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Research Paper

Cyproterone Acetate Loading to Lipid Nanoparticles for Topical Acne Treatment: Particle Characterisation and Skin Uptake

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Purpose. Topical cyproterone acetate (CPA) treatment of skin diseases should reduce side effects currently excluding the use in males and demanding contraceptive measures in females. To improve skin penetration of the poorly absorbed drug, we intended to identify the active moiety and to load it to particulate carrier systems.
Materials and Methods. CPA metabolism in human fibroblasts, keratinocytes and a sebocyte cell line as well as androgen receptor affinity of native CPA and the hydrolysis product cyproterone were determined. CPA 0.05% loaded solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), a nanoemulsion and microspheres were characterized for drug-particle interaction and CPA absorption using human skin *ex-vivo*.
Results. Native CPA proved to be the active agent. Application of CPA attached to SLN increased skin penetration at least four-fold over the uptake from cream and nanoemulsion. Incorporation into the lipid matrix of NLC and microspheres resulted in a 2–3-fold increase in CPA absorption. Drug amounts within the dermis were low with all preparations. No difference was seen in the penetration into intact and stripped skin.
Conclusion. With particulate systems topical CPA treatment may be an additional therapeutic option for acne and other diseases of the pilosebaceous unit.

KEY WORDS: cyproterone acetate; lipid particles; nanostructured lipid carriers; parelectric spectroscopy; pharmacological effects; skin absorption.

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ABBREVIATIONS: BSA, bovine serum albumin; CP, cyproterone; CPA, cyproterone acetate; DHT, dihydrotestosterone; EC_{50}, concentration of drug specifically deplacing ^{3}H-DHT by 50%; EDTA, ethylenediamine tetraacetic acid; FCS, fetal calf serum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GR+/29+, mouse fibroblasts cell line GR+/29+; LD95%, maximum particles size of 95% of the particles; mRNA, messenger-RNA; MS, microspheres; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NLC, nanostructured lipid carrier(s); M (Miglyol® 812), O (oleic acid) added as indicated to the lipid component; NE, nanoemulsion; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PCS, photon correlation spectroscopy; PI, polydispersity index; PS, parelectric spectroscopy; rph, rotation per hour; SD, standard deviation; SLN, solid lipid nanoparticle; SZ95, immortalised sebocyte line; f_0, dipole mobility; $\Delta\varepsilon$, dipole density; $\bar{x}$, arithmetic mean value.

INTRODUCTION

Acne vulgaris belongs to the most frequent skin diseases. Almost 85% of people aged 12–25 years suffer from this disease and still 3% of those aged 35–44 years (1). The manifestation of the multifactorial disease results from enhanced dihydrotestosterone (DHT) formation by 5α-reductase I during adolescence (2,3) stimulating sebocyte proliferation and sebum production. Hypercornification of the upper hair follicle infundibulum interferes with sebum excretion leading to the formation of comedones which may transform into inflammatory lesions by colonising anaerobic bacteria. Other DHT dependent diseases of the pilosebaceous duct include androgenetic alopecia and hirsutism, since dermal papilla cells forming the hair express 5α-reductase II (4). Thus, DHT formation and signal transduction are relevant therapeutic targets. In fact, considering the benefit/risk ratio, the 5α-reductase inhibitor finasteride applied orally has become first choice to delay androgenetic alopecia (5,6) while severe acne and hirsutism in females are clearly more responsive to the oral application of antiandrogens, e.g. combined cyproterone acetate (CPA)/ethinyl estradiol which reduces sebum secre-

tion rate and acne lesion count by up to 65% (7,8). Moreover, the contraceptive combination excludes teratogenic effects of CPA which mean feminisation of a male fetus. In males, CPA side effects encompass loss of libido, gynecomastia, vasomotor flushing and loss of bone mineral density which is acceptable when used for metastatic prostate cancer, yet not in patients with acne or alopecia.

To avoid systemic antiandrogen effects, topical application has been looked for. While early studies failed (9,10), CPA 0.5% alcohol lotion has recently been described to improve total acne lesion count (11). Moreover, major progress obtained in the field of drug carrier systems in topical dermatotherapy [for review see: (12,13)] has stimulated loading of steroidal and non-steroidal antiandrogens to liposomes (14,15) and solid lipid nanoparticles (16). Due to favourable drug penetration into the skin, clinical efficacy and reduced side effects of some carrier-loaded drugs [for review see: (13,17)], we aimed at developing a nanoparticular system for the gold standard of antiandrogens which is CPA. In contrast to liposomes, solid lipid nanoparticle preparations (SLN) can be very stable (18,19) and may deliver the drug at defined rates (20–22). Particles produced using mixtures of solid and fluid lipids (nanostructured lipid carriers, NLC), may be even superior to SLN because of higher loading capacity and stability (23). Drug loading to SLN and NLC increases skin penetration (24–26). Moreover, attachment to the SLN surface can even induce drug targeting to superficial skin strata (25,26). Particles of larger size may have the potential to deliver loaded agents preferentially to the hair follicle (27–29) offering the option of antiandrogen targeting to the pilosebaceous duct which might further improve the benefit/risk-ratio of acne treatment. Thus we compared various CPA loaded nano- and microparticular systems for topical application to the skin.

To ensure loading of the active moiety as well as to study if prodrug formation (16) might be a valid option, cutaneous CPA metabolism as well as receptor binding of CPA and the hydrolysis product cyproterone (CP) which may be formed by skin esterases (30,31) were studied, too. In fact, CPA effects on sebum formation are not yet described at the molecular level. Early reports comparing CPA to other antiandrogens (32,33) have been followed by the study demonstrating CPA induced enzyme inhibition in the sebocyte line SZ95 (3).

MATERIALS AND METHODS

Chemicals. Precirol® ATO 5, a mixture of 8–22% monoacyl, 40–60% diacyl, and 25–35% triacyl glyceryl palmitate and stearate (each 40–60%; melting interval 53–57°C) was a gift of Gattefossé (Weil a. Rh., Germany). Miglyol® 812 (caprylic/capric triglycerides), oleic acid and base cream (cremor basal; oil in water cream prepared as described in Deutscher Arzneimittel Codex 2004, containing: glycerol monostearate 60 4.0 g, cetyl alcohol 6.0 g, medium chain triglycerides 7.5 g, white vaselin 25.5 g, macrogol 20-glycerolmonostearate 7.0 g, propylene glycol 10.0 g, water 40.0 g) were obtained from Caelo (Minden, Germany). Poloxamer 188 (Lutrol F68) was purchased from BASF (Ludwigshafen, Germany). [1,2,6,7]-^{3}H-DHT and ^{3}H-thymidine were provided by Amersham (Freiburg, Germany), RU58841 from Roussel-Uclaf (Romainville, France). Cyproterone acetate (CPA), dihydrotestosterone (DHT) and other chemicals were purchased from Sigma (Deisenhofen, Germany).

Cell Cultures. Human immortalized sebaceous gland cells SZ95 (34) were initially grown in Sebomed® sebocyte basal medium (cc pro, Neustadt, Germany) complemented with epidermal growth factor (hEGF, 0.1 ng/ml), amphotericin B (50 ng/ml), gentamicin (20 μg/ml) and 10% fetal calf serum (FCS) or charcoal stripped FCS. For the proliferation experiments serum-free medium was used as well. Medium and supplements were obtained from Biochrom (Berlin, Germany). GR+/29+ cells (mouse fibroblast cell line overexpressing the androgen receptor, obtained from Prof. A. Wellstein, Washington, DC) were plated in Iscove's modified Dulbecco's Medium (Clonetics, San Diego, CA) supplemented with glutamine (2 mM), amphotericin B (50 ng/ml) and gentamicin (20 μg/ml). With growth medium 10% FCS was added. Human keratinocytes [isolated from juvenile foreskin (35)] were grown in keratinocyte basal medium (Cambrex, Verviers, France) supplemented with bovine pituitary extract (BPE, 30 μg/ml), hEGF (0.1 μg/ml), hydrocortisone (0.5 μg/ml), amphotericin B (50 ng/ml) and gentamicin (50 μg/ml), obtained from Cell Systems (St. Katharinen, Germany). Fibroblasts were grown in fibroblast growth medium consisting of Dulbecco's Modified Eagle's Medium supplemented with FCS 10%, glutamine (2 mM), amphotericin B (50 ng/ml) and gentamicin (20 μg/ml), all from Sigma.

Cells were maintained at 37°C in 5% CO_2 and regularly subcultured at a split ratio 1:3 using 0.25% trypsin and 0.2% EDTA (Sigma) in phosphate buffered saline (PBS). For the experiments keratinocyte and fibroblast passages 2–5 were used.

Synthesis of Cyproterone. Cyproterone acetate (120 mg) was stirred with potassium hydroxide (1.09 g) in a mixture of methanol 40 ml, aqua dest. 20 ml and dichloromethane 10 ml for 2 h at 40°C. Then, the mixture was extracted three times with chloroform 50 ml, the organic phases were removed, combined and evaporated under reduced pressure. Cyproterone was recrystallised with water, dried with $MgSO_4$ and weighed. The identity of the product was confirmed to be cyproterone using ^{1}H NMR-spectroscopy, mass analysis (EI-MS, ThermoFinniganMat, Bremen, Germany) and C-H-N analysis (Elementaranalyzer 240 B, 240 C, Perkin-Elmer, Rodgau-Jügesheim, Germany).

Receptor Binding. Binding affinities of CPA and CP to the androgen receptor were determined by competitive binding assays as described (16) using 29+/GR+ cells (36). The cells were grown in 24 well plates (Nunc, Wiesbaden, Germany) in supplemented Dulbecco's medium. To remove endogenous steroids, growth medium was replaced by basal medium 3 days before the binding experiments. To determine the relative binding affinities of CPA and CP, confluent 29+/GR+ cells were incubated with 0.5 nM ^{3}H-DHT alone and in the presence of nonlabelled DHT (0.05–5 nM), CPA (0.5 nM–10 μM) or CP (1 nM–150 μM) for 1 h under standard conditions (5% CO_2, 37°C). Subsequently, cells were washed three times with PBS and with 0.5 ml lysis buffer containing 2% sodium dodecyl sulfate (SDS), 10% glycerol and 10 mM Tris HCl (pH 6.8). Then radioactivity of ^{3}H-DHT was measured by liquid scintillation counting (1450 Microbeta Plus

Liquid Scintillation Counter, Wallac, Turku, Finland) as described (16) taking non-specific binding into account.

Cutaneous Metabolism. CPA metabolism was followed in cultured human foreskin fibroblasts, keratinocytes and SZ95 sebocytes (16). Three days after seeding the cells (10^5/well) in 6-well plates (Nunc, Wiesbaden, Germany), cells were washed with PBS, and fresh medium containing CPA at final concentrations of 0.1 μM or 1 μM was added. After incubation at 37°C in 5% CO_2 for 24 h or 48 h, 0.5 ml of the culture media were removed. Following the addition of the internal standard (19-nortestosterone) the steroids were extracted with 0.5 ml chloroform and the organic phases transferred to fresh tubes. The extraction was repeated twice and the combined chloroform extracts were dried under vacuum (Universal Vacuum System, Life Science, Frankfurt, Germany), redissolved in 100 μl ethanol and subjected to HPLC. Cell free medium served for negative control.

Cell Viability. The reduction of a tetrazolium dye (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MTT) which occurs only in functional mitochondria (31) served to verify cell viability during CPA and vehicle exposure. 10^5 cells seeded into 6-well plates were incubated with CPA 0.1 and 1 μM for 24 h at 37°C/5% CO_2. 200 μl MTT solution added for another 4 h resulted in the formation of a blue formazan dye. Following the removal of medium, the dye was extracted into 1 ml of DMSO. 200 μl were transferred into 96-well plates for absorbance reading at 540 nm. Viability was related to solvent (ethanol) treated control.

Proliferation of SZ95 Sebocytes. 10^5 SZ95 cells were seeded in 24 well plates (Falcon, Sigma, Deisenhofen, Germany) and grown for 3 days in medium with 10% FCS. Then the medium was changed to serum-free medium or medium with charcoal stripped FCS. DHT (100 pM, 100 nM ethanolic solution) alone as well as in the presence of CPA (10, 100 nM) was added. RU58841 (0.1, 1 μM) was studied in comparison to CPA. Ethanol treated cells served as control. The cells were incubated for 7–14 days changing drug loaded medium every 2–3 days. Then, 5 μl ^{3}H-thymidine (1 mCi) was added for another 6 h. Cells were washed three times with 250 μl PBS, lysed by addition of 250 μl 5% trichloroacetic acid and 200 μl sodium hydroxide solution (0.3 M) and then shaken at 300 rpm for 1 h. The amount of the ^{3}H-thymidine incorporated was measured in the cell suspension following the addition of scintillation cocktail 1.8 ml as described above.

Reverse Transcription and Amplification by Polymerase Chain Reaction (PCR). The mRNA of human keratinocytes, SZ95 sebocytes and 29+/GR+ cells was isolated by QuickPrep Micro mRNA Purification Kit (Amersham Biosciences, Freiburg im Breisgau, Germany) according to the manufacturer's instructions. Aliquots of the mRNA preparation were kept frozen at −80°C until used. 1 μg of mRNA was reversely transcribed (Superscript reverse transcriptase, Invitrogen, Karlsruhe, Germany) in the presence of 1 pmol of a 25–30 mer oligo(dT) primer. Two primers representing specific oligonucleotides were designed based on the nucleotide sequences of the androgen receptor. Oligonucleotide primer pairs were synthesized by Roth (Karlsruhe, Germany): forward primer A (FP) 5′-GAA GAC CTG CCT GAT CTG TG, reverse primer (RP) 5′-AAG CCT CTC CTT CCT CCT GT (269 bp) (37); forward primer B (FP) 5′-TAC CAG CTC ACC AAG CTC CT, reverse primer (RP) 5′-GCT TCA CTG GGT GTG GAA AT (195 bp). PCR amplification was carried out in a thermocycler (T Gradient, Whatman Biometra, Göttingen, Germany) using the Thermoprime plus polymerase (Advanced Biotechnologies, Columbia, MD) under the following cycling conditions: (1) 94°C for 1 min; (2) 94°C for 30 s; (3) 55°C for 30 s; (4) 72°C for 1 min; (5) repeat of steps 2–4 for 35 cycles; (6) 72°C for 2 min; and (7) 4°C for 1 s. Polymerase chain reaction products were size-fractionated in a 2% agarose gel, and visualized by ethidium bromide staining.

Method of Drug Loading to Lipid Carriers. The solid lipid Precirol was used for SLN and NLC production. Nanostructured lipid carriers contained oleic acid (NLC-O) or Miglyol (NLC-M) as the liquid phase. Miglyol was used for the nanoemulsion, too (Table I). All the respective nanoparticle dispersions were made up from 10% lipid phase and stabilized by 2.5% surfactant (Poloxamer 188). In general, the preparations contained 0.05% CPA (or 0.5% with respect to the lipid).

For nanoparticles and the nanoemulsion the drug was dissolved in the hot lipid phase (85°C) which was then dispersed in the surfactant solution. The premix formed using an Ultra turrax® (30 s, 8,000 rpm; IKA, Staufen, Germany) was passed through a high pressure homogenizer (Lab 40® APV Gaulin, Lübeck, Germany) performing three cycles at 85°C and 500 bar (38). Microspheres (MS, Table I) were prepared as described from Precirol by stirring of the premix with Ultra turrax® for 3 min, at 8,000 rpm yet omitting high pressure homogenisation. All dispersions were stored at 4°C in the dark.

Table I. Composition of Lipid Carriers

Carrier	Precirol	Liquid Lipid	% CPA		Crystallisation Degree (%)
			Dispersion	Water Phase	
SLN	10%	–	97.5 ± 11.3	0.52 ± 0.05	112.1
NLC-O	8%	Oleic acid 2%	102.7 ± 4.1	0.45 ± 0.08	107.8
NLC-M	8%	Miglyol 2%	107.2 ± 5.1	0.20 ± 0.03	117.2
NE	–	Miglyol 10%	99.5 ± 11.6	0.83 ± 0.27	–
MS	10%	–	111.0 ± 2.6	0.12 ± 0.004	83.8
cream	–	–	106.7 ± 5.7	n.s.	–

All preparations were stabilized by 2.5% Poloxamer 188 and loaded with CPA 0.05%. CPA content (% of theoretical, mean ± SD) in the formulation and in the separated water phase was quantified by HPLC. Crystallinicity was determined by differential scanning calorimetry. n.s.: not studied

For drug carrier interaction studies by parelectric spectroscopy (PS), at least two batches per CPA concentration (0–0.1%) were produced and evaluated.

CPA Cream. To produce CPA cream, small amounts of base cream (Cremor basal) were added to CPA and homogenised at ambient temperature using a pistil until the final concentration of CPA 0.05% was obtained.

Physicochemical Characterization of the Formulations. Particulate dispersions and the cream were characterized as described previously (38). The declared CPA content of all preparations was verified. Following the addition of the internal standard, 19-nortestosterone, 80 µl of the water phase or the diluted (1:10) preparation was extracted three times with chloroform 100 µl. The combined organic phases were dried under vacuum, redissolved in 100 µl ethanol and subjected to HPLC. To exclude re-crystallization of the drug within the water phase of the dispersion, lipid and water phases were separated by centrifugation using a filter (0.22 µm pore size, Schleicher& Schuell, Dassel, Germany) for 2.5 h at 1,000 g. CPA adsorption to the filter was excluded since the concentration measured by HPLC did not decline by filtration of 80 µl CPA 5.10^{-6} M solution.

Re-crystallization of the drug and the structure of the preparations were followed using light microscopy. This allowed the detection of crystals and aggregates exceeding 5 µm in size. To verify the existence of solid particles differential scanning calorimetry (DSC 821e, Mettler, Giessen, Germany) measurement was carried out. The area of the melting peak of the bulk lipids was set 100% to quantify crystallinity of CPA loaded particles. Particle size analysis was performed by laser diffractometry (LD, Coulter LS 230, Coulter, Miami, FL) and photon correlation spectroscopy (PCS, z-average; Coulter N4 Plus, Coulter Electronics, Krefeld, Germany). LD describes the maximum size of 95% of the particle population in a given volume and thus is influenced by larger particles. PCS gives the mean particle diameter and reflects the homogeneity of the distribution (polydispersity index, PI). Since the range of PCS measurements is limited to particles sized 3 nm to 3–5 µm, this method is not applicable to microspheres.

Characterization of Cyproterone Acetate Lipid Interaction by Parelectric Spectroscopy (PS). PS measurements were performed as described (24,26) using a commercial frequency analyser (type ZVRE; Rohde & Schwarz, München, Germany). The dependence of the parameters mobility f_0 and density $\Delta\varepsilon$ of the dipole-carrying lipids on the drug concentration gives insight into the type of drug loading: incorporation of the drug molecules into the lipid matrix or adsorption to the particle surface.

Drug Penetration into and Permeation of Intact Human Skin. Human skin was obtained from the abdominal and breast region of women aged 20–66 subjected to plastic surgery. After washing with aqua dest., subcutaneous fat tissue was carefully removed avoiding a contamination of the skin surface by subcutaneous lipids. The skin was used immediately or cryoconserved for up to 6 months. Fresh or thawed skin was cut with a dermatome (Aesculap, Tuttlingen, Germany) horizontally to obtain 500 µm split skin, and samples of 15 mm diameter were punched and mounted to static Franz diffusion cells (9 mm in diameter, Crown Scientific, Somerville, NJ) the horny layer facing the air and the dermis making contact with the receptor fluid PBS containing 5% bovine serum albumin (BSA) kept at 37°C. 200 µl (200 µg) of preparations containing CPA 0.05% were applied to the skin surface 9 mm in diameter for 6 or 24 h, respectively. At the end of the experiment the skin was rinsed with water, gently dried with a cotton swab and stripped five times with self-adhesive tape (Tesafilm, Beiersdorf, Hamburg, Germany). Details of skin preparation and Franz cell experiments are described elsewhere (39).

Penetration into Damaged Skin. Before mounting to Franz diffusion cells, the skin was stripped with self-adhesive tape (Tesafilm) 20 times by applying defined pressure using a roller as described by Jacobi *et al*. Moreover, the amount of horny layer removed was read by UV absorbance of the tape strips (40). Uptake into damaged skin was related to the uptake into intact skin from the same donor, which was tested in parallel.

Drug Quantification. Tapes obtained by stripping of the horny layer after CPA application were extracted immediately using chloroform 1 ml by vortexing for 5 s. Then the internal standard was added, mixed and the solvent was evaporated under reduced pressure, redissolved in ethanol 200 µl and subjected to HPLC.

Skin samples were cut horizontally in a freeze-microtome (Frigocut™ 2800 N, Leica Mikrosysteme, Bensheim, Germany) to obtain eight 50 µm slices. Two consecutive slices were pooled. Following the addition of aqua bidest. 500 µl and the internal standard, the slices were subjected to five freeze thaw cycles and then extracted with chloroform 500 µl. The organic phase was transferred to fresh tubes and the extraction was repeated twice. The combined extracts were exsiccated by vacuum rotation, the remainder was dissolved in ethanol 100 µl and subjected to HPLC.

Moreover, 12 ml of the receptor fluid was supplemented with internal standard and extracted three times with chloroform (5 ml). The evaporated combined extract was redissolved in ethanol 200 µl and CPA was quantified by HPLC.

HPLC (Bio-Tec Instruments, Neufahrn, Germany) with a Multohyp column 250×4.6 ODS-5 plus pre-column 20×4 Multohyp ODS-3 (CS-Chromatographics, Langerwehe, Germany) served to quantify CPA and CP concentrations. The internal standard was 19-nortestosterone. The mobile phase consisted of acetonitrile/water (61:39), the flow rate was 1.2 ml/min. UV absorption of CPA and CP was read at 284 nm, and at 246 nm for the internal standard. The limits of detection were CPA 160 ng/ml and CP 180 ng/ml, respectively. The limits of quantification were CPA 208 ng/ml and CP 230 ng/ml. The parameters for accuracy, precision and recovery of the method were determined at three representative CPA concentrations (4.3 to 17.3 µg/ml). Recovery from PBS plus 5% BSA was $85.6 \pm 4.6\%$ and exceeded 94% (SD: 2.0 to 13.7%) from cell media. Recovery was less than $71.5 \pm 15.4\%$ with the skin. Accuracy was 6.5–11.6%, intraday variability 4.7–7.7% and interday variability 5.1–7.4%.

Statistics. All data (% of dose absorbed and penetration ratios relative to cream set 1) are presented as arithmetic mean values including the standard deviations (±SD) of at least three independent experiments using skin from at least three donors and primary cells from three pools. The increased uptake over cream as well as differences of penetration ratios between the particulate preparations or between the skin

layers were analyzed using the *t*-test for independent samples. Normal distribution was proven using the Shapiro-Wilk-test, the homogeneity of variance using the *F* test. If variance inhomogeneity was recognized, the Welch-test was performed. If the requirement for the *t*-test was not fulfilled, the non-parametric Wilcoxon-Mann-Whitney test was carried out. The probability of error α was set to be 5%.

RESULTS

Cutaneous Metabolism. Neither cyproterone nor other metabolites (15β-hydroxy-cyproterone acetate) were detectable in human fibroblasts, keratinocytes and SZ95 sebocytes (10^5 cells/well) exposed for 24 h to CPA 10^{-5} M or 10^{-6} M. This holds also true, if applying 0.05% of CPA dispersion (cream, particulate systems) to the surface of viable human skin (Fig. 1). When doubling the cell number and incubation time we identified CP at approximately 1% of CPA added to human fibroblast cultures. MTT test did not indicate any reduction of cell viability by CPA concentrations of interest (10^{-6} and 10^{-5} M) since viability was 96.8 ± 5.2%. Thus cyproterone acetate is less subject to hydrolysis than described for glucocorticoids (25,30,31).

CPA and CP Binding to Androgen Receptor and Effects in SZ95 Sebocytes. The competitive binding assay in GR+/29+ cells resulted in EC_{50} values of 0.36 ± 0.41 nM for 5α-dihydrotestosterone, 63.8 ± 31.7 nM for CPA and 442.5 ± 90.6 nM for CP. Thus, activity of native CPA is close to the one of RU58841 (88.1 ± 69.3 nM). While we failed with RU58841 loading to SLN, we received a sufficiently stable SLN preparation when introducing an ester side chain into the non-steroidal antiandrogen. The respective prodrug RU 58841 myristate is cleaved rapidly by human keratinocytes and fibroblasts as well as by SZ95 sebocytes (16). Yet CP prodrug formation to improve particle loading is not a valid option with the steroidal antiandrogen because of a major loss in activity as compared to native CPA.

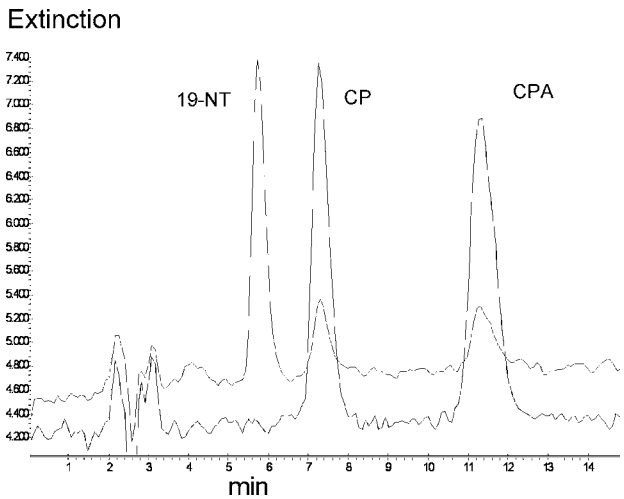

Fig. 1. Representative HPLC chromatogram of 19-nortestosterone (19-NT), CP and CPA. The *lower line* presents recordings at 284 nm (CPA, CP), the *upper one* at 246 nm (19-NT).

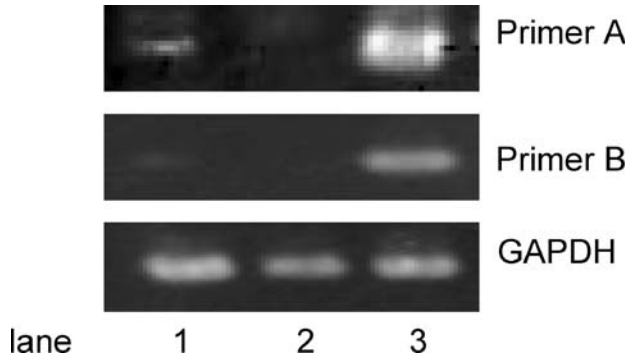

Fig. 2. Androgen receptor expression. Using two different primers (A, B) a high amount of androgen receptor mRNA was detected in GR+/29+ cells (lane 3, positive control) and a lower one in keratinocytes (lane 1), while no expression was seen in SZ95 sebocytes (lane 2). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression served for reference.

Next we aimed to study the influence of CPA on sebocytes which is not yet described in detail. Zouboulis *et al.* established the sebocyte line SZ95 for testing of potential anti-acne drugs (34) and described various inhibitors of androgen synthesis including CPA (3). Here we aimed to investigate androgen and antiandrogen effects on SZ95 sebocytes growth. Cells were grown in serum-free medium, medium supplemented with FCS and charcoal-stripped FCS for 7–14 days. Under serum-free conditions, cell morphology appeared damaged. Grown in the presence of FCS and charcoal-stripped FCS, ^{3}H-thymidine uptake remained constant with increasing DHT concentration. This result is well in accordance with the lack of androgen receptor expression as derived from polymerase chain reaction (Fig. 2). In contrast to SZ95 sebocytes, the androgen receptor is expressed in high amounts in GR+/29+ cells and in lower amounts in human keratinocytes, which served for positive control. Expression of the androgen receptor in the stable transfected GR+/29+ cell line (36) as well as the lower expression in keratinocytes is known (41), while Fritsch *et al.* detected androgen receptor mRNA in SZ95 cells and transformed human keratinocytes (3). Thus, we additionally studied antiandrogen effects on SZ95 sebocytes growth. Yet, CPA 10 nM, 100 nM and RU 58841 0.1, 1 μM used for reference did not inhibit cell proliferation in DHT (0.1 nM, 100 nM) exposed SZ95 sebocytes (data not shown) either.

Facing minor CPA metabolism in keratinocytes and fibroblasts and the strong androgen receptor binding of native CPA, it is obvious that we have to load native CPA to carriers.

Preparation and Characterization of CPA-loaded Lipid Carriers. SLN, NLC-O, NLC-M and MS dispersions as well as a nanoemulsion containing 0.05% of CPA (0.5% of the lipid phase) were prepared and characterized with respect to the amount of CPA in the formulation and within the water phase, formation of solid particles as well as particle morphology and size over time.

Mean amounts of CPA found in the nanodispersions and the cream were 97.5–111.0% of theoretical. In the water phase we detected only 0.12–0.83% of the drug (Table I). Since CPA crystals or aggregates were not detected, at least 99% of CPA is associated with the lipid matrix. As with the bulk lipid which was studied for reference thermoanalytical diagrams of SLN,

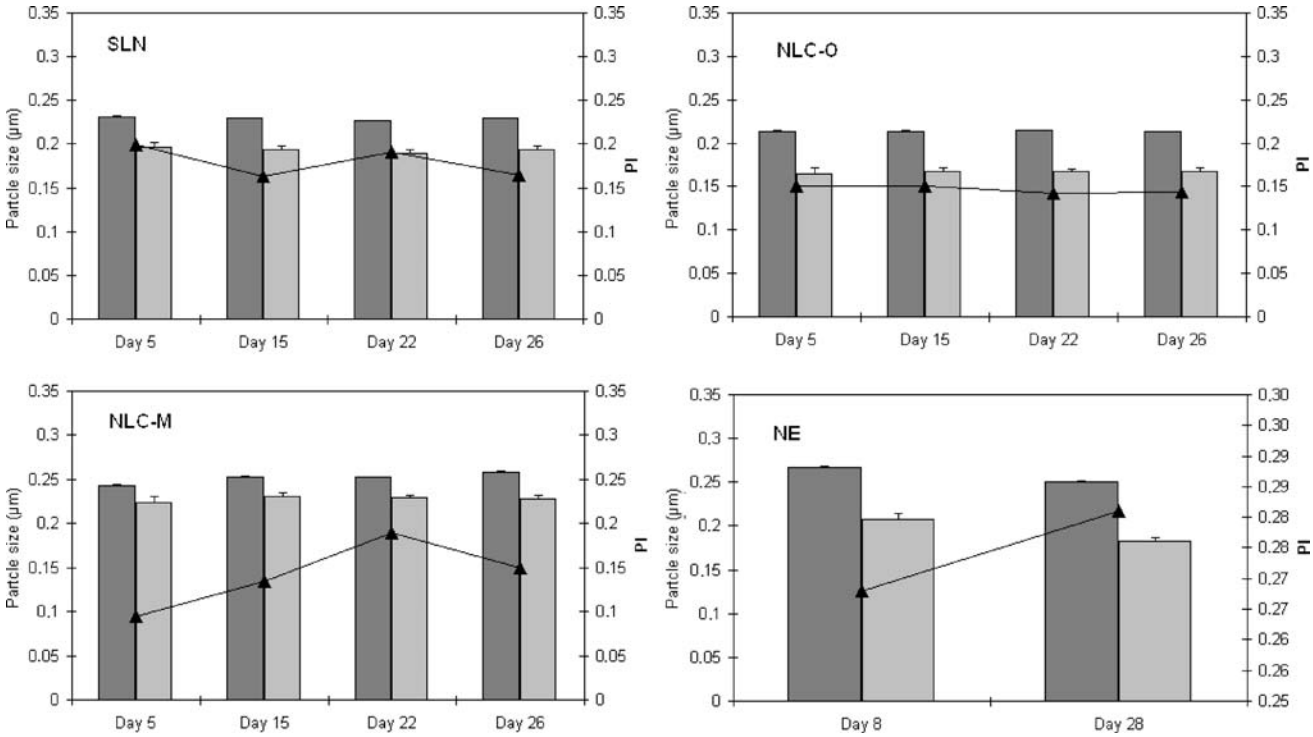

Fig. 3. Characterization of CPA 0.05% nanoparticular carriers. The systems (solid lipid nanoparticles, SLN; nanostructured lipid carriers made up from Miglyol, NLC-M; or oleic acid, NLC-O; as well as nanoemulsions, NE) have been investigated for particle size using laser diffractometry (*black columns*), photon correlation spectroscopy (*grey columns*) and polydispersity index (PI, *black line*) when stored for up to 28 days at 4°C (one representative formulation, mean ± SD).

NLC and MS showed a melting peak of Precirol between 53–57°C, which confirmed the existence of solid particles in the formulation. The degree of crystallization lay between 83.1–112.1%.

Particle size and homogenity of size distribution (PI) were followed for up to 28 days of storage at 4°C. All nanoparticulate preparations (SLN, NLC-O, NLC-M and NE) showed a particle size in the range of 200–250 nm, reproducibility was acceptable (polydispersity index <0.2; Fig. 3). Storage for up to 4 weeks did not change particle sizes and their distribution. Stability, with respect to re-crystallization of the drug, was highest with the nanoemulsion (up to 4 months) followed by NLC-M (up to 8 weeks) >NLC-O (up to 5 weeks) >SLN (up to 2 weeks). Microspheres had a particle size of 2–3 µm (LD95%), re-crystallization of CPA was not observed, while aggregation of the particles was detected after 7 days. Therefore drug-particle interaction as well as CPA skin penetration and permeation were studied within the first weeks after preparation of the nanoparticles, while microspheres were tested within 3 days after production.

Parelectric Spectroscopy. PS allows to describe drug particle interaction (24,26), the respective drug free dispersions served as control (Fig. 4). PS measurements were performed using 2–3 independent series for each type of particle produced from lipids and the tensid purchased over 2 years. Batches with increased CPA content (0–0.1%, at least two batches per concentration) were produced and subjected to PS measurement while stable. In fact, reduced values of f_0 result in augmented $\Delta\varepsilon$, thus proving the consistency of the systems. As, however, except for SLN all these curves obviously follow a linear dependence on c (Fig. 4), we have to assume an almost complete incorporation of the agent into MS and NLC (NLC-M, NLC-O). In contrast, CPA is attached to the SLN surface.

CPA Penetration into and Permeation of Human Skin Ex-vivo. First we studied CPA uptake and metabolism with fresh human skin (from three donors). Since we could not detect any metabolites of CPA, cryoconserved skin (from up to five donors per preparation) was used for further experiments (run in triplicate) comparing the uptake when applying particulate systems and a conventional cream. Two independent series of experiments were performed studying CPA penetration when applied for 6 h to normal skin only (series 1, Fig. 5) and to normal and stripped skin of the same donors (series 2). For the permeation study (series 3), CPA treatment was 24 h to allow sufficient drug transport via the skin.

CPA uptake increased when loaded to nanoparticles, differences in the uptake from SLN and NLC were seen in all series, while stripping the skin did not enhance CPA uptake (series 2; $p > 0.05$) with any preparation. This, however, may result from the fact that we removed less horny layer (UV absorbance 0.0039) than described recently [UV absorbance 0.0065; (40)]. Applying CPA-loaded SLN for 6 h, we observed a high CPA amount within the skin, predominantly within the first 100 µm, exceeding the amount following the cream at least four-fold (Fig. 5). With NLC-O, NLC-M and microspheres the CPA amount increased 2–3-fold in the first 100 µm layer containing the epidermis. CPA uptake from NLC made up from Miglyol and oleic acid did not differ. CPA amounts in

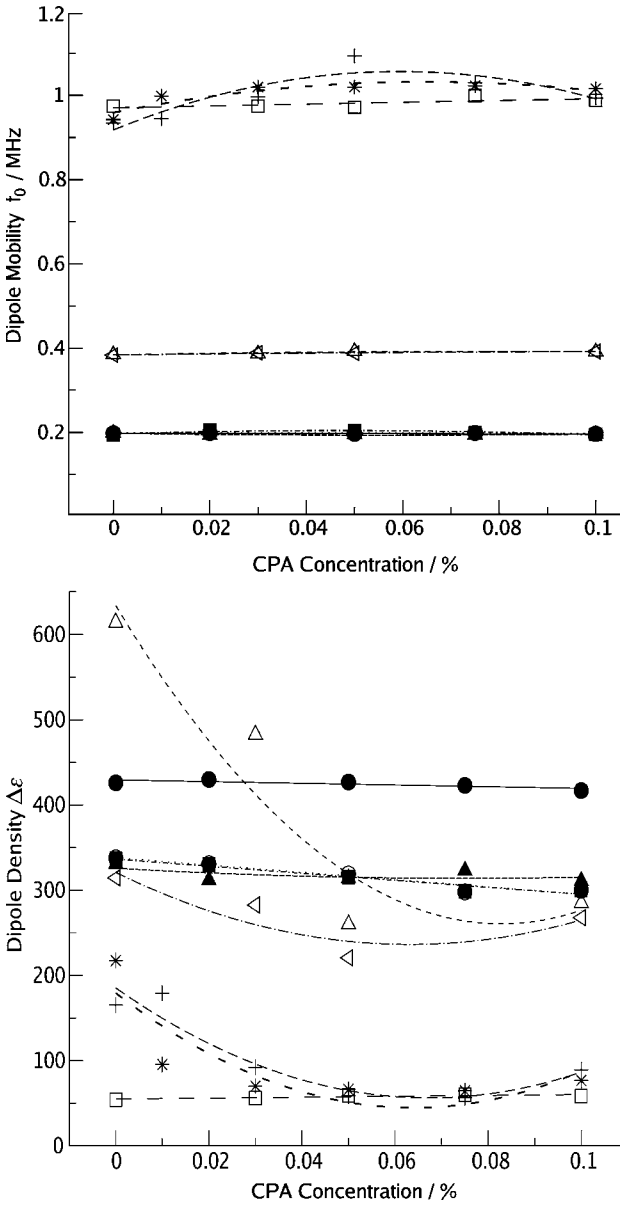

Fig. 4. Influence of CPA on f_o and Δε of the lipid dipole. Particles were loaded with increasing drug concentrations (0–0.1% in the dispersion) as described in Materials and Methods and then subjected to parelectric spectroscopy varying the frequency from 0.01 to 100 MHz at 305 K. Results are given for f_0(c) and Δε(c) for SLN (+, *), NLC-O (*open circle*, *closed circle*), NLC-M (Δ, *right triangle*, *closed triangle*) and MS (*open square*, *closed square*) dispersions. For each type of preparation at least two series of batches were produced within 2 years span, the series are characterised by the respective symbols.

the dermis declined sharply as to be seen from skin layers 100–400 μm ($p < 0.05$).

When comparing the ratios of CPA amounts within the various layers following solid nanoparticular systems as related to the cream (Fig. 5), epidermal targeting is seen with SLN. In the first 100 μm layer CPA concentrations increased four-fold ($p \leq 0.05$), while CPA concentrations surmounting the values for cream are not found in deeper layers. To exclude that the observed CPA enrichment in the epidermis may be an artifact due to a strong adherence of SLN to the most superficial skin layers, we stripped treated skin (five strips) prior to slicing and extracted CPA from the removed tape strips. In fact, CPA amounts in tape strips and the first layer (0–100 μm) were similar following the cream, SLN, and following NLC-O and NLC-M. This should preclude an overestimation of CPA penetration into the 0–100 μm layer due to carrier-associated drug adhering more to the uppermost parts of the stratum corneum. Thus CPA targeting by SLN to the epidermis appears very likely. With MS and NE, however, CPA amounts in the tape strips exceeded amounts in the layer 0–100 μm about 2.5-fold (Fig. 5), which suggests CPA retention of these formulations in the upper stratum corneum. Moreover, penetration did not increase following the nanoemulsion while a two-fold increase was seen when loaded to microparticles.

Following the application for 24 h, permeation was 0.19 ± 0.001% of the applied dose with the cream, 1.30 ± 0.27% following SLN, 0.51 ± 0.007% with NLC-O and 0.67 ± 0.05% with NLC-M. CPA permeation of split skin exceeded permeation following the cream by about six-fold, which is well in accordance with penetration enhancement after 6 h. Thus the drug is not confined to outermost skin and can reach sebocytes and dermal papilla cells. When loaded to microspheres permeation was 0.36 ± 0.02% of dose. Yet, we were unable to study to which extent microspheres may deliver CPA to the hair follicle.

DISCUSSION

Before developing particulate carriers for CPA to induce high and reproducible antiandrogen effects to the skin we first aimed to verify the activity of native CPA and to quantify CP effects. If CP should be as active as CPA this should allow us to form CP ester prodrugs of long chain fatty acids facilitating drug loading to SLN (16). In fact, due to the sterical hindrance of the ester group CPA proved to be very stable in human keratinocytes, fibroblasts and sebocytes as well as in human skin *ex-vivo*.

Next we aimed to study the influence of CPA on sebocytes which is not yet described in detail. Zouboulis *et al.* established the sebocyte line SZ95 for testing of potential anti-acne drugs (34) and described various inhibitors of androgen synthesis including CPA (3). While CPA efficiently binds to the androgen receptor, this is not true with CP. In contrast to our expectations, however, we failed to demonstrate antiandrogen-induced inhibition of proliferation in SZ95 sebocytes and we could not detect androgen receptor expression either (Fig. 2). This may be due to the fact that our growth medium was not hormone supplemented as described only recently (37). Nevertheless it is proven, that we have to load CPA. Prodrugs of CP are no valid options, which is in contrast to the nonsteroidal antiandrogen RU 58841 (16).

Since CPA is highly lipophilic (logP 3.28), we aimed to load the antiandrogen to lipid carriers. Besides SLN, NLC, and a nanoemulsion all of which are of nanosize, we also studied microspheres. In fact, microsize polymers can favour drug penetration into hair follicle and sebaceous gland (27–29). Particles were sufficiently stable except for MS (Table I, Fig. 3). Next the particles were subjected to PS, which allows the description of the mode of interaction

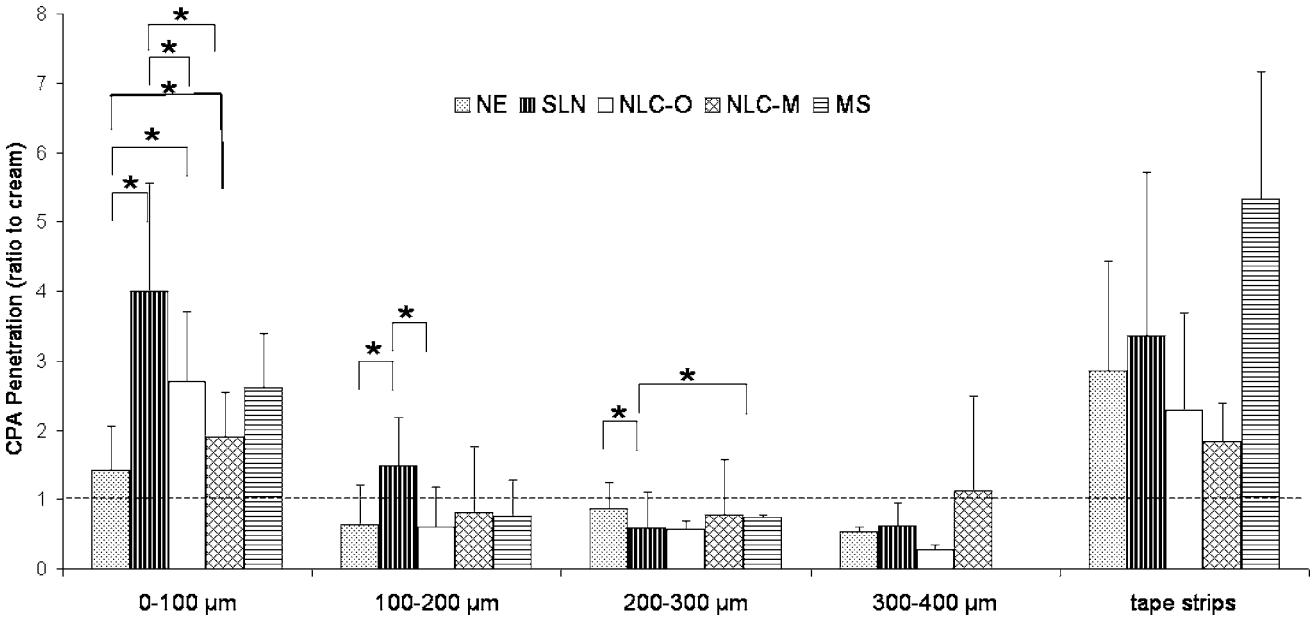

Fig. 5. CPA penetration into human skin. Dispersions of drug loaded carrier system (0.05%) were applied to cryoconserved human skin for 6 h (NE, $n=3$ donors, SLN $n=7$ donors, NLC-O, $n=5$ donors, NLC-M, $n=3$ donors, MS, $n=2$ donors). Particulate dispersions were tested in parallel to o/w cream ($n=7$ donors) using 2–4 skin tissue specimen per donor for each preparation. Except for NE, CPA amounts in the first layer exceed amounts following the cream, moreover penetration ratios of the layers 0–100 µm and 100–200 µm differed for all preparations except for NLC-M ($p \leq 0.05$). *Differences in CPA-ratios (over cream) between the particulate systems ($p \leq 0.05$).

between drug and particle. The principles of the method as well as the experimental set-up have been reported in the detail previously. Successfully applied for glucocorticoids (26) and nile red (24), PS now served to study CPA loading to SLN, NLC-O, NLC-M and MS. Once more a highly lipophilic agent when added in low amounts (≤1% of the amount of lipid) proved to be enclosed in the liquid lipid phase of NLC as it was observed with nile red (24). Moreover, CPA was also entrapped into the particle matrix of MS. This contrasts with SLN (Fig. 4), Δε curves obtained with independently produced batches passing through a minimum have to be interpreted as CPA attached to particle surface.

Next we studied the influence of nanoparticulate and microparticulate carriers on the dermal absorption of CPA. Previous experiments with nile red showed an increased uptake rate, especially when loaded to SLN (24) but also when applying polymer nanoparticles (42). Once more we observed that solid particles favoured drug absorption, SLN more than NLC ($p \leq 0.05$ versus cream; Fig. 5). Total CPA absorption (layers 0–400 µm) was 0.24% of the dose applied for 6 h following the cream. The rather poor penetration is well in accordance with the failure of early clinical studies (9,10) and the high concentration in the alcohol lotion (0.5 and 1%) efficacious in acne patients (11). Preliminary studies in acne patients with liposomal CPA indicated CPA serum levels to be about 10% of serum levels following oral treatment (15). Therefore topical CPA application is a relevant option, given the formulation is stable and allows sufficient penetration to the target site. As expected, uptake increased (1.64%) when applying SLN and with NLC (0.86%). In fact, almost the same increase was seen with nile red incorporated into the lipid matrix of SLN and NLC (24). Yet we failed to detect a difference with NLC containing Miglyol or oleic acid as liquid phase (Fig. 5), although oleic acid is reported to enhance dermal drug absorption (43). To the best of our knowledge CPA concentrations in human skin have not been published yet. Thus a clinical experiment is needed to prove efficacy of systems described here. Studying topical glucocorticoids, we demonstrated SLN to increase cutaneous uptake about four-fold over approved commercial creams (25,26). If this holds also true if compared to the alcoholic CPA formulation (11) is still open.

Making close contact with superficial junctions of corneocyte clusters and furrows between corneocyte islands SLN and NLC may allow drug release for several hours—as described for liposomes (17). Moreover, drug dissolved or finely dispersed within the lipid matrix of the carrier or attached to the carrier surface should facilitate drug dissolution within epidermal lipids. Finally, a lipid film covering the skin surface may further enhance dermal absorption because of an additional occlusive effect.

For the first time, we also included microparticles into our studies. Particle size (2–3 µm) exceeded the sizes of nanoparticles ten-fold, yet CPA penetration was close to the uptake from NLC (Fig. 5) and the drug was incorporated into the lipid matrix with either system.

The major enhancement of CPA amounts in the first 100 µm skin layer following SLN application over the concentrations obtained with the cream as compared to the respective increase seen in deeper skin layers (Fig. 5) suggests epidermal CPA targeting by SLN. This corresponds to targeting of prednicarbate attached to SLN as described previously (25,26) while such an effect was not seen with nile red incorporated into SLN (24). Therefore epidermal targeting appears to be associated with drug loading to the particle surface. This association as well as the basic mechanism is to be elucidated in future studies covering a broader spectrum of drugs or model drugs.

While a thirty-fold enhancement of the absorption of the lipophilic drugs sufentanil and methylprednisolone aceponate

was described when 20 tape strips were taken (44,45), we failed to detect an improved CPA uptake by this approach which is reported to remove the horny layer by about 50% (44). Yet this appears to be less under our experimental conditions. Thus, the influence of skin damage on CPA absorption needs further studies.

CONCLUSION

Given that CPA loading to nanoparticulate carrier systems or microspheres enhancing skin absorption results in therapeutic drug levels within the target tissue while reducing systemic availability of the antiandrogen as compared to the oral route, this may stimulate the development of a commercial topical formulation.

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REFERENCES

1. W. F. Bergfeld. The evaluation and management of acne: economic considerations. *J. Am. Acad. Dermatol.* **32**:S52–S56 (1995).
2. H. Akamatsu, C. C. Zouboulis, and C. E. Orfanos. Control of human sebocyte proliferation *in vitro* by testosterone and 5-alpha-dihydrotestosterone is dependent on the localization of the sebaceous glands. *J. Invest. Dermatol.* **99**:509–511 (1992).
3. M. Fritsch, C. E. Orfanos, and C. C. Zouboulis. Sebocytes are the key regulators of androgen homeostasis in human skin. *J. Invest. Dermatol.* **116**:793–800 (2001).
4. Y. Ando, Y. Yamaguchi, K. Hamada, K. Yoshikawa, and S. Itami. Expression of mRNA for androgen receptor, 5alpha-reductase and 17beta-hydroxysteroid dehydrogenase in human dermal papilla cells. *Br. J. Dermatol.* **141**:840–845 (1999).
5. D. Van Neste. Natural scalp hair regression in preclinical stages of male androgenetic alopecia and its reversal by finasteride. *Skin Pharmacol. Physiol.* **19**:168–176 (2006).
6. D. Van Neste, V. Fuh, P. Sanchez-Pedreno, E. Lopez-Bran, H. Wolff, D. Whiting, J. Roberts, D. Kopera, J. J. Stene, S. Calvieri, A. Tosti, E. Prens, M. Guarrera, P. Kanojia, W. He, and K. D. Kaufman. Finasteride increases anagen hair in men with androgenetic alopecia. *Br. J. Dermatol.* **143**:804–810 (2000).
7. W. A. van Vloten, C. W. van Haselen, E. J. van Zuuren, C. Gerlinger, and R. Heithecker. The effect of 2 combined oral contraceptives containing either drospirenone or cyproterone acetate on acne and seborrhea. *Cutis* **69**:2–15 (2002).
8. C. C. Zouboulis. Therapie der Akne mit Antiandrogenen—Eine evidenzbasierte Übersicht. *J. Dtsch. Dermatol. Ges.* **1**:535–546 (2003).
9. J. Fiet, R. Morville, D. Chemama, J. M. Villette, B. Gourmel, J. L. Brerault, and C. Dreux. Percutaneous absorption of 5 alpha-dihydrotestosterone in man. I. Plasma androgen and gonadotrophin levels in normal adult men after percutaneous administration of 5 alpha-dihydrotestosterone. *Int. J. Androl.* **5**:586–594 (1982).
10. R. T. Scott Jr., B. Ross, C. Anderson, and D. F. Archer. Pharmacokinetics of percutaneous estradiol: a crossover study using a gel and a transdermal system in comparison with oral micronized estradiol. *Obstet. Gynecol.* **77**:758–764 (1991).
11. F. Iraji, A. Momeni, S. M. Naji, and A. H. Siadat. The efficacy of topical cyproterone acetate alcohol lotion versus placebo in the treatment of the mild to moderate acne vulgaris: a double blind study. *Dermatol. Online J.* **12**:26, 2006 (2006).
12. M. J. Choi and H. I. Maibach. Liposomes and niosomes as topical drug delivery systems. *Skin Pharmacol. Physiol.* **18**:209–219 (2005).
13. M. Schäfer-Korting, W. Mehnert, and H. C. Korting. Lipid nanoparticles for improved topical application of drugs for skin diseases. *Adv. Drug Deliv. Rev.* (in press).
14. E. Bernard, J. L. Dubois, and J. Wepierre. Importance of sebaceous glands in cutaneous penetration of an antiandrogen: target effect of liposomes. *J. Pharm. Sci.* **86**:573–578 (1997).
15. D. M. Gruber, M. O. Sator, E. A. Joura, E. M. Kokoschka, G. Heinze, and J. C. Huber. Topical cyproterone acetate treatment in women with acne: a placebo-controlled trial. *Arch. Dermatol.* **134**:459–463 (1998).
16. U. Münster, C. Nakamura, A. Haberland, K. Jores, W. Mehnert, S. Rummel, M. Schaller, H. C. Korting, C. Zouboulis, U. Blume-Peytavi, and M. Schäfer-Korting. RU 58841-myristate-prodrug development for topical treatment of acne and androgenetic alopecia. *Pharmazie* **60**:8–12 (2005).
17. G. Cevc. Lipid vesicles and other colloids as drug carriers on the skin. *Adv. Drug Deliv. Rev.* **56**:675–711 (2004).
18. A. A. Date, B. Naik, and M. S. Nagarsenker. Novel drug delivery systems: potential in improving topical delivery of antiacne agents. *Skin Pharmacol. Physiol.* **19**:2–16 (2006).
19. C. Freitas and R. H. Müller. Stability determination of solid lipid nanoparticles (SLN) in aqueous dispersion after addition of electrolyte. *J Microencapsul.* **16**:59–71 (1999).
20. V. Jenning, A. F. Thunemann, and S. H. Gohla. Characterisation of a novel solid lipid nanoparticle carrier system based on binary mixtures of liquid and solid lipids. *Int. J. Pharm.* **199**:167–177 (2000).
21. B. Siekmann and K. Westesen. Sub-micron sized parenteral carrier systems based on solid lipid. *Pharm. Pharmacol. Lett.* **1**:123–126 (1992).
22. A. zur Mühlen, C. Schwarz, and W. Mehnert. Solid lipid nanoparticles (SLN) for controlled drug delivery-drug release and release mechanism. *Eur. J. Pharm. Biopharm.* **45**:149–155 (1998).
23. R. H. Müller, M. Radtke, and S. A. Wissing. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Adv. Drug Deliv. Rev.* **54**(Suppl 1):S131–S155 (2002).
24. S. Lombardi-Borgia, M. Regehly, R. Sivaramakrishnan, W. Mehnert, H. C. Korting, K. Danker, B. Röder, K. D. Kramer, and M. Schäfer-Korting. Lipid nanoparticles for skin penetration enhancement-correlation to drug localization within the particle matrix as determined by fluorescence and parelectric spectroscopy. *J. Control. Release* **110**:151–163 (2005).
25. C. Santos Maia, W. Mehnert, M. Schaller, H. Korting, A. Gysler, A. Haberland, and M. Schäfer-Korting. Drug targeting by solid lipid nanoparticles for dermal use. *J. Drug Target.* **10**:489–495 (2002).
26. R. Sivaramakrishnan, C. Nakamura, W. Mehnert, H. C. Korting, K. D. Kramer, and M. Schäfer-Korting. Glucocorticoid entrapment into lipid carriers-characterisation by parelectric spectroscopy and influence on dermal uptake. *J. Control. Release* **97**:493–502 (2004).
27. R. M. Hoffman. Topical liposome targeting of dyes, melanins, genes, and proteins selectively to hair follicles. *J. Drug Target.* **5**:67–74 (1998).
28. J. Lademann, H. Weigmann, C. Rickmeyer, H. Barthelmes, H. Schaefer, G. Mueller, and W. Sterry. Penetration of titanium dioxide microparticles in a sunscreen formulation into the horny layer and the follicular orifice. *Skin Pharmacol. Appl. Skin Physiol.* **12**:247–256 (1999).
29. R. Toll, U. Jacobi, H. Richter, J. Lademann, H. Schaefer, and U. Blume-Peytavi. Penetration profile of microspheres in follicular targeting of terminal hair follicles. *J. Invest. Dermatol.* **123**:168–176 (2004).
30. A. Gysler, B. Kleuser, W. Sippl, K. Lange, H. C. Korting, H. D. Höltje, and M. Schäfer-Korting. Skin penetration and metabolism of topical glucocorticoids in reconstructed epidermis and in excised human skin. *Pharm. Res.* **16**:1386–1391 (1999).
31. A. Gysler, K. Lange, H. C. Korting, and M. Schäfer-Korting. Prednicarbate biotransformation in human foreskin keratinocytes and fibroblasts. *Pharm. Res.* **14**:793–797 (1997).
32. M. M. Bouton, D. Lecaque, J. Secchi, and C. Tournemine. Effect of a new topically active antiandrogen (RU 38882) on the rat

sebaceous gland: comparison with cyproterone acetate. *J. Invest. Dermatol.* **86**:163–167 (1986).
33. C. Luderschmidt, J. Jawny, and W. Eiermann. Relative binding affinity at metribolone androgenic binding sites of various antiandrogenic agents. *Arzneimittelforschung* **37**:1262–1265 (1987).
34. C. C. Zouboulis, H. Seltmann, H. Neitzel, and C. E. Orfanos. Establishment and characterization of an immortalized human sebaceous gland cell line (SZ95). *J. Invest. Dermatol.* **113**:1011–1020 (1999).
35. K. Lange, B. Kleuser, A. Gysler, M. Bader, C. Maia, C. Scheidereit, H. C. Korting, and M. Schäfer-Korting. Cutaneous inflammation and proliferation *in vitro*: differential effects and mode of action of topical glucocorticoids. *Skin Pharmacol. Appl. Skin Physiol.* **13**:93–103 (2000).
36. H. J. List, C. Lozano, J. Lu, M. Danielsen, A. Wellstein, and A. T. Riegel. Comparison of chromatin remodeling and transcriptional activation of the mouse mammary tumor virus promoter by the androgen and glucocorticoid receptor. *Exp. Cell Res.* **250**:414–422 (1999).
37. E. Makrantonaki, J. Adjaye, R. Herwig, T. C. Brink, D. Groth, C. Hultschig, H. Lehrach, and C. C. Zouboulis. Age-specific hormonal decline is accompanied by transcriptional changes in human sebocytes *in vitro*. *Aging Cell* **5**:331–344 (2006).
38. W. Mehnert and K. Mäder. Solid lipid nanoparticles: production, characterization and applications. *Adv. Drug Deliv. Rev.* **47**:165–196 (2001).
39. S. Schreiber, A. Mahmoud, A. Vuia, M. K. Rübbelke, E. Schmidt, M. Schaller, H. Kandarova, A. Haberland, U. F. Schäfer, U. Bock, H. C. Korting, M. Liebsch, and M. Schäfer-Korting. Reconstructed epidermis versus human and animal skin in skin absorption studies. *Toxicol. In Vitro* **19**:813–822 (2005).
40. U. Jacobi, H. J. Weigmann, M. Baumann, A. I. Reiche, W. Sterry, and J. Lademann. Lateral spreading of topically applied UV filter substances investigated by tape stripping. *Skin Pharmacol. Physiol.* **17**:17–22 (2004).
41. S. Inui, S. Itami, H. J. Pan, and C. Chang. Lack of androgen receptor transcriptional activity in human keratinocytes. *J. Dermatol. Sci.* **23**:87–92 (2000).
42. R. Alvarez-Roman, A. Naik, Y. N. Kalia, R. H. Guy, and H. Fessi. Enhancement of topical delivery from biodegradable nanoparticles. *Pharm. Res.* **21**:1818–1825 (2004).
43. I. M. Schneider, W. Wohlrab, and R. Neubert. Fettsäuren und Epidermis. *Hautarzt* **48**:303–310 (1997).
44. S. J. Bashir, A. L. Chew, A. Anigbogu, F. Dreher, and H. I. Maibach. Physical and physiological effects of stratum corneum tape stripping. *Skin Res. Technol.* **7**:40–48 (2001).
45. H. Weigmann, J. Lademann, H. Meffert, H. Schaefer, and W. Sterry. Determination of the horny layer profile by tape stripping in combination with optical spectroscopy in the visible range as a prerequisite to quantify percutaneous absorption. *Skin Pharmacol. Appl. Skin Physiol.* **12**:34–45 (1999).