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Research paper

The influence of topical formulations on the permeation of 5-aminolevulinic acid and its *n*-butyl ester through excised human stratum corneum

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Abstract

The limited permeation of 5-aminolevulinic acid (ALA) through excised human stratum corneum could be improved by using 5-aminolevulinic acid-*n*-butyl ester (ABE). Furthermore drug permeation could be increased by choice of a permeation enhancing formulation. In this study, permeation of ALA and ABE was investigated from various formulations. In addition, differential scanning calorimetry (DSC) and wide angle X-ray diffraction (WAXD) experiments were performed in order to reveal an interaction between the tested formulations and stratum corneum lipid structure. Drug incorporation into Dolgit® Mikrogel showed the highest increase in permeability with both ALA and ABE. Especially, ABE together with Dolgit® Mikrogel was the most promising combination. Further permeation studies with poloxamer based ABE formulations, partially enriched with ibuprofen acid and medium chained triglycerides showed that both compounds promote permeation. The permeation coefficients of either drug from Excipial® Creme and Basiscreme DAC were found to be very similar. These results were in accordance with those of DSC and WAXD experiments. Interaction between formulation and stratum corneum lipid structure resulting in an increased drug permeation only occurred after pretreatment with formulations enriched with ibuprofen acid. After pretreatment with Excipial® Creme, Basiscreme DAC or Excipial® Fettcreme stratum corneum structure and subsequently permeability remained unchanged. Nevertheless permeation of ALA from Excipial® Fettcreme is slower than from the tested hydrophilic formulations and therefore believed to be influenced by the affinity of ALA to the vehicle and stratum corneum.

Keywords: 5-Aminolevulinic acid; 5-Aminolevulinic acid-*n*-butyl ester; Stratum corneum; Permeation studies; Permeation enhancement; DSC experiments; WAXD experiments

1. Introduction

5-Aminolevulinic acid (ALA) acts as a precursor in the biosynthetic pathway of porphyrins, especially protoporphyrin IX (Pp IX), and heme in human body [1]. ALA is applied topically as a prodrug of Pp IX and thus sensitizer in photodynamic therapy (PDT) to treat special skin tumours or cancer of the bladder, oesophagus, and lung [2,3]. PDT uses the increase in Pp IX concentration in abnormal cells after topical or systemic application of exogenous ALA. The exposure of this tissue to light of an appropriate wavelength initiates photodynamic activation of singlet oxygen and free radicals which cause the destruction of the sensitized tissue [4–6]. Topical application of ALA is performed with water in oil as well as oil in water emulsions, enriched with ALA in concentrations between 10 and 20% [3,7].

ALA is a hydrophilic molecule. Due to its hydrophilicity ALA shows only limited permeation through stratum corneum, the lipophilic barrier of the skin. To increase lipophilic properties and improve dermal bioavailability a variety of ALA esters were synthesised [8–12]. More lipophilic ALA derivatives such as methyl, butyl and hexyl esters exhibited a higher Pp IX synthesis in in vitro studies, while the Pp IX formation rate induced by the longer-chained esters was highest [13]. The ALA esters also led to

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a more homogeneous Pp IX localisation in comparison to free ALA as shown by in vivo studies [14,15]. Further studies with the hexyl ester showed that this derivative is an excellent precursor of Pp IX synthesis in bladder cancer [16]. Based on their positive effects on Pp IX synthesis, the hexyl ester and the methyl ester have been approved by regulatory offices in Europe and the United States, respectively. Metvix® cream (methyl 5-aminolevulinate) has been approved for the treatment of actinic keratosis and basal cell carcinoma, Hexvix® (hexyl 5-aminolevulinate) for the detection of bladder cancer, respectively. Both products are marketed by Photocure ASA (Oslo, Norway).

Although comparative in vivo and in vitro studies of ALA hexyl ester and ABE showed a superior Pp IX synthesis of the hexyl ester [9,14], skin penetration of ALA could be increased by using the butyl ester as well. Comparative permeation studies with Excipial® Fettcreme enriched with ALA and ABE, respectively, revealed a 10-fold higher permeation coefficient of ABE compared with that of ALA [17].

Another strategy to improve skin permeation of ALA refers to a careful selection of an appropriate formulation. In vitro permeation studies with an oil in water emulsion containing 10% ALA, 3% ethylenediamine-tetraacetic acid disodium (EDTA) and 20% of the penetration enhancer dimethylsulphoxide (DMSO) demonstrated an improved ALA permeation. In addition, the generation and accumulation of Pp IX also increased as shown by in vivo studies [18]. Auner et al. [19] reported that ALA permeation through porcine skin increased threefold upon dermal application of a 6-ketocholestanol containing cream formulation. Furthermore, variations in barrier integrity of nude mouse skin have been reported to influence ALA dermal availability and the generation of Pp IX [20]. Lieb et al. [21] developed thin self-adhesive films as a topical delivery system for ALA. A pressure sensitive adhesive which contained Eudragit NE and the plasticiser acetyl tributyl citrate in the ratio of 1–2 was superior to topical bases such as Psoralon® lipophilic cream regarding the delivery of ALA through human stratum corneum plus epidermis. Furthermore the authors showed that acetyl tributyl citrate acted as a permeation enhancer.

Another disadvantage of ALA is its well investigated instability in aqueous solution at a pH above 5.2. Deterioration increases with increasing pH. This instability is recognized by an increasingly yellow colour and a decrease in pH of the solution due to a formation of pyrazin derivatives [22–24]. At low temperature and a pH of 5.0, which is similar to the pH of stratum corneum yet undergoing interindividual variations [25–27], stability can be guaranteed for several days [24].

The aim of the present study was a comparison between different formulations enriched with ALA and its *n*-butyl ester (ABE). Although ABE seemed to be suboptimal with respect to the induction of Pp IX synthesis [13], this derivative has been chosen as an example of a middle chain length ester with regard to the methyl and hexyl ester. The question was whether permeation of either substance through excised human stratum corneum increases by means of a more appropriate formulation than Excipial® Fettcreme [17]. Formulations studied were Excipial® Creme, Basiscreme DAC and Dolgit® Mikrogel. The latter formulation has already shown an increased permeation coefficient of ibuprofen acid compared with Ibutop® Creme [28]. Oil in water emulsion Excipial® Creme and Basiscreme DAC were chosen as examples of hydrophilic formulations in contrast to lipophilic Excipial® Fettcreme. As recently shown in our group a change in stratum corneum lipid arrangement could be detected by differential scanning calorimetry (DSC) and wide angle X-ray diffraction (WAXD) [29]. Hence, the interaction between formulation and stratum corneum was investigated by means of DSC and WAXD experiments in addition to the permeation studies.

2. Materials and methods

2.1. Materials

ALA hydrochloride was provided by Medac GmbH (Wedel, Germany). ABE hydrochloride was synthesised according to Ref. [8]. *n*-Butanol was purchased from Heraeus GmbH (Karlsruhe, Germany), diethylether (reagent grade) and *o*-phthalaldehyde (OPA, HPLC grade) from Fluka (Neu-Ulm, Germany). Thionylchloride (reagent grade), mercaptoethanol (pro analysi) and all buffer substances (pro analysi) were purchased from Merck KGaA (Darmstadt, Germany). Acetonitrile, methanol and acetic acid (all chemicals were HPLC grade) were purchased from J.T. Baker (VA Deventer, Netherlands). Boric acid was purchased from Carl Roth GmbH (Karlsruhe, Germany), absolute ethanol (HPLC grade) from Riedel de Haën (Seelze, Germany). Water was used in bidistilled quality.

Excipial® Fettcreme and Excipial® Creme were provided by Hans Karrer GmbH (Königsbrunn, Germany). Dolgit® Mikrogel was purchased from a retail pharmacy. Basiscreme (DAC 2000) was prepared with glyceryl monostearate 60, PEG-20-glyceryl stearate (both were purchased from Caelo, D-Hilden), cetylic alcohol (Henkel, D-Düsseldorf), white petrolatum (Merkur Vaseline, D-Hamburg), propylene glycol (BASF, D-Ludwigshafen), medium chain triglycerides (Hüls, D-Witten) and purified water according to instructions of Deutscher Arzneimittelcodex (DAC) 2002. A poloxamer containing formulation enriched with ibuprofen acid (=PIA) was prepared according to a European patent [30] using Lutrol® F127 (=poloxamer 407), dimethylisosorbide (both were provided from Dolorgiet, D-St Augustin/Bonn), ibuprofen acid (Caelo, D-Hilden), isopropylic alcohol and purified water. In addition, a formulation with further incorporation

Table 1
Composition of the poloxamer based formulations, amounts in g

Substance	IFP	PIA	PIT
Ibuprofen acid	–	2.5	2.5
Isopropylic alcohol	8	8	8
Dimethylisosorbide	5	5	5
Lutrol® F127	8	8	8
Water	26	26	26
MCT	–	–	1

of medium chain triglycerides (MCT) was prepared and named PIT. The quantitative composition of this formulation was similar to that of Dolgit® Mikrogel but small amounts of lavender and bitter orange oil were lacking. In order to reveal the influence of ibuprofen acid on stratum corneum structure an ibuprofen acid free poloxamer containing formulation was also prepared (=IFP) which remained liquid after preparation in contrast to PIA and PIT. The quantitative compositions of these three formulations are presented in Table 1.

2.2. Methods

2.2.1. Preparation of excised human stratum corneum

Skin donations from the abdominal regions of healthy females originated from plastic surgeries with written consent of the donors and ethical approval according to the Declaration of Helsinki of the World Medical Association. Directly after biopsy the subcutaneous fat was trimmed away. Then the skin was frozen in liquid nitrogen and stored at −25 °C until separation of stratum corneum by trypsination [31]. For this purpose a skin sheet was spread with its dermal side on filter paper which was soaked with 2% aqueous trypsin solution and incubated at 37 °C. After 24 h stratum corneum was peeled off from deeper skin layers using a blunt pair of tweezers. Immediately thereafter stratum corneum was bathed in 0.01% aqueous solution of trypsin inhibitor and washed several times in water in order to prevent further enzymatic degradation. The isolated horny layer was dried and stored in a desiccator at room temperature to protect it from humidity.

2.2.2. In vitro permeation studies

In vitro permeation studies (n=5–8) were performed with a modified Franz cell [32]. The used Donor and receiver compartments were separated by stratum corneum. The surface area of the used cells was between 0.650 and 0.739 cm^2 (corresponding to diameters between 0.910 and 0.970 cm). Before starting permeation experiments each stratum corneum sheet was completely hydrated in water, placed on a polycarbonate filter (Isopore® membrane filters, type TMTP, 5.0 μm, Millipore, Ireland) for a higher mechanical stability and mounted on the diffusion cell. The donor compartment was filled with one of the chosen formulations enriched with ALA and ABE, respectively. Drug concentration of 10% was chosen in agreement with previous investigations of other groups, who used cream bases enriched with 10–20% ALA [3,7]. The donor was prepared freshly prior to the permeation studies. Drug dissolution in the formulation was performed with a Cito Unguator® (GAKO Konietzko GmbH, Bamberg, Germany). Homogenization was done at 2000 rpm for 2.5 min at room temperature in the case of Basiscreme DAC, Excipial® Creme and Excipial® Fettcreme. Dolgit® Mikrogel, PIA, IFP and PIT, however, were stirred at 1000 rpm for 2 min only to prevent a loss of isopropylic alcohol during heating.

The receiver contained 4.5–6.0 ml of phosphate buffer of pH 5.0 (Ph. Helv. 8) to guarantee the stability of ALA and ABE. During the experiment the receiver compartment was maintained at 37 °C in a waterbath. The homogeneous distribution of permeated drug within the receiver was achieved by stirring with a magnetic bar at 350 rpm.

Samples of 250 μl were taken from the receiver up to 35 h as shown in Figs. 1–3 and replaced by fresh buffer. The permeated amounts of both substances were measured by HPLC with fluorometric detection after precolumn derivatization with OPA [33,34].

2.2.3. HPLC analysis

Analysis was performed with an HPLC system consisting of a fluorescence HPLC monitor RF-353 (Shimadzu, Kyoto, Japan) and a Spectroflow 400 solvent delivery system (Kratos Analytical, New Jersey, USA) equipped with a Rheodyne 7125 syringe loading sample injector (Rheodyne, Cotati, USA). The injector was fitted with a 20 μl loop. The analytical column (250×4.6 mm) was connected in line with a guard column (10×4.6 mm). Both columns were packed with Hypersil ODS (particle size 5 μm, Grom, Herrenberg, Germany).

ALA and ABE were eluted with different mobile phases, because of the higher lipophilicity of ABE. Elution of ALA was performed at ambient temperature with a mixture of

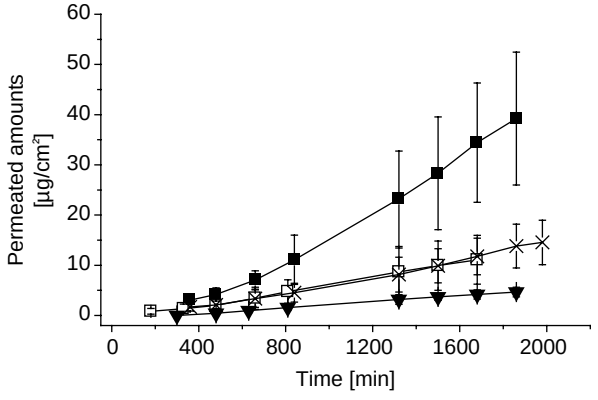

Fig. 1. ALA permeation profiles from Dolgit® Mikrogel (-■-, n=6), Excipial® Creme (-□-, n=6), Basiscreme DAC (-×-, n=8) and Excipial® Fettcreme (-▼-, n=6) through stratum corneum; graph represents mean values and standard deviation; donor: female, abdomen, 35 years.

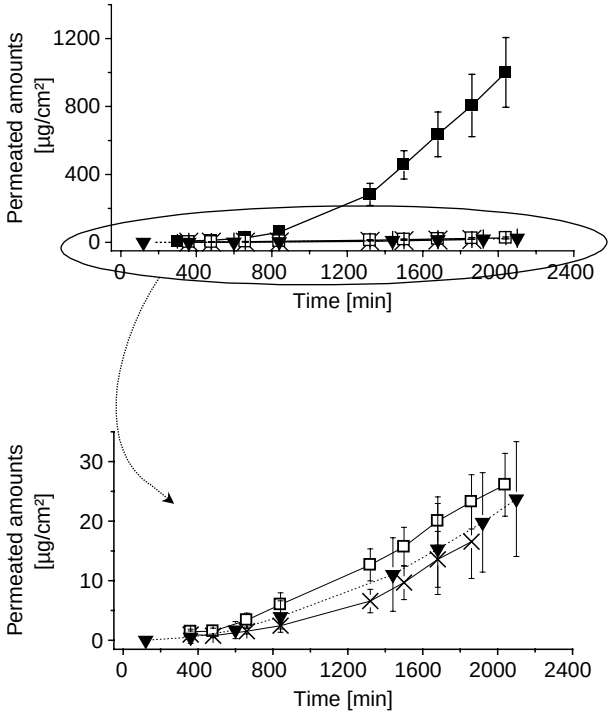

Fig. 2. ABE permeation profiles from Dolgit® Mikrogel (-■-, $n=6$), Excipial® Creme (-□-, $n=6$), Basiscreme DAC (-×-, $n=6$) and Excipial® Fettcreme (···▼···, $n=6$, donor: female, abdomen, 59 years) [17] through stratum corneum (including an extended representation); graph represents mean values and standard deviation; donor: female, abdomen, 35 years.

an aqueous sodium acetate buffer (22 mM) and methanol in the ratio of 7.5–5.0, adjusted to pH 3.38 with acetic acid according to Ref. [33,35]. The flow rate was 1.3 ml/min. Under these conditions, the retention time of derivatized ALA was 8.3 min. This HPLC method was calibrated repeatedly in the concentration range of 0.1–5 and 1–200 μg/ml. The resulting correlation coefficients r were always higher than 0.999.

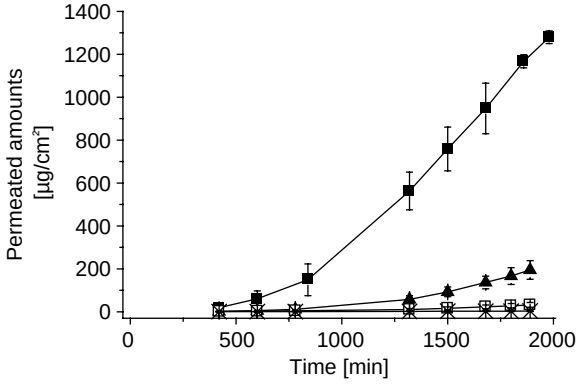

Fig. 3. ABE permeation profiles from Dolgit® Mikrogel (-■-, $n=7$), PIA (-□-, $n=6$), IFP (-×-, $n=6$) and PIT (-▲-, $n=5$) through stratum corneum; graph represents mean values and standard deviation; donor: female, abdomen, 32 years.

The mobile phase for ABE analysis was a mixture of an aqueous monobasic potassium phosphate buffer (12.5 mM, pH 7.2) and acetonitrile in the ratio of 5.5–5.0 [17,36]. The retention time of derivatized ABE was 11.0 min at a flow rate of 1.5 ml/min and ambient temperature. Linear correlation between peak area and ABE concentration was obtained within concentration ranges of 0.1–20 and 1–200 μg/ml. The resulting correlation coefficients r were always higher than 0.999.

Prior to the measurement, derivatization of ALA or ABE was performed according to Ref. [35]. Hundred microliters of the derivatization reagent, i.e. a sodium borate buffer of pH 9.5 enriched with OPA and mercaptoethanol, were mixed with 100 μl of the sample. Immediately after a reaction time of exactly 2 min 100 μl monobasic potassium phosphate buffer (0.1 M) were added to the mixture to stop the reaction. Twenty microliters of this solution were injected onto the column. The fluorescence of the reaction products was monitored at an excitation wavelength of 330 nm and an emission wavelength of 418 nm.

2.2.4. Treatment of excised human stratum corneum for DSC and WAXD experiments

Stratum corneum was hydrated to a water content of 20% by incubating it in a desiccator with saturated sodium chloride solution (rel. humidity: 75.2%) for 48 h to achieve sharper transitions. Afterwards stratum corneum sheets were treated with each drug-free formulation for 30 min. In addition, pieces of stratum corneum were immersed in an aqueous solution of ABE and ALA (in both cases 5% (w/v)) for 30 min, respectively. In order to reveal the influence of *n*-butanol residue originating from synthesis, a stratum corneum sheet was treated with *n*-butanol (1% (v/v))/water mixture. Subsequently, excess of formulation or solution was removed carefully. The cleaned stratum corneum sheets were carefully folded in aluminium pans for DSC analysis or inserted into an amorphous glass capillary for WAXD experiments.

2.2.5. DSC experiments

Human stratum corneum as well as ALA- and ABE-free formulations were thermally analysed using a Differential Scanning Calorimeter DSC 220 C with a disc station 5200 H (Seiko, J-Tokyo). Treated stratum corneum sheets (Section 2.2.4) were folded in aluminium pans, which were then fused on cold. Afterwards the samples were measured against an empty reference pan over a temperature range of −20 to 140 °C with a heating rate of 5 °C/min. DSC experiments with drug-free formulations were performed to reveal the presence of any interfering peaks. The disadvantage of using excised human stratum corneum are inter- and intra-individual variations in permeability and in lipid arrangement between different skin samples. Since the size of a skin donation with similar properties was limited measurements could not be replicated. Therefore, all determinations were performed once.

2.2.6. WAXD experiments

WAXD experiments were done using a Debye-Scherrer Camera (circumference: 360 mm). Treated stratum corneum pieces (Section 2.2.4) were carefully brought into an amorphous glass capillary with a diameter of 0.5 mm and were measured for 24 h. Measurements were performed with X-ray generator PW 1830 (Philips, D-Kassel) and X-ray tube PW2253/11 (Philips, D-Kassel; accelerating voltage: 40 kV, anode current 40 mA; radiation: Cu Kα, $\lambda =$ 0.154 nm). Diffraction rings were visualized by blackening of X-ray specific film material Fuji 100 (Fuji, J-Tokyo). The inner and outer diameters of each diffraction ring as well as its width were measured by using a pair of dividers while the negative films were candled on a strong light source. Afterwards, the distance between the dividers points was measured with a calliper rule (accuracy 0.05 mm). The angle of diffraction was calculated from the mean out of inner and outer diffraction ring diameter with further consideration of width of the respective diffraction ring. The accuracy of 0.05 mm in measuring the diffraction ring diameter resulted in a variation in interlayer spacing of 0.0005 nm. The interlayer spacings were calculated using Bragg's Eq. (1).

$$n\lambda = 2d \sin \theta \quad (1)$$

λ, wavelength; d, interlayer spacing; θ, angle of diffraction; n, order of diffraction.

As mentioned above (Section 2.2.5) stratum corneum with similar properties is only available from skin donations of the same donor. Thus, availability is limited. Therefore, all WAXD experiments were carried out once for each formulation on stratum corneum of a single donor to elucidate the effects of different formulations on stratum corneum lipid arrangement.

3. Results and discussion

3.1. Permeation of ALA and ABE from different formulations

In a previous study with Excipial® Fettcreme, a higher permeation coefficient through excised human stratum corneum was found for ABE in comparison to ALA [17]. In contrast to penetration enhancement with more lipophilic prodrugs, increased permeation coefficients may be obtained by using formulations, which reduce barrier properties of stratum corneum via direct interaction with stratum corneum structure. It has already been reported in the literature [18,37] that the topical delivery of ALA and the Pp IX synthesis could be enhanced by DMSO containing formulations. In addition, papillomas were treated with a cream formulation of ALA-hexyl ester which induced a similar porphyrin concentration in comparison to an ALA formulation which contained the permeation enhancer DMSO [36]. However, in the case of ALA-hexyl ester a cream formulation without DMSO was more efficient than one with DMSO. To yield optimal effects both the drug itself and the vehicle could vary. Therefore two different hydrophilic formulations (Basiscreme DAC and Excipial® Creme) and a permeation enhancing formulation (Dolgit® Mikrogel [28]) were chosen as vehicles for ALA and ABE to compare the results with those of water in oil emulsion Excipial® Fettcreme. In addition to Dolgit® Mikrogel, poloxamer based formulations named PIA, PIT and IFP (Section 2.1) were used as vehicles. Dolgit® Mikrogel, PIA, PIT and Excipial® Creme liquefied, when ABE was dissolved in these formulations. Incorporation of ALA and ABE resulted in complete dissolution of either drug in each of the tested formulations, a process which was controlled by light microscopy. Neither ALA nor ABE crystals could be detected in the respective samples. Enriching Excipial® Creme, Basiscreme DAC or Dolgit® Mikrogel with ALA decreased the pH of the formulations to about 2. In the case of Excipial® Fettcreme the pH of the formulation was 3.3 after the incorporation of ALA. In the case of ABE incorporation the pH decreased to 2.5–4.0 depending on the formulation used. Since the pH of each tested formulation was fairly below 5.0, the ABE and ALA stabilities could be guaranteed during the permeation experiments in accordance with the literature [22–24].

Permeation studies resulted in partially different permeation profiles for ALA and ABE as shown in Figs. 1 and 2, respectively. For each permeation study the linear ascent of the curve was used to determine the flux J. The permeation coefficient P was calculated as the quotient of flux and drug concentration. Permeation data are summarised in Tables 2 and 3. The results of previous permeation studies with ALA and ABE dissolved in Excipial® Fettcreme [17] which were performed with stratum corneum from a different donor are also listed in the tables. To compare the recent results with those from former studies ALA permeation from Excipial® Fettcreme (10% (w/w)) was repeated in the present study. Stratum corneum

Table 2
Comparison of permeation data for ALA from tested formulations, mean ($\pm$standard deviation)

Donor: ALA 10% (w/w) in:	Flux J (g/cm^{2}s)	Permeation coefficient P (cm/s)	Lag-time (min)	n
Excipial® Fettcreme	5.08×10^{-11} ($\pm8.15\times10^{-12}$)	5.37×10^{-10} ($\pm8.62\times10^{-11}$)	152	6
Excipial® Crème	1.20×10^{-10} ($\pm2.55\times10^{-11}$)	1.16×10^{-9} ($\pm2.45\times10^{-10}$)	205	6
Basiscreme DAC	1.51×10^{-10} ($\pm2.84\times10^{-11}$)	1.41×10^{-9} ($\pm2.65\times10^{-10}$)	510	8
Dolgit® Mikrogel	4.90×10^{-10} ($\pm9.83\times10^{-11}$)	4.09×10^{-9} ($\pm8.19\times10^{-10}$)	561	6
Excipial® Fettcreme [17]	2.85×10^{-11} ($\pm7.04\times10^{-12}$)	3.01×10^{-10} ($\pm7.44\times10^{-11}$)	215	9

Table 3
Comparison of permeation data for ABE from tested formulations, mean (±standard deviation)

Donor: ABE 10% (w/w) in:	Flux J (g/cm^{2}s)	Permeation coefficient P (cm/s)	Lag-time (min)	n
Excipial® Creme	3.05×10^{-10} ($\pm3.33\times10^{-11}$)	2.88×10^{-9} ($\pm3.14\times10^{-10}$)	500	6
Basiscreme DAC	3.72×10^{-10} ($\pm1.02\times10^{-10}$)	3.35×10^{-9} ($\pm9.18\times10^{-10}$)	968	6
Dolgit® Mikrogel	1.61×10^{-8} ($\pm4.40\times10^{-9}$)	1.42×10^{-7} ($\pm3.89\times10^{-8}$)	1020	6
Excipial® Fettcreme [17]	3.19×10^{-10} ($\pm8.54\times10^{-11}$)	3.00×10^{-9} ($\pm8.04\times10^{-10}$)	825	6

of the present investigation was twice as much permeable for ALA from Excipial® Fettcreme than stratum corneum of the previous study.

Using the o/w-emulsions Excipial® Creme and Basiscreme DAC the permeation coefficient of ALA was 2–2.5-fold higher compared with Excipial® Fettcreme. Highest ALA permeability was achieved with Dolgit® Mikrogel in terms of a nearly 7.5-fold increase of the permeation coefficient. The incorporation of ABE in Dolgit® Mikrogel resulted in the highest permeated amounts. However, Dolgit® Mikrogel and Excipal® Creme became liquid, when ABE was dissolved in these formulations. Previous studies with Excipial® Fettcreme showed a nearly 10-fold higher permeation coefficient for ABE than for ALA [17]. In the case of Dolgit® Mikrogel ABE permeated nearly 35-fold faster than ALA. The permeation coefficients for Basiscreme DAC and Excipal® Creme were nearly the same. Permeation of ALA from Excipial® Creme began earlier than from Basiscreme DAC. ABE permeation coefficient from the previous study (ABE/Excipial® Fettcreme) [17] is in accordance with ABE permeation coefficients from the present study, although stratum corneum from different donors was used as mentioned above.

In order to evaluate the influence of MCT and ibuprofen acid which is the active substance of Dolgit® Mikrogel, additional permeation studies with ABE were performed using PIA, IFP and PIT as vehicles. The study with ABE and Dolgit® Mikrogel was repeated since skin from different donors was used for the experiments with poloxamer containing formulations and for those with the other tested cream bases. Permeation graphs and permeation data are summarised in Fig. 3 and Table 4, respectively. The permeation coefficient of ABE was 16-fold higher in the case of PIA than that with IFP. It is obvious that ibuprofen acid combined with a poloxamer based formulation promoted permeation of ABE. Comparing permeation data for PIA with those for PIT shows that MCT increased the permeation coefficient further. Nevertheless permeation coefficient for PIT was 4-times smaller than that for Dolgit® Mikrogel. The difference is likely due to variations in quantitative composition of PIT and Dolgit® Mikrogel which is unfortunately unknown. Although lavender and bitter orange oil incorporated in Dolgit® Mikrogel are known to enhance drug permeation, fluidization of stratum corneum lipids by these ingredients is unlikely to occur due to their very small amounts.

As recently shown in previous studies from other groups permeation of ALA or ALA derivatives as well as Pp IX generation were affected by penetration enhancers within the vehicle [18–20,37]. The enhancing effect varied for free ALA and ALA derivatives. According to Ref. [37], DMSO/ethanol increased ALA delivery to papillomas whereas a cream without this penetration enhancer was the best vehicle for ALA hexyl ester. In contrast to these results both ALA and ABE revealed an increased permeation through excised human stratum corneum from Dolgit® Mikrogel. The observed increase was even higher for the butyl ester than for free ALA. As has been demonstrated by Tsai et al. [20] a variation in barrier properties as well as ALA concentration affected ALA delivery and thus generation of Pp IX. The authors found reliable correlations between Pp IX concentrations in both the epidermis and dermis in vivo and ALA's transdermal flux in vitro. In this context higher permeated ABE amounts of the present study could lead to increased porphyrin generation.

As mentioned in the introduction, the ALA butyl ester was chosen exemplarily. With regard to different ALA ester chain lengths, ester derivatives of a longer chain length such as the hexyl ester hold promise in optimisation of ALA-PDT, as has been suggested by De Rosa et al. [38]. Both the methyl ester and the *n*-butyl ester proved to be inferior regarding the affinity towards stratum corneum, drug permeation through the skin, retention in the epidermis, and Pp IX synthesis in comparison to the hexyl ester [9,13,14,38,39]. However, ALA methyl ester was the first marketed active compound in Metvix® cream. A comparison of the results of ALA methyl ester and ABE revealed a similar ranking [38].

Table 4
Comparison of permeation data for ABE from tested poloxamer based formulations, mean (±standard deviation)

Donor: ABE 10% (w/w) in:	Flux J (g/cm^{2}s)	Permeation coefficient P (cm/s)	n
IFP	3.96×10^{-11} ($\pm1.33\times10^{-11}$)	3.70×10^{-10} ($\pm1.25\times10^{-10}$)	6
PIA	6.51×10^{-10} ($\pm1.42\times10^{-10}$)	5.92×10^{-9} ($\pm1.29\times10^{-9}$)	5
PIT	4.19×10^{-9} ($\pm9.98\times10^{-10}$)	3.85×10^{-8} ($\pm9.15\times10^{-9}$)	6
Dolgit® Mikrogel	1.79×10^{-8} ($\pm3.64\times10^{-9}$)	1.59×10^{-7} ($\pm3.22\times10^{-8}$)	7

In contrast to an inferior delivery of ABE through hairless mouse skin and its inferior retention in the epidermis in comparison to ALA, the results of the present study revealed a superior ABE permeation through excised human stratum corneum in comparison with free ALA, especially in the case of Dolgit® Mikrogel as vehicle. Hence a higher ABE retention within the epidermis and conversion to Pp IX has to be expected in accordance with the literature [20]. Since ALA ester hydrolysis via esterases is suggested to limit the subsequent conversion to porphyrins Pp IX [40], saturation of esterase activity is an important rate-limiting parameter in Pp IX synthesis. The determinination of the drug concentration at esterases' saturation would be an interesting aim to know about drug transport under nonsaturating conditions [40]. As described in the literature [14,15], the advantages of using ALA derivatives refer to increased lipophilic properties and higher selectivity of porphyrin accumulation. Penetration enhancers could increase skin permeability in order to decrease the ALA derivative concentration in the formulations. Nevertheless it has been demonstrated that enzymatic hydrolysis of ABE was slower than that of ALA methyl ester or ALA hexyl ester [9] and in consequence porphyrin generation should be suboptimal. On the basis of these results it would be interesting to study stratum corneum permeation of ALA hexyl ester and methyl ester from Dolgit® Mikrogel as the vehicle.

3.2. Influence of ALA, ABE and formulations on structure of stratum corneum

The relation between the barrier function of stratum corneum and its structure is well known. Thus, alterations in the unique composition of stratum corneum lipids and their complex structural arrangement affect permeability. With regard to permeation data for ALA and ABE from the chosen formulations, interactions between the formulation and stratum corneum structure were investigated using differential scanning calorimetry (DSC) and wide angle X-ray diffraction (WAXD). Previous studies from our group have shown that it is possible to detect an alteration of the lipid arrangement after treatment of stratum corneum with appropriate substances [29].

3.2.1. DSC of stratum corneum after treatment with ALA, ABE and formulations selected

A DSC thermogram for excised human stratum corneum normally shows four characteristic endothermic transitions [41–43]. The first and second transition (T1 and T2) at about 40 and 70 °C are lipid-based. The third transition (T3) at about 80 °C is considered by several investigators to represent a phase transition of lipids which are associated with proteins [42,44]. The forth transition (T4) at 105 °C is due to the denaturation of intercellular keratin [41,43]. In contrast to T2 and T3 the first peak, which is small, is not easily detectable as well as the forth peak which requires a water content of the sample of at least 15% to become

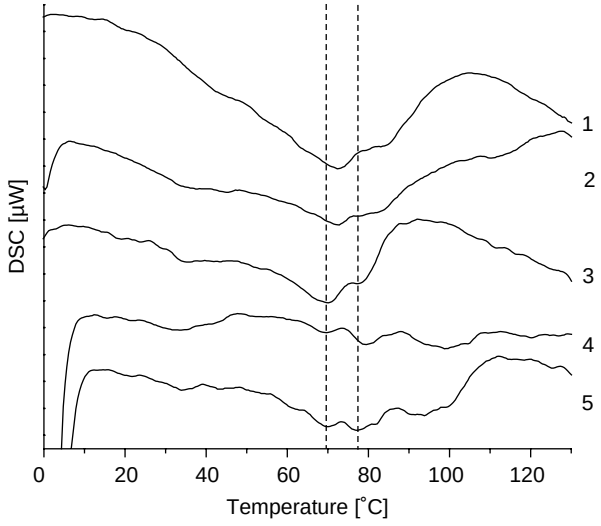

Fig. 4. DSC thermograms for excised human stratum corneum after treatment with water and aqueous solutions of ALA, ABE and *n*-butanol; donor: female, abdomen, 42 years (1: untreated, 2: *n*-butanol 1% (v/v), 3: ALA 5% (m/v), 4: ABE 5% (m/v), 5: water).

apparent [43]. Therefore, only the peak minimum temperatures of T2 and T3 at about 70 and 80 °C were determined in of the present study, respectively. DSC thermograms of stratum corneum sheets after treatment with an aqueous solution of either drug or with each tested formulation were recorded. They are summarised in Figs. 4 and 5 together with DSC curves of stratum corneum sheets treated with purified water and untreated stratum corneum. The peak minimum temperatures of T2 and T3 for pre- and untreated stratum corneum are summarised in Tables 5 and 6.

Comparing data for untreated and completely hydrated stratum corneum reveals a shift of 2.4 and 2.0 °C for T2 and T3, respectively. Since water content of the tested solutions

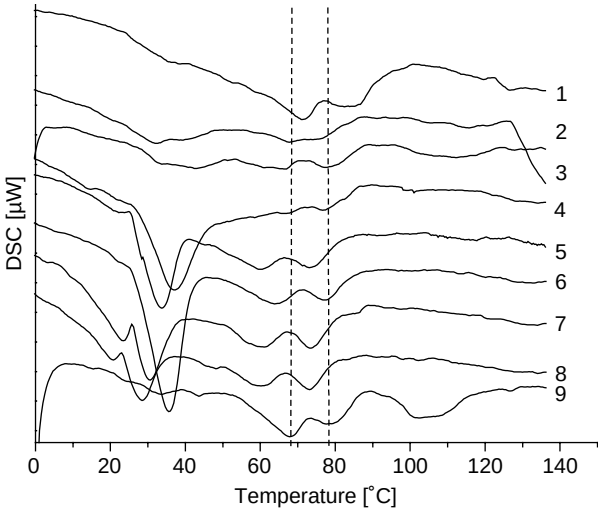

Fig. 5. DSC thermograms for excised human stratum corneum after treatment with tested formulations and water; donor: female, abdomen; 42 years (1: untreated, 2: Excipial® Fettcreme, 3: Excipial® Creme, 4: Basiscreme DAC, 5: Dolgit® Mikrogel, 6: IFP, 7: PIA, 8: PIT, 9: water).

Table 5
Peak minimum temperatures of the second (T2) and third (T3) phase transition of stratum corneum after treatment with aqueous drug solutions (∅ = untreated), donor: female, abdomen, 42 years

Stratum corneum treated with:	T2 (°C)	T3 (°C)
∅	72.4	79.4
Water	70.0	77.4
ABE (5%) (m/v)	69.5	79.3
ALA (5%) (m/v)	70.0	77.2
n-Butanol (1%) (v/v)	72.6	77.3

and formulations is high, hydration of stratum corneum has to be taken in account besides the effects of the respective drug or formulation ingredients. Hence, DSC data of stratum corneum after treatment with water were used as reference to evaluate formulation and drug effects.

Fig. 4 shows DSC thermograms while Table 5 summarises peak minimum temperatures of the transitions after treatment with the tested drug solutions. The maximum peak shift was 2.6 °C (T2) after treatment with an aqueous *n*-butanol (1%) solution. Such a small shift is considered to be insignificant. Leopold and Lippold [45] reported a statistically significant shift to be more than 3 °C. Therefore, none of the tested drug solutions yielded significant changes in the lipid phase transitions of stratum corneum.

DSC thermograms of the tested cream bases, summarised in Fig. 6, were performed to exclude interfering transitions in the range from 60 to 90 °C. Only Excipial® Fettcreme produced a transition in that range, interfering with the transitions of stratum corneum. Yet, peak minimum temperatures could be determined. Fig. 5 and Table 6 reveal that peak minimum temperatures of T2 and T3 changed significantly after stratum corneum treatment with Dolgit® Mikrogel, PIA and PIT, respectively. The shifts to lower temperatures were 7.7 °C (T2) and 5.4 °C (T3) for Dolgit® Mikrogel, 6.7 °C (T2) and 5.1 °C (T3) for PIA and 7.7 °C (T2) and 5.3 °C (T3) for PIT. These results suggest that MCT do not affect stratum corneum structure additionally. The treatment of stratum corneum with IFP showed a shift of more than 3 °C to a lower temperature for

Table 6
Peak minimum temperatures of the second (T2) and third (T3) phase transition of stratum corneum after treatment with tested formulations (∅ = untreated), donor: female, abdomen, 42 years

Stratum corneum treated with	T2 (°C)	T3 (°C)
∅	71.1	81.3
Water	67.8	78.4
Excipial® Fettcreme	67.5	75.6
Excipial® creme	66.8	77.3
Basiscreme DAC	66.5	76.7
Dolgit® Mikrogel	60.1	73.0
IFP	63.9	77.2
PIA	61.1	73.3
PIT	60.1	73.1

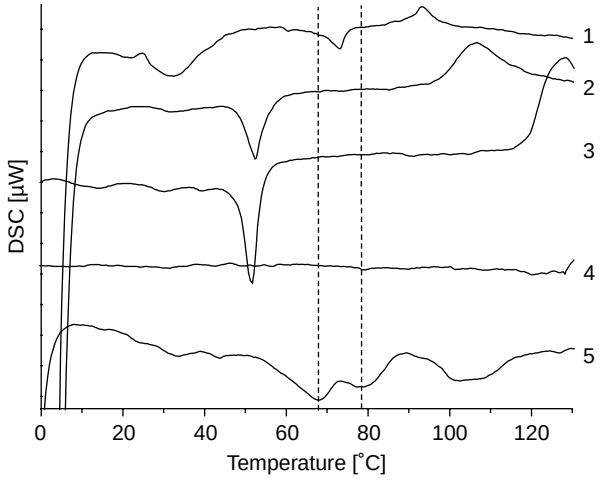

Fig. 6. DSC thermograms for the tested formulations (1: Excipial® Fettcreme, 2: Excipial® Creme, 3: Basiscreme DAC, 4: Dolgit® Mikrogel, 5: water).

T2 (3.9 °C) only which is considered to be significant with regard to reported data [45]. A comparison of DSC shifts of Dolgit® Mikrogel treatment and PIA treatment with those of IFP treatment reveals that the T2 shift was very small. Hence, it is difficult to evaluate the DSC data of stratum corneum after treatment with IFP clearly at this point. Therefore these data will be discussed later in context with results from WAXD experiments (Section 3.2.2).

The other investigated formulations produced minor changes, since the maximum peak shift was just 2.8 °C (T3) after Excipial® Fettcreme treatment. These results indicate an alteration of the lipoidal structure of stratum corneum after treatment with Dolgit® Mikrogel as well as with PIA and PIT. The comparison with an ibuprofen acid free formulation suggests that the combination of a poloxamer containing formulation with ibuprofen acid is most effective in terms of an increased fluidity of stratum corneum lipids. Thus, barrier properties of stratum corneum, which are related to the composition, and complex structural arrangement of its lipids are decreased after treatment with these three formulations subsequently leading to an increase in stratum corneum permeability.

3.2.2. WAXD of stratum corneum after treatment with ALA, ABE and formulations selected

Untreated stratum corneum shows two characteristic sharp and intense reflections at 0.378 and 0.417 nm which are assigned to crystalline lipids [46,47]. These diffraction rings correspond to an orthorhombic crystal structure, while the reflection at 0.417 nm additionally belongs to gel phase lipids with a slightly looser hexagonal alkyl-chain packing arrangement [47]. Minor variations in reflection positions are reported by other WAXD studies with eight different skin donors detecting the first interference in a range from 0.374 to 0.371 nm and the second from 0.416 to 0.412 nm depending on the respective donor [48]. Besides these

Table 7
Wide-angle X-ray diffraction of stratum corneum untreated and treated with aqueous solutions of ALA-HCl and ABE, donor: female, abdomen, 42 years, values in brackets were calculated with regard to the width of each diffraction ring

Stratum corneum treated with:	Diffraction rings (nm)	
∅	0.370 (±0.03)	0.410 (±0.05)
ABE (5% (m/v))	0.370 (±0.03)	0.410 (±0.05)
ALA (5% (m/v))	0.370 (±0.03)	0.410 (±0.05)

reflections two diffuse diffraction rings at 0.46 and 0.98 nm are observed in stratum corneum, normally. The reflection at 0.46 nm is likely to correspond to hydrocarbon chains in the liquid state as well as to soft keratin located in corneocytes. The interference at 0.98 nm is attributed to soft keratin only [47]. These two diffuse reflections were not detected in the present work with the exception of the 0.46 nm interference after treatment with Dolgit® Mikrogel, PIA, PIT and IFP, respectively. The results of the WAXD experiments are summarised in Tables 7 and 8. The values in brackets which are put in brackets were calculated with regard to the width of each diffraction ring (Section 2.2.6).

As shown in Tables 7 and 8 WAXD of stratum corneum from a female donor yielded two reflections at 0.370 and 0.410 nm. Neither the tested drug solutions nor the creams such as Excipial® Fettcreme, Excipial® Creme and Basiscreme DAC induced any changes of the two diffraction rings. In this context, the results of the WAXD experiments are in agreement with those of the DSC experiments. Treatment of six different stratum corneum sheets of the same donor with the different cream bases, aqueous solutions of ALA or ABE and with water resulted in the same values, emphasising the good reproducibility of the interlayer spacings determined by WAXD. Furthermore, the diffraction ring width were the same (0.370 nm (±0.03)) in all cases with the exception of Excipial® Creme treatment (0.370 nm (±0.04)) which showed a rather slight increase in reflection width. The influence of the calliper rule accuracy on the results is negligible since the accuracy of 0.05 mm in measuring the diffraction ring diameter resulted in a variation in interlayer spacing of 0.0005 nm. In the case of IFP treated stratum corneum a change in stratum corneum lipid arrangement from 0.370 to 0.376 nm as determined from WAXD results did not coincide with distinct shifts of T2 and T3 in DSC experiments and increased drug permeation compared with PIA, PIT or Dolgit® Mikrogel. The effect of IFP on the lipid arrangement and subsequently on the barrier properties of stratum corneum is difficult to evaluate and seems to be minor in comparison with that of formulations enriched with ibuprofen acid.

Table 8
Wide-angle X-ray diffraction after treatment with tested formulations (∅ = untreated) donor: female, abdomen, 42 years, values in brackets were calculated with regard to the width of each diffraction ring

Stratum corneum treated with:	Diffraction rings (nm)		
∅	0.370 (±0.03)	0.410 (±0.05)	–
Excipial® Fettcreme	0.370 (±0.03)	0.410 (±0.05)	–
Excipial® Creme	0.370 (±0.04)	0.410 (±0.05)	–
Basiscreme DAC	0.370 (±0.03)	0.410 (±0.05)	–
Dolgit® Mikrogel	0.372 (±0.03)	0.412 (±0.05)	0.462 (±0.09)
IFP	0.376 (±0.04)	0.410 (±0.05)	0.462 (±0.07)
PIA	0.372 (±0.03)	0.412 (±0.05)	0.462 (±0.09)
PIT	0.372 (±0.03)	0.412 (±0.05)	0.462 (±0.09)

It is obvious that shifts of the reflections at 0.370 and 0.410 nm were achieved only after the treatment with Dolgit® Mikrogel, PIA or PIT which also showed changes in stratum corneum lipid structure with regard to the results of the DSC experiments. The reflections shifted to 0.372 and 0.412 nm whereas the diffraction ring width remained unchanged in all cases as to ±0.03 and ±0.04 nm, respectively. Therefore, these results suggest with further consideration of the results from tested drug solutions, Excipial® Fettcreme, Excipial® Creme and Basiscreme DAC, that a shift of 0.02 nm reveals a change in stratum corneum lipid arrangement. This change goes along with a decrease in barrier properties and subsequently with an increase in drug permeation.

4. Conclusion

The limited permeability of excised human stratum corneum for the hydrophilic substances ALA and ABE can be improved by appropriate formulation. Drug incorporation into Dolgit® Mikrogel shows the highest increase in permeability with both, ALA and ABE. In particular, ABE and Dolgit® Mikrogel is the most promising combination. Permeability results are in agreement with those of DSC and WAXD experiments in so far as just Dolgit® Mikrogel induces a fluidizing effect on stratum corneum lipid structure. Neither Excipial® Creme, Excipial® Fettcreme and Basiscreme DAC nor ABE or ALA interact with stratum corneum structure. Although these formulations do not influence stratum corneum permeability, ALA permeation from lipophilic Excipial® Fettcreme is slower than that from the hydrophilic cream bases such as Excipial® Creme and Basiscreme DAC which show similar permeation profiles. These results suggest that ALA permeation from hydrophilic formulations is controlled by structure integrity of stratum corneum alone and strongly depends on the type of cream base. Decreased ALA permeation from Excipial® Fettcreme is influenced by interactions between drug and vehicle, drug and stratum corneum as well as between stratum corneum and vehicle, respectively, in addition the premeation is to be controlled by the integrity of stratum corneum structure.

The fluidizing and subsequent permeation enhancing effect of Dolgit® Mikrogel is mainly due to the active substance ibuprofen acid. Furthermore a 6.5-fold increased

permeation coefficient for PIT compared with PIA reveals that permeation enhancement potential of this formulation goes along with the addition of medium chain triglycerides (MCT) which are known to improve permeation [49]. However, MCT induced permeation enhancement is not accompanied by further changes in DSC-thermograms or WAXD diffraction rings. An alteration of stratum corneum lipid structure by Dolgit® Mikrogel resulted in a higher increase in ABE permeation than in ALA permeation. ABE was chosen as an example of a middle chain length ester derivative with regard to the methyl ester and hexyl ester. Therefore further experiments dealing with these esters together with Dolgit® Mikrogel will be the subject of future research in order to evaluate the impact of ester lipophilicity on permeation through stratum corneum lipid structure, which could simultaneously be modified by the vehicle. Variations in the quantitative composition also seem to influence ABE permeation from vehicles with ibuprofen acid and MCT. Therefore, current studies include an optimisation of the quantitative composition of the poloxamer based formulations. The aim is to achieve comparably high permeation coefficients without ibuprofen acid as active compound.

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References

[1] D. Shemin, On the synthesis of heme, Naturwissenschaften 57 (1970) 185–190.
[2] Q. Peng, K. Berg, J. Moan, M. Kongshaug, J.M. Nesland, 5-Aminolevulinic acid-based photodynamic therapy: principles and experimental research, Photochem. Photobiol. 65 (1997) 235–251.
[3] R.-M. Szeimies, P. Calzavara-Pinton, S. Karrer, B. Ortel, M. Landthaler, Topical photodynamic therapy in dermatology, J. Photochem. Photobiol. B 36 (1996) 213–219.
[4] J.C. Kennedy, R.H. Pottier, D.C. Pross, Photodynamic therapy with endogenous protoporphyrin IX: basic principles and present clinical experience, J. Photochem. Photobiol. B 6 (1990) 143–148.
[5] J.C. Kennedy, R.H. Pottier, Endogenous protoporphyrin IX, a clinically useful photosensitizer for photodynamic therapy, J. Photochem. Photobiol. B 14 (1992) 275–292.
[6] F.S. De Rosa, M.V.L.B. Bentley, Photodynamic therapy of skin cancers: sensitizers, clinical studies and future directives, Pharm. Res. 17 (2000) 1447–1455.
[7] R.M. Szeimies, S. Karrer, A. Sauerwald, M. Landthaler, Photodynamic therapy with topical application of 5-aminolevulinic acid in the treatment of actinic keratoses: an initial clinical study, Dermatology 192 (1996) 246–251.
[8] J. Kloek, G.M.J. Beijersbergen van Henegouwen, Prodrugs of 5-aminolevulinic acid for photodynamic therapy, Photochem. Photobiol. 64 (1996) 994–1000.
[9] J. Kloek, W. Akkermans, G.M.J. Beijersbergen van Henegouwen, Derivatives of 5-aminolevulinic acid for photodynamic therapy: enzymatic conversion into protoporphyrin, Photochem. Photobiol. 67 (1998) 150–154.
[10] H. Takeya, Preparation of 5-aminolevulinic acid alkyl esters as herbicides, Jpn. Kokai Tokkyo Koho JP 0409, 360(1992) cited in Chem. Abstr. 116 (1992) 189633m.
[11] A. Casas, A.M. del C. Batlle, A.R. Butler, D. Robertson, E.H. Brown, A. MacRobert, P.A. Riley, Comparative effect of ALA derivatives on protoporphyrin IX production in human and rat skin organ culture, Br. J. Cancer 80 (1999) 1525–1532.
[12] J.-M. Gaullier, K. Berg, Q. Peng, H. Anholt, P.K. Selbo, L.-W. Ma, J. Moan, Use of 5-aminolevulinic acid esters to improve photodynamic therapy on cells in culture, Cancer Res. 57 (1997) 1481–1486.
[13] P. Uehlinger, M. Zellweger, G. Wagnières, L. Juillerat-Jeanneret, H. van den Bergh, N. Lange, 5-Aminolevulinic acid and its derivatives: physical chemical properties and protoporphyrin IX formation in cultured cells, J. Photochem. Photobiol. B 54 (2000) 72–80.
[14] S. Gerscher, J.P. Conelly, J. Griffith, S.B. Brown, A.J. MacRobert, G. Wong, L.E. Rhodes, Comparison of the pharmacokinetics and phototoxicity of protoporphyrin IX metabolized from 5-aminolevunic acid and two derivatives in human skin in vivo, Photochem. Photobiol. 72 (4) (2000) 569–574.
[15] T. Kormeili, P.S. Yamauchi, N.J. Lowe, Topical photodynamic therapy in clinical dermatology, Br. J. Dermatol. 150 (2004) 1061–1069.
[16] A. Marti, P. Jichlinski, N. Lange, J.-P. Ballini, L. Guillou, H.J. Leisinger, P. Kucera, Comparison of aminolevulinic acid and hexyl ester aminolevulinate induced protoporphyrin IX distribution in human bladder cancer, J. Urol. 170 (2003) 428–432.
[17] A. Winkler, C.C. Müller-Goymann, Comparative permeation studies for δ-aminolevulinic acid and its *n*-butyl ester through stratum corneum and artificial skin constructs, Eur. J. Pharm. Biopharm. 53 (2002) 281–287.
[18] F.S. De Rosa, J.M. Marchetti, J.A. Thomazini, A.C. Tedesco, M. Lopes, B. Bentley, A vehicle for photodynamic therapy of skin cancer: influence of dimethylsulphoxide on 5-aminolevulinic acid in vitro cutaneous permeation and in vivo protoporphyrin IX accumulation determined by confocal microscopy, J. Control Rel. 65 (2000) 359–366.
[19] B.G. Auner, E. Petzenhauser, C. Valenta, Influence of 6-ketocholestanol on skin permeation of 5-aminolevulinic acid and evaluation of chemical stability, J. Pharm. Sci. 93 (11) (2004) 2780–2787.
[20] J.-C. Tsai, I.-H. Chen, T.-W. Wong, Y.-L. Lo, In vitro/in vivo correlations between transdermal delivery of 5-aminolevulinic acid and cutaneous protoporphyrin IX accumulation and effect of formulation, Br. J. Dermatol. 146 (2002) 853–862.
[21] S. Lieb, R.-M. Szeimies, G. Lee, Self-adhesive thin films for topical delivery of 5-aminolevulinic acid, Eur. J. Pharm. Biopharm. 53 (2002) 99–106.
[22] B. Franck, H. Stratmann, Condensation products of the porphyrin precursor 5-aminolevulinic acid, Heterocycles 15 (1981) 919–923.
[23] A.R. Butler, S. George, The nonenzymatic cyclic dimerisation of 5-aminolevulinic acid, Tetrahedron 48 (1992) 7879–7886.
[24] M. Novo, G. Hüttmann, H. Diddens, Chemical instability of 5-aminolevulinic acid used in the fluorescence diagnosis of bladder tumours, J. Photochem. Photobiol. B 34 (1996) 143–148.

[25] H. Öhman, A. Vahlquist, In vivo studies concerning a pH gradient in human stratum corneum and upper epidermis, Acta Derm. Venereol. 74 (1994) 375–379.
[26] E. Berardesca, F. Pirot, M. Singh, H. Maibach, Differences in stratum corneum pH gradient when comparing white caucasien and black african–american skin, Br. J. Dermatol. 139 (1998) 855–857.
[27] D. Wilhelm, P. Elsner, H.I. Maibach, Standardized trauma (tape stripping) in human vulvar and forearm skin, Acta Derm. Venereol. 71 (1991) 123–126.
[28] C. Specht, I. Stoye, C.C. Müller-Goymann, Comparative investigations to evaluate the use of organotypic cultures of transformed and native dermal and epidermal cells for permeation studies, Eur. J. Pharm. Biopharm. 46 (1998) 273–278.
[29] I. Brinkmann, C.C. Müller-Goymann, An attempt to clarify the influence of glycerol, propylene glycol, isopropyl myristate and a combination of propylene glycol and isopropyl myristate on human stratum corneum, Pharmazie 60 (2005) 215–220.
[30] M. Löhner, H.H. Wagener, Transdermal resorbierbare, wasserhaltige Zubereitungen von Arylpropionsäurederivaten und Verfahren zur Herstellung derselben, European Patent EP 0 215 423 B1 (1991).
[31] A.M. Kligman, E. Christophers, Preparation of isolated sheets of human stratum corneum, Arch. Dermatol. 88 (1964) 702–705.
[32] T.J. Franz, Percutaneous absorption. On the relevance of in vitro data, J. Invest. Dermatol. 64 (1975) 190–195.
[33] M. Roth, Fluorescence reaction for amino acids, Anal. Chem. 43 (1971) 880–882.
[34] J. Ho, R. Guthrie, H. Tieckelmann, Quantitative determination of porphyrins, their precursors and zinc protoporphyrin in whole blood and dried blood by high performance liquid chromatography with fluorimetric detection, J. Chromatogr. 417 (1987) 269–276.
[35] J. Ho, R. Guthrie, H. Tieckelmann, Detection of δ-aminolevulinic acid, porphobilinogen and porphyrins related to heme biosynthesis by high performance liquid chromatography, J. Chromatogr. 375 (1986) 57–63.
[36] T.A. Graser, H.G. Godel, S. Albers, P. Földi, P. Fürst, An ultra rapid and sensitive high-performance liquid chromatographic method for determination of tissue and plasma free amino acids, Anal. Biochem. 151 (1985) 142–152.
[37] A. Casas, C. Perotti, H. Fukuda, L. Rogers, A.R. Butler, A. Batlle, ALA and ALA hexyl ester-induced porphyrin synthesis in chemically induced skin tumours: the role of different vehicles on improving photosensitization, Br. J. Cancer 85 (11) (2001) 1794–1800.
[38] F.S. De Rosa, A.C. Tedesco, R.F.V. Lopez, M.B.R. Pierre, N. Lange, J.M. Marchetti, J.C.G. Rotta, M.V.L.B. Bentley, In vitro skin permeation and retention of 5-aminolevulinic acid ester derivatives for photodynamic therapy, J. Control Rel. 89 (2003) 261–269.
[39] A. Casas, H. Fukuda, G. di Venosa, A. Batlle, Photosensitization and mechanism of cytotoxicity induced by the use of ALA derivatives in photodynamic therapy, Br. J. Cancer 85 (2) (2001) 279–284.
[40] C. Perotti, H. Fukuda, G. DiVenosa, A.J. MacRobert, A. Batlle, A. Casas, Porphyrin synthesis from ALA derivatives for photodynamic therapy. In vitro and in vivo studies, Br. J. Cancer 90 (2004) 1660–1665.
[41] B.F. Van Duzee, Thermal analysis of human stratum corneum, J. Invest. Dermatol. 65 (1975) 404–408.
[42] G.M. Golden, D.B. Guzek, A.H. Kennedy, J.E. McKie, R.O. Potts, Stratum corneum lipid phase transitions and water barrier properties, Biochemistry 26 (1987) 2382–2388.
[43] J.A. Bouwstra, M.A. de Vries, G.S. Gooris, W. Bras, J. Brussee, M. Ponec, Thermodynamic and structural aspects of the skin barrier, J. Control Rel. 15 (1991) 209–220.
[44] B.W. Barry, Mode of action of penetration enhancers in human skin, J. Control Rel. 6 (1987) 85–97.
[45] C.S. Leopold, B.C. Lippold, An attempt to clarify the mechanism of the penetration enhancing effects of lipophilic vehicles with differential scanning calorimetry (DSC), J. Pharm. Pharmacol. 47 (1995) 276–281.
[46] D.M. Small, Handbook of Lipid Research; vol. 4: The Physical Chemistry of Lipids: From Alkanes Alkanes to Phospholipids, Plenum Press, New York, 1986.
[47] J.A. Bouwstra, G.S. Gooris, M.A. Salomons-de Vries, V.A. Van der Spek, W. Bras, Structure of human stratum corneum as a function of temperature and hydration: a wide-angle X-ray diffraction study, Int. J. Pharm. 84 (1992) 205–216.
[48] P.A. Cornwell, B.W. Barry, C.P. Stoddart, J.A. Bouwstra, Wide-angle X-ray diffraction of human stratum corneum: effects of hydration and terpene enhancer treatment, J. Pharm. Pharmacol. 46 (1994) 938–950.
[49] R. Neubert, U. Wohlrab, C. Huschka, Wirkstoffpermeation in die Haut und deren Modulation, Pharm. Ztg. 141 (17) (1996) 11–23.