Research letter

Hexyl-5-aminolaevulinate 0·2% vs. methyl-5-aminolaevulinate 16% daylight photodynamic therapy for treatment of actinic keratoses: results of a randomized double-blinded pilot trial

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Dear Editor, Photodynamic therapy (PDT) is an effective treatment for actinic keratoses (AK). Pain during the treatment and adverse reactions lower the tolerability.[1] Recently, there have been various attempts to improve the tolerability of PDT.[2,3]

Currently used topical photosensitizers used in dermatological PDT include 5-aminolaevulinate (5-ALA) and its short chain methyl-ester (MAL).[4] A lipophilic long-chained hexyl-ester (HAL) has improved skin penetration and enables the use of low concentrations.[5–7]

This prospective randomized double-blinded nonsponsored pilot study compared 0·2% HAL and 16% MAL in the treatment of AKs with daylight PDT (DL-PDT). The study was approved by the local ethics committee. All patients gave their written informed consent. Inclusion criteria were at least two AKs ≥ 6 mm in diameter symmetrically on the faces or scalp and equally clinically graded. Exclusion criteria are detailed elsewhere.[8] We assumed 30% differences in histological lesion clearance between the two photosensitizers.[8] With an alpha error of 0·05, power of 0·80 and sigma value of 0·26, we arrived at a sample size of 12 subjects.

The topical photosensitizers were HAL (Hexvix® powder; Photocure ASA, Oslo, Norway), prepared to a 0·2% concentration using a lipid-rich cream base (Unguentum M; Allmiral, Madrid, Spain), and a 16% MAL cream (Metvix®; Galderma, Paris, France). The HAL-cream concentration and 6-week stability were verified using an ion-trap mass spectrometer (Esquire 6000 Plus; Bruker Daltonics GmbH, Fremont, CA, U.S.A.).

A web-based validated program (Research Randomizer) generated a randomized list to define the treatment sides. The randomization results were kept blinded from the investigators who conducted the follow-up visits, and from the pathologist and patients.

Lesions within symmetric fields were graded (I–III),[9] and drawn on a plastic sheet. Equally graded ≥ 6 mm AKs (two

Table 1 Clinical baseline and clinical lesion clearance and histological baseline and clearance, reduction in p53 expression

	HAL	MAL	P-value
Clinical			
Baseline			
Treatment field size, mean (range) mm^2	5800 (3500–9400)	5800 (3500–9400)	
Total number of lesions	103	98	0·837
Grade I lesions	82	81	0·893
Grade II–III lesions	21	17	0·453
Lesions per patient, mean (range)	7·4 (3–14)	7 (3–10)	
3 months			
Complete response all (mean % per patient)	73·4	77·8	0·754
Grade I	82·5	75·6	0·374
Grade II–III	34·8	85·8	**0·017**[a]
New lesions	1	0	
Histological			
Baseline total	13	13	0·625
Grade I	2	4	
Grade II–III	11	9	
p53 mean (%)	39	41	0·600
3 months			
Complete response total (%)	38·5	69·2	0·289
Grade I	50	50	1·00
Grade II–III	36·4	77·8	0·092
Mean reduction in p53 expression (%)	30	34	0·861

[a]HAL was significantly less effective for grade II-III AKs compared with MAL. For grade I AKs the clearance was equal.

per patient) were biopsied with a 3-mm punch bilaterally before treatment and at 3-month follow-up. Biopsy specimens were stained for haematoxylin and eosin and p53 immunohistochemistry.[8] Patients received one treatment covering the whole field, and grade II–III AKs received a retreatment 6–7 days later. An organic sun cream (P20®; Riemann & Co. A/S, Hilleroed, Denmark) was applied for 15 min. Then the treatment area was superficially curettaged with a 7-mm ring-curette (Stiefel, GSK, Brentford, U.K.) to remove crusts, followed by application of a 0·025-mm-photosensitizer layer (treatment area mm^2; 0·25 mg/mm^{-2}) and 2-h daylight-exposure. Treatments were conducted in June 2014 between 09·00 h and 17·00 h. (average temperature 24 °C, range 20–28 °C) and postponed on very dark or rainy days. The weather was sunny on 12 daylight exposures, partly cloudy on 10 and cloudy on one. Patients recorded pain during and after treatment using a visual analogue scale (VAS, 0–10).

All outcome measurements were measured by blinded investigators (M.G., T.T.T, T.K.) who took no part in the treatments which were all conducted by N.N.P. The primary outcome measurement was lesion clearance (mean % of baseline lesions per patient who was 100% cleared). Secondary outcome measurements included histological clearance, adverse effects and cosmetic outcome. A pathologist blinded to the randomization interpreted the histological samples. The presence of AK dysplasia was categorized into grades I–III.[10] Samples not fulfilling the criteria of an AK were defined as healthy or completely cleared. The p53 reactivity expressed as average percentage of positive nuclei in three consecutive high power fields from the region of highest reactivity (< 10% normal).[11] Local adverse reactions (erythema, crusting; one minimal, two mild, three intermediate, four severe) were assessed 1 week after the treatment.

Wilcoxon signed rank test was used to compare baseline characteristics, clinical and histological lesion clearance, p53 baseline reduction, per patient half-face clearance and pain scores. McNemar's test was used to analyse complete histological clearance. P-values < 0·05 were regarded as statistically significant.

Sixteen patients were screened for enrolment. Of these, two were excluded, one due to diffuse photodamage and the other due to only one AK being present. All the 14 patients included (eight males and six females aged 66–88 years, mean 78·5) completed the study (Table 1).

In the per patient half-face analysis, clearance on the MAL and HAL sides was equal (P = 0·79). HAL and MAL cleared grade I lesions as effectively (82·5% vs. 75·6%), P = 0·374. However, HAL cleared thicker AKs less effectively (34·8%) compared with MAL (85·8%), P = 0·017 (Table 1). The majority (52·4%) of the residual HAL lesions were partially cleared.

One patient was excluded from the histological analysis because one biopsied lesion clinically taken as an AK appeared histologically to be seborrhoeic eczema. Histological clearance is shown in Table 1 and Figure 1.

Both treatments were painless with mean maximal VAS scores ≤ 1 (no significant difference). The adverse reactions were minimal in 10 HAL and three MAL sites, mild in three HAL and nine MAL sites, and intermediate in one HAL and two MAL sites. At their 3-month follow-up, 10 patients expressed no preference while one favoured HAL and three MAL. The cosmetic outcome, assessed by a blinded observer (M.G.), was equal in seven patients, better for the HAL site in two and for the MAL site in five cases.

Low concentration HAL resulted in equal clearance of thin AKs compared with MAL, and the results are in concordance with earlier reports of DL-PDT using MAL.[12,13] The complete clearance of grade II–III AKs treated twice was lower for HAL. Thus, low-concentration HAL-PDT should only be considered for thin lesions. Also histologically, HAL and MAL cleared thin

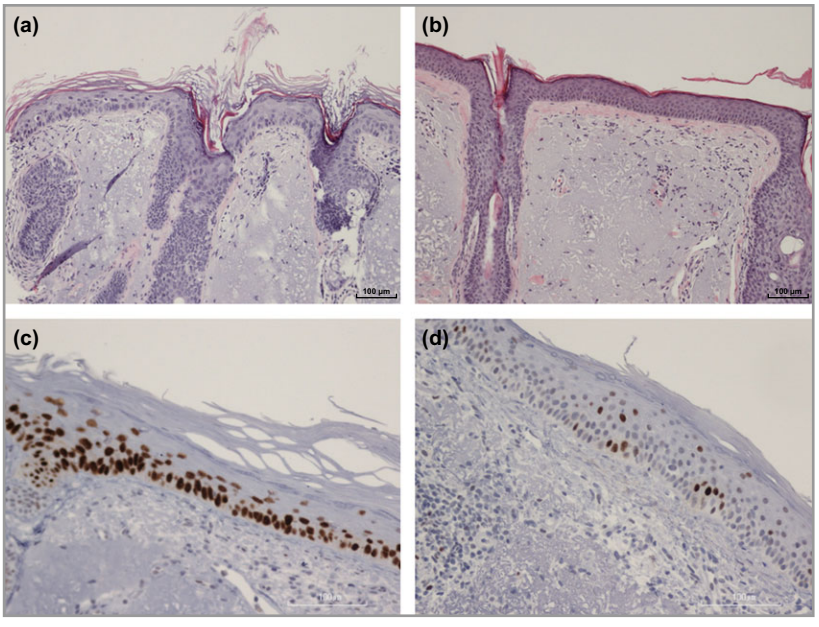

Fig 1. Histological clearance of hexyl-5-aminolaevulinate daylight photodynamic therapy. (a) Haematoxylin and eosin-staining showing grade II actinic keratoses (AK) before treatment: atypical keratinocytes in lower two-thirds of the epidermis, solar elastosis; (b) the same area 3-months post treatment: no signs of an AK evident; (c) same lesion in p53 staining showing increased p53 expression (59%); (d) same lesion 3 months post treatment showing p53 expression decreased to normal level (< 10%).

AKs as effectively while a trend for improved clearance of thicker lesions was detected with MAL. Interestingly, both treatments resulted in similar reduction in p53 expression indicating the reversal of carcinogenesis.

As far as we know, this is the first trial using HAL in treatment of human skin cancer precursors. Several preclinical reports of HAL are available.[14,15] As the study was conducted as a single centre study, the results should be confirmed in a larger trial also valuing the patient complete response rates. In addition, the use of multiple blinded investigators could have further increased the reliability of the results. A further limitation was that we did not record light doses.

Our preliminary findings show that HAL at very low concentration is a promising topical photosensitizer for DL-PDT. The use of low-concentration photosensitizers could reduce the adverse reactions and lower the costs of PDT. Further studies should be conducted to confirm the results.

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