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Research paper

Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin

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Abstract

To evaluate the potential use of solid lipid nanoparticles (SLN) in dermatology and cosmetics, glyceryl behenate SLN loaded with vitamin A (retinol and retinyl palmitate) and incorporated in a hydrogel and o/w-cream were tested with respect to their influence on drug penetration into porcine skin. Conventional formulations served for comparison. Excised full thickness skin was mounted in Franz diffusion cells and the formulations were applied for 6 and 24 h, respectively. Vitamin A concentrations in the skin tissue suggested a certain drug localizing effect. High retinol concentrations were found in the upper skin layers following SLN preparations, whereas the deeper regions showed only very low vitamin A levels. Because of a polymorphic transition of the lipid carrier with subsequent drug expulsion following the application to the skin, the drug localizing action appears to be limited for 6–24 h. Best results were obtained with retinol SLN incorporated in the oil-in-water (o/w) cream retarding drug expulsion. The penetration of the occlusion sensitive drug retinyl palmitate was even more influenced by SLN incorporation. Transepidermal water loss (TEWL) and the influence of drug free SLN on retinyl palmitate uptake exclude pronounced occlusive effects. Therefore enhanced retinyl palmitate uptake should derive from specific SLN effects and is not due to non-specific occlusive properties. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Solid lipid nanoparticles; Vitamin A; Porcine skin; Percutaneous absorption; Drug localization in the skin

1. Introduction

Early work on solid lipid nanoparticles (SLN) dispersions mainly focused on the parenteral route of drug administration, sustained release and drug targeting were the primary objectives [1–5]. Only recently have the peroral or dermal use [6] been evaluated. Peptides like cyclosporin A were encapsulated into SLN [7]. These peptide formulations showed a reduced peak plasma concentration and prolonged therapeutic drug level following oral administration. Moreover, cyclosporin bioavailability appeared more reproducible as compared to a commercial formulation [8].

Because SLN are based on non irritative and non toxic [9] (often GRAS status) lipids they seem to be well suited for use on damaged or inflamed skin. Sustained drug release properties can also be useful for dermal formulations. For example, for topical antibiotics it is desirable to maintain high drug concentrations over a prolonged time on the skin surface and within the skin tissue [10]. Moreover, irritant drugs like benzoyl peroxide [11] or tretinoin [12,13] turned out to be less irritating if applied in a controlled release device. This also reduces systemic uptake of the active agent [14]. With respect to their use as carriers for drugs and cosmetics, further favourable properties of SLN include an occlusive effect due to film formation on the skin surface which reduces transepidermal water loss (TEWL) [15,16]. Increasing the water content in the skin reduces the symptoms of atopic eczema and also improves the appearance of healthy human skin [17]. Occlusion also favours drug penetration into the skin. The high specific surface area of nanometer sized SLN facilitates contact of encapsulated drugs with the stratum corneum.

The present study investigates the interactions between SLN and porcine skin. A SLN dispersion was compared to a nanoemulsion, which is a submicron fat emulsion (in this case 200 nm in diameter). Additionally SLN containing hydrogels and oil-in-water (o/w) cream were evaluated. Release properties were assessed by monitoring drug distribution in the skin following a 6 and 24 h incubation period. Vitamin A – retinol and its ester retinyl palmitate – served as test drugs because of their high lipophilicity which make them excellent candidates for SLN encapsulation. Both

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molecules differ in physicochemical properties like molecular weight (M_r (retinyl palmitate): 468.8, M_r (retinol): 286.4) and chemical structure (ester vs. alcohol). Furthermore, dermal absorption of retinol is not sensitive to occlusion whereas an increased penetration of retinyl palmitate due to occlusion is reported [18]. Because of these differences in physico-chemical properties and bioavailability the obtained results and mechanisms are not restricted to a single drug but can be discussed more generally.

2. Materials

Compritol 888 ATO (glyceryl behenate, tribehenin) is a mixture of mono-, di- and triglycerides of behenic acid (C22) and was a gift of Gattefossé (Weil a. R., Germany). Fatty acids other than behenic acid, mainly of shorter chain length, account for less than 15%. Retinol and retinyl palmitate were donated by BASF (Ludwigshafen, Germany). Miglyol 812 (caprylic/capric triglycerides) was provided by Hüls AG (Witten, Germany), Xanthan gum by Caelo (Hilden, Germany). All other chemicals were obtained from Sigma (Deisenhofen, Germany) and were of the highest quality available.

3. Methods

3.1. Preparation of SLN and nanoemulsion

SLN and nanoemulsions with 5% vitamin A (with respect to the lipid) were prepared. Details for glyceryl behenate SLN were described elsewhere [19]. Briefly, Compritol (glyceryl behenate) was melted at 85°C and the respective drug was added. The hot lipid phase was dispersed in a surfactant solution and a premix was formed using an ultra turrax (IKA, Staufen, Germany). The premix was passed through a Lab 60 high pressure homogenizer (APV Gaulin, Lübeck, Germany). Two cycles at 500 bar and 85°C were performed.

Nanoemulsions were prepared in the same manner as the SLN dispersion only replacing the solid lipid Compritol by the oil Miglyol 812. The final concentrations of the drugs were 0.5% in the formulations.

3.2. Preparation of hydrogels and oil-in-water creams

Ten percent glycerol (85%), 69.5% water and the gelling agent (0.5% Xanthan gum) were weighed in a beaker and stirred (Cito Unguator, Konictzko, Bamberg, Germany) at approximately 1000 rev./min for 5 min. Finally, the SLN dispersion or nanoemulsion (20%), respectively, was added under continuous stirring.

The lipid and aqueous phases of the cosmetic o/w-cream were heated separately to 90°C. The hot water phase was added to the lipid phase under stirring. The SLN dispersion or nanoemulsion, respectively was added to the emulsion cooled down to 40°C. Stirring was continued until the o/w-cream reached ambient temperature. The final preparations contained retinol or retinyl palmitate at a concentration of 0.1%.

3.3. Particle size analysis

Particle size analysis was performed by photon correlation spectroscopy (PCS) (Coulter N4 Plus, Coulter Electronics, Krefeld, Germany). PCS yields the mean particle size and the polydispersity index (PI) as a measure of the width of the distribution.

3.4. Penetration experiments

Skin samples were obtained from adult Yucatan pigs from the axiliar-breast region because of the lower density of hair follicles in this area. After removing subcutaneous fat tissue, samples of 15 mm diameter ($n = 3$ for each experiment) were punched and mounted to Franz flow-through (average flow 1.0 ml/h) diffusion cells (0.9 cm in diameter, Crown Scientific, Sommerville, NJ). Phosphate-buffered saline (PBS) containing 1.5% bovine serum albumin (BSA) thermoregulated at 37°C served as receptor fluid. Then 100 μl of liquid SLN dispersion and nanoemulsion or 50 mg of the semisolid preparations were applied to the skin surface. At the end of the experiment the skin was rinsed with water and gently dried with a cotton swab. This procedure was repeated twice.

3.5. Drug extraction

Skin samples were cut in a freeze-microtome (Frigocut™ 2800 N, Leica, Bensheim, Germany) to five 100 μm horizontal slices. Following the addition of 1.5 ml acetone each slice, as well as the residual skin, was subjected to ultra turrax (10 000 rev./min, 5 min) and an ultrasonic (Sonorex RK 100 H, Bandelin, Berlin, Germany) treatment at elevated temperatures (50 ± 5°C, 5 min). Subsequently, the suspension was centrifuged at 15 000 rev./min (Biofuge 22R, Heraeus Sepatech, Osterode, Germany). Then 1.4 ml from the supernatant was exsiccated by vacuum rotation and the remainder resolved in 200 μl acetone.

To ensure sufficient extraction and reliability of the results a treated skin sample was divided into two equal parts. One part was extracted as described above, the other powdered in a mortar mill (RMO, Retsch, Haan, Germany) under liquid nitrogen. The resulting material was suspended in acetone and treated as described above. Both ultra turrax and mortar mill method led to similar results (retinol ±2.8%, retinyl palmitate: ±5.1%). Because of its convenience the ultra turrax extraction was used routinely.

3.6. HPLC analysis

HPLC (Kontron Instruments, Neufahrn, Germany) and a LiChrospher 60 RP select B column (Merck, Darmstadt,

Germany) served to quantify retinol and retinyl palmitate concentrations. The mobile phases consisted of acetonitrile/water (80:20) plus 0.1% phosphoric acid for retinol and 100% acetonitrile for retinyl palmitate, respectively. The retention times were 6.1 min (retinol) and 4.2 min (retinyl palmitate). UV absorption was read at 325 nm and drugs were identified by their UV spectrum using a diode array detector. The limits of detection (signal to noise ratio 3:1) were 14.7 ng/ml retinol and 32.7 ng/ml retinyl palmitate. Reproducibility was 2.1%.

For light microscopy frozen skin specimens were cut in a freeze-microtome vertically in 10 μm slices. Sections were stained with hematoxylin and eosin.

3.7. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed on a Mettler DSC 821^e (Mettler Toledo, Gießen, Germany). Samples containing 14–16 mg stratum corneum were accurately weighted in 40 μl aluminium pans. DSC scans were recorded at a heating rate of 5°C/min. Melting points correspond to the maximum of the heating curve.

3.8. Transepidermal water loss (TEWL)

For TEWL measurements porcine skin was punched to circles of 15 mm in diameter and transferred onto a perforated (circles of 10 mm diameter) plate. Surplus receptor medium was filled beneath the plate. 4 mg/cm^2 of the respective formulations were applied to the skin ($n = 3$). TEWL was measured in a climate chamber (21 ± 1°C/50% humidity, WTB™, Binder, Tuttlingen, Germany) with an evaporimeter (Evaporimeter™ EP 1, Servomed, Kinna, Sweden) keeping a measurement interval of 10 s.

3.9. Statistics

All data are presented as arithmetic mean values ± standard deviation ($\bar{x}$ ± SD). Significance of differences was analyzed using Shapiro–Wilk-, F-, and Student's *t*-tests, $P \leq 0.05$ was considered significant.

4. Results

4.1. SLN dispersions: retinol penetration characteristics

Vitamin A penetration into porcine skin was determined after 6 or 24 h treatment by slicing the frozen skin specimens in horizontal sections. According to light microscopy the upper 100 μm represent mainly the stratum corneum and upper layers of viable epidermis. The second slice (100–200 μm skin depth) consists basically of viable epidermis, the further slices divide mainly the dermis. Residual skin comprises dermis and small parts of subcutaneous fat tissue. Reifenrath et al. [20] confirm in their study with pigs that the upper 100 μm of porcine skin consist mainly of stratum corneum and epidermal layers. The thickness of porcine skin and human skin is very similar. For human skin a thickness of approximately 700 μm from the top (stratum corneum) to the middle area of the dermis layers has been reported [21]. Because porcine skin is structurally closest to that of humans [22] it is well suited for representing the permeability of human skin [23].

First, the original SLN dispersion and a nanoemulsion of approximately the same size were compared with respect to their ability to influence the drug penetration and distribution characteristics within the skin. The PCS mean diameter of the SLN dispersion was 224 nm and the PI 0.205. The nanoemulsion possessed a size of 186 nm and a PI of 0.113. While the nanoemulsion contained retinol in a liquid oil phase, the solid matrix of the SLN immobilized vitamin A molecules making differences in release kinetics and subsequently in drug distribution characteristics likely. In both experiments, 500 μg retinol were applied to the skin. Following the SLN dispersion for 6 h a high retinol concentration was found in the stratum corneum, and upper epidermis (approximately 3400 ng in the first slice). The nanoemulsion, however, only transported 2500 ng into the upper skin strata (Fig. 1; upper graph). The difference in the stratum corneum penetration of SLN encapsulated retinol and retinol applied via the nanoemulsion demonstrates the relevance of the carrier system for cutaneous drug absorption. Above that for 6 h the SLN formulation proved their ability to localize retinol in the upper skin as there was no parallel increase in drug concentrations in the dermis and subcutaneous tissue. Total drug amounted to 4000 and 3100

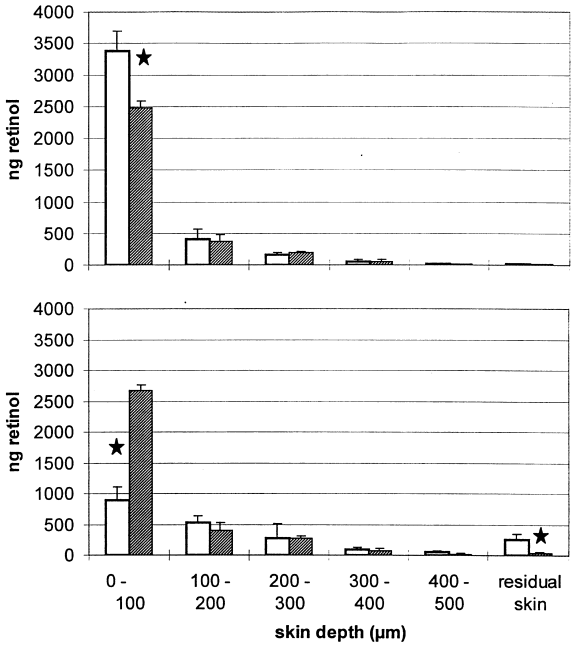

Fig. 1. Distribution of retinol in porcine skin. The drug was applied as a SLN dispersion (open bars) or nanoemulsion (filled bars) for 6 h (upper graph) and 24 h (lower graph). Data give retinol mean values ± SD ($n = 3$) in the individual skin slices. Significant differences ($P < 0.05$) are marked with asterisks.

ng following the SLN preparation and the nanoemulsion, respectively.

The improved retinol penetration into the upper skin should result from the solid matrix of the particles. SLN may penetrate into follicular openings of the skin or may stick tightly to the stratum corneum which prevents removal by the washing procedure. Thus, the recovered retinol in the first slice may include drug encapsulated in SLN and freely dissolved retinol.

Clues for the presence of SLN are derived from DSC experiments. SLN treated skin showed an increased endothermic peak at 68.1°C whereas untreated stratum corneum displayed the expected transition at 64.5°C. For porcine stratum corneum lipids (e.g. ceramides) a transition temperature of 60–66°C depending on the hydration has been reported [24]. The increased melting point of 68.1°C is explained by the presence of lipid nanoparticles (melting point 64–70°C, depending on the polymorph). The interaction of SLN with stratum corneum is currently under further investigation.

A surprising distribution pattern appeared after treatment for 24 h (Fig. 1, lower graph). Following the SLN dispersion the drug concentration in the first slice declined considerably (900 ng) and the amount in the deeper layers appeared to increase. With the nanoemulsion, however, retinol distribution pattern did not change. The surprising result for the SLN dispersion may result – at least in part – from the instability and gelation of the SLN. In contrast to the nanoemulsion, water evaporation induced SLN transformation to a rigid semisolid gel. The thick gel film on the skin surface might reduce transepidermal water loss and by enhancing occlusion enables retinol penetration into the deeper skin layers as well as permeation through the skin. This is reflected by the increase in retinol concentration in the residual skin and in the receptor fluid. Moreover, because of a change of the polymorphic form of the lipid lattice [25], drug expulsion occurs and sustained release properties of the carrier system are lost [26]. Depending on the penetrability of expulsed drug, the process may further increase but also decrease cutaneous penetration.

The concentrations of retinol in the receptor were – except for the SLN preparation after 24 h – below the detection limit. This can be partly explained by the high lipophilicity of the drug preventing diffusion from the skin into the receptor medium. Obviously, permeation of retinol through porcine skin is, despite the high concentration applied to the skin, very low. Since pig skin and human skin are very similar with respect to permeability [23], this result is very important for the evaluation of the risk potential and safety of topical applied vitamin A. According to literature reports, topical retinol is partly metabolized to retinyl esters and stored in the viable human skin [27]. The main ester is retinyl palmitate. However, the porcine skin used was not able to metabolize retinol to retinyl palmitate in this ex vivo model. After 6 and 24 h no retinyl palmitate could be detected by HPLC.

4.2. Retinol penetration: hydrogel and oil-in-water-cream

4.2.1. Hydrogel

The interactions of SLN and hydrogel components were described in detail elsewhere [26]. In this study we focused on the penetration characteristics of these formulations. Two hydrogel formulations were compared which contained SLN-encapsulated retinol or 'freely' dispersed drug in emulsion droplets. The concentration of drug in the formulations applied to the skin surface was 0.1% and thus 20% of the former experiment. Total retinol in the skin was about 25% of the SLN dispersion. In accordance with this experiment, at 6 h the SLN containing hydrogel yielded a higher active concentration in the upper layers of the skin as compared to the conventional formulation. The differences of retinol concentrations in deeper layers were less pronounced (Fig. 2). After 24 h retinol concentrations in the first slice increased with both preparations and the second section, mainly viable epidermis, showed a slight increase for the SLN formulation, too. Therefore, superiority of retinol penetration into the viable epidermis following the SLN formulation is still observed.

As with the SLN dispersion, the SLN hydrogel follicular penetration or sticking of the particles to the horny layer may contribute to the higher drug levels in the stratum corneum layer at 6 h. Once more the SLN performed a polymorphic transformation with subsequent drug expulsion. However this transformation was in the hydrogel slower as compared to the fluid dispersion [26]. This should

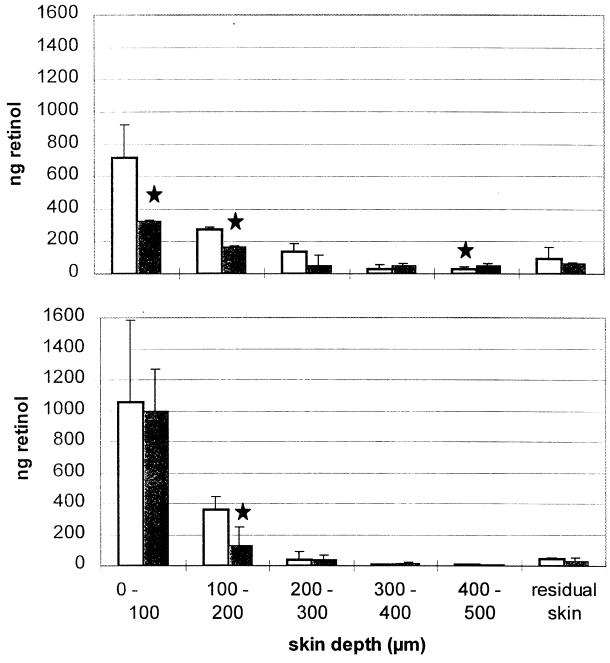

Fig. 2. Distribution of retinol in porcine skin at 6 h (upper graph) and 24 h (lower graph). A sample of 50 mg of hydrogels containing retinol in SLN (open bars) or nanoemulsion (filled bars) were applied. Data give retinol mean values ± SD ($n = 3$) in the individual skin slices. Significant differences ($P < 0.05$) are marked with asterisks.

explain the relatively more pronounced increase in retinol concentrations following the dispersed drug (Fig. 2).

4.2.2. Oil-in-water cream

The possibilities and limitations of the incorporation of SLN in creams are addressed in [6,15,16,26,28]. O/w-creams are preferred in cosmetics since they combine a pleasant application and skin care. Yet the possibilities to achieve sustained release or drug targeting with a conventional formulation are limited. Incorporation of particulate carriers like SLN, however, might be helpful to modify release and drug targeting properties of an o/w cream.

Fig. 3 shows retinol distribution in the skin after 6 and 24 h. As with the former formulations, more retinol was found in the upper layers of SLN treated skin at 6 h. Moreover, Fig. 3 depicts lower retinol concentrations in the dermis (>300 μm) following the SLN preparation. Other than with the SLN dispersion and the hydrogel, however, the retinol concentration in the stratum corneum and viable epidermis exceeded the concentrations following the reference product not only after 6 h but also after 24 h. This is in good accordance with our data [26] showing protective effects of the cream components against polymorphic transition of the carrier and drug expulsion. Therefore, the drug localising ability is prolonged over a 24-h period. Moreover, as the ratio of retinol levels following the SLN o/w-preparation and the o/w-cream does not decline from 6 to 24 h as much as with the retinol expulsing SLN dispersion and SLN hydrogel, drug expulsion appears to reduce retinol absorption.

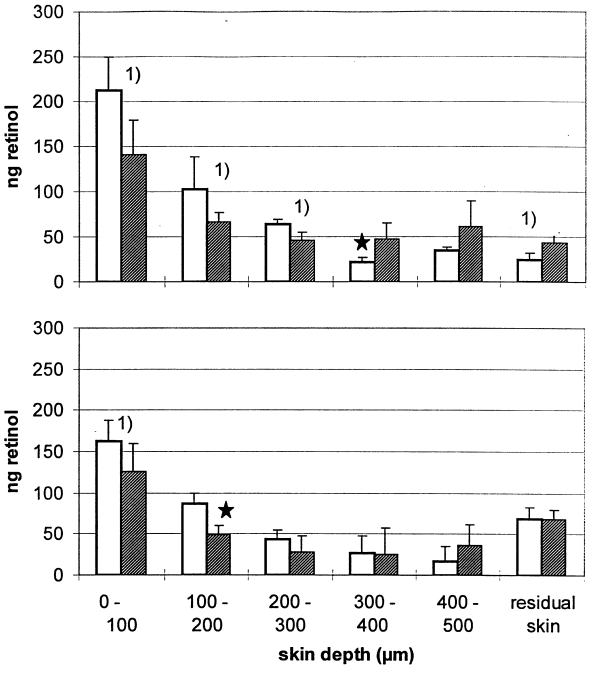

Fig. 3. Distribution of retinol in porcine skin at 6 h (upper graph) and 24 h (lower graph). A sample of 50 mg of o/w-cream with retinol in SLN (open bars) or in nanoemulsion containing (filled bars) were applied. Data give retinol mean values ± SD ($n = 3$) in the individual skin slices. Significant differences ($P < 0.05$) are marked with asterisks. P values are between 0.05 and 0.20.

Comparing 6 and 24 h data, drug levels increased only within the residual skin. The retinol concentrations in the horny layer appeared to decline slightly. After 6 h incubation the stratum corneum was no longer accumulating retinol. This result is in good agreement with literature reports [29] showing increasing retinol amounts in stratum corneum for the first 8 h and essentially stable levels thereafter for another 22 h.

Retinol penetration into the skin was only about 35% as compared to the hydrogel. Because the oil phase of the vehicle is a good solvent for vitamin A, thermodynamic activity of retinol is lower in this vehicle. Therefore a reduced penetration, as observed, can be expected [30].

4.3. Occlusive properties

Small particles like SLN possess a high specific surface area and therefore adhesive properties. Film formation of SLN on filters (e.g. membrane filters) was observed which reduced evaporation of water through these filters. Therefore occlusive properties have been claimed [28]. In this study three different methods have been employed for a further investigation of occlusive properties: transepidermal water loss (TEWL), light microscopy and the penetration of an occlusion sensitive drug (retinyl palmitate) into the skin.

4.3.1. Transepidermal water loss

Occlusion facilitates drug penetration by enhancing hydration of the stratum corneum [31] due to the inhibition of water evaporation. In our experiments not only the TEWL of treated porcine skin was quantified under standardized conditions but also the water loss from the preparations themselves. To obtain this information the formulations were applied on a glass surface. All preparations lost their own water almost completely within 1 h. Values of approximately 12 g/m^2 per h were recorded at 15 min. After 1 h water loss declined to less than 0.5 g/m^2 per h. After 4 h, the first time point for the measurement of TEWL from porcine skin, there was no detectable evaporation of the preparations anymore. Therefore, the TEWL values after 4 and 24 h can be attributed solely to the transepidermal water loss.

With untreated skin constant TEWL values were obtained (8 g/m^2 per h). All applied formulations reduced the TEWL, most pronounced effects were seen with the SLN dispersion reducing the TEWL to 6 g/m^2 per h on average. Differences between SLN dispersion and the other formulations, however, were small (Fig. 4). Therefore, the results of TEWL measurements do not indicate a relevant skin occlusion by the SLN.

4.3.2. Microscopy

Occlusive effects were further evaluated by hematoxylin/eosin staining of vertical skin slices after 24 h incubation with the respective formulations. Fig. 5 shows microscopic

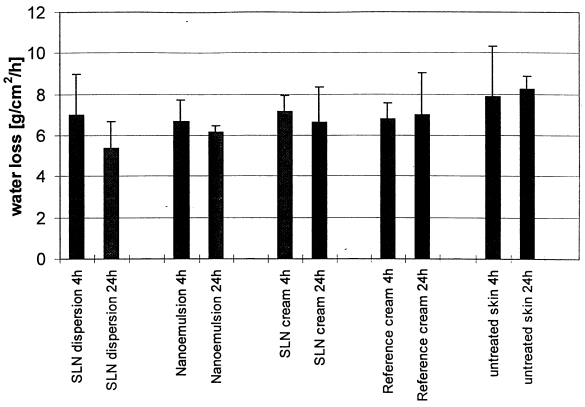

Fig. 4. Transepidermal water loss of untreated porcine skin and 4 and 24 h after treatment. Values were recorded at 21°C and 50% humidity ($n = 3$).

pictures of untreated skin (Fig. 5A), skin treated with SLN-free cream (Fig. 5B) and skin treated with SLN cream (Fig. 5C). Untreated skin showed a compact stratum corneum with corneocytes layers closely conjugated. Application of the conventional cream only slightly changed the structure. A different result was obtained for the SLN cream. The stratum corneum appeared swollen and overall thickness had increased. SLN in a cream obviously possess the ability to induce structural changes. Other than TEWL measurements light microscopy does not rule out that SLN may induce occlusion and improve a relevant skin hydration. The skin slice shown in (Fig. 5C) is very similar to the skin before the experiment. Obviously the skin dehydrates during the experiment if untreated. Application of a conventional cream or the SLN cream reduced or prevented this dehydration, respectively. Yet nanoparticles themselves may increase stratum corneum thickness by disturbing the lamellar arrangement of the lipids. Such a result was obtained with oleic acid or azone application [31]. Anionic surfactants like sodium dodecylsulphate induce swelling of the stratum corneum by protein or lipid interaction [32] which leads to an increase of TEWL and a broadened DSC endotherm related to the lipids at a lower temperature [24]. Such effects were not detected here which excludes skin damage by SLN application. Therefore, explanation of experimentally evidenced improved hydration of stratum corneum requires that mechanisms other than occlusion or damage have to be considered.

4.3.3. Penetration characteristics of retinyl palmitate

Because TEWL measurement and microscopy failed to clarify occlusive effects, a third method was used. In contrast to retinol, retinyl palmitate penetration into the skin is sensitive to occlusion. If applied without occlusion biological response is low which is not so if the same preparation is applied under occlusion [18]. In the following, three different retinyl palmitate formulations of the o/w type were compared (A) o/w-cream with freely dispersed drug, (B) cream with dispersed drug and drug free SLN and (C) cream with SLN encapsulated drug.

Table 1 summarizes the experimental results. Following the conventional cream retinyl palmitate accumulated in the upper skin (slices 1–3) over 24 h as expected. A much smaller but also increasing amount was found in the deeper skin layers. Retinyl palmitate concentrations in the deeper skin were much below retinol concentrations (Fig. 3) in spite of identical amounts applied, demonstrating the low penetration ability of retinyl palmitate.

In contrast to our expectations, however, the addition of SLN did not increase retinyl palmitate penetration. At 6 h the highest drug level in lower skin was obtained with the conventional cream (14.6 ng as compared with 7.9 ng for drug free SLN and 5.5 ng for drug if encapsulated in SLN). When drug free SLN were added to the conventional formulation, a remarkable reduction of the amount of drug in the upper compartment resulted (75.5 ng as compared with 150.4 ng at 6

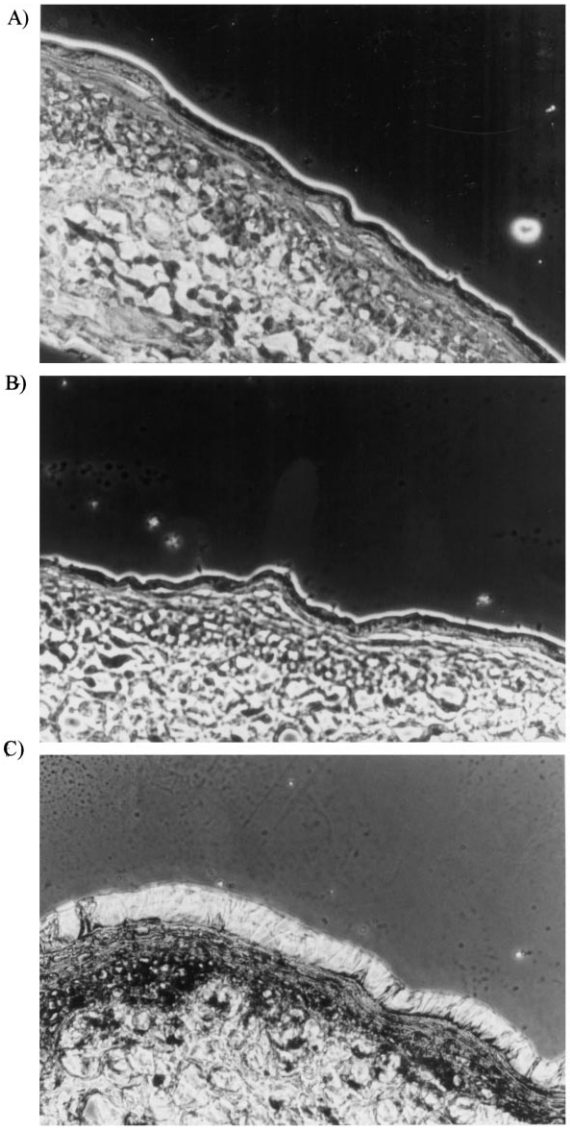

Fig. 5. Light microscopy (230×) of vertical sections of porcine skin (H/E staining). The skin was treated for 24 h with SLN-free o/w cream (B) or with a SLN containing o/w cream (C). (A) depicts untreated skin.

Table 1
Amount (ng) of retinyl palmitate in the upper (sections 1, 2 and 3) and lower (sections 4, 5 and residual skin) skin after 6 and 24 h incubation[a]

O/w-cream		6 h		24 h	
		Upper skin	Lower skin	Upper skin	Lower skin
A	Reference	150.4 ± 14.1	14.6 ± 6.2	211.7 ± 19.9	38.5 ± 6.7
B	Drug free SLN	75.5 ± 18.5	7.9 ± 4.1	92.7 ± 14.4	46.9 ± 9.2
C	SLN encapsulated drug	178.4 ± 10.2	5.5 ± 3.9	115.6 ± 9.3	20.4 ± 4.9

[a] $X \pm$ SD, $n = 3$.

h). Only after the prolonged treatment for 24 h the recovered drug in the lower region is (as expected) highest for formulation B. The impaired retinyl palmitate uptake by the addition of drug free SLN indicates that the barrier properties of the stratum corneum were indeed altered by SLN treatment. This is the consequence of the microscopically detected structural changes (Fig. 5). In accordance with TEWL measurements (Fig. 4) an occlusive effect favouring retinyl palmitate uptake is not observed, the barrier is improved.

As with retinol encapsulated in SLN, the retinyl palmitate nanoparticles (formulation C) led to a drug localization in the upper skin. As with retinol the drug concentration in the upper skin decreased after 24 h indicating a partial drug expulsion from the carrier. Retinyl palmitate levels in the deeper skin increased but still remained lower than with the other formulations. Therefore drug targeting is also obtained with retinyl palmitate.

Comparing retinol and retinyl palmitate the first showed the better penetrability. The stratum corneum is the main barrier for the highly lipophilic retinyl palmitate. This might explain the comparable low efficacy of topical retinyl palmitate formulations. Different lipophilicities of the two drugs can be evidenced by monitoring their distribution between the reversed phase of the HPLC column and the water containing mobile phase. Using a mobile phase of 80% acetonitrile and 20% water and a C8 reverse phase column, the retention time of retinol is 6.1 min. Under these conditions retinyl palmitate is strongly adsorbed to the reversed phase and is not eluting even after 1 h. This shows the much higher lipophilicity of retinyl palmitate compared to retinol

5. Discussion

In dermatological treatment improving the efficacy and/or the benefit/risk ratio demands high drug levels in specific strata of the skin [33]. This holds true especially with topical glucocorticoids which can induce irreversible striae by the negative influence on collagen formation in the dermis while high drug levels in the epidermis are longed for [34].

Our experiments show that retinol and retinyl palmitate encapsulated in SLN can be effectively delivered to the upper skin layers. Drug levels in the deeper skin strata appear not to increase in parallel. By means of DSC measurements differences in the melting behaviour of stratum corneum lipids in treated and untreated sites were observed. The shift to higher temperatures is attributed to the existence of lipid nanoparticles in the upper stratum corneum. A penetration of small particles into the stratum corneum was shown for titanium dioxide (30 nm) [35] and drug crystals of several micrometers [36] by a tape stripping method, too. Most likely these particles, as well as SLN, penetrate using the follicular route [35].

Yet our results clearly show that the drug localising effect can be only obtained for the first few hours. Later on, less drug is absorbed as compared to conventional nanoemulsions or hydrogels (Figs. 1 and 2). With time polymorphic transition and subsequent drug expulsion from SLN becomes more likely. After the drug is expelled from the nanoparticles the penetration characteristics are changing. Most interestingly, prolonged targeting was obtained with SLN-retinol incorporated into an o/w cream slowing down the polymorphic transition and thus drug expulsion (Fig. 3).

Distribution characteristics of retinol and retinyl palmitate are influenced most interestingly as the drugs appear rapidly to reach high levels in viable epidermis without a parallel increase in deeper layers. Using SLN encapsulation a drug localizing effect in the skin seems possible. The addition of drug-free SLN to a conventional cream had the opposite effect increasing drug levels in the deeper skin.

Metabolism of topical retinol is well investigated for viable human skin [27,37]. The ratio of retinol to its metabolites is very constant within 24 h in human skin [37]. In contrast, in our ex vivo model the main metabolite retinyl palmitate was below the detection limit. Nevertheless our results should correlate with in vivo results in humans because the esterfication of retinol in human skin is approximately a linear function of the retinol concentration [37].

Occlusive properties were investigated by three different methods. A relevant difference between SLN containing and SLN free preparations were not seen with respect to TEWL. The high standard derivation of TEWL, however, may mask minor occlusive effects. On the other hand damage of the horny layer with subsequently increased TEWL could be excluded as well. Application of a conventional o/w-cream did not change skin structure whereas application of a SLN containing cream increased thickness of the stratum corneum. The investigation of retinyl palmitate penetration even indicated an improvement of the penetration barrier due to the nanoparticles.

In conclusion the present study shows that release profile, and penetration characteristics of drugs as well as stratum corneum properties can be altered by utilizing SLN technology. Decisive for the further fate of an active is whether the drug is encapsulated within the SLN matrix or outside the nanoparticle.

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