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Permeation of cyproterone acetate through pig skin from different vehicles with phospholipids

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Abstract

The permeation of cyproterone acetate (CPA) from Derma Membrane Structure (DMS) creams and liposomal formulations was investigated. Standard diffusion experiments with dermatomed porcine skin were performed. The cumulative CPA amount permeated of the DMS creams was between 2.9 and 6.8 μg/cm^2 within 48 h. By addition of a phospholipid concentrate, the CPA permeation could be 2.6-fold further increased compared to the control DMS. A working temperature of 60 °C resulted in a change of the preparation and a higher permeation which could be confirmed by additional differential scanning calorimetry studies. In case of the liposomal formulations, the CPA permeation was strongly dependent on the lipid content. The higher the lipid content, the higher was the CPA permeation. Extruding procedures for decreasing the particle size of the liposomes resulted in a two-fold increase in CPA permeation compared to the unextruded liposomes.

It is possible to control the CPA permeation by combining various formulations containing different phospholipids with saturated and unsaturated fatty acids.

Keywords: Cyproterone acetate; Phospholipids; Dermatomed pig skin; Differential scanning calorimetry

1. Introduction

Anti-androgens can have their effect at many sites in the body. The hypothalamus, pituitary, adrenal glands, gonads and their target tissue such as prostate, sebaceous glands and hair follicles can each be the targets for anti-androgens. The term anti-androgen usually refers to androgen receptor blockers in target tissue, of which there are two types: steroidal such as cyproterone acetate (CPA) and non-steroidal such as flutamide (Shaw, 1996a).

CPA has been used since the early 60s and represents the first specific anti-androgen of clinical interest (Hammerstein et al., 1975; Ebling et al., 1977). The drug competitively binds to androgen receptor sites in peripheral target tissues and has a strong progestogen effect (Shaw, 1996a). It has been shown that acne and hirsutism can be successfully treated with orally applied CPA (Miller et al., 1986; Pugeat et al., 1991; Shaw, 1996b). The disadvantage of the oral route are systemic side effects which can occur with high doses such as lassitude breast tenderness, loss of libido and nausea (Hammerstein et al., 1975). It would be of great benefit to develop a topical CPA formulation which is able to permeate in sufficient high doses to the target side such as the sebaceous glands and which should produce low plasma concentrations. It has been shown

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that topical CPA packed in phosphatidyl-liposomes, which were not further specified, is as effective as oral CPA in combination with ethinylestradiol for the treatment of acne in women (Gruber et al., 1998).

The aim of the present study was to test new formulations on one hand on the basis of phosphatidyl-liposomes showing a high skin permeation and to compare the CPA permeation of these vehicles through excised pig skin. And, on the other hand, it seems very interesting to prepare other formulations with lecithins not only liposomes. For this purpose, DMS creams were used with different additional lipids. They are a new development in the field of topical preparations and compositions with hydrogenated soybean–phosphatidylcholine by Derma Membrane Structure (DMS) technology (Kutz, 1997). DMS stands for basic gels containing hydrogenated soybean–phosphatidylcholine, sebum compatible medium chain triglycerides, shea butter and squalane. The goal was to determine an optimised formulation with a high CPA permeation for further stability and in vivo studies.

2. Material and methods

2.1. Materials

CPA was a gift from Schering (A). DMS creams for normal, fatty and dry skin were gifts from Synopharm (Ge). Phosphatidylcholine (L-α-phosphatidylcholine) solution, 100 mg/ml in chloroform (PC), was purchased from Sigma (St. Louis, MO). A liposome concentrate containing 20% of a purified phospholipid fraction with high PC content and approximately 18% propylene glycol and water was used (Natipide II PG). Microcrystalline cellulose corresponded to Elcema P100.

Table 2
Liposomes with CPA

Code	Name	Lipid content (mg/ml)	Particle size (nm)
D	PC	1	>120
D1	PC	1	110–120
D2	PC	2	>120
E	PC	∼140	200–300

Each formulation contained 0.2% (w/w) CPA. PC, L-α-phosphatidylcholine.

2.2. Formulations

2.2.1. Formulations with DMS-basis creams

The composition of the formulations and the corresponding codes (A, A1, A2, A3, B, C) are listed in Table 1. For formulations A, B and C, the CPA (0.2%, (w/w)) was dispersed in an equal amount of propylene glycol and mixed with the DMS cream. In the end products A, B and C, the CPA is suspended. A1 was prepared by mixing 4% Natipide II PG and CPA at 21 °C and filled up with DMS cream for normal skin.

A2 and A3 were prepared by mixing 4 and 8% Natipide II PG, respectively, and CPA at 60 °C and afterwards DMS cream for normal skin and water were added.

2.2.2. Formulations with liposomes

The liposomes and the corresponding codes (D, D1, D2, E) are listed in Table 2.

2.2.2.1. Preparation D. CPA was dissolved in a 3:1 chloroform:methanol solvent. Two hundred microlitres of the PC solution and 500 μl CPA solution were mixed in a round flask. The mixture was then carefully dehydrated, using a gentle steam of argon, until a thin homogenous lipid film was formed inside the flask. The film was stored, under vacuum, in

Table 1
CPA formulations with DMS creams

Code	Name	Natipide II PG (%)	Additional water (%)	Temperature of preparation (°C)
A	DMS normal skin	–	–	
A1	DMS normal skin	4	30	21
A2	DMS normal skin	4	30	60
A3	DMS normal skin	8	30	60
B	DMS dry skin	–	–	–
C	DMS fatty skin	–	–	–

Each formulation contained 0.2% (w/w) CPA.

a desiccator for 48 h to ensure that all the solvents had evaporated. The lipid film was rehydrated with 2 ml of 10 mM phosphate buffer, adjusted to pH 7.2. The resulting multilamellar liposomes were used for the permeation experiments; the lipid content was 1 mg/ml.

2.2.2.2. Preparation D1. In a first step, the multilamellar liposomes D were frozen and re-thawed five times. Then they were extruded 10 times through a 100 nm diameter polycarbonate Nuclepore membrane and support (Nuclepore, CA). Nitrogen gas was used to extrude the mixture using a pressure ranging from 200 to 500 psi (LiposoFast; Avestin). The 10-fold repetition of extrusion produced a virtually homogenous population of unilamellar vesicles with diameters of 110–120 nm range. This method is a standard technique for producing unilamellar liposomes. Each batch of filters available from Whatman Nuclepore has been tested (protocol included with each test certificate) and the diameter of the vesicles measured by quasi-elastic light scattering (application manual).

2.2.2.3. Preparation D2. CPA was dissolved in a 3:1 chloroform:methanol solvent. Afterwards, 500 μl of this CPA solution and 400 μl of the PC solution were mixed in a round flask. The ongoing procedure was perfomed in the same way as D. The lipid content in the end product was 2 mg lipid/ml.

2.2.2.4. Preparation E. E was prepared by mixing CPA and an equal amount of Natipide II PG at 60 °C and then completed with water and the rest of the phospholipid concentrate. The lipid content of the end product was approximately 140 mg/ml.

2.3. HPLC analysis

The flow rate was 1.5 ml/min at 280 nm. The stationary phase was an ODS-2, 5 μm column (150 mm × 3.6 mm). Any impurities were held back on a pre-column of the same material (40 mm × 3.6 mm). A mobile phase of 420 g acetonitrile and 600 g water (w/w) was used. Twenty microlitres of samples were injected. The retention time for CPA was about 14 min. Calibration curves were calculated on the basis of peak area measurements.

2.4. Solubility

In order to identify a suitable receptor medium, the saturation solubilities of different media were analysed. For this purpose, an excess of CPA was added to the media at 37 °C and stirred for 14 h. After filtering (Minisart; Sartorius 0.45 μm), the CPA content was analysed by HPLC.

2.5. Monitoring of crystal formation

The appearance of drug crystals was monitored visually and microscopically (Nikon 104 Microscope; GE) of different parts of the tubes of A1, A2 and A3. For this purpose, 0.1–0.5 g of the samples were applied onto a microscope slide and any observed particles or crystals were counted and measured using a measuring ocular. In this manner, 10 visual views were analysed per sample on the date of manufacture and weekly for a time period of 6 weeks.

2.6. Diffusion cell preparation

Permeation of CPA was investigated using Franz-type diffusion cells. Of all preparations (A–E), 1.0 g or 1.0 ml was applied, respectively. The receptor compartment was filled, thermostatted at 37 °C and continuously stirred using a magnetic bar. The effective area available to diffusion was about 1.13 cm^2. The excised skin was mounted on the cell, stratum corneum uppermost, with the dermal side facing the receptor compartment. At defined time points, the receptor medium was removed for analysis and replaced with fresh receptor medium.

2.7. Skin preparation

Split thickness porcine abdominal skin was obtained by removal of approximately 1 mm of dermis from full thickness skin by use of a dermatome.

2.8. Differential scanning calorimetry (DSC)

The following additional preparations for DSC analysis were prepared: CPA and Natipide II PG (1:5) at 21 and 60 °C. In the same manner, CPA and microcrystalline cellulose (1:5) were prepared at 21 and 60 °C. DSC curves were obtained by a Perkin-Elmer DSC-7.

Aliquots of 10–20 mg of each preparation were placed in an aluminium pan and sealed with an aluminium cover. Reference was an empty sample pan. Nitrogen is passed through the cell with a constant flow rate of 35 ml/min. Thermograms were measured by heating the samples from 25 to 230 °C at a scan rate of 5 °C/min.

2.9. Statistical analysis

Results are expressed as the means of at least three or four parallel experiments ± S.D. as indicated in the text. Statistical data analysis was performed using a non-parametric Kruskal–Wallis test. All tests have $P < 0.05$ as a minimal level of significance.

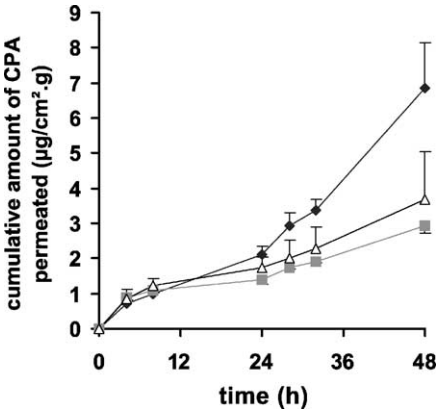

Fig. 1. Cumulative amount of CPA permeated through excised pig skin from different vehicles: A (◆); B (■); and C (△). $n = 3$.

3. Results and discussion

3.1. Solubility

In order to identify the optimal receptor medium, the solubility of CPA in different possible solvents was analysed (Table 3). As a general rule, the concentration of the permeant should not be allowed to exceed 10% of saturation solubility (Shah et al., 1998). Therefore, for the present study, PG/phosphate buffer (4 + 6) was used. The advantage of the dermatomed skin was the constant thickness of 1 mm. The time period of the diffusion experiments for all preparations was 48 h.

3.2. DMS creams

Semisolid bases such as DMS contain hydrogenated soybean–phosphatidylcholine, sebum compatible medium chain triglycerides, shea butter and squalane. The lipid fraction consists of saturated fatty acids such as stearic acid (18C) and caprylic–capric acid (8C; 10C). In addition to conventional liposomes and nanoemulsions, the DMS is a third way to formulate phosphatidylcholine with hydrophilic and lipophilic compounds free of further emulsifiers (Lautenschläger, 2001).

Table 3
Saturation solubility of CPA in different solutions

Medium	Solubility at 37 °C (μg/ml)
P	0.9 ± 0.3
P and 1% CD	8.90 ± 0.19
EtOH/P (2 + 8)	27.3 ± 2.6
PG/P (4 + 6)	63.3 ± 8.1

CD, γ-cyclodextrin; EtOH, ethanol; PG, propylene glycol; P, phosphate buffer (pH 7.2; 0.01 M).

Firstly, the CPA permeation of the formulations A, B and C was investigated. As indicated in Fig. 1, the highest cumulative amount of CPA within 48 h could be seen from DMS cream for normal skin (formulation A). One reason may be seen in the lipid content, which is 20.8% for normal, 36% for dry and 16.1% for fatty skin. Because of the highest CPA permeation from A, we tried to further increase the permeation from preparation A by combination with unsaturated lipids. We increased the lipid content by phosphatidylcholine in form of a liposomal concentrate (Natipide II PG) which is rich of linoleic acid. As indicated in Fig. 2, the cumulative amount of CPA decreased by this lipid addition at room temperature. However, when the processing temperature was increased to 60 °C, the cumulative amount of permeated CPA was in the range of formulation A without any additives. If the amount of liposome concentrate (Natipide II PG) was increased to 8% at 60 °C, the cumulative amount of CPA could be increased 2.6-fold compared to A. The higher processing temperature seems to influence the solubility and distribution of CPA in the preparation. This could be confirmed by a microscopic technique. In A1, few small crystals of about 5 μm could be observed, whereas in A2 and A3, no crystals could be detected. Beyond it, no further crystal growth could be detected

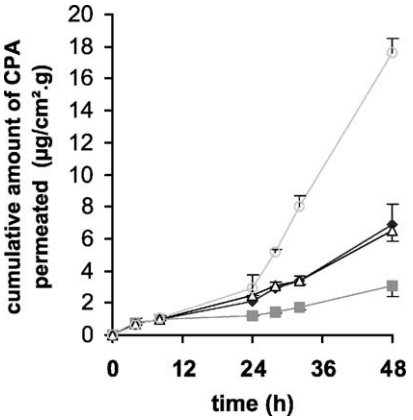

Fig. 2. Cumulative amount of CPA permeated through excised pig skin from different vehicles: A (◆); A1 (■); A2 (△); and A3 (○). $n = 3$.

microscopically over a 6-week period in all three formulations.

DSC studies were performed in order to find out whether the temperature of preparation has any influence on the characteristic phase transition temperature of phospholipid concentrate (Natipide II PG) and CPA. For this purpose, we prepared CPA–phospholipid concentrate mixtures in a different mass ratio than for the preparations of the diffusion studies, because 0.2% CPA would be too less to see the CPA peak at 202 °C, the melting point. Therefore, we prepared mixtures of CPA and Natipide II PG in a mass ratio 1:5 at 21 °C as well as at 60 °C. After comparing the DSC curves of the two preparations, cold prepared and warm prepared, it could be clearly seen that one characteristic peak for the preparation produced at room temperature was 116.2 °C whereas the peak of the formulation produced at 60 °C was 133.5 °C (Table 4).

Table 4
Thermal changes following different preparations of CPA formulation samples: CPA:Natipide II PG (1:5); CPA:elcema (1:5)

Sample	T_m (°C)	T_m (°C; CPA)	Enthalpy
Sample cold	116.2 ± 1.9	202.5 ± 1.7	4.23 ± 2.3
Sample warm	133.5 ± 0.7	202.6 ± 1.1	1.4 ± 1.8
Elcema cold	135.3 ± 10.9	205.5 ± 0.85	7.53 ± 0.37
Elcema warm	144.1 ± 5.6	204.3 ± 0.28	7.47 ± 0.6

Temperature of the transitions maximum, T_m; $n = 4$.

This indicates a possible change of the structure of the vehicle. Independent of the working temperature, the mass ratio of CPA to phospholipid concentrate for both preparations was 1:5, the enthalpy of the CPA peak at 202 °C of the product prepared at 60 °C was three-fold lower. This indicates, beside of the structural change, a partly higher sort of inclusion of CPA into the phospholipid concentrate when prepared at 60 °C instead at 21 °C. For a further confirmation, we prepared analogous mixtures of CPA and the inert microcrystalline cellulose (1:5) at room temperature and at 60 °C and used these samples as controls. As seen in Table 3, no significantly different peaks could be observed and the enthalpies of the CPA peak were in the same range, independent of the processing temperature. We found characteristically step transitions of microcrystalline cellulose which are confirmed by recently performed studies (Picker and Hoag, 2002).

3.3. Liposomal formulations

There is one publication presenting in vivo data in which CPA with unspecified liposomes are shown to have a positive effect on acne in men (Gruber et al., 1998). We wanted to be more systematic in this point. Therefore, the CPA diffusion of PC-liposomes with different lipid contents (Table 2) was compared. The preparation E is more viscous than D, D1 and D2. The release profiles of CPA of the different liposomes are presented in Fig. 3. As can be seen, the highest CPA amount was released by formulation E. In this case, the very high lipid content (approximately 140 mg/ml) seems to be responsible, this is confirmed by other studies that the penetration of dithranol or isotretinoin have been enhanced by the same liposomal concentrate (Gehring et al., 1992; Tschan et al., 1997). A comparison of the multilamellar liposomes D and D2 with different lipid content (Table 2) showed a three-fold increase of the CPA permeation within 48 h for D2 with the higher lipid content.

In contrast to the multilamellar liposomes D, the preparation D1 has been extruded. This preparation technique resulted in an average particle size of 110–120 nm and an unilamellar shape. Therefore, the two-fold increase of the CPA permeation of D1 is due to the smaller particle size. Moreover, D1 has only a single bilayer which could have been altered more easily than those of multilamellar vesicles with the

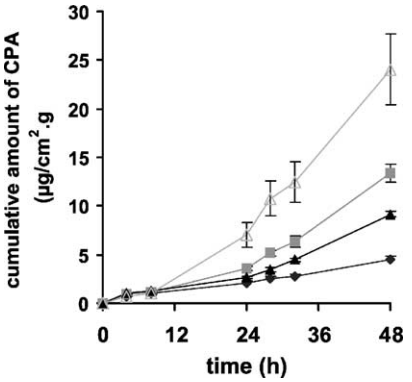

Fig. 3. Cumulative amount of CPA permeated through excised pig skin from different liposomal preparations: D (1 mg lipid/ml; ◆), size >120 nm; D1 (1 mg lipid/ml; ▲), size 110–120 nm, extruded; D2 (2 mg lipid/ml; ■), size >120 nm; E (△) prepared by a concentrate (∼140 mg lipid/ml), size 200–300 nm. $n = 3$.

same composition. This finding is in agreement with recently performed studies (Manconi et al., 2002). In summary, the CPA permeation rates from liposomes were dependent on the lipid content and on the particle size.

In most of the reports, an accumulation of drugs is observed in stratum corneum (Braun-Falco and Korting, 1992; Touitou et al., 1994; Lasic, 1997). In general, liposomes are not expected to penetrate into viable skin, but there are publications in which a vesicle transport is discussed (Mezei, 1992).

Another reason might be the penetration-enhancing effect of soya-lecithin itself, which has been investigated of various phospholipids of different drugs through the dorsal skin of guinea pigs (Yokomizo, 1996a,b; Yokomizo and Sagitani, 1996) or rat skin (Valenta et al., 2000). The results suggested that the phospholipids directly influence the lipid bilayer of the cell membrane in the stratum corneum. It has been found out that lecithin inhibited the peroxidation of skin lipids, but did not affect the release of sulfhydryl groups from skin protein. Similar drug absorption enhancement was observed for piroxicam, indomethacin and diclofenac preparations. It was concluded that lecithin has a high affinity for epidermal tissue, changes the fluidity of tissue by hydration of stratum corneum and enhances the percutaneous absorption of drugs (Natsuki and Takabatake, 1987a,b). Fourier Transform Infrared Spectroscopy (FTIR) and DSC studies indicated that lecithin affects the stratum corneum lipid organisation (Dreher et al., 1997). The phospholipid concentrate (Natipide II PG) bilayers as well as the L-α-phosphatidylcholine are at the temperature of the experiments in the liquid crystalline state which is related to the fact that its chief constituent, phosphatidylcholine contains mainly linoleic acid, a double unsaturated fatty acid (Betz et al., 2001a,b). The DSM bilayer, on the other hand, is at the temperature of the experiment in the gel state because of the content of stearic acid and caprylic acid, saturated fatty acids. This consideration may be responsible for the different fashion in which the liposome formulation interacts with the stratum corneum. Van Der Bergh et al. (1998) showed, by freeze substitution electron microscopy, that gel state liposomes aggregate fuse and adhere on the stratum corneum surface, thereby depositing stacks of lamellar sheets and forming lipid bilayer networks. On the other hand, liquid crystalline liposomes did not aggregate or fuse on the surface of the stratum corneum but were reported to interact with intercellular lipids in deeper stratum corneum layers. This is confirmed by other scientific studies (Zellmer et al., 1995; Van Kuijk-Meuwissen et al., 1998).

4. Conclusion

It is possible to control the permeation rate of CPA by various formulation components. It depends mainly on the type of lipid and lipid content. According to the afforded consistency, a DMS cream and an additional lipid can be recommended. In a previous study (Morganti et al., 1997), the vehicle alone consisting of phosphatidylcholine-rich linoleic acid showed a satisfactory anti-acne activity; in this way, the anti-acne effect of CPA could be increased.

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