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## Photodynamic therapy using $\delta$ -aminolevulinic acid and intensity modulated diode laser light

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### INTRODUCTION

Photodynamic therapy (PDT) has been a developing tumour treatment modality since decades. The administration of a tumour-sensitizing agent is followed by its excitation with light, initiating photochemical reactions leading to the formation of singlet oxygen and free radicals. In addition to the oxidative cell damage, ischemic necrosis from vascular damage of the tumour, induction of apoptosis [1,2] and anti-tumour immune reactions [3] are also involved in the process of tumour damage. During years of development, various systemically administered chemical compounds have been used as photosensitizers. Clinical results from topically applied  $\delta$ -aminolevulinic acid (ALA) were first presented in 1990 [4], and has been extensively used since then, because of the absence of the systemic adverse effects, mainly general skin phototoxicity, observed after the use of some intravenously administered sensitizing agents. In the haem cycle, the photodynamically non-active ALA is biosynthetically converted into the fluorescent and photodynamically active protoporphyrin IX (PpIX). When an excessive dose of exogenous ALA is administered, PpIX accumulation is favoured in diseased tissues because of differences in enzymatic activity and damage to the keratin layer of the skin. As a result of a higher concentration of PpIX, a selective necrosis of hyperproliferative cells is obtained following therapeutic

light delivery. Various lasers are used as light sources for PDT, such as gold vapour lasers or dye lasers, emitting at approximately 630 nm. The main disadvantage of these light sources is their unwieldiness, which makes their use in clinical practice inconvenient. Various lamps have also been used. Some authors suggest that fractionated light delivery might be advantageous [5-9]. In recent experimental studies, an enhanced photodynamic effect with pulse-modulated light at 652 nm was reported [10,11]. In this work we used a diode laser emitting light at 652 nm, which is within the absorption band of PpIX. The light source used also allowed computer-controlled intensity modulation of the light.

Laser-induced fluorescence (LIF) is a spectroscopic method used to reveal the chemical components in tissue. Due to its non-invasiveness and sensitivity for tissue components it has been used for tissue characterization. By irradiating tissue with violet light, some of the tissue molecules, *i.e.* the tissue fluorophores, will emit radiation at wavelengths longer than the excitation wavelength. By studying the spectral shape of this autofluorescence, it is possible to extract information on various fluorophores present in the tissue. PpIX has a very distinctive dual-peaked fluorescence in the red wavelength region (635 and 705 nm). The intensity of the red fluorescence peak at 635 nm is related to the concentration of PpIX and thus the

latter can easily be monitored by LIF. During the treatment, PpIX disintegrates and forms photoproducts with a fluorescence emission around 670 nm.

The aim of this study was to investigate the efficacy of ALA-PDT at 652 nm in various skin lesions, and the possibility to improve the clinical treatment result by using intensity modulated light instead of continuous light delivery. Laser-induced fluorescence was used to monitor the photobleaching of the drug during the treatment.

## STUDY DESCRIPTION

### *Patients*

Fourteen patients (7 females and 7 males) with 39 lesions were included in the study. Out of these, 21 were basal cell carcinomas (BCCs), 17 actinic keratoses (AK) and 1 plantar wart. More than half of the lesions (26) were localized on the face and scalp, 7 on the trunk, and 6 on the extremities. The median age of the patients was 65 years (range: 39-88 years). All lesions were photodocumented before and after treatment. At the 3-months follow-up visit all lesions were photographed, evaluated visually and by palpation, and from each patient at least one biopsy was taken for histopathological evaluation of the treatment result. Usually the lesion that showed the least response to the treatment on clinical examination was biopsied. According to the study protocol, the lesions showing poor clinical response could be retreated with the 652 nm laser and later with a laser emitting at 635 nm. The therapeutic effect was evaluated at the 3-months follow-up visit as follows: complete response (CR) - no tumour clinically evident; partial response (PR) - tumour size reduced 50% or more; and no response (NR) - tumour size reduction less than 50%. The investigation was performed at the Lund University Medical

Laser Centre, with ethical approval from the local hospital.

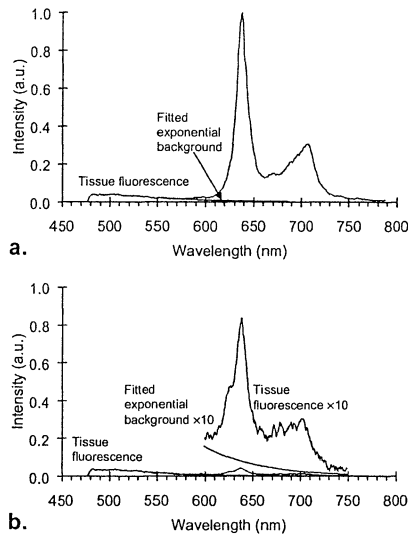
### *Drug*

For the sensitization of the lesions, PpIX induced by topically applied ALA (Porphyrin Products # 091295, 20% in Essex cream) was used. Before application of ALA, all lesions were washed with ethanol in order to facilitate ALA penetration. Crusts from ulcerative lesions were removed and in cases of nodular growth of the tumour, curettage was used as a pre-treatment. Then ALA was applied to the lesion with a margin of 5-10 mm into the visibly normal skin and covered with a waterproof dressing (Tegaderm, 3M). ALA was applied over day (time range from 4 to 8 hours) in 3 lesions and over night in 36 lesions (time range from 18 to 26 hours). After that time period the dressing and remaining ALA-cream were removed and laser irradiation was performed.

### *Light source*

The lesion and surrounding normal skin were irradiated by using a fibre-coupled diode laser (Photo Dynamic Therapy HGeSmbH, Vienna, Austria) emitting light at 652 nm. The total light dose was 100 J/cm<sup>2</sup> for BCCs. Due to its superficial nature, the light dose for AK was set to 75 J/cm<sup>2</sup>. The output power from the fibre was limited to 1 W, in order not to damage the microlens attached at the distal end of the fibre. The illumination spot was 4 cm in diameter, which resulted in an homogeneous fluence on the skin surface of 80 mW/cm<sup>2</sup>. In cases of smaller lesions, the illumination spot was decreased and a fluence rate of 100 mW/cm<sup>2</sup> was used. In all cases the fluence rate was kept below 110 mW/cm<sup>2</sup> to avoid hyperthermic effects of the tissue.

The light was given in three modes; continuous wave (CW), and two different pulse-modulated modes. One of these was a mode with 100-ms-long light pulses



**Fig. 1.** Typical fluorescence spectra recorded from a BCC 25 hours after topical application of 20% ALA. The autofluorescence from the tissue fluorophores can be seen between 470 and 770 nm with maximum around 500 nm.. The dual peak at 635 and 705 nm comes from PpIX. The peak intensity at 635 nm is extracted by fitting a exponential curve to the autofluorescence curve. **a.** Measurement inside the lesion before treatment **b.** Measurement inside the lesion after 100 J/cm<sup>2</sup> applied energy.

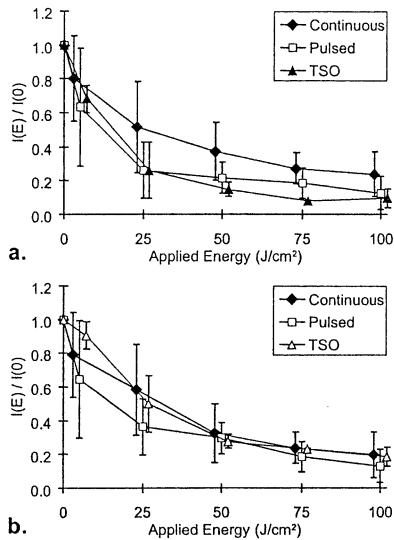
being separated by 100-ms-long dark periods (pulsed, PU), and the other with fast intensity variations around 5-30 Hz, according to a pre-determined pattern (named TSO by the laser manufacturer). Continuous wave irradiation was used in a total of 20 lesions (10 BCCs and 10 AK), PU in 17 lesions (9 BCCs, 7 AK and 1 wart) and TSO in 2 BCCs. The peak power of the intensity modulated light was the same as the power of the CW light. In order to deliver 100 J/cm<sup>2</sup> with a fluence rate of 80 mW/cm<sup>2</sup>, the illumination time was around 20 minutes for CW, and 30-40 minutes for the intensity modulated light, due to the reduced average power. In the

presence of multiple lesions of one patient the lesions were treated with different light modes to reduce the effect of biological variability in the evaluation of the treatment results. In cases where the lesions were located close to the eye, fractions of the light were diffusely scattered through the tissue onto the retina and were there visible to the patient. The varying intensity of the pulsed and TSO modes was irritating and uncomfortable for the patient, and could also constitute a risk for inducing epileptic attacks [12]. Thus, only CW light was used in the treatment of eye-close lesions.

#### *Laser-induced fluorescence measurements*

The laser-induced fluorescence measurements were performed with a point-monitoring optical multichannel analyser (OMA). The system has previously been described in detail [13]. The laser pulses from a nitrogen laser (337 nm) were transformed in a dye solution to 405 nm laser emission, delivering a few  $\mu$ J per pulse at a repetition rate of approximately 10 Hz. The light was guided by a 600  $\mu$ m optical quartz fibre, which was held perpendicularly to, and in light contact with, the skin. The laser-induced tissue fluorescence emission was collected by the same fibre, guided back through a dichroic mirror (transmitting light with wavelengths longer than 405 nm) and a cut-off filter (Schott GG 435), and was then focused onto the entrance slit of a grating spectrometer. The fluorescence spectra between 475 and 790 nm were captured with a cooled and gated image-intensified CCD detector. The fluorescence from 30 laser pulses were accumulated to create one spectrum.

The treatment light was delivered in fractions with pauses of about 2 minutes after light doses of 5, 25, 50, and 75 J/cm<sup>2</sup>. Fluorescence measurements were



**Fig. 2.** The figures show the average ( $\pm$  standard deviation) of the PpIX fluorescence intensity at 635 nm relative to the intensity before treatment measured in BCCs for different treatment light doses applied. **a.** Measurements inside the lesion. **b.** Measurements 5 mm outside visible border of the lesion.

performed before treatment, during these pauses, and after the full light dose was given. At each fluorescence measurement, two spectra were recorded in the centre of the lesion and two 5 mm outside the visible border of the lesion.

#### Data analysis

The fluorescence spectra were analysed by a computer program developed at the centre [14]. These spectra were, due to the non-uniform spectral response of the detection system, corrected with a spectrum taken from a NIST-traceable white light calibration lamp. To evaluate the effect of the photodynamic treatment, the photobleaching was calculated by measuring the PpIX fluorescence peak at 635 nm. The tissue fluorescence was subtracted by fitting an exponential curve at three different points (530, 580 and 800

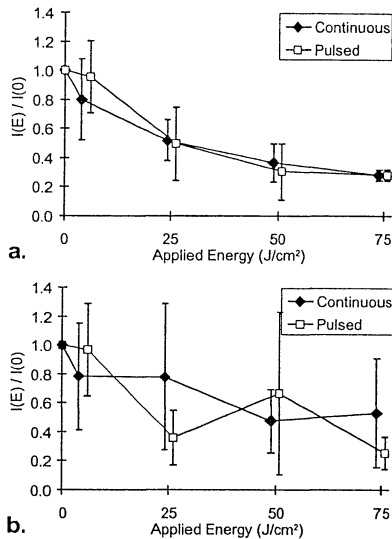
nm). The background-free peak intensity of PpIX before treatment was normalized to unity, and the relative peak intensity decrease during treatment was calculated. In this way, the PpIX synthesis variations among patients was eliminated. The average values and standard deviations of the bleaching after different applied energies for the different light structures and lesion types were calculated and plotted. The bleaching rate was evaluated by fitting an exponential curve to the bleaching curve of each lesion. The fitted  $k$ -values in the expression  $e^{-kE}$ , where  $E$  equals the applied energy and  $e$  is the base for the natural logarithms, were compared for lesions treated with different light structures in the same patient.

## RESULTS

### Photobleaching

A typical fluorescence spectrum recorded from a BCC can be seen in Fig. 1a. The endogenous fluorophores (lipids, collagen, elastin, NADH, etc.) generate a blue-green fluorescence peaking around 500 nm. The PpIX gives rise to a dual-peaked fluorescence in the red part of the wavelength range and during the treatment, the intensity of these peaks decreases. The normalized photobleaching was averaged for all BCCs and AK, which are shown in Figs. 2 and 3, respectively. In Fig. 2a the bleaching curves indicate that while irradiating BCCs with the pulse-modulated light, PpIX is bleached more efficiently than during the irradiation with the CW light. Outside the lesions there is no difference in bleaching using different light modes (Fig. 2b). The bleaching for the actinic keratoses is more ambiguous due to not very well demarcated border of this type of lesion (Fig 3).

The photobleaching curves for each patient and lesion were fitted to an exponential curve and the  $k$ -values for the



**Fig. 3.** The figures show the average ( $\pm 1$  standard deviation) of the PpIX fluorescence intensity at 635 nm relative to the intensity before treatment measured in AK for different treatment light doses applied. **a.** Measurements inside the lesion. **b.** Measurements 5 mm outside visible border of the lesion.

patients with multiple lesions treated with different light modes were compared for each patient in order to eliminate variations among patients. The results for the BCCs and AK can be seen in Figs. 4a and 4b, respectively. The  $k$ -values did not show any significant difference between the different light modes.

#### Clinical results

All lesions were initially evaluated 1 week after treatment. At that time all lesions were photographed and the necessity of retreatment was evaluated. As a result of visual inspection, two nodular BCCs and one plantar wart were retreated with the laser at 652 nm using the CW mode.

At the 3-months follow-up visit, clinical evaluation of BCCs revealed that the CR rate was 20% for lesions treated with CW

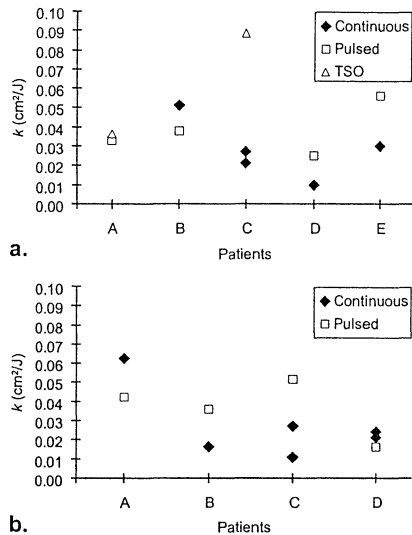
light. The PU mode showed no CR, and treatment with TSO resulted in 1 CR out of 2 lesions, which gives a total CR rate of 14%. Partial response rates were 40% for CW, 33% for PU, and 0% for TSO, yielding a total PR rate of 33%. Forty percent of the lesions treated with CW illumination showed NR, while for PU and TSO the NR rates were 67% and 50%, respectively. Actinic keratoses showed higher CR (CW - 30% and PU - 14%, in total 24%) as well as PR rates (CW - 50% and PU - 72%).

At the same 3-months visit 11 BCCs (4 treated with CW, 6 with PU and 1 with TSO) and 3 AK (2 - CW and 1 - PU) were biopsied. One of the 4 BCCs treated with CW showed no tumour, 1 decreased tumour size, and 2 no changes in tumour growth. One lesion showed no tumour after treatment with the PU mode and 1 after TSO. Actinic keratoses had the following results: after treatment with CW 1 lesion was healed, and 1 showed a decreased tumour size; after PU mode 1 lesion with decreased tumour size.

After obtaining the results of the 3-months follow-up, all lesions with poor clinical response were retreated with ALA-PDT, using laser light at 635 nm.

#### DISCUSSION

The presence of oxygen is essential for the PDT process and therefore fractionation of the treatment light could increase tumour damage, allowing transport of oxygen to the treatment site during pauses, to replace the oxygen that was consumed during the irradiation. In structural models it has been shown that fractionation increases the  $^1\text{O}_2$  dose to the cells relatively remote from the capillary walls and fractionating the light dose should result in an improved therapeutic ratio for PDT [15]. Photodynamic therapy with fractionated light has previously been performed in cell cultures as well as *in*



**Fig. 4.** *k*-values for different patients with multiple lesions where the lesions have been treated with different light modes. **a.** BCC **b.** AK

*vivo*. The treatment protocols have been variable, with pauses between fractions being from 24 hours to less than a minute. In addition to that, various pulsed lasers have been used. The effect of 24-hour-long fractions is shown to be poorer than a single delivery of the same light dose [16]. In an early study, Moan and Christensen showed an increased cell-killing using two fractions instead of a single irradiation {Moan & Christensen 1979 ID: 2163}. According to Berg *et al.* [5] the optimal length between two fractions is 30-90 min. In another study, van Geel *et al.* [6] found that 1 h dark periods between 6 fractions of light delivery is superior to a single illumination with the same light dose for both Photofrin and *meta*-tetrahydroxyphenylchlorin (*m*-THPC). The use of alternating light and dark periods of 30 s improved the tumour response even further for *m*-THPC, but did not result in any improvement using Photofrin. During the first fraction some

vasoconstriction occurs which relaxes during the first break permitting reoxygenation. This suggests that even a single short interruption of light may reduce the light dose needed to achieve extensive necrosis, while the optimal time for the pause is after 50 s of irradiation [7]. Foster *et al.* [8] showed also a better response with Photofrin when using 30-second fractions separated by 30-s-long dark periods and claimed reoxygenation being responsible for the enhanced effect. Similar effects were observed for aluminium phthalocyanine tetrasulfonate (AlPcS<sub>4</sub>) when 15-second-long fractions were used and the effect was thought to be due to the fact that the photosensitizing dye changes the localisation in the tumour during the first seconds of exposure [9]. In a recent study, Müller *et al.* [11] used 50-ms-long irradiation fractions, separated by a dark phase of the same length, at 652 nm to treat cells incubated with *m*-THPC. An increased cell killing could be found. Similar results were also found in a study using the PU-mode from the same type of diode laser system as used in this study [10]. The authors speculate on possible photochemical and photobiological phenomena that might be responsible for such an effect.

As PpIX is selectively accumulated in hyperproliferative cells, the fluorescence spectra from the tumour area are characterized by a dual-peaked signal at about 635 and 705 nm. At the same time normal skin shows very low porphyrin-related fluorescence, but has high tissue autofluorescence peaking around 490 nm. During the irradiation with light emitted at 652 nm, ALA-induced PpIX bleaches and this leads to a decrease in the intensity of the peak of PpIX. Concurrently several photoproducts, mostly of chlorin-type, are formed, which form a new broad fluorescence peak around 670 nm. In the irradiation process singlet oxygen and free radicals are formed which cause the

oxidative damage to the cells. Thus, the bleaching shows that PpIX is activated and that photochemical reactions take place which should result in cell damage.

The fluorescence spectra collected from the BCCs are quite uniform and represent usual bleaching tendencies. The border of a BCC is quite distinct and normal skin is measured outside the lesion. The variability of the photobleaching measured outside AK can be due to the nature of the lesion. The actual border of the diseased tissue is not always coinciding with the visible border of the lesion. Thus, measurements performed 5 mm outside the visible border, might be infiltrated by diseased tissue, giving rise to a higher PpIX fluorescence. By measuring slightly different locations at different times, artifacts in the bleaching curves are obtained (Fig. 3b).

Broad-band lamps as light sources for PDT have been used with the rationale not only to hit the absorption peak of PpIX, but also to take advantage of photoproducts with photosensitizing properties, mainly photoporphyrin, that has an absorption at around 670 nm. Such lamps are inefficient sources of red light, which is considered to be optimal for PDT [17]. In this study we show CR rates of only 14% (BCC) and 24% (AK) which are much lower than previously reported average results (87% and 92%, respectively) [18]. It is also lower than the average values, 50% - 100% and 80% - 100%, respectively, reported in a review based on the outcome of several recent studies [19]. This indicates that the use of an excitation source only about 17 nm off the absorption peak of PpIX decreases the treatment efficiency significantly. In this study the total light dose was increased by 67% (from the usually used dose of 60 J/cm<sup>2</sup> at 635 nm up to 100 J/cm<sup>2</sup>) to compensate for the lower PpIX absorption at 652 nm. According to Peng *et al.* [18]

the absorption cross-section at 652 nm is about 63% of that at 635 nm. The increase in total light dose did obviously not compensate for the shift in the treatment light wavelength.

According to Svaasand *et al.* [20], irradiation of 100 mW/cm<sup>2</sup> at 635 nm for 20 minutes gives an adequate light dose to a depth of 3 mm. The time required for ALA to reach that depth will vary between 3 and 15 hours, depending on the diffusivity of the tissue [20]. As the penetration depth is time dependant, we applied ALA overnight, not for 6 hours as usual, to allow more time for penetration as the laser light is supposed to penetrate deeper at 652 nm in comparison to 635 nm. Additionally, it has been reported that pulsed light may [21] or may not [22] have an effectively deeper penetration than CW light.

The use of laser irradiation at 652 nm, either when continuous wave or intensity modulated light was used, did not result in tumour damage to the extent obtained when using irradiation at 635 nm. A lower effectiveness of that wavelength was also observed as a lower bleaching rate of PpIX. The bleaching fluence (defined as  $1/k$ ) has previously been reported to be 17.2 J/cm<sup>2</sup> by Svaasand *et al.* [20,23], and 20 J/cm<sup>2</sup> by Andersson *et al.* [24]. This corresponds to a  $k$ -value between 0.04 and 0.05 cm<sup>2</sup>/J, which is slightly higher than the average values in this study. Further, pain and other sensations related to the treatment were less intense at 652 nm [25], possibly indicating a less pronounced photochemical reaction. No significant difference was obtained using CW or pulse-modulated light. It should be noted that our statistical material is limited. However, our results are consistent with the outcome of a very recent cell culture study [26] where CW and pulse-modulated treatment with 652 nm light were found to be nearly



equivalent. In view of the inconsistent results from different studies [5,6,19], it would be interesting to pursue further studies with light of different modulation patterns in search of possible advantages. Such studies are facilitated with easily modulated diode laser sources, like the one used in this study, which now are replacing previously used lasers.

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