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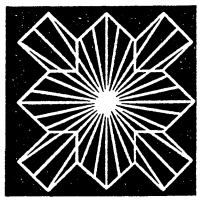

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Photodynamic therapy of non-melanoma skin malignancies with topical δ-amino levulinic acid: diagnostic measurements

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ABSTRACT

Photodynamic therapy (PDT) using topical application of the Protoporphyrin IX (PpIX) precursor δ-amino levulinic acid (ALA) in various malignant skin tumours is a new promising treatment modality. We have treated an extensive number of non-melanoma malignancies of the skin over the past three years with very satisfying initial results. For superficial, shallow lesions one treatment session is sufficient. In thicker lesions, such as nodular basal cell carcinomas, complete treatment response is achieved after two or three treatment sessions. In conjunction with the treatment procedure the tissue fluorescence and the superficial blood flow have been investigated during and after the treatment procedures. The PpIX build-up has been detected *in vivo* and the degree of tumour selectivity has been evaluated using laser-induced fluorescence. Also changes in the bloodflow in tumours compared to normal skin before, during and after the treatment procedure has been followed using a laser-Doppler perfusion imaging system. Results from the measurements in basal cell carcinomas (BCCs), Mb. Bowen lesions (squamous cell carcinoma *in situ*) and cutanous T-cell lymphoma lesions are presented.

Keywords: ALA, laser-induced fluorescence, laser Doppler imaging, skin malignancies, photodynamic therapy, Protoporphyrin IX sensitisation.

1. INTRODUCTION

Photodynamic therapy (PDT) utilising topical δ-amino levulinic acid (ALA) induced Protoporphyrin IX (PpIX) for sensitisation is a new alternative in the treatment of non-melanoma skin tumours.[1-3] The conventional treatment modalities for these tumours include surgery, cryotherapy, diathermy and ionising radiation[4]. PDT utilising systemic administration of different hematoporphyrin derivatives has been investigated for at least a decade. The modality has become more convenient with the introduction of ALA-induced PpIX sensitisation mainly because of the transient PpIX sensitisation with a clearing time of about 24 hours.[5,6] In cases of superficial skin tumours ALA can be applied topically. Thus, the general skin sensitisation is totally avoided.

ALA is the first step in the haem cycle, which takes place in a number of cell types in the body. The haem production within the cycle is regulated by different feed-back systems and the synthesis of the different products is enzymatically dependent. The enzyme pattern involved in the haem cycle varies in different tissue types. Thus, the PpIX production is enhanced in malignant tissue due to higher enzymatic activity

at the beginning of the cycle. Furthermore, the insufficient amount of ferrochelatase in malignant tumour tissue results in a PpIX accumulation with only a low haem production.[7,8] When ALA is distributed excessively the feed-back mechanisms are overridden and PpIX is produced in malignant tissue and to a certain degree in tissues originating from the ecto- and entoderm; i.e. the epidermis, the mucous membranes etc.[2,5,6]

The build-up of the photodynamically active and highly fluorescent PpIX from the inactive and non-fluorescent ALA takes place within a period of hours. The light absorption profile for PpIX with the Soret band shows an absorption peak in the red wavelength region at 635 nm suitable for PDT. Laser light from a tuneable dye laser pumped by a frequency-doubled Nd:YAG laser was used for the induction of the photochemical reaction. The fluence rate was kept below 110 mW/cm^2 in order to avoid hyperthermic activity in conjunction with the PDT procedure. As the PpIX accumulates to a higher degree in tumour tissue as compared to normal tissue, a selective tumour necrosis is achieved.

In order to investigate the kinetics of the PpIX production, laser-induced fluorescence was used for the *in vivo* point monitoring of the lesion surface and the surrounding normal skin. For the fluorescence studies, laser light at 405 nm was used and the fluorescence emission spectra were detected with a fibre-based fluorosensor equipped with an optical multichannel analyser (OMA) system. Important information about the build-up of the PpIX and the tumour selectivity in the superficial layers of the area planned for treatment was obtained.

A laser Doppler imaging system (LDI) was utilised for monitoring of the tissue reaction induced by the photodynamic therapy. The superficial bloodflow was investigated before and at different time intervals after the treatment procedure.

2. MATERIALS AND METHODS

2.1. Patients

About 300 lesions of non-melanoma skin malignancies in 100 patients were treated with ALA-PDT during the period December-91 until August-94. The main part of the tumours are basal cell carcinomas (BCCs) with superficial, nodular and morphea-like characters. Out of these BCCs, a small number of lesions were located in the immediate surrounding of the eye. The rest of the tumours were squamous cell carcinomas *in situ* (Mb. Bowen) and a few examples of other skin tumours, such as cutanous T-cell lymphomas and Mb. Paget of the vulva region. In 17 patients a total of 24 lesions (12 superficial BCCs, 7 nodular BCCs, 3 Mb. Bowen and 2 cutanous T-cell lymphoma lesions) were monitored by means of laser-induced fluorescence. In 23 patients a total of 51 lesions (21 superficial BCCs, 19 nodular BCCs, 6 Mb. Bowen and 5 cutanous T-cell lymphoma lesions) were measured with LDI. The measurements were performed on one to five occasions for each lesion, before and immediately after treatment and at follow up occasions, 1,2-3 weeks and several months after treatment.

2.2. Photodynamic therapy

The treatment procedure was initiated by applying a water-in-oil emulsion containing 20 or 25 % ALA (Porphyrin Products, Logan, Utah, USA) onto the lesions. In some cases 2% Na-EDTA was added to the cream in order to chelate the Fe^{2+} ions and further prevent the transfer of PpIX to haem.[9] A thin water-

resistant plastic film was used on the area and a thicker dressing on top to protect from light. After 4-6 hours the covering dressing and the cream were removed and the area was illuminated with laser light at 635 nm. The laser light was delivered from a frequency doubled Nd:YAG pumped dye laser (Technomed, Bron, France) with a repetition rate of 5 kHz mimicking a continuous wave laser. To achieve an even distribution of the light, a microscope objective lens was placed in front of the 600μm fibre from the laser, resulting in a "top-hat" distribution at the lesion. The fluence rate was kept below 110 mW/cm^2 in order to avoid hyperthermic effects. The total delivered light dose was 60 J/cm^2 which resulted in an illumination period of normally 10-12 minutes. Standard safety goggles were used. When treating lesions in the face, the eyes of the patients were shielded with special lead protections shaped for the orbital area. For treatment of the eye-close lesions a specially fitted thinner lead lens was placed into the eye slid. The non-treated side was covered with the lead protection placed over the closed eye. The only reason for using protective covers out of lead was their availability from the radiotherapy unit. Clearly, the only important aspect in this context was that they were opaque to visible light.

In the cases when the patients reported pain-like sensations during the illumination procedure, different kinds of local anaesthetics (Emla® creme for 30-60 min, xylocain spray and infiltration) were used. Another way of handling the tickling and in some cases burning sensation was to spray or pour ordinary water during the laser procedure in order to "cool" off the surface.

2.3. Laser-induced fluorescence measurements

The laser-induced fluorescence studies were performed immediately before the illumination procedure 4-6 hours after the application of the ALA cream. For the tissue excitation, a Laser Science Model VSL 337 pulsed nitrogen laser, operating at 337 nm, was used to pump a Laser Science dye laser, tuned to 405 nm. The excitation light was brought to the tissue through a 600 μm quartz fibre that was held vertically onto the site measured. The fluorescence emission was conducted back through the same fibre to the entrance slit of a Jobin Yvon 0.25 m grating monochromator equipped with an EG&G Model 1421 gated and intensified linear array detector connected to an EG&G OMA (Optical Multichannel Analyser) mainframe.

Point monitoring of the fluorescence was performed at the surface of the lesion and 2, 5 and sometimes 10 mm outside the visual border of the lesion. The background-free fluorescence peak at 635 nm was evaluated in order to achieve the specific PpIX-related signal. The tissue autofluorescence was evaluated at 490 nm. A ratio between the two fluorescence intensities was formed. Such a ratio has the advantage of minimising the effects of variations in parameters that are hard to control clinically. Such influences could be a fluctuating distance between the tissue and the probe, variations in the excitation and detection sensitivity and angular light deviations. The numbers presented are average values of the evaluation for all spectra recorded within the tumour and outside the lesion, respectively.

2.4. Laser-Doppler perfusion imaging

The superficial bloodflow in the tumour and surrounding skin was detected before the treatment in order to monitor the tumour growth activity. The measurements were then repeated immediately after the laser procedure for the investigation of the immediate vascular response as an indication of treatment effect. The healing process was followed at different time intervals with measurements 1 week, 2-3 weeks and several months after the treatment procedure.

Using laser Doppler imaging (LDI)[10,11] (Lisca, Linköping, Sweden) the surface including the tumour and the surrounding skin was scanned in a non-contact mode measuring 64x64 pixels stepwise. The laser illumination was delivered from a helium-neon laser (632.8 nm, 1 mW output). The instrument detects the frequency shift in the back-scattered laser light. The wavelength shift is caused by the moving blood cells and the read-out from the equipment is given as a value of the tissue perfusion. The sampling depth is a few hundred micrometers and the displayed perfusion number is a function of the product of the concentration and average velocity of the capillary blood cells. The distance between the scanning head with the light source and the detection unit and the tissue was kept constant at 16 cm, which results in a measured area of 10x10 cm. The scanning head was kept in parallel with the studied tissue to allow a 90 degrees angle between the light beam and the tissue surface. The measurement time was about 4.5 minutes.

Evaluation of the images obtained was performed by taking the average over the largest possible area inside the lesion and an area as large as possible and as far away as possible from the lesion. Both areas were chosen to be representative for the different tissue types, i.e. they did not include crust formation, which blocks off the light totally and results in an artificial read out showing no bloodflow. The number of pixels in each area varied between 40 and 150.

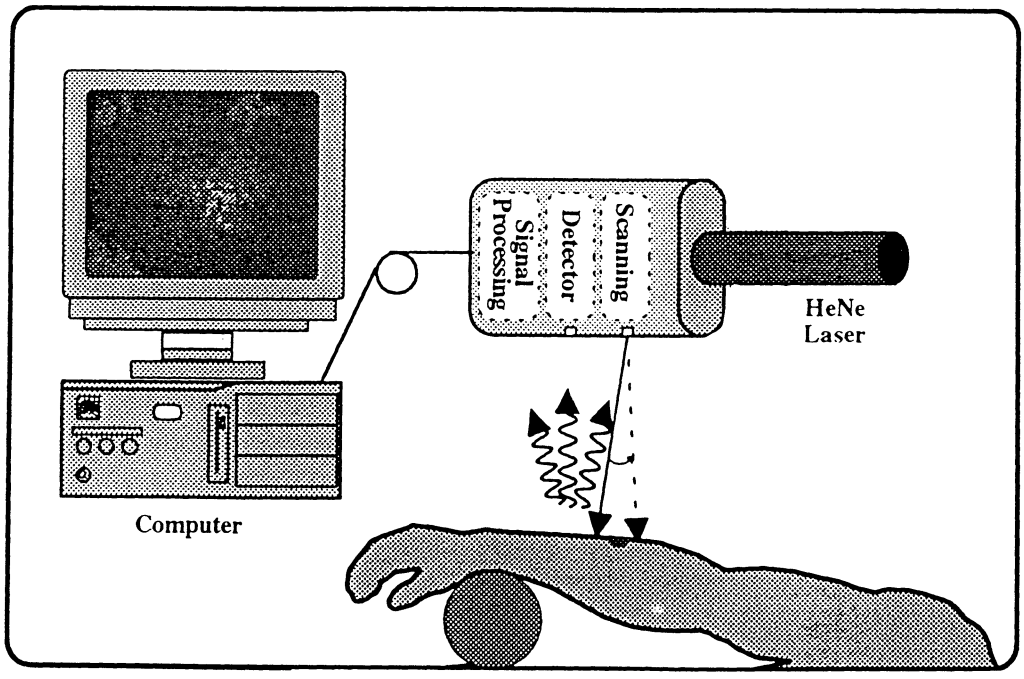

Figure 1. Set-up of the laser Doppler imaging system. A colour image is processed from the signals detected in all the 64x64 points measured and displayed on the screen.

3. RESULTS

3.1. Laser-induced fluorescence

All tumours showed a clear sign of PpIX with a marked dual-peaked fluorescence at about 635 and 705 nm 4-6 hours after the ALA application. The fluorescence data from all tumours are in detail presented elsewhere.[12] A typical tumour spectrum is shown in Figure 2 together with a spectrum recorded from normal surrounding skin. The tumour is a nodular basal cell carcinoma and the ALA cream was applied to the tumour and the surrounding skin 5 hours *prior* to the fluorescence investigation. The tumour centre shows a high PpIX-related fluorescence in the red wavelength region, while the normal surrounding skin 5 mm outside the tumour border only exhibits a small signal. Beside the marked PpIX fluorescence, the tumour is also marked by a lower autofluorescence intensity in the blue-green part of the spectrum as compared to the normal skin.

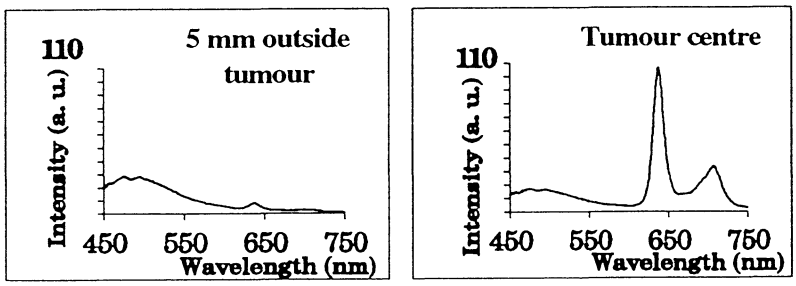

Figure 2. Fluorescence spectra recorded from a nodular basal cell carcinoma and surrounding normal skin. The tumour and the skin 5 mm outside the lesion had been exposed to the same amount of ALA cream during the same time period (5 hours). The tumour is marked by the dual-peaked PpIX-related fluorescence at 635 and 705 nm. The tumour autofluorescence in the blue-green part of the wavelength spectrum shows a lower intensity as compared to the surrounding skin.

The PpIX-related fluorescence at 635 nm showed in all cases a higher intensity in the lesion as compared to the surrounding skin at the time of measurement of 4-6 hours after the ALA application. Figure 3 shows the ratio tumour/normal skin for the fluorescence intensity at 635 nm evaluated with the subtraction of the background fluorescence and expressed in terms of an internal reference. For superficial BCCs the ratio tumour/normal skin was about 10:1 (SD=9.1), for nodular BCCs about 12:1 (SD=3.8) and for Mb. Bowen about 9:1 (SD=3.5). In the cases of cutanous T-cell lymphoma lesions the demarcation ratio was about 15:1 (SD=2.0).

If the autofluorescence is incorporated in the tumour demarcation criterion a further contrast enhancement of a factor of 7-10 is achieved. As seen in Figure 4, the ratio for superficial BCCs is 68:1 (SD=60), for nodular BCCs 123:1 (SD=33), for Mb. Bowen 47:1 (SD=4) and for the cutanous T-cell

lymphomas 127:1 (SD=29). The superficial BCCs showed the highest standard deviations in both types of ratios.

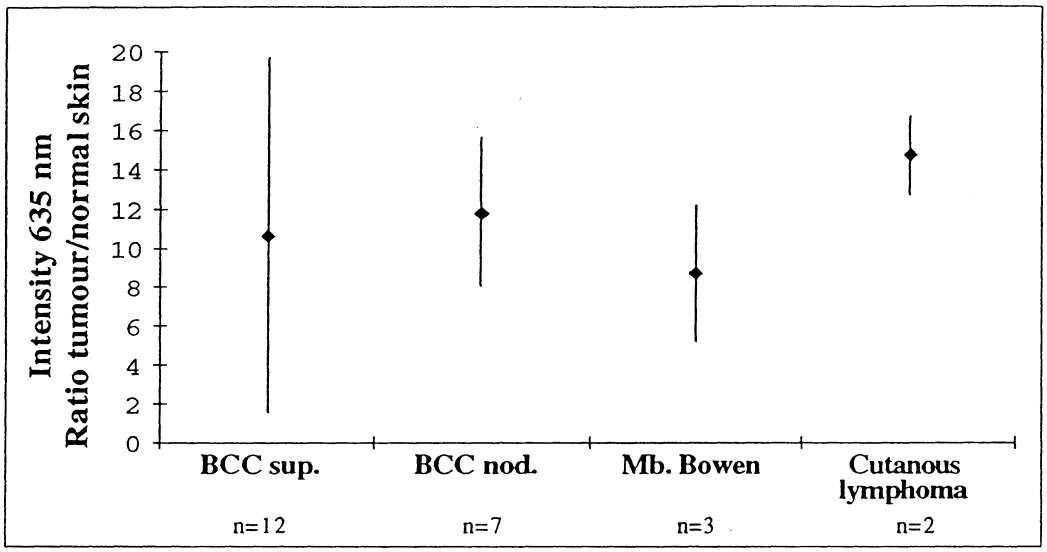

Figure 3. The background-free PpIX-related fluorescence intensity at 635 nm expressed as a ratio tumour/surrounding skin for different kinds of non-melanoma skin malignancies.

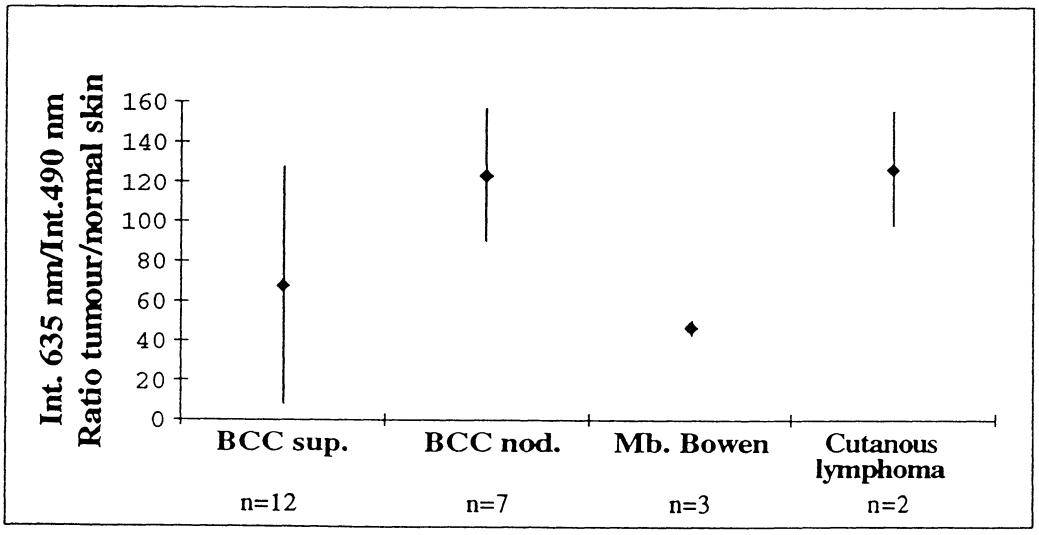

Figure 4. Contrast ratio tumour/surrounding skin when utilising the ratio of the background-free fluorescence intensity at 635 nm and the autofluorescence intensity at 490 nm for different kinds of non-melanoma skin malignancies.

3.2. Laser-Doppler perfusion imaging

The LDI measurements performed before the ALA-PDT showed an increased blood perfusion in all the malignant skin lesions as compared to the normal surrounding skin. A detailed presentation of the LDI measurements in the skin tumours is presented elsewhere.[13] In Figure 5 the ratio tumour to normal skin of the evaluated laser Doppler values is shown for 43 cases. As can be seen in the figure the ratio of the blood flow was about 4-5 times higher in tumour as compared to normal surrounding skin. Superficial BCCs showed a ratio 4.9:1 (SD=2.5), nodular BCCs 4.1:1 (SD=2.6), Bowen's disease lesions 5.1:1 (SD=2.5) and the cutanous T-cell lymphomas 5.2:1 (SD=2.6).

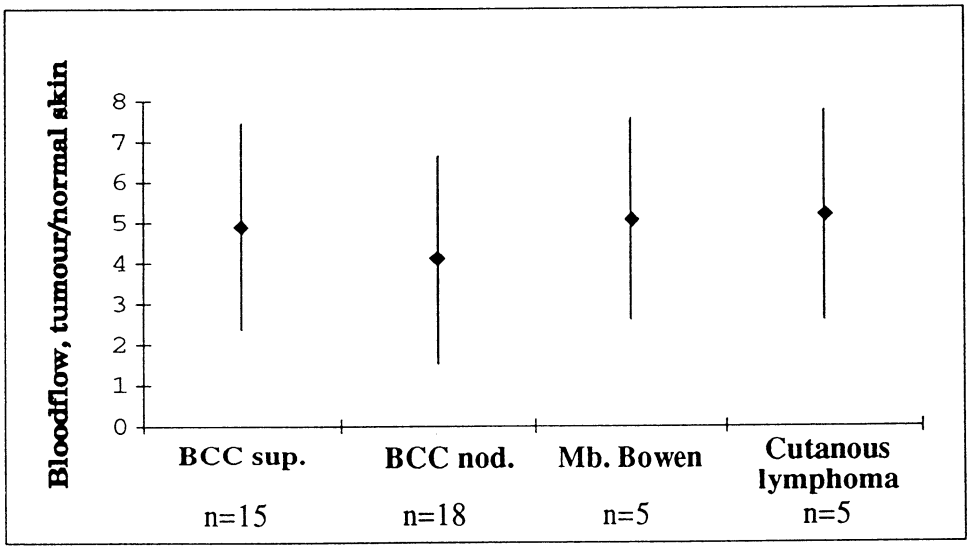

Figure 5. Superficial bloodflow in malignant skin tumours and normal surrounding skin monitored by means of LDI. The evaluated data are expressed as a ratio between the tumour tissue and the normal skin. The recordings were performed before the ALA-PDT procedure.

Figure 6 shows the bloodflow before and at different time intervals after the PDT procedure for superficial BCCs and surrounding skin. Immediately after the PDT procedure an increased blood perfusion was recorded in the tumour region and also in the immediate surrounding of the tumour. The LDI ratio tumour to normal tissue for superficial BCCs increased from about 5:1 before PDT to about 8:1 immediately after the laser illumination procedure. One week after the treatment the blood flow had decreased as compared to the immediate response. At 2-3 weeks the ratio is still elevated in the treated tumour reflecting the ongoing healing in the tissue. After 3 weeks, the bloodflow ratio is nearly 1:1, which indicates that the healing is almost completed. At that time the visual inspection and palpation of the former tumour area also indicted tumour treatment remission. The same pattern was also seen for the other tumour types.

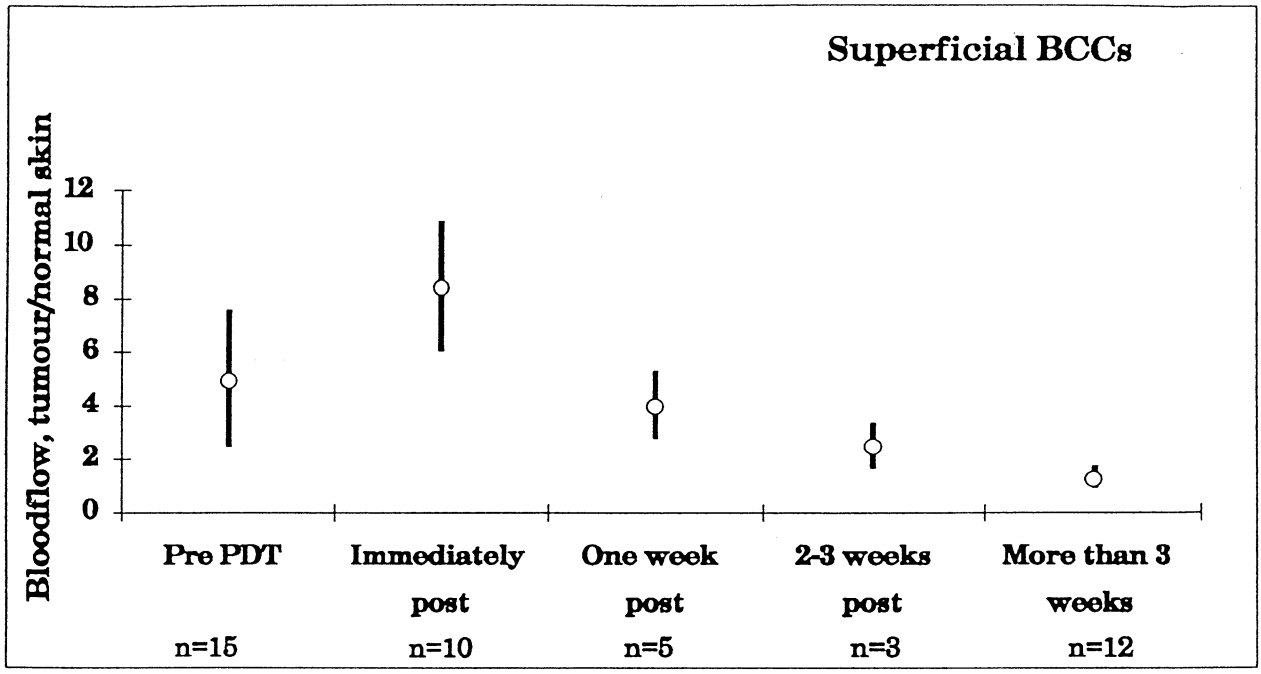

Figure 6. The LDI ratio tumour to normal surrounding skin in a number of superficial basal cell carcinomas. The recordings were performed before the ALA-PDT and at different time intervals after the treatment. At the times 3 weeks and more after the visual inspection and manual palpation indicated treated tumour remission.

4. DISCUSSION

Photodynamic therapy with topical application of ALA for the induction of PpIX sensitisation is a treatment modality with promising initial results for non-melanoma malignant skin tumours. The method offers a selectivity in the treatment leaving normal surrounding skin unaffected. which is advantageous compared to other conventional treatment modalities, including surgery, cryotherapy or ionising radiation therapy. In some locations, such as the pretibial area with poor blood perfusion, the ear, the nose or the eye lids ALA-PDT offers a particularly interesting alternative. The conventional treatment methods might in some of these cases be difficult to apply as normal tissue impairment may be the result with alterations of the shape of the tissue or the function in the treated area. The healing process is comparatively fast and less traumatic as compared with e.g. cryosurgery. Normally, the healing process after ALA-PDT is without any adverse effects, such as secondary infections, leakage or pain. As visible light is used there is no risk of the development of new malignancies, which is a concern in the use of ionising radiation. As compared with surgery, cryotherapy and diathermy, the cosmetic results are also superior in ALA-PDT. This is important since the lesions are often multiple with a spread in easily visible regions, including the face, neck and arms. The long-term efficacy of the treatment cannot be evaluated at the present time as the follow-up period is comparably short with the longest intervals between treatment and follow-up about two and a half years. Recurrences in the border of the tumour might be expected to occur less often with PDT because of the possibility to treat wide margins without compromising the outcome, regarding function and cosmetics.

The selectivity in the treatment is easy to foresee with the PpIX fluorescence recordings. Typically, the fluorescence measurements showed a tumour selectivity towards immediate surrounding skin of about 10-15:1. The relatively higher error bars for the superficial BCCs may be due to the fact that these tumours often show an undefined border and might contain malignant tissue stretching out into the surrounding skin not clearly visible to the eye. In such cases the real-time *in vivo* fluorescence diagnostic measurements may help in the treatment planning with fewer border line recurrences resulting. By including the autofluorescence the tumour demarcation is increased by a factor of about ten due to correlation with the naturally occurring chromophores.

As no heat is generated in the PDT procedure the only tumour treatment effect is the photochemical action, the influence of which on the bloodflow was monitored by means of LDI. In all tumours treated with topical ALA an immediate inflammatory response was seen after the laser procedure with a reddening of the skin. The blood perfusion measured by means of LDI showed an elevated level as compared to the blood flow before the treatment. This is in contradiction with the common knowledge about PDT utilising intravenous injected sensitisers where part of the treatment effect seems to originate from hypoxia following vasoconstriction and thrombi formation.[14-16] In the case of topical application of ALA this study suggests that the treatment effect is related to a direct cell kill.[13]

5. ACKNOWLEDGEMENTS

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