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HOT CLINICAL STUDY

Fluorescence characteristics and pharmacokinetic properties of a novel self-adhesive 5-ALA patch for photodynamic therapy of actinic keratoses

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Abstract Actinic keratosis (AK) can be treated by photodynamic therapy (PDT), which is becoming a well-established tool in dermatology. Normally a precursor of the photosensitiser is applied topically and converted into protoporphyrin IX (PPIX) in the cells. By activating PPIX with light, the dysplastic cells will be destroyed. We report the results of two clinical studies investigating the properties of a novel self-adhesive 5-ALA-patch (PD P 506 A) intended for PDT of mild to moderate AK on the face and head. The studies investigated the influence of patch application duration on PPIX-specific fluorescence and the pharmacokinetic properties of the 5-ALA patch. The PPIX fluorescence in AK lesions and normal skin after patch application (intraindividual comparison; application for 2, 3, 4, 5 h) was investigated in 13 patients using DYADERM Professional (Biocam). In the subsequent pharmacokinetic study 12 patients were treated with 8 patches each (4 h application). 5-ALA and PPIX were analysed in plasma (over 24 h) and urine (over 12 h). PPIX-specific fluorescence measured immediately after patch removal increased with increasing application duration to a maximum at 4-h application. The fluorescence in AK lesions was more intense than in normal skin. A small increase of 5-ALA plasma concentrations was observed in 10 of 12 patients after applying 8 patches for 4 h, which rapidly declined to normal values after patch removal. The maximum increase was 3.7-fold of the predose 5-ALA plasma concentration. No PPIX-concentrations above the lower limit of quantification were observed. PPIX-specific fluorescence in AK lesions can be steered by application duration of this novel 5-ALA patch. Application is safe and well tolerable. The observed small rise in 5-ALA plasma concentrations is regarded clinically irrelevant. Clinical efficacy of the patch in PDT will be investigated in further clinical trials.

Keywords Aminolevulinic acid · Fluorescence · Pharmacokinetics · PPIX

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Introduction

Actinic keratosis (AK) mainly occurs in people with fair skin. Today, AK is considered as in situ carcinoma, which usually is caused by sun exposure. The head, hands and lower legs, are primarily affected. It is standard of care to treat AK when diagnosed which can be achieved by either physical ablation, chemotherapeutic agents or photodynamic therapy (PDT). For PDT in dermatology, the most frequently used substances are 5-ALA (5-aminolevulinic acid) and its derivative methyl aminolevulinic acid. Studies have proven the effectiveness of topical 5-ALA-PDT in the treatment of actinic keratoses and other superficial skin tumours [3, 8, 14, 15, 18, 19, 25].

The procedure for PDT of AK normally involves the application of 5-ALA or its ester in a dermatological preparation

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(e.g. alcoholic solution or cream) for several hours. After application the lesion has to be occluded and protected from light for the whole application period. 5-ALA penetrates the skin and is metabolised to protoporphyrin IX (PPIX). PPIX concentrations resulting from 5-ALA application are higher in tumour cells than in normal cells [10]. PPIX is the actual photosensitiser and will, when excited by light of an appropriate wavelength, lead to the generation of singlet oxygen, which will damage tumour cells and finally lead to cell death [4].

In the clinical studies presented here we tested a newly developed self-adhesive thin 5-ALA patch (PD P 506 A), which has the capability to bypass current handling disadvantages in PDT procedures due to its easy use. The patch has the advantage to deliver 5-ALA to the lesion and protect the newly formed PPIX from bleaching at the same time [16]. The primary aim of the fluorescence analysis study (study code AK 01) was the assessment of the influence of application duration of PD P 506 A on the PPIX specific fluorescence in AK lesions. The second study (study code AK 05) aimed at evaluating the pharmacokinetic parameters of 5-ALA and PPIX when applying eight 5-ALA patches simultaneously to AK lesions for 4 h.

Methods

Both studies were performed as prospective open monocentre study. The fluorescence analysis study (FA study) was designed as intra-individual comparison of different application times of the study patch. The FA study was clinically conducted from February 2004 to April 2004 and the pharmacokinetic study (PK study), from April 2005 to June 2005. The study centre, which was the same for both studies (PROVERUM), is located in Münster, Germany. PROVERUM holds a department, which specialises on the conduct of clinical phase I studies in dermatology. Dermatologists from the surrounding areas send suitable patients to the study centre.

Ethics

Both study protocols and associated information had been submitted to and approved by the Independent Ethics Committee responsible for the principal investigator prior to the starting of the study. The investigations were carried out in accordance with the German Drug Law, national and international GCP-Guidelines and the Declaration of Helsinki.

Study medication

PD P 506 A is a rectangular, skin coloured, self-adhesive patch with rounded corners. One patch contains 2 mg 5-aminolevulinic acid (present as 5-aminolevulinic acid hydrochloride) per cm^2 and has a size of 4 cm^2. Four patches, all on a single removable protective film, are packed in a heat-sealed pouch. The pouch provides protection against moisture, light and environmental contamination.

Study population

The aim of each study was to involve 12 patients with mild to moderate AK on the head or face. The following is a list of the most important inclusion and exclusion criteria. Females of childbearing potential were not eligible for the studies. Known or suspect, acute or chronic-hepatic diseases or renal dysfunction led to exclusion of the patients. Patients with dermatologic conditions jeopardising the aim of the study (e.g. scalp psoriasis) were excluded. Any treatment being able to influence the disease status of AK was not permitted 4 weeks prior to or during the study, urea and salicylic-acid containing formulations, 2 weeks prior to or during the study. Patients suitable for the PK study had furthermore to be in good condition with pulse and blood pressure reading within the normal ranges. The patient's alcohol and caffeine consumption characteristics were defined by the study protocol. Post organ transplantation condition, any major concomitant disease or any indication of drug abuse disqualified the patients. In both studies, the studied AK lesions had to be either of mild (defined as flat, pink maculae or patches on sun-damaged skin, background mottling, no roughness or hyperkeratosis) or moderate grade (defined as pink to red papules or plaques with rough, hyperkeratotic surface, variable induration) [5]. The identification of suitable AK lesions and the classification of their severity were done on clinical grounds.

Fluorescence analysis (FA) study

The primary aim of the study was the assessment of the influence of patch application duration (2, 3, 4 or 5 h) on the PPIX-specific fluorescence in AK. The primary parameter was the fluorescence of the patched areas determined directly after patch removal.

In the FA study, four distinct AK study lesions were chosen per patient. The application times of the 5-ALA patch (2, 3, 4, 5 h) were randomly allocated to the lesions. Two lesions of non-affected skin were patched (2, 5 h). No preparation of the study lesions prior to patch application as e.g. curettage was performed. The fluorescence data of all studied areas were referenced to baseline data (background fluorescence prior to patch application). PPIX-specific fluorescence of the study areas was determined at given intervals for all selected skin areas (directly after removal (0 h)

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and 2, 4, 6, 24 and 48 h after removal of the 5-ALA patch). In the time between the measurements, all study lesions were protected from ambient light by cotton wool and aluminium foil to prevent fluorescence bleaching. A fluorescent foil was used as fluorescence standard.

Fluorescence measurements were performed with DYADERM, a highly sensitive digital fluorescence imaging system (Biocam GmbH, Regensburg, Germany). DYADERM is a diagnostic medical product for fluorescence diagnostics and serves the early diagnosis of dermal changes [1]. The light source emits light in the range of 370–440 nm. A picture of the fluorescence is taken by an integrated CCD camera and stored electronically. The fluorescence data were analysed by a person blinded to the application duration. After correction of shading and determination of the reference value the fluorescence was determined for the AK area, where the study medication was applied.

Pharmacokinetic (PK) study

The primary aim of the study was the evaluation of the pharmacokinetics of 5-ALA after application of 8 patches for 4 h to mild to moderate AK lesions. The primary parameters were $AUC_{0-\infty}$, AUC_{0-t}, C_{max}, t_{max}, $A_{e,ur}$ and urinary recovery for 5-ALA. Secondary aims of the study were the evaluation of the pharmacokinetics of PPIX and safety of the patch application.

In this study, eight AK lesions of each patient were patched with PD P 506 A for 4 h. The application duration of 4 h had been determined on the basis of the results of the FA study. Again, no curettage of the study lesions was performed prior to patch application. For the determination of the PK parameters, blood and urine samples were collected prior to and during 12 h after start of the patch application. Body weight, body temperature, pulse and blood pressure were measured prior to administration, pulse and blood pressure also at 2, 4 and 8 h after administration. A final blood sample was taken 24 h after the application of the study medication.

For the analysis of 5-ALA in human plasma and human urine and for the analysis of PPIX in human plasma, a chromatographic method (HPLC/fluorescence method) developed and validated by CRS-Mannheim GmbH (Grünstadt, Germany) was used [23]. The bioanalytical procedures of the laboratories were GLP-certified. 5-ALA was determined in plasma by derivatisation with acetylacetone/formaldehyde and separation by high-performance liquid chromatography. The limit of quantification was 11.69 µg/l for 5-ALA. PPIX was isolated from plasma by acidic protein precipitation followed by high performance liquid chromatography with fluorescence detection. The limit of quantification was 4.47 µg/l for PPIX.

Statistical analysis

FA study

The influence of the application duration of the 5-ALA patch on the PPIX fluorescence was statistically evaluated by regarding the observed value of fluorescence directly after patch removal (F_{0h}). The log-transformed fluorescence characteristic F_{0h} was submitted to analysis of variance including the effects of patient and application (four levels with regard to the AK areas) in the model. In order to assess the influence of application duration in a confirmative manner, the multiple test procedure REGWQ was applied. Point estimates with 95% confidence intervals were calculated for the following pair-wise differences of application duration: “3 h minus 2 h”, “4 h minus 2 h”, “5 h minus 2 h”, “4 h minus 3 h”, “5 h minus 3 h” and “5 h minus 4 h”. An additional analysis was run in an exploratory manner by extending the above-mentioned ANOVA to six treatments levels, i.e. the results obtained for normal skin were incorporated.

PK study

All patients having an evaluable treatment period were included in the PK analysis. Calculation of the pharmacokinetic characteristics was based on actual blood-sampling time [h] (relative to the corresponding administration time) rounded to two decimal digits and negative pre-dose times set to zero. Descriptive statistics of concentrations were calculated if at least half of the individual data were quantifiable, concentrations below the LLOQ were calculated as half of the LLOQ. For calculation of the pharmacokinetic characteristics the following rules were applied: At time points in the lag-time between time zero and the first concentration equal or above LLOQ, concentrations below LLOQ were calculated as zero. Concentrations below LLOQ between two quantifiable concentrations were calculated with half the LLOQ. Trailing concentrations below LLOQ were not used in calculations. The 5-ALA plasma concentrations were corrected for pre-dose concentrations ($C_t - C_0$, in the case of $C_0 < \text{LLOQ}$ C_0 was replaced by LLOQ). The primary model-independent pharmacokinetic characteristics for 5-ALA in plasma were calculated using non-compartmental procedures.

Furthermore, a subgroup with an absorption of 5-ALA with C_{max} concentrations $> 1.5*C_0$ was evaluated statistically in addition to the evaluation of the 5-ALA plasma pharmacokinetics of all 12 patients.

Safety and tolerability

In both studies adverse events were documented. The investigator judged their relation to the study medication.

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Local phototoxic reactions were prevented by applying a cover to the study lesions for the 48–72 h after removal of the 5-ALA-patch.

Results

The patient population as well as the selected study lesions shows comparable characteristics for both studies (Tables 1, 2). The majority of study lesions were located on the scalp and forehead. Most of the study lesions (70%) were of mild intensity.

Fluorescence analysis study (FA study)

Thirteen patients were recruited into the study. PPIX fluorescence data are based on the analysis of 48 AK study lesions and 24 normal skin areas (12 patients) which had been patched with the 5-ALA patch as specified in the study protocol and which provided valid fluorescence data. The patient who did not show valid fluorescence data was identified being a "PDT non-responder" retrospectively. All 13 patients were valid for safety evaluation.

Table 1 Summary of patient characteristics of the safety samples of both studies

	FA study $N = 13$ (safety sample)	PK study $N = 12$ (safety sample)
Age (years)		
Mean ± SD	69.6 ± 9.87	69.8 ± 7.78
Median (range)	73 (49–80)	71 (50–81)
Sex		
Female	2 (15%)	2 (17%)
Male	11 (85%)	10 (83%)

FA Fluorescence Analysis; *PK* pharmacokinetic; *SD* Standard deviation

Table 3 FA Study: fluorescence at the moment of patch removal (F_{0h}; $N = 12$) and summary statistics for primary variable

Study area	Application duration (h)	Fluorescence mean (SD)	Fluorescence median (range)
AK lesions	2	1.558 (0.403)	1.635 (0.81–2.17)
	3	2.496 (0.856)	2.580 (0.69–3.76)
	4	3.183 (1.041)	3.220 (1.55–4.95)
	5	3.407 (0.980)	3.260 (1.42–4.79)
Normal skin	2	0.823 (0.157)	0.805 (0.56–1.15)
	5	1.598 (0.649)	1.430 (0.75–2.77)

Baseline fluorescence was well comparable between study lesions. Mean values ranged from 0.66 to 0.70 for all except one AK lesions as well as normal skin areas.

The analysis of variance and multiple test procedure for treatment comparisons yielded the following results for the PPIX fluorescence in AK lesions (Tables 3, 4):

- The 2 h-duration is significantly different from each of the 3, 4 and 5-h durations
- The 3-h duration is significantly different from each of the 4 and 5-h durations
- The 4 and 5-h durations do not show a statistically significant difference.

By applying a repeated measurement model of analysis of variance, a statistically significant interaction between application duration and time was detected. Differences in mean fluorescence levels between the application durations changed over time. The mean profiles for fluorescence data are presented in Fig. 1a. Figure 1b presents the data in reference to the start of patch application. Since there is no difference in the fluorescence profiles depicted from start of the application for the application durations 3, 4 and 5 h, it can be postulated that a saturation of the biological system responsible for the conversion of 5-ALA to PPIX takes place.

Table 2 Summary of lesion characteristics of the safety samples of both studies

Application duration (h)	FA study $N = 13$ (52 lesions)				PK study $N = 12$ (96 lesions)
	2 h	3 h	4 h	5 h	4 h
Site					
Scalp	5 (38%)	6 (46%)	4 (31%)	6 (46%)	7 (7%)
Forehead	6 (46%)	4 (31%)	9 (69%)	5 (38%)	68 (71%)
Cheek	2 (15%)	3 (23%)	0	1 (7%)	19 (20%)
Ear	0	0	0	1 (7%)	1 (1%)
Temple	0	0	0	0	1 (1%)
Severity					
Mild	10 (77%)	8 (62%)	11 (85%)	9 (69%)	66 (69%)
Moderate	3 (23%)	5 (38%)	2 (15%)	4 (31%)	30 (31%)

FA Fluorescence Analysis; *PK* pharmacokinetic; *SD* Standard deviation

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Table 4 FA Study: fluorescence at the moment of patch removal (F_{0h}; $N = 12$): comparison of application durations

Comparison of AK study areas (application duration)	*p* value
3–2 h	0.0005
4–2 h	<0.0001
5–2 h	<0.0001
4–3 h	0.0200
5–3 h	0.0036
5–4 h	0.4932

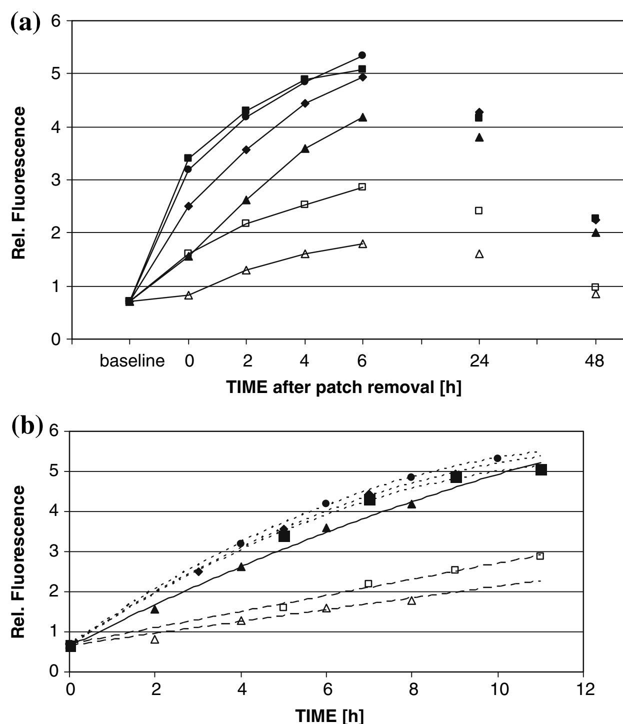

Fig. 1 FA study. **a** Mean profiles for PPIX fluorescence values. **b** Fluorescence values referenced to start of patch application. *Dotted lines*: 3, 4, 5 h application on AK lesions; *solid line*: 2 h application on AK lesions; *dashed line*: 2, 5 h application on normal skin (Normal skin 2 h: *open triangle*; normal skin 5 h: *open square*; AK lesion 2 h: *closed triangle*; AK lesion 3 h: *closed rhomb*; AK lesion 4 h: *closed circle*; AK lesion 5 h: *closed square*)

The fluorescence ratio between AK lesions and normal skin at the time of patch removal is about 2:1 for the 2 and 5-h applications. Exploratory statistical analyses for the comparison of the two application durations 2 and 5 h, on normal skin, provided significant differences at each of the time-points after patch removal besides the 48-h value which was not statistically significantly different ($p = 0.36$). Fluorescence values on AK lesions, 48 h after patch removal, still significantly exceeded the baseline value for all application durations ($p < 0.001$) showing at least 3-fold mean differences between 48 h and baseline (Fig. 1a).

Pharmacokinetic study (PK study)

In the PK study, none of the 144 blood samples (pre-dose samples excluded) deviated from the planned schedule by more than 10 min. The actual blood-sampling times were used for calculations. There were no missing concentration-time data for 5-ALA and PPIX**.**

5-ALA plasma concentrations increased from endogenous 11.23 µg/l (measured pre-dose) to a maximum of 27.57 µg/l (geometric means of 12 patients; values not baseline corrected). A t_{max} of 3.99 h after start of the application and an AUC_{0-t} of 393.8 µg h/l (median value of 12 patients) were calculated. Descriptive statistics of plasma concentrations of 5-ALA as well as summaries of the results for the pharmacokinetic parameters for 5-ALA (with and without baseline correction) are given in Tables 5, 6, 7. The primary parameter $AUC_{(0-\infty)}$ could not be calculated for non-baseline corrected values due to the endogenous 5-ALA concentrations mimicking a longer elimination half-life. Figure 2 presents the synoptic plots of the geometric mean concentration-time profiles of 5-ALA plasma concentrations and individual profiles.

Table 5 PK Study: descriptive statistics of 5-ALA plasma concentrations (µg/l) ($N = 12$)

Nominal time after application (h)	Geometric mean (SD)	Median (range)
0.00	11.23 (1.49)	13.75 (<LLOQ−15.42)
0.25	14.22 (1.10)	14.26 (12.04–16.49)
0.50	14.06 (1.36)	15.06 (<LLOQ−20.11)
0.75	15.99 (1.14)	15.86 (12.86–20.92)
1.00	16.37 (1.44)	16.81 (<LLOQ–27.95)
1.50	18.59 (1.31)	17.20 (12.94–32.67)
2.00	21.65 (1.38)	21.47 (12.77–37.63)
3.00	25.16 (1.46)	22.22 (15.29–50.69)
4.00	27.57 (1.47)	24.87 (16.98– 53.08)
6.00	19.75 (1.31)	20.31 (13.56–31.96)
8.00	16.83 (1.24)	17.44 (12.34–23.48)
12.00	14.81 (1.39)	15.65 (<LLOQ−20.98)
24.00	11.93 (1.60)	13.72 (<LLOQ−25.10)

Table 6 PK study: descriptive characteristics of pharmacokinetic parameters for 5-ALA ($N = 12$)

Parameter	Geometric mean (SD)	Median (range)
C_{max} (µg/l)	28.36 (1.44)	25.64 (18.05–53.08)
$AUC_{(0-t)}$ (µg*h/l)	350.00 (1.60)	393.81 (109.37–565.64)
T_{max} (h)	4.58 (1.73)	3.99 (3.00–23.97)

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