at least 99% related to the dry weight. Fluconazole was a generous gift from Pfizer Corporation, Vienna, Austria. Praseodymium(III)chloride hydrate was purchased from Sigma Aldrich (Vienna, Austria). All chemicals used in this study were of analytical reagent grade and used as received without any further purification.

Synthesis of Oligolysines

Lys-5 and Lys-7 (oligomers of lysine with five or seven amino acid residues) were prepared using conventional techniques of solid-phase peptide synthesis. Fmoc-Lys(Boc)-OH was used as amino acid derivative.[6] The synthesis was carried out on Wang resin (Fluka, Vienna, Austria) applying the batchwise process. The final hydrolysis with trifluoroacetic acid (99%), which included the cleavage of peptide from the resin as well as the deprotection of the side chains, provided the unprotected oligolysines. The structure of the peptides was confirmed by electrospray ionization–mass spectrometry.

Liposome Preparation and Characterization

Liposomes were prepared by a modified film hydration method.[7] Briefly, DPPC was dissolved in chloroform. Subsequently, a thin film was prepared using a rotary evaporator. In order to ensure a complete removal of the organic solvent, the dry film was dried in vacuo for 2 h. The film was then hydrated with distilled water and the flask agitated in a water bath, well above the main transition temperature of DPPC at 65°C for 3 h, resulting in a highly viscous white emulsion. This emulsion was then treated with a Bandelin Sonopuls HD 70 ultrasound device (operating frequency: 20 kHz, power output: 70 W; Berlin, Germany) for 15 min, in order to form liposomes. Afterwards, they were characterized in terms of size and PDI using a Zeta Sizer Nano ZS (Malvern Instruments, Malvern, UK). All liposomes were characterized in terms of these physicochemical properties on the day of production and selected formulations weekly for a time period of 12 weeks. In the case of the stability test, measured parameters also included zeta potential and conductivity. All samples were diluted 1:10 with distilled water.

Formulations

The percentages of oligolysines included in the liposomes are mol %. In contrast, all other percentages mentioned in this work are wt %. Fluc is used as an abbreviation for fluconazole in Tables and Figures.

Liposomes were prepared with an initial DPPC content of 7.5%. Subsequently, they were diluted to a lipid content of 3.75%. Simultaneously, 0.5% of fluconazole and/or oligolysine (Lys-5 or Lys-7, 5% or 10%) were added. For this dilution step the formulations were agitated at 65°C for 16 h.

Liposomes for nuclear magnetic resonance (NMR) analysis were prepared as described above but hydrated with a mixture of 90% H_2O/10% D_2O.

Encapsulation Efficiency

The encapsulation efficiency of fluconazole was determined using a modified differential centrifugation method.[8] In short, liposomes were centrifuged at 4700×g for 90 min at 25°C. The supernatant was removed and the pellet was resuspended in distilled water. The fluconazole content was determined in both the supernatant and the suspension by high-performance liquid chromatography (HPLC) as previously reported.[9] The concentration of the standard solution ranged from 1.4 to 1144 μg/mL.

Micro Differential Scanning Calorimetry

The thermotropic phase transitions of DPPC liposomes were studied using a Setaram III microcalorimeter (Setaram Instrumentation, Caluire, France). Samples of all prepared formulations were scanned with purified water as reference. The scanning rate was set at 1.0°C/min in a temperature range from 15°C to 65°C.

NMR Spectroscopy

Nuclear magnetic resonance experiments were performed on a Bruker Avance DRX 600 NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) operating at 600.13 MHz for ^{1}H, 564.69 MHz for ^{19}F, and 242.94 MHz for ^{31}P. A 5 mm quadruple observe probe (^{1}H, ^{13}C, ^{19}F, and ^{31}P) equipped with *z*-axis gradient coil was used. For spectrometer stability

 DOI 10.1002/jps