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ScienceDirect
Chemistry and Physics of Lipids 148 (2007) 84–90
CPL
CHEMISTRY AND PHYSICS OF LIPIDS
www.elsevier.com/locate/chemphyslip

The influence of selected steroid hormones on the physicochemical behaviour of DPPC liposomes

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Received 12 February 2007; received in revised form 17 April 2007; accepted 21 April 2007
Available online 27 April 2007

Abstract

The physicochemical properties of DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine) liposomes used for topical application are pharmaceutically important. Therefore the aim of our study was to establish rapid and efficient methods for the exact characterisation of the physicochemical properties of extruded DPPC liposomes containing low concentration (0.5%, w/w) of different, therapeutically interesting steroid hormones, named 17-β-estradiol, cpa (cyproterone acetate) and finasteride. In a first step it could be shown that all drugs influenced the liposome size and changed the zeta potential compared to the placebo formulations. Our further analytical strategy was to use micro-calorimetry and ATR-FTIR (Fourier transformed infrared spectroscopy), two powerful and non-destructive methods to confirm the drug incorporation into the liposomes by proving interactions between the phospholipids and the steroids. Thereby it was even possible to localize the location of interaction. The characteristic phase transition temperatures of the phospholipid were decreased by the hormones which was detected by micro-DSC (differential scanning calorimetry). The results of the ATR-FTIR measurements indicated shifts of the specific lipid peaks, the C=O stretching bands and PO_2^- antisymmetric double stretching band, in the gel and liquid crystalline phase. A polar as well as a non-polar interaction could be proven. It could be shown that the investigated steroid hormones changed the physical properties of the phospholipid bilayers.

Keywords: Phospholipids; Steroid hormones; Size; Zeta potential; Micro-DSC; ATR-FTIR

1. Introduction

Steroid hormones are very efficient drugs. They generally posses a non-genomic and a genomic mechanism of action (Korkmaz and Severcan, 2005). In case of the genomic mechanism the hormones bind to specific intracellular receptors. In the non-genomic case the cell membrane acts as mediator and interactions take place with membrane bound receptors or intercalations into the membrane. Previous studies showed an interaction of progesterone with the membranes through phospholipids by changing the lipid fluidity, protein mobility and increasing the calcium-ATPase activity (Korkmaz and Severcan, 2005).

Liposomes are promising vehicles for the dermal application of steroid hormones (Biruss and Valenta, 2006). However, numerous factors such as the lipid composition, the lamellarity, the charge and the size of the liposomes are important concerning drug deposition (Cevc and Blume, 1992; Verma et al., 2003). Referring to a recently study in which the influence of progesterone

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0009-3084/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.chemphyslip.2007.04.009

on the physicochemical behaviour of phospholipid liposomes was indicated (Biruss and Valenta, 2007) our aim was to analyse in what extent other interesting steroid hormones like 17-β-estradiol, cyproterone acetate and finasteride would be able to modify the physicochemical behaviour. Therefore the liposome size and the zeta potential were primarily evaluated. Additionally a successful incorporation of the selected hormones should be proven and exactly localized. Therefore two non-destructive methods named micro-DSC (differential scanning calorimetry) and ATR-FTIR (Fourier transformed infrared) spectroscopy measurements should be used. The idea was to analyse the interactions between the steroid hormones and the phospholipids in the gel and liquid crystalline phase and thereby localize the location of interaction. To date phospholipid interactions between progesterone, 17-β-estradiol, vitamin D_2 and melatonin with DPPC liposomes have been matter of investigations by these techniques (Korkmaz and Severcan, 2005; Boyar and Severcan, 1997; Toyran and Severcan, 2003; Kazanci et al., 2001; Severcan et al., 2005). In the following study the interaction of cyproterone acetate and finasteride within phospholipid membranes was studied the first time by ATR-FTIR spectroscopy and micro-DSC. The innovation behind the following study was to establish rapid and efficient methods for exact characterisation of the physicochemical properties of the steroid hormones 17-β-estradiol, cyproterone acetate and finasteride within DPPC liposomes. The advantage of an exact characterisation of the vehicle would make it easier to predict its behaviour in terms of releasing properties and stability.

2. Materials and methods

2.1. Materials

17-β-Estradiol was purchased from Sigma (St. Louis, USA). Cyproterone acetate (cpa) and finasteride were purchased from Kemprotec (UK). DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine) was obtained from Lipoid (Ge). The chemical structures are presented in Fig. 1A–D. All other chemicals were of analytical reagent grade and used without any further purification.

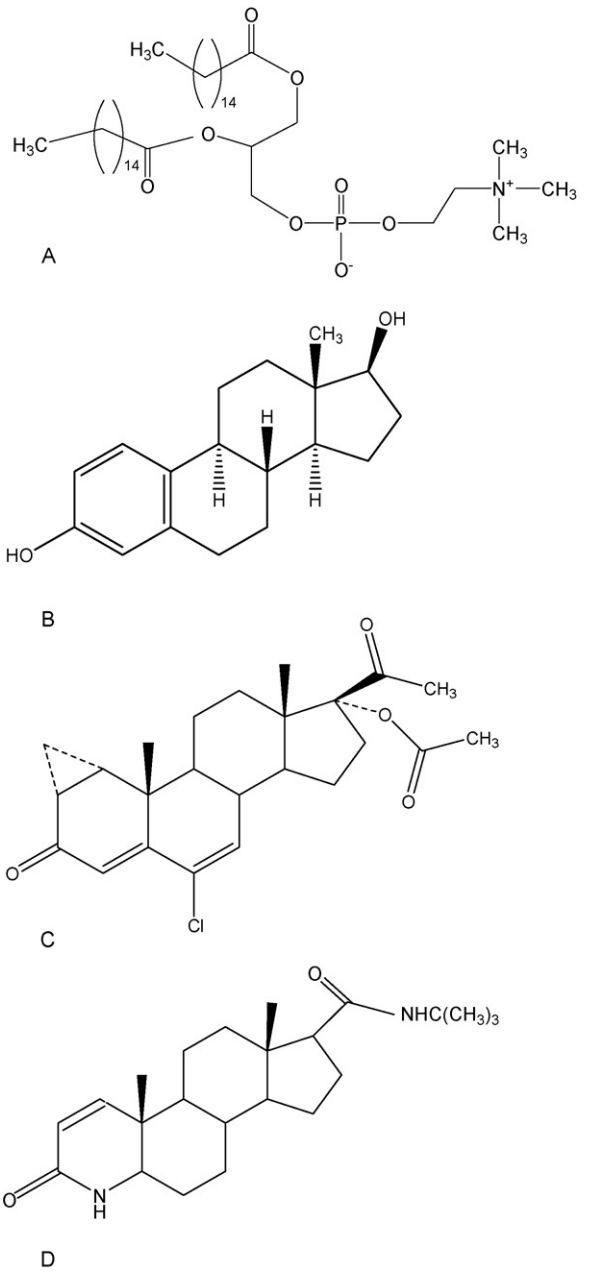

Fig. 1. A–D Chemical structures—(A) DPPC; (B) 17-β-estradiol; (C) cpa; (D) finasteride.

2.2. Formulations

2.2.1. Multilamellar liposomes

Multilamellar vesicles (MLV) were prepared by the traditional Bangham method (Bangham et al., 1974). Consequently 0.5% (w/w) of the selected drugs 17-β-estradiol, finasteride and cpa and DPPC were dissolved by a chloroform/methanol solvent in round-bottomed flasks. One charge without drugs was produced. The flasks were evaporated and the films were re-hydrated with deionised water in order to achieve a lipid content of 2 mg/ml. The resulting vesicles were shaken for 15 min and annealed for 2 h at 60 °C (above the phase transition temperature).