European Journal of Pharmaceutics and Biopharmaceutics 79 (2011) 68–75

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

In vitro penetration properties of solid lipid nanoparticles in intact and barrier-impaired skin

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ARTICLE INFO

Article history:
Received 1 December 2010
Accepted in revised form 24 May 2011
Available online 31 May 2011

Keywords:
Solid lipid nanoparticles
SLN
Barrier-impaired skin
Tape stripping
Skin penetration
Corticosteroid

ABSTRACT

Treatment of skin diseases implies application of a drug to skin with an impaired epidermal barrier, which is likely to affect the penetration profile of the drug substance as well as the carrier into the skin. To elucidate this, the effect of skin barrier damage on the penetration profile of a corticosteroid applied in solid lipid nanoparticles (SLN) composed of different lipids, varying in polarity, was studied. The studies were carried out *in vitro* using impaired and intact porcine ear skin, and the SLN were compared with a conventional ointment. It was shown that a significantly higher amount of corticosteroid remained in the skin, intact as well as barrier impaired, when SLN was used as a vehicle. In general, the penetration profile of the drug substance into the skin was affected by the type of lipid used in the formulation and related to lipid polarity and drug substance solubility. When formulated in SLN and applied to intact skin, the permeation of the drug substance across the skin was significantly reduced, as compared to the ointment. Altogether, in both barrier-impaired and intact skin, a higher amount of drug substance remained in the skin during application of SLN for 6, 16, and 24 h, as compared to the ointment. These results emphasize the applicability of SLN to create a drug reservoir in skin, with the drug localized distinctively in the stratum corneum.

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1. Introduction

The objective of topical treatment of skin diseases is local and directed delivery of an active substance to diseased cells in the skin. Directed delivery limits adverse effects resulting from unspecific delivery and systemic exposure. Local treatment of skin diseases often implies that the drug is applied to skin in which the main protective barrier, the stratum corneum, is not intact. When new drug delivery systems or drug substances intended for treatment of skin disorders are tested on skin *in vitro*, the studies are almost always carried out using intact healthy skin. However, the results obtained with intact skin may be quite different from results obtained with diseased skin [1,2]. Including *in vitro* studies in models that simulate diseased skin can improve the understanding of how the skin barrier affects penetration into and permeation across the skin. Treatment of the skin disease may resolve the initial condition, which can change the skin barrier properties during the period of the treatment. This change can also affect the penetration profile of the administered drug substance [3]. To study the effect of an impaired barrier, tape stripping can be used. Tape stripping is a classic method commonly employed for pharmacokinetic evaluation of drug absorption into the stratum corneum, but it may also be used to damage the skin barrier *in vitro* and *in vivo* [4–6].

Conventional topical treatment of the skin implies the use of ointments or creams. The delivery from these systems is often unspecific, and the skin penetration can be very low with high variation [7]. One way of optimizing topical drug delivery to the skin is to use particulate carriers. Lipid carriers (e.g., liposomes, nano- and microemulsions and lipid nanoparticles) in particular have received attention for their ability to improve penetration across the stratum corneum and for their targeting properties [7]. SLN have been subject to various studies regarding their properties as a topical drug delivery system [8,9]. Thus, SLN were shown to facilitate retention of the loaded drug substance in the upper skin layers and to possess occlusive properties [10,11]. The occlusive effect of SLN results from the small size and strong adhesive properties of the particles, which may lead to film formation on the skin [12,13]. The film formation will reduce the transepidermal water loss (TEWL) and possibly help to physically restore the barrier.

Atopic dermatitis (AD) is a skin disease involving an impaired epidermal barrier. AD is one of the most common inflammatory skin disorders, as it affects 10–20% of all children. There is a high prevalence in early infancy but AD also affects 1–3% of adults [14]. The typical clinical features are dry skin, itching, and an

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doi:10.1016/j.ejpb.2011.05.012

impaired epidermal barrier resulting in an increased TEWL [15–17]. Studies have shown an increased systemic effect of hydrocortisone in the acute phase of AD compared with that of the remission phase [3,18]. Even in visibly healthy looking AD skin, the barrier is impaired causing increased percutaneous penetration [19]. First line treatment in AD is topical moisturizers, e.g., creams with high lipid content or ointments, which can relieve the dryness. Moisturization as treatment in AD should also be reflected in the choice of vehicle for the drug treatment, and SLN, therefore, seems to be an appropriate choice of drug delivery system for the treatment of AD [20]. As a first line drug treatment, topical corticosteroids (TC) are used to treat the acute stages of AD, and in general, TC is the most frequently used group of drugs to treat skin diseases [21]. The effect of TC is broadly based, e.g., they have anti-inflammatory, immunosuppressive and vasoconstrictive effects. The site of action is cells in the lower epidermis and dermis, and therefore, the rate limiting step is the penetration across the stratum corneum [21]. The performance of SLN containing corticosteroids has been studied, and it has been shown that an actual skin-targeting effect was likely to be correlated with corticosteroid molecular structure (e.g., side chain structure) and to corticosteroid interaction with the SLN lipid component [11,22–25].

The aim of the present studies was to evaluate how SLN affect the drug substance penetration profile into and across the skin and to study any reservoir effect in the skin when the barrier was impaired. SLN containing the corticosteroid betamethasone-17-valerate (BMV) was applied to intact and barrier-impaired porcine skin *in vitro*. The influence of SLN lipid component, application time, and occlusive conditions were studied in detail.

2. Material and methods

2.1. Materials

Lipids were glycerol distearate (Precirol® ATO 5) and cetylpalmitate purchased from Gattefossé (Genas, France); glycerol tripalmitate (Dyanasan® 116) was kindly donated by Sasol (Witten, Germany). Polysorbate 80 was from Croda AB (Limhamn, Sweden). White, soft paraffin and liquid paraffin were from LEO Pharma A/S (Dublin, Ireland). BMV (purity 99.8%) was from SICOR SpA (Milano, Italy), tritiated [1,2(n)-3H]-betamethasone 17-valerate (1070 GBq/mmol, 10,1 MBq/ml) was prepared by RC TRITEC AG (Teufen, Schwitzerland), 1-^{14}C tripalmitate (2.22 GBq/mmol, 3.7 MBq/ml) was from Biotrend Chemikalien GmbH (Köln, Germany), and Soluene® 350 and Hionic-Fluor were purchased from Perkin Elmer (Skovlunde, Denmark). Kleptose Crysmeb (methyl-β-cyclodextrine) was purchased from Roquette (Lestrem, France); sodiumchloride and sodium acetate trihydrate were from Merck (Darmstadt, Germany). All organic solvents were of analytical quality and purchased from Sigma Aldrich (Broendby, Denmark) and VWR International ApS (Herlev, Denmark).

2.2. Preparation of formulations

SLN was prepared by hot high pressure homogenization as described previously [26], but modified in an initial step in order to mix the radiolabeled substances with the unlabeled substances. This initial step did not influence the particle size or the crystallinity of the lipid. First, 10% (w/w) lipid and 0.1% (w/w) unlabeled BMV were dissolved in chloroform together with ^{14}C-labeled tripalmitate (final strength 0.2 MBq/g) and ^{3}H-labeled BMV (final strength 1 MBq/g). Chloroform was used to ensure complete dissolution of the lipid. After evaporating chloroform overnight, the lipid mixture was melted at 80 °C. An aqueous 80 °C 2.5% (w/w) polysorbate 80 solution was added to the lipid mixture, and the mixture was homogenized for 2 min at 6000 rpm using an Ultrathurrax (IKA Labortechnik, Staufen, Germany) to create a coarse emulsion. This emulsion was subsequently high pressure-homogenized using an EmulsiFlex C5 (Avestin, Ottawa, Canada). The homogenizer was placed in a water bath to maintain a high temperature. The coarse emulsion was processed at 600 bar applying 4 homogenization cycles. These processing conditions were selected from preliminary studies showing them to be favorable for preparing SLN with a small particle size (<250 nm) and a low polydispersity (<0.25) [25]. The dispersions were cooled at room temperature and protected from light. The compositions of the SLN formulations are shown in Table 1.

The ointment was prepared by dissolving ^{3}H-labeled BMV and unlabeled BMV in ethanol after which the ethanol was evaporated in an ultrasound water bath. Ultrasound ensured a small size (<25 μm) of the BMV crystals. The ^{3}H-labeled BMV crystals were then dispersed in an ointment using a mortar. The ointment was composed of 98.9% (w/w) white soft paraffin, 1% (w/w) liquid paraffin, and 0.1% (w/w) BMV. All formulations were stored at 5 °C protected from light.

2.3. Characterization

Initially, the solubility of BMV in the lipids for SLN was determined by hot stage microscopy as described previously [25]. All formulations were quantitatively analyzed with liquid scintillation ($n = 3$) in a Tri-Carb 2100 TR Liquid Scintillation Analyzer from Packard Instrument Company (Meriden, USA). Twenty milligrams of SLN was accurately weighed out and dissolved in 10 ml of ethylacetate; 1 ml was withdrawn and mixed with 10 ml of Hionic-Fluor before analyzing with liquid scintillation. Twenty milligrams of ointment was dissolved in 10 ml of heptane/ethanol (30:70) instead of ethylacetate; otherwise, the same procedure as for SLN was followed.

Particle size analysis was performed on SLN by DLS on a Zetasizer Nano ZS (Malvern, Worcestershire, UK) equipped with a 633 nm laser and 173° detection optics. The samples ($n = 3$) were adequately diluted with purified water before measurement, i.e., the viscosity of water was used for the measurement. Measurements were taken at 25 °C. Malvern DTS v 5.10 software was applied for data acquisition and analysis. Particle size distribution was described by the polydispersity index (PdI) and the mean hydrodynamic diameter (*Z*-average). Differential scanning calorimetry was applied for the study of lipid crystalline structures and melting transitions of SLN with and without BMV. DSC analysis was performed with a VP-DSC (MicroCal, Milton Keynes, UK). The scan rate was 1.5 °C/min going from 20 to 80 °C. VPViewer 2000 and Ori-

Table 1
Composition of the SLN formulations, the fatty acid chain length given as the number of C atoms (C), the calculated lipid solubility parameter, and the observed BMV solubility in the SLN lipid component.

SLN lipid component	Lipid trade name	Fatty acid chain length (C)	Solubility parameter(calculated)	BMV solubility % (w/w)
Distearate	Precirol® ATO 5	16–18	9.5	1.8–1.9
Tripalmitate	Dynasan® 116	16	8.9	0.1–0.3
Cetylpalmitate	Cetylpalmitate	16	8.7	<0.1

All SLN formulations contained 0.1% (w/w) BMV, 10% (w/w) lipid component, and 2.5% (w/w) polysorbate 80 as surfactant.

gin®7 scientific plotting software was used for data analysis. To achieve signals that could be appropriately analyzed, the SLN was diluted 1:100 with purified water before the DSC analysis was carried out ($n = 3$). The solubility, as well as visual inspection of the final formulations, was evaluated microscopically employing a Nikon Eclipse 80i microscope equipped with a Linkam PE94 heater, both from DFA Instruments (Glostrup, Denmark). The software used was Image Pro Plus®. Ultrafiltration was performed applying Centrisart® Centrifugal Ultrafiltration Unit, with a 20 kDa cutoff. Five hundred microliters of SLN ($n = 2$) was added to the vial and centrifuged at 4000 rpm for 60 min. The aqueous filtrate was analyzed with liquid scintillation, and the concentration in the aqueous phase was used as an indirect measure of entrapment efficiency.

Microscopy and DSC analysis were carried out on unlabeled formulations only. Quantitative analysis and DLS ($n = 3$) were always repeated on the day the formulations were to be used in order to ensure homogeneity and stability of the formulation.

2.4. Skin sample preparation

The porcine ears were obtained from newly slaughtered pigs from the Danish Meat Trade College (Roskilde, Denmark). The ears were stored at −20 °C and thawed slowly at 4 °C before gently cutting the hairs with an animal hair clipper from Oster (Tennessee, USA) and removing full-thickness skin from the back of the ears using a scalpel. Subcutaneous tissue was carefully removed with a scalpel, and the skin was cut into appropriate pieces before freezing at −20 °C until use (after no more than 14 days). Two skin pieces were obtained from each ear, and they were balanced with respect to intact and barrier-impaired skin.

Skin barrier impairment was induced by 25 successive tape strippings applying D-Squame® tape disks (Cuderm Corp., Dallas, USA); 225 g/cm^2 pressure on the tape was applied with a D-Squame® tape applicator for 5 s. (Cuderm Corp., Dallas, USA). The skin was mounted on a cork plate with small pins, stretching it to overcome problems with skin furrows when tape stripping. The method was adapted from Simonsen et al. who created a skin model to simulate barrier properties of AD skin [5]. The effect of tape stripping fresh versus frozen and thawed skin was validated by an initial study comparing fresh and thawed skin ($n = 6$) and tape stripping 0, 5, 15, 25, and 40 times. Punch biopsies were taken and fixed in 10% (w/w) formalin followed by hematoxylin–eosin staining before examination by microscopy.

2.5. Skin penetration and permeation studies

Penetration profiles of BMV and lipid were evaluated. ^{3}H-labeled BMV and ^{14}C-labeled lipid were used to study the penetration of BMV and lipid particles (distearate and tripalmitate) into intact and barrier-impaired skin.

The skin was mounted on Franz type diffusion cells with the dermis side facing the receptor medium (diffusion area 3.14 cm^2, recipient volume 10 ml, constant stirring, temperature kept at 32 °C). A solution of 1% (w/w) methyl-β-cyclodextrin in isotonic acetate buffer pH 5.5 (15 mM sodium acetate, 100 mM sodium chloride) was used as receptor medium. The choice of receptor medium was based on the sufficiently high BMV solubility in buffer containing methyl-β-cyclodextrin, which ensured sink condition throughout the study. Further, the solubilizing capacity was not affected by the presence of skin, and non-desired effects on the integrity of the skin barrier properties were avoided.

After equilibrating the skin for 30 min with isotonic sodium chloride solution and for 60 min with receptor medium, the receptor medium was renewed and 20 mg formulation (6.4 mg formulation/cm^2) applied evenly on the skin surface using a spatula. The exact amount of formulation applied was determined by weighing the spatula before and after application. After complete incubation, the skin was separated and analyzed. All studies were carried out on intact and barrier-impaired skin, and the formulation application time was varied between 6, 16, and 24 h. Occlusion was created by mounting a glass plug on the top of the skin to ensure complete occlusive conditions.

After complete application time, surplus formulation was removed by wiping the skin twice with a cotton pad. Ten milliliters of ethylacetate was used to extract BMV and lipid from the cotton pads and from the lid of the donor compartment. Heptane/ethanol (30:70) was used to extract BMV from the ointment. The stratum corneum was removed by applying a maximum of 15 tape strips to intact skin using D-Squame® tape disks (Cuderm Corp., Dallas, USA) applying the same technique as when inducing skin damage. For barrier-impaired skin, only a maximum of 3 tape strippings was done. In both cases, the first tape strip was included as surplus formulation. The optimal method for removing stratum corneum was established in preliminary experiments with different numbers of tape strips after 24 h of exposure to different formulations. If the epidermis started to loosen with less than 15 or 3 tape strips, respectively, tape stripping was ended and the last strip included in the epidermis count. Epidermis and dermis were separated by heat (incubation at 5 min at 60 °C and high humidity). The skin surrounding the application area (designated non-applied skin) was cut into small pieces and analyzed as well to include any lateral penetration and to achieve full recovery. Soluene® 350 was added to the tape strips and to the skin samples to solubilize the tissue and extract drug substance and lipid. After 24 h of incubation at 50 °C, 10 ml of Hionic-Fluor was added to the Soluene® 350 samples and analyzed by liquid scintillation in a Tri-Carb 2100 TR Liquid Scintillation Analyzer from Packard Instrument Company (Meriden, USA). The extractions from the lid and the cotton pad and the content in the receptor medium were analyzed by mixing 1 ml with 10 ml of Hionic-Fluor before scintillation counting. The appropriate liquid (i.e., ethylacetate, receptor medium, heptane/ethanol (30:70), and Soluene® 350 mixed with Hionic-Fluor) was used as background measurements.

When comparing 6, 16, and 24 h, the amount of BMV in the different skin layers was pooled to take into account the fact that the skin structure changes during 24 h, so that the efficiency of the separation procedure may also change.

2.6. Data analysis

Solubility parameters were calculated according to Fedors substituent method [27]. All data were plotted in Microsoft Excel or Graph Pad Prism 5.0. Statistical analysis was performed in Graph Pad Prism 5.0. One way ANOVA ($p < 0.05$) followed by Newman Keuls multiple comparison test to compare means was applied.

3. Results

3.1. Characterization

In order to determine the solubility of BMV in the lipid component, hot stage microscopy was used. The data in Table 1 show that BMV solubility in the lipid increases with increasing solubility parameter. Thus, the solubility of BMV was highest in distearate. As can be seen from the solubility data, BMV was dispersed, rather than fully dissolved in the lipids tripalmitate and cetylpalmitate. This was also reflected when studying the final SLN by optical microscopy. Compared with the ointment, few BMV crystals were seen in the tripalmitate and cetylpalmitate SLN but no crystals were detected in the distearate SLN. Microscopy showed the

presence of crystals (<25 μm) homogenously distributed throughout the ointment vehicle. Ultrafiltration showed that trace amounts of the total amount of BMV was present in the aqueous filtrate upon ultrafiltration of the SLN. The amount in the aqueous filtrate was 2.83% ± 0.05, 1.49% ± 0.01, and 0.85% ± 0.04, for distearate, tripalmitate, and cetylpalmitate, respectively. The method is a supplement to the microscopic evaluation as it only detects dissolved and not dispersed BMV in the aqueous phase. Liquid scintillation confirmed homogeneity of the SLN dispersions and the ointment, i.e., the relative deviation of ^{3}H-activity was less than 3% for SLN (n = 3) and less than 10% for the ointment (n = 3). The overall BMV recovery in the penetration studies was 86–106% for SLN and 80–118% for the ointment. Similarly, the recovery of the lipid from SLN was 83–103%. The range of recovery can be explained by sample preparation and by the slight deviation in homogeneity in ^{3}H activity.

Table 2 illustrates the size and the melting properties of the three SLN dispersions. The diameter of the three different SLN studied was within the same range, i.e., 150–212 nm, with a PdI of less than 0.24 (Table 2). The DSC analysis showed that there was a tendency toward a reduction in the SLN lipid melting temperature when BMV was included (Table 2), and no new melting peaks were observed upon application of BMV (thermograms not shown).

3.2. Skin penetration and permeation studies

3.2.1. Skin sample preparation

The effect of tape stripping fresh versus frozen and thawed skin was evaluated by histological examination after tape stripping 0, 5, 15, 25, and 40 times, respectively. The study did not show any significant difference between tape stripping fresh and thawed skin, with regard to the amount of stratum corneum removed by the tape-stripping procedure. Fig. 1 illustrates the effect of tape stripping thawed skin 0, 10, and 25 times. The stratum corneum was gradually removed when the numbers of tape strips were increased. Forty tape strips removed the stratum corneum completely (data not shown), whereas a thin layer remained with 25 tape strips, comparable with what was seen previously [5].

3.2.2. Skin penetration and permeation studies

In vitro penetration studies were carried out on intact and barrier-impaired porcine skin in Franz diffusion cells varying the SLN lipid component, duration of application, and the presence of occlusive conditions in order to evaluate how SLN affect the drug substance penetration profile into and across the skin.

Fig. 2 shows the ratio of the recovered amount of BMV to that of lipid (distearate and tripalmitate SLN, respectively) after application on intact skin for 24 h. There was an overall increase in the ratio of BMV to lipid with skin depth, i.e., more BMV than lipid penetrated the skin. The ratio in the surplus formulation was approximately 1. In stratum corneum, it decreased to below 1 and subsequently increased through epidermis and dermis to reach a ratio of 3.6 and 2.7 in the receptor medium for distearate and tripalmitate SLN, respectively (receptor medium is not included in Fig. 2). The fact that the BMV/lipid ratio increased with skin depth indicates that the lipid stayed mainly on the surface or in the upper layers of the skin and that BMV was released from the SLN vehicle to penetrate into the skin. Table 3 shows the penetration data of lipid into the different skin layers and into the receptor medium. When the skin was barrier-impaired by tape stripping, an increase in the amount of lipid reaching the epidermis, dermis, and receptor medium was seen. The increase in lipid penetration (~3–7-fold) was very low compared with the increase in BMV penetration (~3–60-fold) in barrier-impaired skin, emphasizing that BMV was released from the SLN vehicle and diffused separately through the skin.

The main difference observed between the three SLN and the ointment after skin application for 24 h was that SLN caused a significantly higher total amount of BMV to remain in the skin, both intact and barrier-impaired (Fig. 3). When the barrier was intact, a large amount of BMV administered in all SLN formulations was found in the stratum corneum and less in the receptor medium (Fig. 3a). It was apparent that SLN, and in particular SLN made from distearate, caused significantly more BMV to penetrate deeper into the stratum corneum of intact skin when compared to the

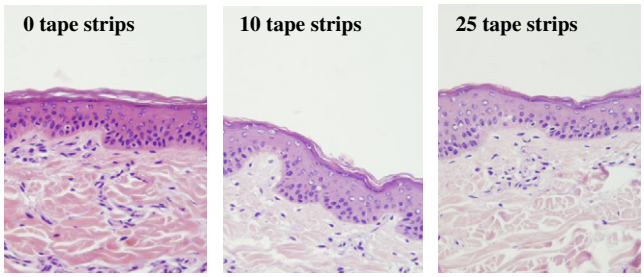

Fig. 1. Visualization of the effect of inducing skin barrier damage by successive numbers of tape strips (n = 6). (a) No tape stripping, (b) 10 tape strips, (c) 25 tape strips. Skin exposed to 25 tape strips was used as barrier-impaired skin. Magnification 400×. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

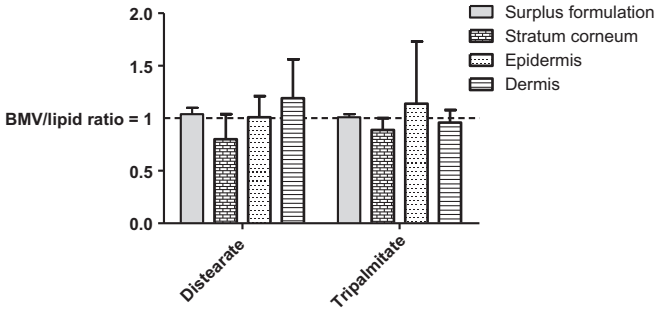

Fig. 2. The BMV/lipid ratio in the different skin layers after application of SLN on intact skin for 24 h. Mean ± SD (n = 6).

Table 2
The hydrodynamic diameter (Z-average), the polydispersity index (PdI), and the lipid melting points determined by DSC for SLN with and without 0.1% (w/w) BMV mean ± SD (n = 3).

	Tripalmitate Incl. BMV	Tripalmitate Placebo	Distearate Incl. BMV	Distearate Placebo	Cetylpalmitate Incl. BMV	Cetylpalmitate Placebo
DLS						
Z-average (nm)	212.5 ± 11.0	222.5 ± 6.0	150.9 ±0.12	186.7±2.1	179.3 ± 1.4	181.3 ± 2.08
PdI	0.16 ± 0.07	0.23 ± 0.04	0.19 ± 0.01	0.21 ± 0.01	0.12 ± 0.02	0.05 ± 0.02
DSC						
T_m (°C)	60.97 ± 0.01	61.02 ± 0.01	52.80 ± 0.04	52.98 ± 0.04	50.56 ± 0.35	51.13 ± 0.41

Table 3
Penetration of the ^{14}C-labeled lipid component into and across the skin layers. The results are given as the percentage of the total amount of ^{14}C-lipid recovered mean ± SD (n = 6).

	Surplus	Stratum corneum	Epidermis	Dermis	Receptor medium	Non-applied skin
Tripalmitate intact skin	74.1 ± 13.1	20.9 ± 10.7	2.0 ± 1.0	1.0 ± 0.5	0.2 ± 0.1	0.9 ± 0.6
Tripalmitate tape stripped skin	78.9 ± 4.0	11.0 ± 2.5	7.5 ± 3.0	0.7 ± 0.3	1.6 ± 0.6	2.0 ± 1.0
Distearate intact skin	70.5 ± 7.3	20.3 ± 6.51	3.0 ± 2.2	1.6 ± 0.9	0.3 ± 0.2	2.6 ± 2.6
Distearate tape stripped skin	71.3 ± 7.2	8.8 ± 3.6	11.4 ± 4.4	1.6 ± 0.8	2.1 ± 1.7	4.2 ± 5.2

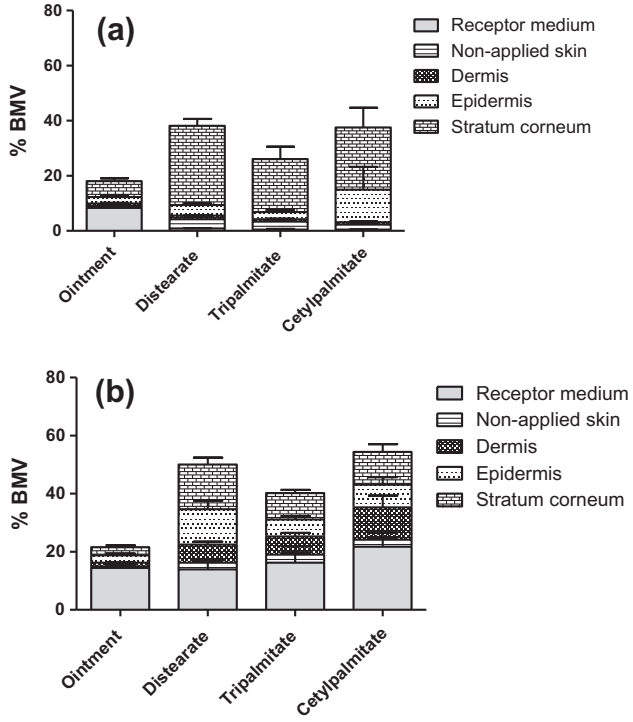

Fig. 3. The relative amount of ^{3}H BMV penetrated into the different skin layers after applying BMV in an ointment and the three different SLN, respectively, onto intact (a) and barrier-impaired (b) skin for 24 h. The results are relative to the total amount of ^{3}H BMV recovered. Mean ± SD (n = 8).

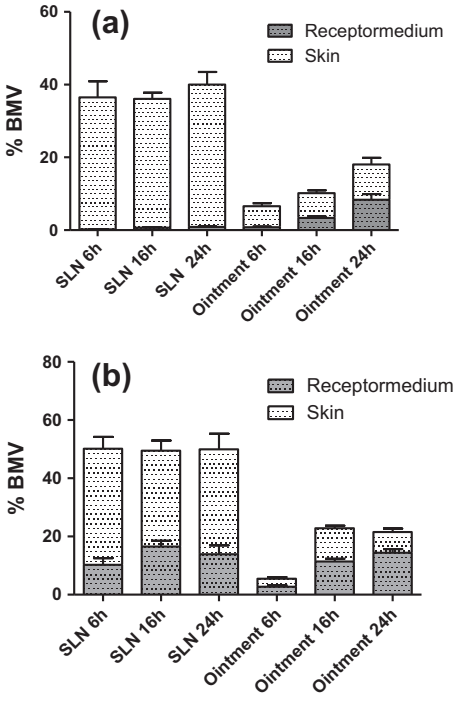

Fig. 4. The relative amount of ^{3}H BMV penetrated into the skin and into the receptor medium, respectively, after applying BMV in an ointment and distearate SLN, respectively, onto intact (a) and barrier-impaired (b) skin for 6, 16, or 24 h. The results are relative to the total amount of ^{3}H BMV recovered. Mean ± SD (n = 6).

ointment ($p < 0.05$). Only a negligible amount of BMV (<0.9%) permeated to the receptor medium when SLN were applied to the intact skin whereas significantly more (8.4 ± 4.0%) permeated the intact skin after using the ointment (Fig. 3a). Upon tape stripping, the skin prior to application of either SLN or ointment, permeation of the BMV from all formulations into the receptor medium was greatly enhanced (13.8–21.8%) (Fig. 3b). Significantly more BMV remained in the barrier-impaired skin upon application of SLN as compared to the ointment ($p < 0.05$), but a similar amount of BMV permeated to the receptor medium (Fig. 3b).

To achieve a better understanding of the results obtained after 24 h of application, two additional time points were included in the study, viz. 6 and 16 h. SLN composed of distearate were chosen for these studies due to the higher solubility of BMV in this lipid. After application for 6, 16, and 24 h, respectively, it was shown that, at all time points, application of SLN resulted in a constant and significantly higher amount of BMV in the skin and that significantly lower amounts of BMV was present in the skin when using the ointment (Fig. 4).

When the skin was immediately occluded for 24 h, after application of SLN, BMV in the receptor medium was significantly increased as compared to non-occluded conditions, and the amount in the skin was lowered (Fig. 5). Upon occlusion and in contrast to the intact skin, the increase in BMV that permeated

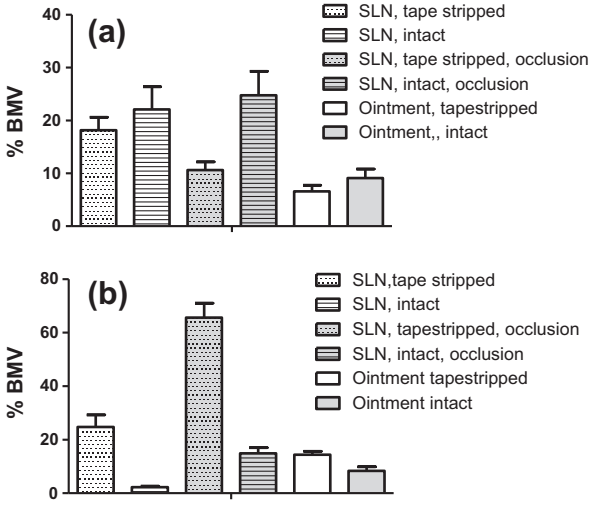

Fig. 5. The relative amount of ^{3}H BMV in the skin (a) and in the receptor medium (b) after application of distearate SLN and ointment, respectively, for 24 h. The skin exposed to SLN was in some cases occluded after application of SLN. The results are relative to the total amount of ^{3}H BMV recovered. Mean ± SD (n = 8).

to the receptor medium was extremely high for barrier-impaired skin; 65.6 ± 15.2% when distearate SLN was applied (Fig. 5b).

4. Discussion

4.1. Characteristics of the SLN

The hot stage microscopy showed that a higher lipid solubility parameter (SP) caused a higher BMV solubility, with more BMV dissolved in the lipid phase and less BMV dispersed in the aqueous phase of SLN. This was in accordance with previous studies of SLN composed of lipids containing varying amounts of monoglycerides [25,28,29]. The microscopy data are also consistent with those of Zhang and Smith, who showed that a higher solubility in the lipid component and a more efficient encapsulation of BMV were achieved with the more polar lipid monostearin compared with the less polar beeswax [24]. To supplement the microscopic data, ultrafiltration was performed on the SLN as well. The analysis indicated that a very low amount of BMV was dissolved in the aqueous phase (<2.9% of the total amount), in accordance with the poor aqueous solubility of BMV. The slight differences seen in the BMV amount in the aqueous filtrate may be related to differences in solubility of BMV in the aqueous phase caused by residuals from the different lipids [25]. Ultrafiltration is an indirect method of determining entrapment efficiency, and besides retaining SLN, the filter may also retain any drug crystals present in the aqueous phase. Moreover, previous studies indicated that BMV is most likely associated with the lipid surface and not efficiently incorporated in the core of SLN [23,25].

Besides affecting the drug solubility, the lipid SP may also affect the release and the skin penetration of BMV as a result of a changed interaction of SLN with skin lipids. Components with a SP close to that of the skin may have good miscibility with the skin, and varying the lipid SP can be a way of controlling the release of BMV from the SLN and partitioning into the skin [30]. The SP is estimated to be 12 for BMV [25] and 10 for porcine skin [31]. The lipids used had solubility parameters between 8.7 and 9.5 (Table 1). SLN composed of distearate, which has a solubility parameter of 9.5, would be expected to be more miscible with the skin lipids than with tripalmitate and cetylpalmitate SLN (Table 1). Additionally, BMV was soluble in distearate and the SLN were smaller in size than the SLN composed of tripalmitate or cetylpalmitate. All of the above can explain the higher amount of BMV in the skin and particularly in the stratum corneum after application of BMV in distearate SLN as compared to tripalmitate and cetylpalmitate SLN.

The mean diameter is correlated with the particle surface area, which is thought to be an important parameter for the SLN interaction with and penetration into the skin as well as any occlusive properties of the SLN [13,32]. It is generally believed that particles above 10 nm do not penetrate the intact skin but that diseased skin may be penetrated by larger sized particles because the barrier is impaired[33,34]. The penetration properties of a particulate system will be dependent not only on the size but also on the polarity and the rigidity of the particles [35,36]. The measured particle size of 150–212 nm indicates that the SLN should not be able to penetrate the intact skin, whereas the barrier-impaired skin may allow particle penetration.

A small reduction in lipid melting temperature was detected in the DSC analysis upon BMV addition to SLN, which can be explained by that addition of a drug substance may reduce the melting temperature due to disturbance of the lipid crystal structure [37].

4.2. Skin penetration and permeation studies

The skin penetration of a drug substance intended to exert a local effect in the skin is a complex process involving three major steps: (1) release of the substance from the vehicle, (2) penetration into the stratum corneum, and (3) partitioning from stratum corneum to target sites in viable epidermis and dermis. The first step is dependent on the physicochemical properties of the drug and the vehicle and may be optimized by the processing. The second and third steps are more complex. Again, the drug substance physicochemical properties and the degree of drug saturation in the vehicle are important for the partitioning of the drug substance between the vehicle and the skin and may be affected by optimization of the vehicle. But in addition, the condition of the major biological barrier for the penetration into the skin – the stratum corneum – is influenced by skin diseases. Here, SLN were evaluated for their properties as a topical drug delivery system for barrier-impaired skin. SLN were compared with a conventional ointment formulation, which, like SLN, is suitable for the delivery of lipophilic compounds to the skin and possess occlusive properties.

4.2.1. SLN remain on the skin surface on intact and barrier-impaired skin

The studies with ^{14}C-labeled lipid and ^{3}H-labeled BMV showed that the lipid mainly stayed on the skin surface and in the stratum corneum in both intact and barrier-impaired skin (Table 3) and that the ratio of BMV to lipid increased down the skin layers to reach a maximum in the receptor medium. For this reason, it was concluded that BMV was not delivered to the target cells in the deeper skin layers by the SLN (Fig. 2), which rather serve as a drug reservoir of drug substance in the upper layers of the skin. Thus, BMV must be released from the SLN vehicle to diffuse further into the skin and reach the target cells in the lower epidermis and dermis. Küchler et al. showed by SEM that SLN composed of glycerolbehenate (Compritol ® 888 ATO) fused with the stratum corneum lipids after application for 4 h [38]. A similar mechanism for SLN delivery BMV to the skin may be relevant here.

Regarding safety, it is beneficial that SLN do not permeate across the skin, even if the barrier is strongly impaired as illustrated here by tape-stripped skin. The fact that the lipid stayed on the surface of the skin also makes it probable that SLN can improve skin hydration and physically strengthen the barrier, a highly relevant property when treating skin diseases involving dry skin such as AD [39,40].

4.2.2. SLN create a drug reservoir in intact and barrier-impaired skin

SLN were superior to the ointment in achieving a high amount of drug substance in the skin. This could be clearly seen from the results in Fig. 3. A large proportion of BMV was found in the upper layer of the skin, intact and barrier impaired, which was most likely to be related to the large surface area and adhesive properties of SLN. The result is in accordance with previous findings of lipophilic substances being delivered in large amounts to the upper skin layers when applied in SLN [23,38,41]. In addition, SLN and drug substance may penetrate hair follicles and skin furrows from where they can act as a drug reservoir [42]. After application of distearate SLN to intact and barrier-impaired skin, a higher amount of BMV was present in the stratum corneum and epidermis compared with tripalmitate and cetylpalmitate, which is thought to be related to differences in lipid polarity and particle size (Figs. 3). Thus, the close interaction with the skin, the fusion with the skin lipids, and the subsequent release of the drug substance in a controlled manner can contribute significantly to a penetration-enhancing effect of SLN.

The higher amount of BMV in the epidermis of intact skin and in the receptor medium for intact and barrier-impaired skin when applying cetylpalmitate SLN can be attributed to the different lipid structure, lower polarity and lower BMV solubility [24,43]. This may cause BMV to leave the cetylpalmitate SLN vehicle more readily and partition into the skin.

The reason why there was no significant difference between the amount of drug substance delivered to the receptor medium of barrier-impaired skin from the ointment and from the SLN, respectively, may be that BMV was associated with the surface layer of the nanoparticles and was readily released from the SLN vehicle upon skin application [23]. Fusion of the SLN with skin lipids may be another explanation [38]. Other studies have indicated that BMV is associated with the surface of SLN and that the lipid must be carefully selected to achieve a high solubility of the drug substance in the vehicle [23–25]. This was reflected in distearate SLN that resulted in the highest BMV solubility and were superior in keeping a high amount of BMV in the skin and a lower amount of BMV in the receptor medium. The level of BMV in the skin was similar for intact and barrier-impaired skin, which indicates that the SLN adhered effectively to the surface of the intact skin as well as to that of the barrier-impaired skin.

The correlation between the skin reservoir effect and the therapeutic effect of the drug applied to the skin in a SLN formulation is currently being studied in an *in vivo* model.

4.2.3. SLN keep a constant amount of BMV in the skin during 24 h

A higher amount of BMV was kept in the skin, intact as well as impaired, during application of distearate SLN for 6, 16, and 24 h, compared with that of an ointment (Figs. 4). The minimal increase in the receptor medium during the 24 h can be explained by surface depletion of the formulation, i.e., when the aqueous phase and the surface layer are depleted upon burst release, then less BMV may diffuse from the SLN to the skin and with a slower rate of release [29]. Another reason for the profile seen is that the skin or the hair follicles are saturated with BMV, i.e., the level of BMV in the skin is very similar at all times [44].The results illustrate that SLN may be used to keep a constant amount of drug substance available for absorption. However, the rate of exchange between SLN and skin may change with time of application because of the drug incorporation [45,46]. SLN may thus have the ability to deliver a drug substance in a biphasic manner – initiated by a burst release from the surface of the particles and the aqueous phase followed by a reservoir effect in the stratum corneum from the drug substance associated more closely with the lipid particles.

4.2.4. Influence of occlusion on BMV penetration profile

The significant increase in penetration of BMV upon occlusion of barrier-impaired skin may have different reasons. First, occlusion almost always enhances the amount of drug substance absorbed in the skin because of the increased diffusion coefficient due to the increased water content in the stratum corneum and the disturbance of the lipid barrier [47]. Furthermore, the occlusion may reinforce the penetration-enhancing effect on the skin barrier of the surfactant present in SLN. Because of the very large amount of drug permeating to the receptor medium upon occlusion of the barrier-impaired skin, BMV must be readily released from the SLN vehicle.

5. Conclusion

The results demonstrate that SLN can be used to increase the amount of drug substance kept in the intact and barrier-impaired skin and to create a reservoir of a drug substance distinctively in the stratum corneum. When the barrier was intact, the reservoir effect was more evident and the drug partitioning into the different skin layers was dependent on the lipid properties of the SLN. It was possible to obtain a constant high level of BMV in the skin when administered in SLN as compared to an ointment. Moreover, the present results emphasize the influence of the properties of the skin barrier on the penetration profiles of a drug substance applied topically.

Acknowledgements

This work was financially supported by the Danish Ministry of Science Technology and Innovation. Louise Langthaler is acknowledged for help with the penetration studies.

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