British Journal of Dermatology 2001; **145:** 467–471.

A follow-up study of recurrence and cosmesis in completely responding superficial and nodular basal cell carcinomas treated with methyl 5-aminolaevulinate-based photodynamic therapy alone and with prior curettage

A.M.SOLER, T.WARLOE, A.BERNER* AND K.E.GIERCKSKY

*Photodynamic Out-Patient Clinic, Department of Surgical Oncology, and *Department of Pathology, The Norwegian Radium Hospital, Montebello, N-0310 Oslo, Norway*

Accepted for publication 27 April 2001

Summary

Background Methyl 5-aminolaevulinate (mALA) is an ester derivative of 5-aminolaevulinic acid (ALA) with increased lipophilicity compared with ALA.

Objectives To assess long-term cure rate, cosmesis, recurrence rate and extent of fibrosis after mALA-based photodynamic therapy (PDT) of superficial and nodular basal cell carcinomas (BCCs) showing early complete response to treatment.

Methods Of 350 BCCs treated, 310 responded completely. These were in 59 patients who were followed for 2–4 years (mean 35 months) after mALA-PDT. Nodular tumours were curetted before PDT, and mALA 160 mg g^{-1} was applied to all tumours for 24 h or 3 h before illumination from a broad-band halogen light source with light doses from 50 to 200 J cm^{-2}. Fibrosis was assessed histologically in 23 biopsies.

Results The overall cure rate for 350 BCCs, including non-responders and recurrences was 79%. Of 310 lesions, 277 (89%) remained in complete response, and the cosmetic outcome was excellent or good in 272 of the completely responding lesions (98%). Histological examination showed dermal fibrosis in one of 23 biopsies.

Conclusions We conclude that mALA-based PDT with prior curettage of nodular lesions is a promising new method for the treatment of BCC.

Key words: basal cell carcinoma, broad-band halogen lamp, methyl 5-aminolaevulinate, photodynamic therapy

5-Aminolaevulinic acid-based photodynamic therapy (ALA-PDT) is a promising therapy for basal cell carcinoma (BCC) and solar keratoses (SK). ALA is a precursor of the highly photosensitive protoporphyrin IX (PpIX), which is formed from succinyl coenzyme A and glycine by 5-ALA synthetase in biosynthesis of haem. This step is regulated by feedback inhibition by haem. PpIX is converted to haem within mitochondria by the incorporation of iron under the action of ferrochelatase. The principle of ALA-PDT is that ALA in excess results in a build-up of intracellular porphyrins, and especially of PpIX, in cancer cells of epithelial origin. Subsequent irradiation of the lesion with visible light leads to selective destruction of tumour tissue.[1] Several clinical studies report high response rates of up to 100% in superficial BCC (sBCC) and SK using topical ALA-PDT.[1–9] For nodular BCC (nBCC) the results are more disappointing, with reported cure rates ranging from 10% to 42%.[2,4,6,10] ALA itself is a charged molecule with hydrophilic properties, and penetration through intact skin covering such tumours, as well as through cell membranes, is often inadequate.[1,11,12] Penetration enhancers such as dimethylsulphoxide (DMSO) in combination with ALA improve skin penetration and subsequent PpIX production in tumour tissue,[11,13] and cure rates for nBCC are

Correspondence: Ana Maria Soler. E-mail: anams@radium.uio.no

Table 1. Tumour response after methyl 5-aminolaevulinate-based photodynamic therapy (mALA-PDT)

BCC subgroup	No. of lesions	Complete response (%)	Recurrence after mALA-PDT (%)
Unclassified	6	6 (100%)	0
Superficial	131	119 (91%)	12 (9%)
Nodular thin	82	76 (93%)	6 (7%)
Nodular thick	86	74 (86%)	12 (14%)
Morpheaform	2	0	2 (100%)
Mixed morphology	3	2 (66%)	1 (33%)
Total	310	277 (89%)	33 (11%)

BCC, basal cell carcinoma.

reported to be up to 77% with ALA and DMSO.[14] However, DMSO aggravates acute side-effects as local pain and redness, probably because selectivity of ALA towards normal skin is reduced. The use of a debulking procedure on nBCC before PDT in addition to DMSO increases cure rates up to about 85–90%.[15,16]

A new sensitizer, methyl 5-aminolaevulinate (mALA), is an ester derivative of ALA and has increased lipophilicity compared with ALA. mALA resembles ALA except that its carboxyl group is methylated and therefore does not carry a negative charge under physiological conditions. mALA is converted into photoactive porphyrins in cancer cells after enzymatic hydrolysis, but only to a limited extent in normal cells. The penetration of mALA is higher than that of ALA both through intact epithelium and cell membranes, with subsequent increased PpIX formation in cells; preliminary studies suggest deeper penetration into tumour tissue than is achieved with ALA.[12,17–19]

We present a long-term follow-up study of BCC successfully treated with mALA-PDT. The aim of the study was to assess the long-term complete response (CR) rate, recurrence rate, cosmetic outcome and extent of dermal fibrosis after mALA-PDT at 2–4 years after treatment.

Materials and methods

This study is a retrospective non-comparative follow-up study to evaluate clinically the response of BCC to treatment with mALA-PDT. The Independent Ethics Committee approved the protocol.

Patients

We studied 310 BCCs (of 350 treated) that showed CR. These were from 59 patients (25 men and 34 women) treated between June 1995 and March 1997. Patients' age ranged from 45 to 89 years (mean 69) and the number of lesions ranged from 1 to 23 per patient. The initial cure rate was 89% at 3–6 months follow-up after the last treatment for all BCCs (40 of 350 BCCs showed a partial response).

According to the inclusion criteria, all lesions should have been treated with mALA only, leading to CR, and previous treatments had to be more than 6 months earlier. Patients with Gorlin's syndrome or patients who had received immunosuppressive treatment before or after mALA-PDT were excluded from the study. Patients with BCC who had received other treatments within the last 6 months before mALA-PDT were also excluded.

Lesions

At least one lesion per patient was verified by cytology or histology before treatment with mALA-PDT. Of a total of 310 lesions, 131 (42%) were sBCC, 82 (26%) were thin nBCC (< 2 mm thickness) and 86 (28%) were thick nBCC (> 2 mm thickness) by clinical evaluation. The clinical evaluation of tumour thickness was assessed during curettage, but we are aware of its limited accuracy compared with histological evaluation of tumour biopsies. Three lesions (1%) had mixed morphology (two nodular thick lesions with morpheaform elements and one nodular thick lesion that showed an infiltrative growth pattern). Two lesions were BCC. Six lesions were not classified into any subgroup (Table 1).

Approximately one-half of the lesions (52%) were located on the trunk or neck, and 31% on the face or scalp, while the rest (17%) were located on the extremities. Looking at the subgroups, most of the sBCCs were located on the trunk, neck or extremities (73%), while nBCCs were divided evenly between the face and the trunk or extremities.

Of the 310 lesions, 31 (10%) were lesions recurring after previous therapy more than 6 months before treatment with mALA. Previous therapy was surgery ($n = 13$), radiation ($n = 9$), ALA-PDT ($n = 8$), cryotherapy ($n = 5$) and 5-fluorouracil ($n = 1$).

Treatment procedure

For all superficial lesions any crusts were gently removed before treatment. A debulking procedure was performed on all nodular lesions by removing only visible parts of the tumours using a small surgical curette (House, 3–9 French, Elcon, Tutlingen,

Table 2. Cosmetic results of lesions showing a complete response after methyl 5-aminolaevulinate-based photodynamic therapy

BCC subgroup	No. of lesions	Excellent	Good	Fair	Poor
Unclassified	6	5 (83%)	1 (17%)	0	0
Superficial	119	94 (79%)	25 (21%)	0	0
Nodular thin	76	67 (88%)	7 (9%)	0	2 (3%)
Nodular thick	74	64 (86%)	8 (11%)	1 (1%)	1 (1%)
Mixed morphology	2	0	1 (50%)	1 (50%)	0
Total	277	230 (83%)	42 (15%)	2 (0·7%)	3 (1·2%)

BCC, basal cell carcinoma.

Germany). This intratumoral curettage spared the underlying dermis and adjacent normal skin. At the most, only a minimum of bleeding took place.

Subsequently, mALA 160 mg g^{-1} in cream (P-1202, PhotoCure ASA, Oslo, Norway) was applied to the lesion area and covered by a semipermeable dressing (3M, St Paul, MN, U.S.A.) for 3 h or 24 h. After removal of the cream, the lesion area was exposed to light from a filtered broad-band halogen light source (PhotoCure ASA) with an emission spectrum of 570–670 nm. The light intensity at the skin surface was 100–180 mW cm^{-2}. The total light dose varied within the range 50–200 J cm^{-2} (mean 73, median 50). Mean light doses for the subgroups of BCC were 64 J cm^{-2} (sBCC), 78 J cm^{-2} (nBCC), 88 J cm^{-2} (morpheaform lesions) and 100 J cm^{-2} (mixed lesions).

The time between application of mALA cream and light exposure ranged from 2·5 to 24 h (mean 4, median 3). A total of 287 lesions (93%) received PDT only once, while 19 lesions (6%), three lesions (1%) and one lesion received PDT in two, three and four treatment sessions, respectively.

Clinical and cosmetic evaluation after the initial methyl 5-aminolaevulinate photodynamic therapy

Initial lesion response was evaluated 3–6 months after the last mALA-PDT session by one of two investigators (T.W., A.M.S.). The initial responses were evaluated as CR (complete disappearance of tumour), partial response (partial remission of tumour) or no response. Only patients with initial CR are included in this report.

The initial cosmetic outcome of CR lesions was classified as excellent (lesion area not visible), good (slightly visible scarring, atrophy or change in pigmentation), fair (moderately visible scarring, atrophy or change in pigmentation) or poor (marked scarring, atrophy or change in pigmentation).

All 310 tumour sites with initial CR were re-examined at our clinic. During the long-term follow-up the same physician (A.M.S.) scored the clinical and cosmetic outcome in all patients using the same classification as for the initial response except for tumour status, which was either continuous CR or recurrence.

Histological examination

A 4-mm punch biopsy was taken from healed lesions in 23 patients. Of these, 22 were located on the trunk or extremities and only one biopsy was taken from the face (to avoid scar formation). The biopsies were fixed in buffered 4% formalin, processed and embedded in paraffin. Sections of 5-μm thickness were stained with haematoxylin and eosin before light microscopic examination.

Cytological examination

If not confirmed by histology, skin scrapings were taken from primary BCCs or apparently recurrent tumours. The scrapings were smeared on glass slides, air dried and Diff-Quick stained before cytological evaluation.

Results

Seven lesions had recurred after another therapy before treatment with mALA-PDT. In total, 350 lesions were treated with mALA-PDT, of which 310 (89%) had shown CR by initial clinical evaluation at 3–6 months. These 310 lesions were followed up for a mean period of 35 months (range 24–48) from PDT.

The overall long-term CR rate for the 310 lesions showing initial CR was 89% (277 lesions) and overall recurrence rate was 11% (33 lesions). Thus, the overall cure rate, including the initial non-responders and recurrent lesions, was 79% (277 of 350 lesions). Response by morphological subgroup is shown in Table 1.

The CR rate for the initially responding 279 primary BCCs in this study was 91%, and for the 31 previously

treated BCCs the response rate was 71%. Fifteen of these 31 recurrent lesions were nBCCs, of which only eight were healed (53%), compared with a CR rate of 93% for primary nBCCs (142 of 153 lesions). For sBCCs, there was no difference in CR between recurrent and primary lesions.

In total, 260 lesions (84%) were located at areas defined as low risk (trunk, neck, extremities, cheek, chin and forehead) and overall response rate was 92%. The remaining 50 lesions were located at high-risk areas (nose, nasolabial area, temple, periorbital region, scalp and ear) and the overall long-term response rate for these lesions was 76%.

Cosmetic results

Cosmetic results of the 277 CR lesions are shown in Table 2. For the 47 lesions that not were scored as excellent, the investigator found depigmentation ($n = 38$), redness ($n = 5$), atrophy ($n = 4$) and hyperpigmentation ($n = 4$).

Histological results

A biopsy was taken from 23 healed lesion sites in this study: one showed dermal fibrosis, and the other 22 revealed normal skin structure without any sign of fibrosis.

Recurrences

In total, 33 lesions (11%) had recurred after mALA-PDT (Table 1). Among these, 12 lesions were thick nBCC, six thin nBCC, 12 sBCC, two morpheaform BCC and the last was of mixed morphology. Most of the lesions (29 of 33) were treated only once, and the other four were treated twice. Nearly half of the recurrent lesions ($n = 15$) were located at high-risk areas and 80% of these lesions were either nodular or morpheaform. Seven lesions had recurred after another therapy before mALA-PDT.

Discussion

The life-table 5-year cumulative recurrence rates for primary BCC treated with excision, cryotherapy, curettage or radiotherapy range from 2 to 20%.[20,21] Mohs' micrographic surgery may be used in difficult cases, with improved long-term response reported.[22,23] The current study reports an overall long-term cure rate of 89% in lesions showing initial CR, including both recurrent and primary tumours, after a mean of 35 months follow-up. The 279 primary BCCs included in the study had a long-term response rate of 91%, which is comparable with cure rates of more traditional treatment modalities. As expected, 31 previously treated lesions had a lower response rate of 71%.

Most of the lesions (93%) were treated in one session only. Total recurrence rate after mALA-PDT was 11% (33 lesions) and the vast majority of these (29 lesions) were treated only once. Several clinical studies report an increase in initial response rate with repeated PDT sessions as part of the primary treatment plan.[3,4,8,10,16,24] Ongoing prospective clinical studies with mALA for difficult or recurrent BCCs are planned to evaluate the hypothesis that repeated treatments can improve cure rates.

Recurrences in this study were confirmed by cytology, which is a reliable diagnostic method and comparable with histology.[25] Cytology is easy to perform, and has one important advantage over histology because no or only minute scarring occurs after the procedure. This is important in PDT, as a biopsy can lead to scarring in tumour tissue, decreased drug penetration and consequently a reduced effect of PDT. In some cases residual BCC growth in a scar after biopsy can mimic an aggressive-growth BCC.[26] The problem with cytology in diagnosing BCC is the inability to determine depth or subgroup classification. In the future this may be solved by the introduction of ultrasound for depth measurement and shave biopsy for subgroup classification.[27]

Some studies report occurrence of dermal fibrosis after ALA-PDT.[2,28,29] However, fibrosis has not been seen in the histological descriptions in several other clinical studies, and most report favourable scar formation after treatment with ALA-PDT.[3–5,8] Our group has published one study where biopsies were examined in previous lesion areas after ALA-PDT, and only in one of seven biopsies was some dermal fibrosis found.[16] In the current study fibrosis was found in only one of 23 biopsies. These results support the impression of favourable cosmetic results after mALA-PDT.

Results in this and our previous study show that mALA-PDT is an efficient method for the treatment of many types of BCC, including recurrent and difficult tumours.[24] mALA is more lipophilic than ALA and preliminary data suggest that mALA has improved penetration and induces a faster, more homogeneously distributed fluorescence with a higher intensity in human nBCC compared with free ALA.[17] Furthermore, in actinic keratoses, selectivity of mALA to dysplastic tissue rather than to normal skin is improved compared

with ALA, causing less pain and local phototoxic reaction.[30] A recent report shows that cell uptake of ALA, but not of mALA, is mediated through γ-aminobutyric acid (GABA) transporters.[31] Uptake of GABA into peripheral nerve endings is known to be related to pain perception. This may explain reports that mALA-PDT is less painful than ALA-PDT, although no studies have been designed to address this issue formally. We conclude that mALA-based therapy, with prior curettage for nodular lesions, is a promising new method in the treatment of BCC.

References

1 Kennedy JC, Pottier RH, Pross DC. Photodynamic therapy with endogenous protoporphyrin IX. Basic principles and present clinical experience. *J Photochem Photobiol B Biol* 1990; **6**: 143–8.

2 Wolf P, Rieger E, Kerl H. Topical photodynamic therapy with endogenous porphyrins after application of 5-aminolevulinic acid. *J Am Acad Dermatol* 1993; **28**: 17–21.

3 Svanberg K, Andersson T, Kilander D *et al.* Photodynamic therapy of non-malignant tumours of the skin using topical delta-aminolaevulinic acid sensitization and laser irradiation. *Br J Dermatol* 1994; **130**: 743–51.

4 Fijan S, Hönigsmann H, Ortel B. Photodynamic therapy of epithelial skin tumours using delta-aminolaevulinic acid and desferrioxamine. *Br J Dermatol* 1995; **133**: 282–8.

5 Lui H, Salasche S, Kollias N *et al.* Photodynamic therapy of nonmelanoma skin cancer with topical aminolevulinic acid: a clinical and histologic study. *Arch Dermatol* 1995; **131**: 737–8.

6 Wennberg AM, Lindholm LE, Alpsten M, Larkö O. Treatment of superficial basal cell carcinomas using topically applied delta-aminolevulinic acid and a filtered xenon lamp. *Arch Dermatol Res* 1996; **288**: 561–4.

7 Stender IM, Wulf HC. Photodynamic therapy with 5-aminolaevulinic acid in the treatment of actinic cheilitis. *Br J Dermatol* 1996; **135**: 454–6.

8 Morton CA, MacKie RM, Whitehurst C *et al.* Photodynamic therapy for basal cell carcinoma: effect of tumour thickness and duration of photosensitizer application on response. *Arch Dermatol* 1998; **134**: 248–9.

9 Soler AM, Angell-Petersen E, Warloe T *et al.* Photodynamic therapy of superficial basal cell carcinoma with 5-aminolevulinic acid with dimethylsulfoxide and ethylendiaminetetraacetic acid: a comparison of two light sources. *Photochem Photobiol* 2000; **71**: 724–9.

10 Wang I, Bauer B, Andersson-Engels S *et al.* Photodynamic therapy utilising topical δ-aminolevulinic acid in non-melanoma skin malignancies of the eyelid and the periocular skin. *Acta Ophthalmol Scand* 1999; **77**: 182–8.

11 Peng Q, Warloe T, Heyerdahl H *et al.* Distribution of 5-aminolevulinic acid-induced porphyrins in nodulo-ulcerative basal cell carcinoma. *Photochem Photobiol* 1995; **62**: 906–13.

12 Gaullier J-M, Berg K, Anholt H *et al.* Use of 5-aminolevulinic acid esters to improve photodynamic therapy on cells in culture. *Cancer Res* 1997; **57**: 1481–6.

13 Ackermann G, Abels C, Baumler W *et al.* Simulations on the selectivity of 5-aminolevulinic acid-induced fluorescence *in vivo*. *J Photochem Photobiol B Biol* 1998; **47**: 121–8.

14 Orenstein A, Kostenich G, Roitman L *et al.* Photodynamic therapy of malignant lesions of the skin mediated by topical application of 5-aminolevulinic acid in combination with DMSO and EDTA. *Lasers Life Sci* 1996; **7**: 49–57.

15 Warloe T, Heyerdahl H, Giercksky KE. Curettage and topical ALA-based photodynamic therapy for nodular ulcerative basal cell carcinomas. Thesis, University of Oslo. ISBN 82–7722–038–3 1995 paper VI.

16 Soler AM, Warloe T, Tausjø J, Berner A. Photodynamic therapy by topical aminolevulinic acid, dimethylsulfoxide and curettage in nodular basal cell carcinoma: a one-year follow-up study. *Acta Derm Venereol (Stockh)* 1999; **79**: 204–6.

17 Peng Q, Warloe T, Moan J *et al.* ALA derivative-induced protoporphyrin IX build-up and distribution in human nodular basal cell carcinoma. *Photochem Photobiol* 1995; **61**: 82S(Abstr.).

18 Peng Q, Moan J, Warloe T *et al.* Build-up of esterified aminolevulinic acid derivative-induced porphyrin fluorescence in normal mouse skin. *J Photochem Photobiol B Biol* 1996; **34**: 95–6.

19 Marti A, Lange N, van den Bergh H *et al.* Optimisation of the formation and distribution of protoporphyrin IX in the urothelium: an *in vitro* approach. *J Urol* 1999; **162**: 546–52.

20 Silverman MK, Kopf AW, Grin CM *et al.* Recurrence rates of treated basal cell carcinomas. Part 1: overview. *J Dermatol Surg Oncol* 1991; **17**: 713–18.

21 Thissen MRTM, Neumann MHA, Schouten LJ. A systematic review of treatment modalities for primary basal cell carcinomas. *Arch Dermatol* 1999; **135**: 1177–82.

22 Roenigk RK, Ratz JL, Bailin PL, Wheeland RG. Trends in the presentation and treatment of basal cell carcinoma. *J Dermatol Surg Oncol* 1986; **12**: 860–5.

23 Rowe DE, Carroll RJ, Day CL Jr. Mohs surgery is the treatment of choice for recurrent (previously treated) basal cell carcinoma. *J Dermatol Surg Oncol* 1989; **15**: 424–31.

24 Soler AM, Warloe T, Tausjø J, Giercksky KE. Photodynamic therapy of residual or recurrent basal cell carcinoma after radiotherapy using topical 5-aminolevulinic acid or methylester aminolevulinic acid. *Acta Oncol* 2000; **39**: 605–9.

25 Berner A, Soler AM, Warloe T. Skin scrape cytology in the diagnosis of nodular basal cell carcinoma for treatment by photodynamic therapy. *Acta Derm Venereol (Stockh)* 1999; **79**: 147–9.

26 Swetter SM, Yaghmai D, Egbert B. Infiltrative basal cell carcinoma occurring in sites of biopsy-proven nodular basal cell carcinoma. *J Cutan Pathol* 1998; **25**: 420–5.

27 Russell BE, Carrington PR, Smoller R. Basal cell carcinoma: a comparison of shave biopsy versus punch biopsy techniques in subtype diagnosis. *J Am Acad Dermatol* 1999; **41**: 69–71.

28 Karrer S, Szeimies R-M, Hohenleutner U *et al.* Unilateral localized basaliomatosis: treatment with topical photodynamic therapy after application of 5-aminolevulinic acid. *Dermatology* 1995; **190**: 218–22.

29 Fink-Puches R, Soyer HP, Hofer A *et al.* Long-term follow-up and histological changes of superficial nonmelanoma skin cancer treated with topical δ-aminolevulinic acid photodynamic therapy. *Arch Dermatol* 1998; **134**: 821–6.

30 Fritsch C, Homey B, Stahl W *et al.* Preferential relative porphyrin enrichment in solar keratoses upon topical application of δσ-aminolevulinic acid methylester. *Photochem Photobiol* 1998; **68**: 218–21.

31 Rud E, Gederaas O, Høgset A, Berg K. 5-Aminolevulinic acid, but not 5-aminolevulinic acid esters is transported into adenocarcinoma cells by system beta transporters. *Photochem Photobiol* 2000; **71**: 640–7.