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Epidermal penetration and protoporphyrin IX formation of two different 5-aminolevulinic acid formulations in ex vivo human skin

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ABSTRACT

Background: Photosensitizer formation and epidermal penetration depth represent basic predictors of drug efficacy in dermatological Photodynamic Therapy (PDT). Different drug formulations and application standards are used to perform PDT in clinical practice.
Methods: Thus, we developed a human ex vivo model suitable to explore drug permeation in human skin and compared in 10 patients the penetration of nanoemulsion formulation (BF-200 ALA) with that of a 20% ALA cream formulation frequently used in clinical practice. Protoporphyrin IX (PpIX) formation was assessed according to different durations of incubation with both preparations.
Results: BF-200 ALA led to more intense PpIX fluorescence than the 20% ALA cream formulation as assessed by fluorescence microscopy: after 12 h of incubation, total measured fluorescence was at 101,995 fluorescence units with BF-200 ALA and 40,960 fluorescence units with 20% ALA cream, respectively. This could be reproduced using quantitative fluorimetric measurements in tissue lysates. After the clinically relevant incubation time of 3 h the PpIX concentration induced by BF-200 ALA was more than three-fold higher than that induced by the 20% ALA formulation (7.1 ± 5.5 and 1.9 ± 1.8 nmol/l, $p < 0.05$, Mann–Whitney *U*-test) and four-fold higher after 12 h (30.0 ± 4.6 and 6.7 ± 2.0 nmol/l, $p < 0.01$, Mann–Whitney *U*-test).
Conclusions: In spite of the 50% lower ALA content BF-200 ALA triggers significantly higher PpIX concentrations than the 20% ALA formulation, indicating that clinical efficacy with BF-200 ALA may be higher. Moreover, the ex vivo eyelid skin model may represent a useful tool to investigate drug permeation in human skin.

1. Introduction

The efficacy of 5-aminolevulinic acid (ALA) Photodynamic Therapy (PDT) for epidermal neoplasias is associated with effective formation and distribution of the photosensitizer protoporphyrin IX (PpIX). To remove epidermal cancers the sensitizer should reach the proliferative basal layer and remove the neoplastic cells located there [1,2]. Both commonly used compounds that drive epidermal PpIX formation (aminolevulinic acid and its methyl ester) are not sufficiently lipophilic to pass through the lipid membranes without specific transporters [3]. While the ALA-methyl-ester (MAL) is slightly more stable in an aqueous environment [4], it is less potent in forming PpIX [5]. Fritsch et al. suggested MAL to display a higher tumor cell selectivity than ALA [6]. However, more recent publications by Novak et al. and Schulten et al. hold evidence for the opposite [5,7]. Despite its advantageous pharmacological profile, the use of ALA has long been hampered by its instability in aqueous environments. Emulsions of lipid vesicles with nanometre sizes are known to enhance compound stability and penetration into the epidermis [8]. It is highly conceivable that preclinical analysis of epidermal PpIX formation may readily predict clinical efficacy. Nevertheless, penetration of different ALA and MAL formations has been determined in porcine skin [9,10] which in structure resembles human facial skin [11] but differs in thickness of the vital epidermis and stratum corneum [12,13]. This difference may hamper translation to the clinical situation [14]. Other authors assessed epidermal PpIX formation in murine skin [15], healthy human skin [16] or human basal cell carcinoma [17]. While the first is of lim-

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ited predictive value due to the differences between human and murine skin, the latter are clinically more relevant, but have other limitations. Using healthy volunteers' skin after photosensitizer application in vivo limits the number of samples that can possibly be obtained and usually shifts the application site from the face to other parts of the body. Assessing PpIX formation in pretreated and subsequently excised tumors (e.g. BCC as in Ref. [17]) is a very relevant approach but limited by the availability of suitable patients. Upper eyelid surgery is a routine intervention in dermatological practice. It yields healthy facial skin that closely resembles the locations where PDT is performed for actinic keratosis and may thus be suitable for in vitro analysis of PpIX formation in human epidermis.

PpIX formation and distribution were observed microscopically in slices and quantitatively determined in tissue lysates of the same specimen. Using this approach we compared PpIX formation after the application of a nanoscale lipid vesicle formulation containing ALA (BF-200 ALA) and a compounded formulation containing 20% ALA frequently used in clinical practice. We found that PpIX formation after application of BF-200 ALA was more rapid and more abundant than with the 20% ALA formulation.

2. Material and methods

2.1. Chemicals

BF-200 ALA, a nanoscale lipid vesicle gel formulation containing 78 mg/g ALA (free base, corresponding to 10% ALA-HCl) was obtained from Biofrontera Pharma GmbH. Placebo was the same gel without active ingredient. A 20% (w/w) ALA cream as routinely used in clinical practice was prepared by mixing the hydrophilic nonionic cream NRF (Bombastus Werke AG, Freital, Germany) and ALA (Biofrontera Bioscience GmbH). 20% ALA cream was freshly prepared for every experimental day. All other chemicals and reagents were purchased from Sigma–Aldrich (Taufkirchen, Germany) or Thermo Fisher Scientific (Waltham, MA, USA).

2.2. Human skin and surgery

Human upper eyelid skin was obtained from ten routine blepharoplastic surgeries performed in one dermatological center. Informed consent was collected from all of the included patients. The experiments were approved by the local ethical committee (Ethical committee: University Witten-Herdecke; number 53/2012). Eyelid material was comprised of either one (2 of 10 patients) or both (8 of 10 patients) upper lids (see Table 1) and was subjected to experimental processes immediately. Surgical procedure was carried out as follows: the incision lines were marked with dots, then local anaesthesia (Scandicain® with epinephrine) was infiltrated subcutaneously. The excision boundaries were put under tension and incisions were made with a number 15 blade starting at the medial lower boundary, followed by the medial upper boundary. Then, using surgical tweezers, the lateral part of the strip of skin was pulled upward and dissected. Hemostasis was performed by an electrocoagulation forceps. Finally, the wound was closed using a 6.0 Prolene® R intracutaneous suture (see Fig. 1A,B)

2.3. Test system

Eyelid skin was superficially disinfected and degreased using 70% ethanol. Test items BF-200 ALA, placebo gel or 20% 5-ALA formulation were topically applied to the epidermis at approximately 1 mm thickness. Test items were allowed to dry for 10 min and 4 mm punch biopsies were performed. Biopsies were immediately transferred to a culture chamber (Fig. 1). Culture chambers (see Fig. 1C) were built using a six well cell culture plate and inserts with 0.4 μm pore PET microporous membrane, into which holes of 3 mm diameter were punched. Tissue biopsies were placed onto these holes with the epidermal side upwards and inserts were transferred to the wells containing DMEM/F12 medium without phenol red (Invitrogen). The dermis had contact to the medium while the epidermis was exposed to air. Ex vivo cultures were incubated at 37 °C and 5% CO_2 in a humidified atmosphere. After incubation times of 0, 1, 3, 5 and 12 h, samples were immersed in Tissue Tek freezing medium (Jung, Germany) and frozen in isopentane on dry ice, using Tissue Tek Cryomolds (1 × 1 × 0.5 cm).

2.4. Cryosectioning and processing of sections

Cryo slices were cut using a cryotome (Leica, Wetzlar, Germany) at a thickness of 20 μm. Care was taken to avoid exposure to intense light. One slice was fixed in 4% formaldehyde and stained with hematoxylin and eosin (H&E) according to standard methods, while a neighboring slice was microscopically analyzed for PpIX fluorescence using a set up as previously described [10].

2.5. Fluorescence microscopy

For PpIX distribution analysis relative to epidermal depth, a plot profile approach was chosen. 16 bit grayscale images (400 nm excitation, 600 nm emission filters, 200-fold magnification, 750 ms exposure time using a PCO. 1400-m-FW-CM camera, PCO AG, Kelheim, Germany on an Axioskop 2, Zeiss, Jena, Germany) were taken. In an initial calibration step before the experimental series, PpIX solutions of nanomolar concentrations (1, 10, 100 nmol/l) and untreated tissue slices were used as standards for fluorescence signals and tissue auto fluorescence, respectively, to establish the microscope and illumination settings used in this study. Exposure time was kept constant at 750 ms throughout all experiments and performance of the illumination light source was routinely monitored using appropriate light dosimetry. Regions (200 × 200 μm) outside the sample were determined as background and detector was set to minimize the effect of background and auto fluorescence. No upper margin or cut-off was selected to avoid trimming of the data. Images were loaded into ImageJ software (v. 1.47; NIH, USA) and a line perpendicular to the basal membrane was drawn through the epidermis. Pixel intensities along this line were recorded and allocated to their distance from basal membrane. Data was grouped into depth categories of 5 μm width (e.g., 0–5 μm; 5–10 μm etc.) and median values and median absolute deviation (MAD) were calculated category-wise.

2.6. PpIX extraction and analysis

For the microfluorimetric quantification of PpIX, ten 20 μm tissue sections from the same eyelid were transferred to a reaction tube and treated with a two-step lysis and PpIX extraction buffer combination as and PpIX was quantified using a fluorescence microplate reader (Mithras LB 940, Berthold) as previously described [5,7]. Measurement values of a lysis/extraction buffer mixture served as blanks and were subtracted from all values. Raw data was converted to PpIX concentrations in lysate by using reference samples. Median values and median absolute deviations were calculated for all time points and treatment conditions.

2.7. Data processing and statistics

Data are expressed as medians. As a measure of variation, median absolute deviation was chosen. For statistical testing, WinSTAT for Microsoft Excel (v. 2007.1) and SigmaStat (v. 3.5) software were used. For comparison of two groups, the Mann–Whitney *U*-test was used, for multiple comparisons, Kruskal–Wallis ANOVA-on-ranks and Dunn's post-hoc tests were applied. Significance

Table 1
Patient demographics.

Patient no.	Age [years]	Gender	Fitzpatrick skin type	Samples taken	Treatment	Epidermal thickness [μm]	Stratum corneum thickness [μm]
#1	58	m	II	2	Placebo/BF-200 ALA	61.8	19.5
#2	63	f	II	2	Placebo/BF-200 ALA	54.8	24.4
#3	56	f	II	2	Placebo/BF-200 ALA	65.2	16.8
#4	46	f	II	1	BF-200 ALA	54.0	23.9
#5	40	f	I	2	BF-200 ALA/20% ALA cream	33.8	18.9
#6	60	f	II	2	BF-200 ALA/20% ALA cream	58.2	25.2
#7	59	m	II	2	BF-200 ALA/20% ALA cream	63.6	23.2
#8	50	f	II	2	BF-200 ALA/20% ALA cream	39.0	15.5
#9	47	f	II	1	20% ALA cream	35.0	14.8
#10	53	f	II	2	BF-200 ALA/20% ALA cream	31.0	15.7

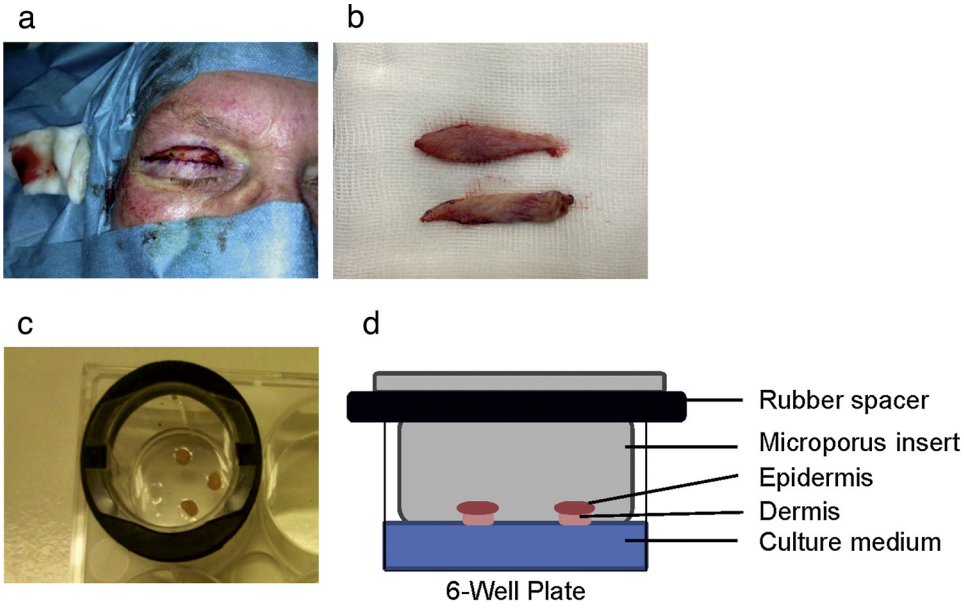

Fig. 1. a–d Set up of the skin culture model. (a) Localization of the upper eyelid skin during blepharoplastic surgery. (b) Representative tissue samples immediately after surgery. (c) 4 mm diameter punch biopsy samples in culture chamber with test item on the epidermis. (d) Schematic drawing of the culturing chamber.

criteria for rejection of the null hypothesis were at $p \leq 0.05$. All evaluations on microscopic pictures were performed using ImageJ (v. 1.47, NIH, USA) without prior alterations of any image parameter.

3. Results

3.1. Patient data and demographics

A summary of patient disposition is presented in Table 1. The median age of the ten patients was 53 (40–63) years, skin of eight female and two male patients was analysed. Except for one patient with Fitzpatrick skin type I, the skin type of all patients was type II. Thickness of the vital layers of the epidermis (stratum basale to stratum granulosum) reached from 31 to 65 μm (mean: 50 ± 13 μm), while stratum corneum thickness varied between 14.8–25.2 μm (mean: 20 ± 4 μm).

3.2. Microscopic evaluation of PpIX formation and intensity plots

Fig. 2 displays representative microscopic pictures of neighboring cryosections subjected to either H&E staining or PpIX-fluorescence analysis. Cytoarchitecture was retained during the culturing process and no signs of epidermal cellular atrophy became apparent in the H&E staining. Fluorescence pictures revealed that the induction of PpIX-fluorescence by both 5-ALA-containing test items was restricted to the epidermis. With placebo, faint fluorescence signals were detected only in the stratum corneum. As exposure times and excitation intensities were held constant over all analyzed conditions, fluorescence pictures taken after 12 h appear nearly saturated. However, pixel intensity analysis revealed no saturation kinetics even in these pictures. No subepidermal PpIX-formation was detectable.

Fig. 3a–d depicts PpIX fluorescence intensities as determined from the microscopic photographs in relation to distance from the basal membrane. Data are presented as mean values of $n = 8$ (BF-200 ALA), 6 (20% ALA cream) and 3 (placebo gel) independent experiments. Fig. 3a illustrates that after 1 h of incubation PpIX fluorescence was not detectable in any of the treatment conditions and a slight and non-significant increase was detectable for both ALA formulations between basal membrane and 35 μm distance after 3 h (Fig. 3b). After 5 h (Fig. 3c), BF-200 ALA induced visibly increased PpIX fluorescence. After 12 h (Fig. 3d) both BF-200 ALA and the 20% ALA cream generated strong signals. After 5 and 12 h the BF-200 ALA induced PpIX signal was significantly different from placebo ($p < 0.05$; Kruskal–Wallis ANOVA-on-ranks with Dunn's post-hoc test) between 15 and 65 μm distance from the basal membrane (5 h) and between basal membrane itself and 55 μm distance (12 h) in the pairwise comparison (Fig. 3). As an additional read-out to the distance-category-wise comparison, areas under the curve (AUCs) were calculated (Fig. 4a) by integrating over the intensity plots shown in Fig. 3. The difference between the AUCs of the 20% cream formulation and placebo could not be assessed as statistically sig-

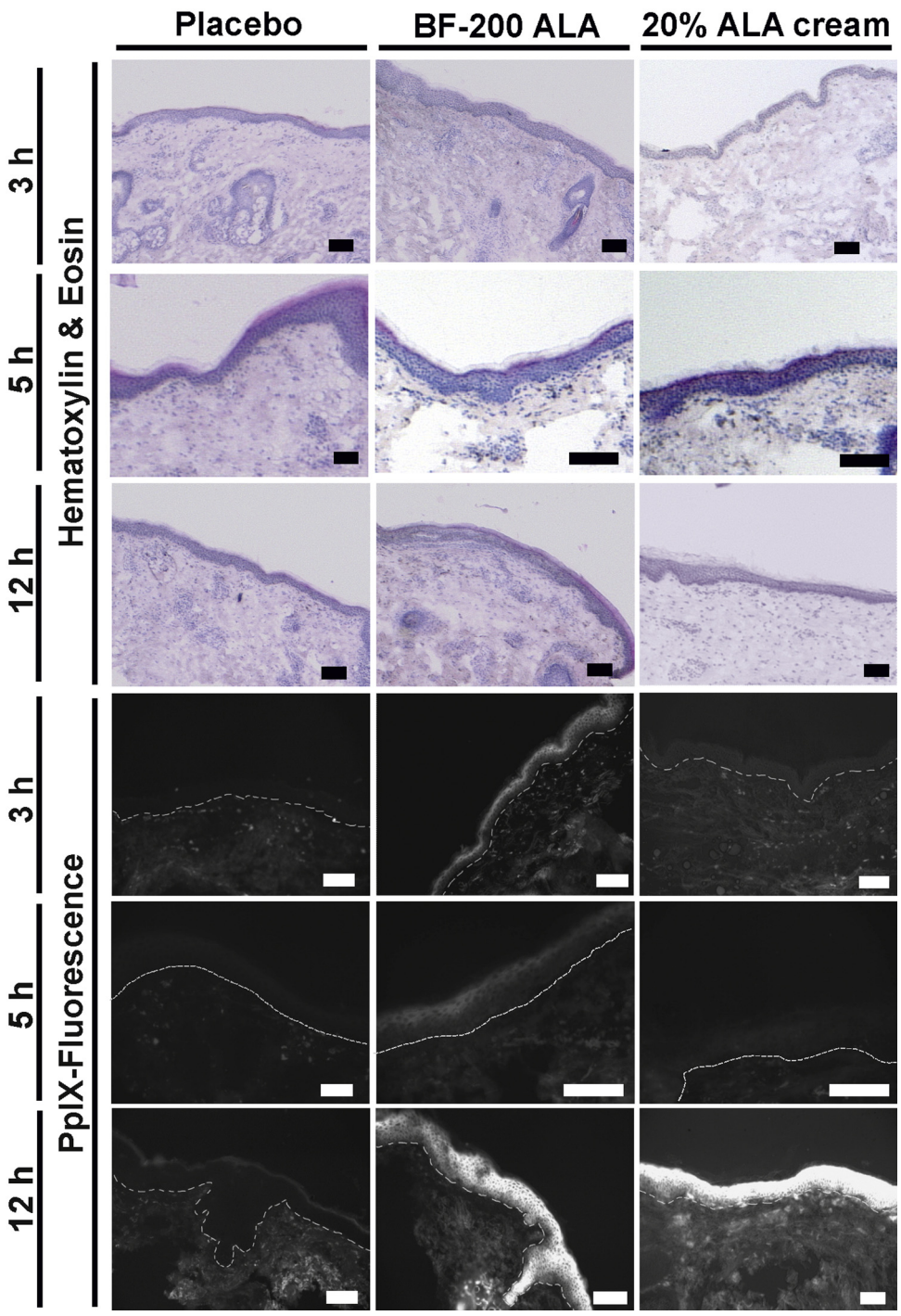

Fig. 2. Representative pictures of 20 μm cryosections of skin explants after application of different drugs and different incubation times. The upper three rows depict H&E staining of the sections. Epidermal areas can be recognized by the more densely packed nuclei. Sections show no signs of cell atrophy. The epidermal boundary is indicated in the fluorescence pictures (lower three rows) by dashed gray lines. The lower two rows are grayscale pictures of PpIX-fluorescence derived from 400 nm excitation and 600 nm (long pass) emission illumination. PpIX-fluorescence intensity is dependent on test sample and incubation time and appears exclusively in the epidermis. Scale bars in all pictures represent 100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

nificant in this test. BF-200 ALA induced statistically superior values of PpIX fluorescence compared to placebo after 5 and 12 h ($p < 0.05$, Mann–Whitney *U*-test). No statistically significant difference could be found between BF-200 ALA and the cream formulation using this read-out, although PpIX fluorescence was stronger with BF-200 ALA than with the 20% cream formulation over almost the entire epidermis. The difference seemed not reach statistical significance due to the poor precision of quantification with this method.

3.3. PpIX concentration in skin tissue lysates

To devise a more quantitative approach to measure PpIX levels in the epidermis we measured PpIX concentrations in lysates of the sections neighboring those analyzed microscopically (Fig. 4a). Only samples treated with BF-200 ALA or 20% ALA cream were subjected to this analysis, as PpIX induction had been absent microscopically in the placebo group. Using BF-200 ALA, median PpIX concentrations increased to 2.6 nmol/l after 1 h, 7.1 and 8.8 nmol/l after 3

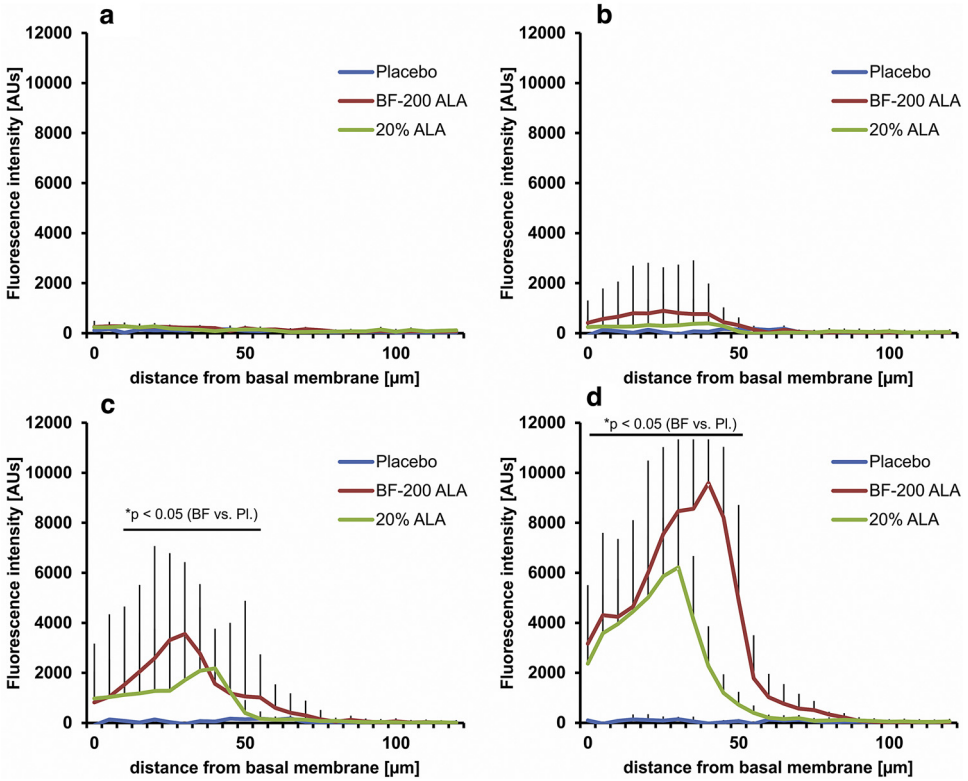

Fig. 3. Spatial distribution of PpIX in the epidermis. PpIX-fluorescence was quantified from microscopic pictures and plotted against the distance from the basal-membrane. a–d show the spatially stratified signal intensities for BF-200 ALA (red line), 20% 5-ALA cream (green line) and placebo gel (blue line) after 1, 3, 5 and 12 h, respectively. Data are expressed as median values of $n = 8$ (BF-200 ALA), $n = 3$ (Placebo gel) and $n = 6$ (20% 5-ALA cream) independent experiments with skin material from 10 different patients. Error bars depict median absolute deviation (MAD). Data points were tested for statistical significance using Kruskal–Wallis ANOVA-on-ranks with Dunn's post-hoc test. $^{*}p < 0.05$, (BF = BF-200 ALA; Pl = Placebo). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

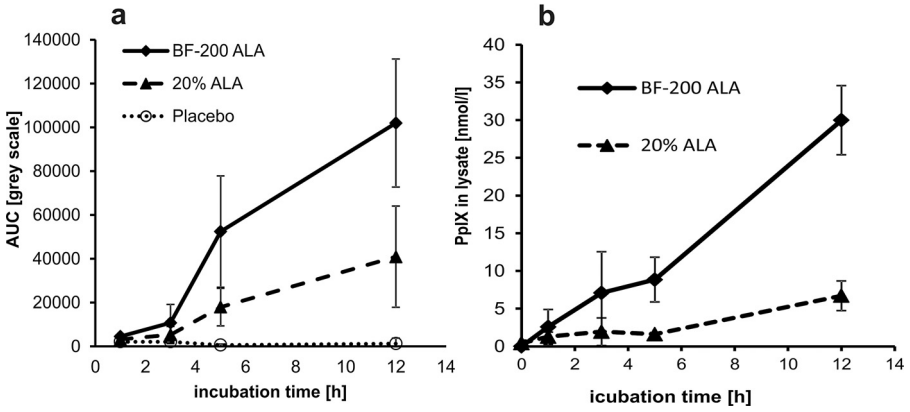

Fig. 4. (a) Area under curve (AUC) calculations from the microscopically assessed fluorescence values. PpIX fluorescence over the whole measurement area was calculated as AUC for the three treatment groups. Data points represent median values (BF-200 ALA ($n = 8$), 20% ALA cream ($n = 6$), placebo ($n = 3$)). Error bars represent median absolute deviation (MAD). $^{*}p < 0.05$ (Kruskal–Wallis ANOVA-on-ranks with Dunn's post-hoc test). (b) PpIX quantification in tissue lysates. PpIX was extracted from 200 [10 × 20] µm of tissue treated with the two ALA containing drugs (BF-200 ALA, solid line; 20% ALA cream, dashed line, respectively), measured fluorimetrically and plotted against the incubation times. Fluorescence values were converted to nmol/l PpIX using PpIX reference values measured in parallel to the test samples. Data points show median values of $n = 8$ (BF-200 ALA) and $n = 7$ (20% ALA cream) independent experiments with eyelids from 10 different patients. Error bars represent median absolute deviation (MAD). $^{*}p < 0.05$ (Mann–Whitney *U*-test); $^{**}p < 0.01$ (Mann–Whitney *U*-test).

Table 2
PpIX concentrations in tissue lysates (median ± MAD).

	Treatment	Incubation time				
		0 h	1 h	3 h	5 h	12 h
PpIX concentration [nmol/l]	BF-200 ALA	0.0 ± 0.0	2.6 ± 2.3	7.1 ± 5.5	8.8 ± 3.0	30.0 ± 4.6
	20% ALA cream	0.4 ± 0.4	1.3 ± 0.6	1.9 ± 1.8	1.6 ± 0.2	6.7 ± 2.0

and 5 h, respectively and further to 30.0 nmol/l after 12 h (Table 2). With the 20% 5-ALA cream, PpIX concentrations also increased with time, albeit slower. With this preparation, concentrations were 1.3 nmol/l after 1 h, 1.9 and 1.6 nmol/l after 3 and 5 h, respectively and 6.7 nmol/l after 12 h of incubation (Table 2). PpIX induction occurred earlier with BF-200 ALA than with the 20% ALA formulation and was significantly higher at time points from 3 h ($p < 0.05$ after 3 and 5 h, and $p < 0.01$ after 12 h, respectively, applying the Mann–Whitney *U*-test).

4. Discussion

The most relevant parameter determining efficacy and sustained disease control in topical PDT of non-melanoma skin cancers is the penetration depth of the photosensitizer. This correlation was confirmed in a recent phase III clinical trial in which BF-200 ALA was compared to a commercial methyl-ALA (MAL) preparation in the treatment of actinic keratosis (AK) while penetration depth and fluorescence intensity were analyzed in a porcine skin model. BF-200 ALA achieved higher clearance rates and better long-term disease control and also triggered higher penetration depth and PpIX fluorescence intensity in the porcine skin model [10,18,19]. As actinic keratosis is due to the presence of mutated keratinocytes at the basal layer of the epidermis [1] in which mitosis occurs, deep penetration seems mandatory for long-term clearance. The concept of field cancerization postulates the existence of subclinical lesions [20] with neoplastic keratinocyte stem cells existing in the basal layer long before AK becomes clinically visible. This is confirmed by observations that even low-grade actinic keratosis can transform to squamous cell carcinoma (SCC) without first progressing through the clinically more advanced stages of AK [21–23].

The current study was designed to compare two formulations of ALA currently used in clinical practice, the BF-200 ALA 10% formulation with nanoemulsion approved as Ameluz® and a 20% ALA cream formulation in a standard ointment (nonionic hydrophilic cream NRF). 20% ALA cream as prepared by compounding pharmacies is one of the most frequently used none approved formulation for PDT [24,25]. To avoid criticism that may derive from the use of porcine skin [10] we designed an in vitro tissue culture model using human facial skin to test PpIX formation. For clinical implications, i.e., higher efficacy, it was of strong interest to determine whether a difference in PpIX formation between BF-200 ALA and the 20% formulation can be found. Limitations of the study were the small number of patients and a low median age of 53 years in our population.

Nanoemulsion formulations of soluble lipid vesicles offer advantages for topical drug application [8]. The nature of the interaction of the drug with nanovesicles depends on the lipophilic properties of the drug. BF-200 is an oil-in-water nanoemulsion containing phosphatidylcholine-coated lipid vesicles of a nanometer scale [10]. ALA is a polar and zwitterionic hydrophilic molecule [3], prone to head-to-head condensation in aqueous solutions [26]. Combining it with the nanoemulsion increased stability of ALA to 36 months. The nonionic hydrophilic cream used in standard formulations is a pharmaceutically relevant comparator, as it is, in spite of an approval, clinically used as pharmacy compounding product. It also represents an oil-in-water emulsion, albeit with a markedly higher vesicle size, providing ALA stability for few days only [4]. As outlined above, epidermal penetration may, in the absence of a side-by-side comparison in clinical studies, serve as an indicator of clinical efficacy.

The design of our test system was inspired by Lebonvallet et al. [27] who described a "Trowell-type organ culture chamber" for skin explants, that mimics a physiological environment and allows survival of the explants for several days [27]. Using this technique, we found the skin explants well-preserved and without signs of atrophy.

Upper eyelids seem well suited because of their rather invariant thickness of both vital and cornified epidermal layers. As observed earlier [28], at an age span from 50 to 70 years thickness of facial epidermis is constant around 50 μm. Since stratum corneum hinders drug penetration low variability is important [9]. Stratum corneum thickness was rather invariant between samples in our experiments, and no correlation was found between stratum corneum thickness and PpIX formation. Thickness of the vital epidermal layers was found to be 50 ± 13 μm. Moreover, PpIX formation in human skin declines with age [29]. Our study population with a median age of 53 (40–63) years differs from usual elderly patients suffering from actinic keratosis. However, we assume that the central findings can be transferred to the elderly as well, even if the absolute values may decline equally for both treatments. In terms of consistency of the data, it is rather an advantage to have patients from a more homogeneous age group, as this may reduce the impact of age on the data within the treatment arms. In consideration of a possible photo damage of the human facial skin we choose the upper eyelid skin, which should carry as little UV damage as possible. Furthermore, two samples (left and right eyelid) were taken per patient in 8 out of 10 cases. These two samples were randomly allocated to different treatment regimes in a side by side experimental setting. This should greatly aid to reduce the impact of inter individual differences.

PpIX formation was greatly superior after application of the nanoemulsion gel compared to the cream formulation, in spite of the 2-fold higher ALA concentration. This was first indicated by quantification from microscopic pictures the quantification of which was, however, hampered by large standard deviations caused by slice quality, background and saturation effects of the CCD camera [30]. To overcome this problem we established a parallel biochemical PpIX read-out to provide quantitative results beyond the microscopical findings on spatial fluorescence distribution. Fluorimeter measurements were performed on the same tissue samples, using slices immediately adjacent to those analyzed by microscopy. At all analyzed time points, PpIX concentrations were 2-fold up to almost 5-fold higher with BF-200 ALA than with the 20% ALA cream. The difference was statistically significant after 3, 5 and 12 h, in full agreement with the visual appearance on the microscopic results.

Using the fluorimetric measurements, the difference between the two preparations was significant already at the clinically relevant time point of 3 h, at which the microscopic analysis did not reveal increased PpIX fluorescence at all. This illustrates the lower sensitivity and the lower limit of quantification of the microscopic readout.

Other authors also concluded that hydrophilic creams are poor 5-ALA vehicles when comparing different ALA formulations [9], among them also ALA in hydrophilic ointment. Even after 24 h the ALA ointment did not trigger PpIX formation at the basal membrane in porcine skin. In our study, the 20% cream formulation did reach the basal membrane but the fluorescence increase was not statistically significant. The most efficient formulation in [11] was ALA in an alcohol/HCl liquid vehicle, which induced PpIX distribution throughout the epidermis after 24 h. Even though this formulation was never directly compared with the ALA nanoemulsion, the latter was analyzed in the same porcine skin model, albeit using different time points [9]. Therefore, only an indirect comparison is possible, indicating a similar penetration efficacy after 3–5 h. It will be of interest to compare these two ALA-drugs in our model. Another study assessed the impact of ablative laser pretreatment on the PpIX formation from ALA cream and solution in an ex vivo porcine skin model. Authors could demonstrate that low-dose continuous and fractional laser pretreatment had an enhancing effect on

speed and intensity of PpIX formation. Such pretreatment modalities would be most interesting to analyze in this human skin model in future studies [31].

In summary, we analyzed PpIX distribution microscopically in a human facial skin epidermal model that may be closer to the clinical situation than previous work with porcine skin and additionally provides the opportunity to analyze diseased skin in future studies. We furthermore precisely quantified PpIX after various times of incubation using tissue lysates. The comparison between a 20% ALA cream and a 10% ALA nanoemulsion gel, two clinically used ALA formulations, indicated that the nanoemulsion gel triggered about 2 to 5 times higher PpIX formation in the epidermis. Based on these results, it may be speculated that the nanoemulsion will yield a better clinical outcome than standard creams.

Consent

Informed consent was obtained from all individual participants included in the study.

Conflict of interest

HL holds a double position, as chair of the department of animal physiology at Ruhr-University Bochum and as CEO of Biofrontera AG, the company which developed BF-200 ALA. BN holds a double position, as visiting scientist of the department of animal physiology at Ruhr-University Bochum and as employee of Biofrontera Pharma GmbH, the company which commercializes BF-200 ALA. TD and LS have been appointed as advisors to Biofrontera Pharma GmbH and took part in clinical trials sponsored by Biofrontera Bioscience GmbH. AKH declares no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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