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ORIGINAL ARTICLE

Deformable liposomes containing alkylcarbonates of γ-cyclodextrins for dermal applications

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Abstract Liposomes made with hydrogenated soya lecithin (HPC) mixed with dodecylcarbonate γ-cyclodextrin (C12CD) at 20:1, 10:1 and 5:1 w/w ratios were prepared by the solvent evaporation method. C12CD had emulsifying properties and the possibility of producing deformable liposomes, as topical delivery system of progesterone (PG), was evaluated. Liposome size, deformability and drug entrapment were determined and the interaction between C12CD and HPC was investigated using differential scanning calorimetry (DSC). The size and the amount of PG loaded in the liposomes depended on the lipid:C12CD ratio: the smallest liposomes were obtained using 20:1 ratio and the maximum drug entrapment at 5:1 ratio. DSC analysis suggested that C12CD interacted with liposomes disrupting and fluidizing the lipid bilayer. PG transepidermal permeation through intact pig skin and PG skin uptake from deformable liposomes were assessed and compared to the values obtained from aqueous suspension and conventional liposomes. The PG permeations were negligible for all systems, while skin uptake increased for liposomes containing C12CD. This was attributed to the deformability and to the increase in the drug entrapment efficiency of these liposomes. The use of C12CD in liposome formulations can improve PG topical therapy.

Keywords Deformable liposomes · Alkylcarbonate cyclodextrins · Progesterone · Skin uptake

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Introduction

The percutaneous route for drug administration has many advantages over other pathways including the continuous drug delivery, the avoidance of the hepatic first pass effect, fewer side effects and improved patient compliance [1].

A major obstacle to percutaneous drug delivery is the permeation characteristics of the stratum corneum that limits the drug transport making this route of administration frequently insufficient for medical uses. Consequently various approaches have been developed in order to enhance the bioavailability of topical applied drugs [2]. One of the possibilities for increasing the penetration of drugs through the skin is the use of vesicular systems such as liposomes. Due to their biocompatibility and capability of incorporating both hydrophilic and lipophilic drugs, liposomes have been investigated as drug delivery systems [3].

The strategy of using liposomes is of interest, but remains controversial owing to their large minimum size compared to the spaces between the corneocytes in the skin, which have an average diameter typically around 30 nm or less.

In the literature elastic liposomes containing phosphatidylcholine and a surfactant have been recently described [4]. These vesicles resemble liposomes in morphology but not in function and they can transport drugs through the

intact skin if applied non occlusively in vivo, by virtue of their self-regulating deformability. Sodium cholate [5], Span 80 or Tween 80 [6], dipotassium glycyrrhizinate [7] were employed as surfactant and an increase of the drug deposition and of the penetration depth could be achieved for some drugs by using these carriers.

In order to achieve optimum efficiency, it is necessary to encapsulate the maximum possible quantity of drug in the carrier. Comparison of this value with the therapeutic dose indicates whether, in principle, liposomes can be used as a delivery system for that drug.

Cyclodextrins are well-known pharmaceutical excipients which can be used to improve the physicochemical and biopharmaceutical properties of drugs (e.g. solubility, stability and bioavailability). Recently, we have synthesized alkylcarbonate γ-cyclodextrin. These derivatives are able to form inclusion complexes with some drugs and to have emulsifying properties [8].

The aim of this work is to evaluate the possibility of producing deformable liposomes using the dodecylcarbonate γ-cyclodextrin to enhance drug encapsulation and its topical release. As one of the major therapeutic applications of liposomes is in the area of topical delivery of steroidal drugs, progesterone (PG) was used as model drug.

Experimental

Materials

Progesterone and γ-cyclodextrin were purchased from Fluka (Buchs, Switzerland), hydrogenated soya lecithin (HPC) was supplied by Phospholipid GmbH (Munich, Germany). Water was freshly bidistilled. All other reagents were of analytical grade.

Dodecylcarbonate γ-cyclodextrin (C12CD) was synthesized in our laboratory according to procedure reported in literature [8]. Briefly, the dodecyl alcohol was activated by reaction with an excess of carbonyldiimidazole in alcohol free chloroform. In the second step, the imidazoil derivative was allowed to react with anhydrous γ-cyclodextrin in anhydrous pyridine at 80°C for 4 h. Once the reaction was over, the residual precipitate was filtrated off and distilled water was added to the organic solution. The solid was recovered by filtration, washed many times with water and then lyophilized. The mean degree of substitution was 3.

Liposome preparation

PG loaded liposomes made of HPC alone or mixed with different amounts of C12CD were prepared by a conventional rotary evaporation method. Appropriate amounts of HPC (150 mg) and PG (7.5 mg) were dissolved in the minimum amount of ethanol. The organic solvent was evaporated under vacuum and solvent traces were removed by maintaining the lipid film under the stream of nitrogen for 30 min. The films were hydrated with water (5 ml) by magnetic stirring for 10 min. The hydrated vesicles were sonicated at 60°C and centrifuged at 10,000 rpm for 10 min to separate the liposomes from the non-entrapped drug. When C12CD was used, the lipid:cyclodextrin weight ratio was 20:1, 10:1 or 5:1.

Size determination

Laser light scattering measurements (LLS) were performed at 25°C (0.1°C to determine the mean size of liposomes using a 90 Plus Particle Size Analyzer (Brookhaven Instrument Corporation, Holtville, NY, USA). Measurements were obtained at an angle of 90° after appropriate dilutions. Scattering intensity data were analysed by a digital correlator and fitted by the method of inverse Laplace transformation. Each system was analysed twice times, and for each sample ten size determinations were made.

Determination of PG content in the liposomes

The PG content in the liposomes was determined by HPLC analysis. An aliquot of the PG loaded liposomes were dissolved in ethanol and analysed. The PG content was expressed as the measured drug content (mg) in 100 mg lipid.

The HPLC system consisted of a pump (LC10-AD), an UV detector (SPD-10, λ = 240 nm), a data station (Shimadzu, Kyoto, Japan) and a 250 × 46 mm RP C18 column (Beckman, Fullerton, USA). The mobile phase comprised methanol, water (90:10 v/v) and was delivered at a flow rate 1 ml/min. The injection volume was 20 μl and the relative retention time was found to be 5.3 min.

DSC analysis

DSC was performed with Perkin Elmer differential calorimeter (DSC7, Perkin Elmer, CT, USA). The liposome suspensions with or without C12CD and PG were placed in conventional aluminium pan and a scan speed of 2°C min^{-1} was employed. The weight of each sample was 12–15 mg.

Determination of liposome deformability

Liposome deformability was determined by measuring the mean size of the liposomes before and after filtra-

tion through a microporous filter with pore diameter 100 nm (Isopore, Millipore, Bedford, MA, USA) using a stainless steel pressure filter holder for 47 mm diameter filters, with 200 ml capacity barrel (Pall Gelman Laboratory, Ann Arbor, MI, USA) connected to 0.5 MPa pressure source. The experiments were performed in triplicate and for each sample ten size determinations were made.

In vitro transepidermal permeation and skin uptake studies

The PG transepidermal permeation and the skin uptake were determined using vertical Franz cell [9] and full-thickness pig ear skin. The skin was rinsed with normal saline and, to maintain an in vivo transepidermal hydration gradient [10], and was pre-hydrated by floating with the stratum corneum upward on 0.002% w/v aqueous sodium azide. The receptor chamber of the cell was filled with 6 ml of PBS pH 7.4/EtOH 90/10. The test formulations were applied to the skin surface, which had an available diffusion area of 2.05 cm^2, and left to dry out. The content of the receptor chamber, continuously stirred at 37°C, was removed at appropriate intervals for PG determination and the cell was immediately refilled with fresh receptor solution. At the end of the permeation experiments (48 h) the surface of the skin was washed five times with 50% ethanol and water to remove excess drug on the surface. The skin was then cut into small pieces. The tissue was further homogenized with ethanol (3 ml) and left for 3 h at room temperature. After 5 min centrifugation at 5,000 rpm, the PG content in the upper phase was determined by HPLC. The skin uptake was expressed as mg PG/cm^2 skin diffusion area.

Results and discussion

Liposomes characterization

Table 1 reports the mean size (±SD) of the liposomes with and without PG. The PG free liposome sizes decreased slightly in the presence of HPC:C12CD 20:1. On the contrary, in the presence of higher amount of C12CD, an increase of the mean size was observed and very large liposomes were obtained at 5:1 ratio. The same behaviour was observed for the PG loaded liposomes.

The mean diameters of HPC and HPC:C12CD liposomes at different w/w ratios, determined by LLS after filtration through 100 nm microporous membrane are reported in Table 1.

The HPC liposomes were not capable of passing through pores smaller than their own diameters showing no deformability. Instead, all the liposome formulations containing C12CD resulted deformable. The size of liposomes containing C12CD before and after pore passage is nearly the same, unless their size is quite large. The passage of these liposomes through pores that are too narrow is nearly complete, even if their size exceeds the pore diameter by a factor up to 4.

The C12CD has emulsifying properties and the localization within the lipid bilayer caused the fluidization of the bilayer itself, permitting to liposomes to cross the pores of the membrane.

The introduction of C12CD in liposome formulations increased their PG loading capacity. In particular, it increased by increasing the amount of cyclodextrin used, reaching the value of 4.58 mg/100 mg lipid for HPC:C12CD 5:1 liposomes.

The thermograms of liposomes prepared with HPC alone and with C12CD in the absence of PG are reported in Fig. 1.

The thermogram of HPC liposomes showed a peak transition (T_m) at 49.61°C and an enthalpy of 7.5 ± 0.4 kcal mol^{-1} in accordance with the values reported in literature [11]. The presence of C12CD reduced the cooperativity of the transition which reflected the presence of a system having different degrees of disruption in the packing characteristics. The decrease in T_m and in enthalpy values by increasing the C12CD content may indicate that the cyclodextrin derivative perturbs the packing characteristics and consequently, fluidizes the lipid bilayer, as previously observed in the deformability studies.

Table 1 Liposome size and drug content

Liposome	PG free (nm)	PG loaded (nm)	PG loaded after filtration (nm)	PG content (%)
HPC	201 ± 14	235 ± 15	–	0.99 ± 0.11
HPC + C12CD 20:1	181 ± 13	200 ± 13	255 ± 21	1.31 ± 0.20
HPC + C12CD 10:1	216 ± 17	257 ± 16	287 ± 23	3.99 ± 0.43
HPC + C12CD 5:1	304 ± 21	404 ± 32	329 ± 28	4.58 ± 0.52

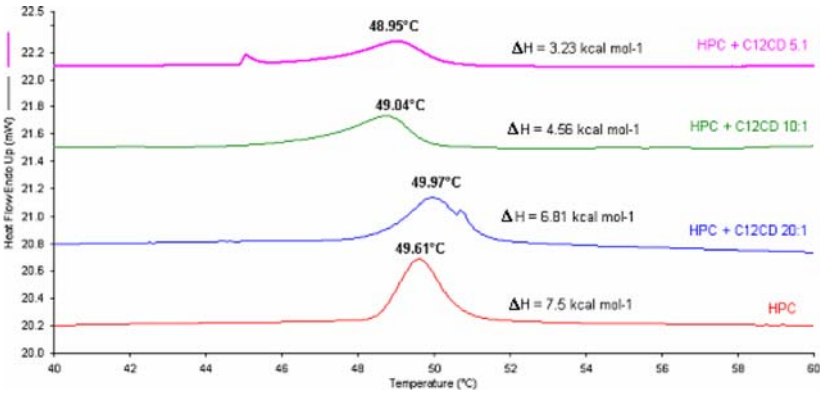

Fig. 1 Thermograms of PG free liposomes

In vitro transepidermal permeation and skin uptake studies

The pig skin permeation and uptake of PG from liposomes and from an aqueous suspension are summarised in Table 2.

All systems showed a negligible permeation suggesting that any liposome was beneficial in delivery of PG through the skin. The PG accumulated into skin was 6.1 $\mu g/cm^2$ for the suspension while it increased up to 15.5 $\mu g/cm^2$ for 20:1 and to 21.4 $\mu g/cm^2$ for HPC:C12CD 10:1 liposomes, respectively. At 5:1 ratio, the skin uptake was only 18.8 $\mu g/cm^2$. Probably these liposomes, even if deformable, were not able to cross the skin barrier because of their large size.

In accordance with what has been reported for other lecithin:surfactant liposomes [12], the main reasons for the function of deformable liposomes may be ascribed to the synergetic effect of lecithin and C12CD on the penetration of the liposomes into the skin; this penetrating capacity through the interstices of the stratum corneum under the influence of transcutaneous hydration force was attributed to the water concentration difference between the skin surface and the skin interior. On the other hand, the enhancement effect could be due to fusion of the vesicles with the skin, facilitated by an increase in the fluidity of the phospholipidic bilayers and/or by an increase in drug entrapment of these liposomes.

Table 2 PG permeation and PG uptake

Sample	PG permeation	Skin uptake ($\mu g/cm^2$)
PG suspension	Negligible	6.1 ± 1.4
HPC liposome	Negligible	10.4 ± 1.9
HPC + C12CD 20:1 lip.	Negligible	15.5 ± 2.2
HPC + C12CD 10:1 lip.	Negligible	21.4 ± 2.6
HPC + C12CD 5:1 lip.	Negligible	18.8 ± 2.4

Conclusions

C12CD can be used as deformant agent to produce deformable liposomes. The introduction of this cyclodextrin derivative increased up to four times the drug loading and more than twice the skin uptake compared to conventional liposomes.

The use of liposomes containing alkylcarbonate derivatives of γ-cyclodextrin could be a new opportunity for the well-controlled and innovative topical medication.

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