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SHORT COMMUNICATION

Safety of Novel Amino-5-laevulinate Photosensitizer Precursors in Photodynamic Therapy on Healthy Human Skin

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Photodynamic therapy (PDT) is a highly effective treatment for superficial skin cancers and skin cancer precursors (1, 2). In PDT, the administration of a photosensitizing drug, followed by its activation by a specific wavelength of light matching the absorbance of the sensitizer, leads to a phototoxic reaction destroying the tumour cells (3). Adverse effects include pain during the illumination, and erythema and crusting after treatment. Recently, several attempts have been made to improve the tolerability of PDT (4–6). Several topical porphyrin-based photosensitizer precursors are available for PDT. Currently, amino-5-laevulinate (5-ALA) and its methyl ester (MAL) are widely used. Novel photosensitizer formulations include 5-ALA nanoemulsion (BF-200 ALA) and a long-chain lipophilic 5-aminolaevulinate hexyl-ester (HAL). These novel formulations can be used at low concentrations, which may increase the tolerability and reduce the costs of the treatment (7, 8). This non-sponsored double-blinded pilot study tested the safety of PDT with BF-200 ALA and HAL at 2 different concentrations compared with MAL on healthy human skin.

MATERIALS AND METHODS

The study was approved by the local ethics committee. We included 7 healthy volunteers, aged between 26 and 34 years, with skin phototypes I–II, to receive PDT on the sun-protected sides of their forearms with 4 different photosensitizer precursors: (*i*) MAL 16% (Metvix®, Galderma, Paris, France), (*ii*) BF-200 ALA (10% 5-ALA, Ameluz®, Biofrontera, Leverkusen, Germany), (*iii*) HAL 2%, and (*iv*) HAL 0.2%. The HAL creams were prepared from commercially available HAL powder (Hexvix®, Photocure, Oslo, Norway) by mixing it into a standard cream base (Unguentum M®, Allmirall, Madrid, Spain). The treatment sites were randomized using a web-based validated Research randomizer© and kept blinded from the subjects. Areas 2 ×1082 cm at distances of 5 cm from each other were marked on the skin and gently curettaged. The photosensitizers were weighed to achieve a 0.5 mm layer on the skin (0.5 mm-layer thickness was determined using the formula: treatment area size (mm^2)* 0.5 (mg/mm^2)) and kept occluded under a light-impermeable cover for 3 h. Afterwards the drugs were wiped off and areas were irradiated using red light-emitting diode (LED) light (Aktilite CL128, Garderma, Paris, France) to achieve a light dose of 37 J/cm^2. Pain was recorded before, during and after the illumination using a visual analogue scale (VAS). Fluorescence images were taken before and immediately after the illumination using Wood's light (Philips Burton®, Somerset, USA) and a digital camera (Canon Ixus 10 megapixels). Fluorescence intensity and photobleaching were calculated from the images using the MatLab© (Mathworks, Natick, MA, USA). The fluorescence index in arbitrary units (AU) was calculated by measuring the mean fluorescence of the treated area and dividing it by the fluorescence of untreated skin of the same patient. The severity of the reactions at 1 and 2 days was evaluated from photographs by a blinded observer (NNP) who was unaware of the randomization. In addition, erythema was measured using a spectrometer (DermaSpectrometer®, Cortex Technology, Hadsund, Denmark) (9). Friedman's test and Pearson's correlation were used for statistical analyses.

RESULTS

Mean maximal pain during the illumination was significantly lower for HAL 0.2% and HAL 2% compared with BF-200 ALA and MAL ($p = 0.043$). No significant difference was found in pain due to BF-200 ALA and MAL.

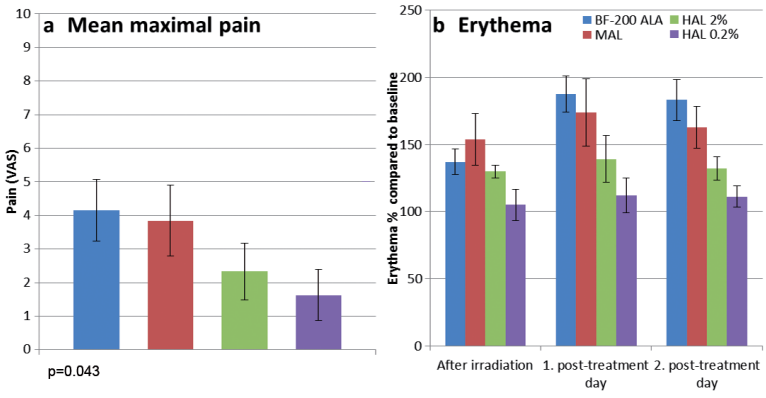

Fig. 1. (a) Mean maximal pain values during the illumination. Hexyl-ester (HAL) 0.2% and HAL 2% caused significantly less pain compared with amino-5-laevulinate (5-ALA) nanoemulsion (BF-200 ALA) and the methyl ester (MAL) of 5-ALA ($p = 0.043$), while no difference was found in the pain between BF-200 ALA and MAL. (b) Erythema% compared with the baseline. Directly after illumination the erythema values differed between all 4 photosensitizers, $p = 0.0041$. During 2 post-treatment days HAL0.2% caused significantly less erythema compared with BF-200 ALA ($p = 0.003$ at the first and $p = 0.001$ at the second post-treatment day) and MAL ($p = 0.043$ at the first and $p = 0.023$ on the second post-treatment day). No significant difference was found between MAL and BF-200 ALA or HAL 2%.

The mean ± SD maximal pain scores (VAS 1–10) were 4.2 ± 2.4 for BF-200 ALA, 3.8 ± 2.8 for MAL, 2.3 ± 2.2 for 2% HAL and 1.6 ± 2.0 for 0.2% HAL (Fig. 1a).

The erythema values are shown in Fig. 1b. A significant difference was found in the erythema between HAL0.2% and BF-200 ALA ($p = 0.003$ on the first and $p = 0.001$ on the second post-treatment day), and between HAL0.2% and MAL ($p = 0.043$ on the first and $p = 0.023$ on the second post-treatment day). No significant difference was found between MAL and BF-200 ALA or HAL 2%.

The treatment reactions of the investigated compounds are summarized in Table I. The reactions were similar on the second post-treatment day (Fig. S1[1]).

Reduction in the initial protoporphyrin fluorescence induced by the photosensitizers, i.e. photo-bleaching, can predict the treatment efficacy (10). After 3 h occlusion the fluorescence was equal in the BF-200 ALA, MAL and HAL2% groups (ns), but lower in the HAL 0.2% group ($p = 0.043$). Photobleaching was equal with BF-200 ALA, MAL and HAL2%, while significantly lower photobleaching was seen with HAL0.2% ($p = 0.003$ and $p = 0.023$ compared with BF-200 ALA and MAL).

There was a strong positive correlation between photo-bleaching and the clinically assessed reaction severity[2], and a weaker positive correlation between photobleaching and erythema measured with the spectrometer[3]. There was no correlation between mean maximal pain and photo-bleaching, erythema or total protoporphyrin IX (PpIX) fluorescence.

Table I. *Treatment reactions*

	BF-200	MAL	HAL 2%	HAL 0.2%
None	0	1	1	4
Mild	1	2	3	2
Intermediate	2	2	0	1
Severe	4	2	3	0

MAL: amino-5-laevulinate methyl ester; HAL: 5-aminolaevulinate hexylester

DISCUSSION

These results show that low-concentration HAL was better tolerated than BF-200 ALA or MAL with regards to pain and erythema. Interestingly, while better tolerated, HAL2% produced similar fluorescence and photobleaching to that of MAL and BF-200 ALA. The fact that all photosensitizers induced pain and erythema on non-photo-damaged healthy skin indicates that the effect of PDT is not completely specific to cancerous tissues.

A limitation of our trial was the small sample size and the lack of an accurate fluorescence imaging system. As our study was limited to healthy human skin, further research is needed into the safety and efficacy of these novel photosensitizers in cancerous skin.

Previously PDT with MAL20% was less painful and caused less erythema compared with ALA20% on healthy sun-exposed human skin (11). We showed that BF-200 ALA was as well tolerated as MAL. This may be due to the lower concentration of 5-ALA in this nano-formulation. In UV-exposed mice, HAL2% induced similar epidermal fluorescence to that of MAL 20% (12). Our finding of strong fluorescence and photobleaching with only 2% HAL supports these findings and further indicates the better tolerability of HAL.

These pilot results show better tolerability of low-concentration HAL compared with BF-200 ALA and MAL on healthy human skin. However, further research is required to determine whether the treatment might be sufficiently effective without erythema and pain.

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[2]r = 0.969 for MAL, $p = 0.007$; r = 0.847 for BF-200 ALA, p = ns; r = 0.940 for HAL2%, $p = 0.018$; r = 0.939 for HAL 0.2%, $p = 0.022$.

[3]r = 0.850 for MAL, $p = 0.015$; r = 0.569 for BF-200 ALA, p = ns; r = 0.906 for HAL2%, $p = 0.005$; r = 0.312 for HAL 0.2%, p = ns.

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