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Photodynamic Therapy in Interplay with Fluorescence Diagnostics in the Treatment of Human Superficial Malignancies

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ABSTRACT

In the present paper we address the question if fluorescence diagnostics can be used to monitor and possibly predict the outcome of Photodynamic Therapy (PDT) using the tumor seeking agents Photofrin and δ-aminolevulinic acid (ALA). The degree of selective uptake may vary from patient to patient and it would be interesting to use the drug-related fluorescence signal as a tool to tailor the treatment strategy. Clearly, the fluorescence intensity cannot be directly related to the tissue drug contents because of varying absorption and scattering properties of the tissue. However, because of the real-time capability of fluorescence it is interesting to see how far the fluorescence information can be utilized for optimizing the light delivery.

Patients with basal cell carcinoma and spread metastatic breast cancer in the skin were treated. Two different doses, 1 and 2 mg/kg b.w. of Photofrin (Quadra Logic, Vancouver, Canada) were used. The treatment laser was a Nd:YAG pumped dye laser (Multilase Dye 600, Technomed International, Paris/Bron, France). The system provides 1064 nm IR and 532 nm green light from the Nd:YAG laser as well as red light in the region 620-670 nm from a dye laser. The treatment procedure was preceded by fluorescence measurements for allowing comparisons between the diagnostic signals and the treatment outcome. At the end of the treatment, fluorescence was again monitored to assess the degree of bleaching manifested by the appearance of an additional red peak. Our data on the connection between fluorescence signals, delivered dose and observed treatment outcome are presented and the potential of imaging fluorescence monitoring in PDT dosimetry is discussed.

Introduction

Photodynamical therapy (PDT) is a malignant tumor treatment modality relying on a selective transfer of triplet to singlet oxygen mediated by a laser excited sensitizing drug. The drug is normally injected intravenously and is selectively retained in malignant tissue. Thus, a selective necrosis of cancer lesions can be achieved. Laser-induced fluorescence (LIF) can be utilized to localize the tumor based on specific drug fluorescence and native chromophore fluorescence. It has been found

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that the prime mechanism for tumor destruction is damage to the vessels supplying the tumor. The only agent presently in more common clinical use is a haematoporhyrin derivative, available under the trade mark of Photofrin. The drug is normally injected 48 hours prior to the laser treatment which follows at a wavelength of 630 nm. Good clinical results have been obtained by different groups for a variety of malignant tumors [1,2]. The only side effect of PDT using Photofrin is a skin sensitization for about 4 weeks calling for the patients to stay out of strong ambient light during such a time period. The first PDT treatments in Scandinavia were performed by our group in 1987 using a laboratory laser set-up consisting of an argon-ion laser pumping a dye laser [3,4]. One of the early patients treated for multiple basal cell carcinoma now has a follow-up time of more than four years showing no recurrences in the treated areas.

Recently, a different PDT procedure for treating superficial tumors has been applied by Kennedy *et al.* [5]. In this case δ-aminolevulinic acid (ALA) prepared in 20% concentration in an oil-water base is topically applied on the lesions 3-6 hours prior to the treatment. The small ALA molecules pass the cell walls of the tumor cells and are intra-cellularly transformed to protoporphyrin following the normal heme cycle in the mitochondria [6]. Following laser irradiation at 630 nm the photodynamic action is initiated. Due to the different nature of administration no problems due to photosensitization to ambient light occur.

In the present paper we report case studies of PDT in three patients with multiple lesions of basal cell carcinoma or metastatic breast cancer. Conventional PDT using Photofrin as well as PDT using topical application of ALA was applied. A new treatment laser based on a high repetition rate Nd:YAG laser frequency-doubled to 532 nm to pump a dye laser was employed. Extensive laser-induced fluorescence (LIF) measurements on tumors and normal tissue were performed before and after PDT using a fluorosensor, described in [7]. The main purpose of the present study is to relate the LIF results to the treatment outcome and to study the bleaching of the sensitizer as studied by LIF to infer the deposited dose of light. The bleaching or photodegradation of the sensitizer molecules is manifested in a reduction in the fluorescence light intensity accompanied by the appearance of a further characteristic fluorescence peak in the red spectral region. Utilization of the drug bleaching has been proposed for achieving a deeper treatment effect in the irradiated tissue [8]. Because of the stronger photodegradation of the agent in the superficial tissue layers subject to the strongest light intensity, the upper layer is protected from overtreatment, provided that the correct initial drug concentration is chosen. Further irradiation will continue the treatment in the deeper layers with continuing bleaching.

Materials and Methods

Below three case studies will be presented. In the LIF investigations and the PDT treatment we used the agents and the equipment as follows.

Drugs - For intravenous injection we used Photofrin (Quadra Logic Technologies, Vancouver, Canada), Lot # B90-0020. The drug was injected at a concentration of 1 or 2 mg/kg body weight. For topical application we used δ-aminolevulinic acid hydro chloride (ALA) (Porphyrin Products Inc., Logan, Utah), catalogue # A167, Lot # 072791. The powder (0.5 g) was dissolved in 0.5 g sterile water and was then mixed with 1.5 g Essex cream (Schering Corp., Kenilworth, New Jersey), to yield a 20 % ALA cream. The cream was applied to the lesions and their close surroundings and was kept in place for a time period of 6 hours using an occlusion cover.

Laser equipment for PDT - In the present treatments we used a Technomed Multilase 2500 Nd:YAG laser system operating on its second harmonic (532 nm) to pump a Technomed Dye 600 laser tuned to 630 nm. The red radiation was conducted through a 400 μm quartz fibre. The polished end face of the fibre was imaged using a 40 times magnification microscope objective onto the lesion in a slightly diverging beam. By adjusting the distance between the microscope objective and the lesion a "top hat" uniform intensity distribution of the desired diameter could be obtained. Typically, a total energy of about 1 W was delivered onto the target resulting in irradiation times of typically 15 minutes for achieving a dose of 50 J/cm^2 in a circle of 5 cm diameter. Normally, a margin of 5 mm was allowed in the treatment of the tumors. The power of the projected beam was measured by a calibrated large-area power meter before and after a tumor irradiation.

Laser fluorosensor for LIF - For recording tissue fluorescence spectra we used the clinical fluorosensor described in [7]. This equipment uses a single quartz fibre of diameter 600 μm for transmitting the exciting light and collecting induced fluorescence light. Normally, the probe is kept in contact with the tissue. 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. Fluorescence was conducted to the entrance slit of a Jobin Yvon 0.25 m grating monochromator equipped with an EG&G Model 1460 gated and intensified linear array detector connected to an EG&G OMA mainframe. Normally, the signal from 100 laser pulses were averaged during a period of 10 seconds. The signals were spectrally corrected using a calibrated standard lamp.

3 case studies of superficial tumor PDT and LIF analysis

Case 1. A 69 year old woman was treated with PDT following intravenous injection of 2 mg/kg b.w. Photofrin for basal cell carcinoma on her lower limbs. The tumors appeared 2 years ago and were histopathologically verified in multiple biopsies. Conventional surgical removal of the lesions was contra-indicated due to poor circulation, related to a diabetical condition. 5 lesions of diameters ranging from 1 to 4 cm were treated with light doses ranging between 25 and 50 J/cm^2. The laser light was given as a single treatment.

All five lesions were eradicated. The healing period lasted for about 10 weeks. Immediately after PDT a slight oedema appeared in the area corresponding to the laser light border. The oedema disappeared after about 6 hours and a halo could be seen in the skin surrounding the tumors. Two days post PDT the surface of the tumors started to leak small amounts of clear liquid. This continued for about 2 weeks. After this period the tumor surface started to dry out and a necrotic crust was formed. The underlying tissue went through a granulation. The crusts fell off about 6-8 weeks post PDT and the reepitalization took another week.

Case 2. A 68 year old woman was treated for multiple basal cell carcinoma lesion on the capellitium. The tumors first appeared 5 months before the laser therapy and were first treated by cryo therapy with curettage. That treatment left border zones of viable tumor. PDT with topical application of ALA was judged to be appropriate in this case. In the treatment, the central part of the tumor already treated by the cryo therapy was blocked with black paper. The border zones of three larger lesions with diameters between 3 and 5 cm and two smaller lesions with diameters ranging from 1 to 2 cm were treated. Doses varying between 30 and 40 J/cm^2 with a fluence rate of 70 mW/cm^2 were applied.

Immediately after PDT a slight oedema and a reddening of the skin surrounding the tumors was seen. No halo formation was observed afterwards. Six hours after the laser treatment small amounts of clear liquid leaked from the tumor surface. Already two days post PDT the leaking stopped and a dry necrosis appeared in the tumor regions, while the normal surrounding skin was unaffected. Eight days after the treatment the necrotic thin crusts started to fall off. Photographs of two treated lesions are shown in Fig. 1.

Case 3. A 55 year old woman was treated for local recurrences of breast cancer on the thoracic wall. Three different treatment procedures were performed. In the two initial procedures intravenously injected Photofrin (1 mg/kg b.w.) was used, whereas the last one was performed after topically applying ALA. All procedures were performed with a light dose of 50 J/cm^2 in a single light administration procedure.

During the two first procedures three areas ranging in diameter from 5-10 cm were treated on the thoracic wall and in the axilla. Immediately after the treatment a slight oedema appeared in the treated areas. Six hours later the oedema had disappeared and a halo was formed with its outer border corresponding with the border line of the laser light. The tumors started to leak clear liquid from day two after the treatment and continued for about eight days. Thereafter the tumors were covered with a black necrotic crust which stayed for about 6 weeks on the two smaller lesions and 12 weeks on the larger area. When the crusts fell off the granulation and part of the reepitalization was already finished. Three months after the PDT fine needle cytology was performed on the healed lesions and no vital tumor cells could be recognized.

The third treatment procedure with topically administered ALA included four lesions of a diameter of 5 cm each. The therapy effect was less in all four lesions compared with the treatment following intravenously administered Photofrin. In one of the lesions a slight necrosis was formed three days post PDT.

LIF results in relation to treatment outcome and dose dependent bleaching

Two examples of fluorescence spectra recorded immediately before and after treatment of a basal cell carcinoma (case 2, ALA) are shown in Fig. 2. The background-free fluorescence intensities at about 630, 670 and 700 nm and the total fluorescence intensities at about 470 and 600 nm are denoted A, Y, C, B and D, respectively. The tumor was exposed to ALA cream for 6 hours before the treatment. As can be seen from the two curves, the decrease of the porphyrin fluorescence was in this case, as in many others, about ten-fold whereas the blue fluorescence, denoted B, did not show any apparent reduction. The spectrum recorded after PDT is also shown in five times magnification in the lower part of Fig. 2, where the appearance of an additional peak at 670 nm (denoted Y) is quite clear. The Y peak seems to result from the photodegradation of the porphyrins or may reflect a photoinduced displacement of the porphyrins in the cells. A wavelength shift for the red peaks is observed for ALA as compared to Photofrin. For ALA the three porphyrin peaks, including the bleaching peak, are found at 635, 670 and 705 nm, whereas in the case of Photofrin the same peaks are found at 630, 660 and 695 nm, respectively. This may be a result of different mechanisms for drug retention in the tissue.

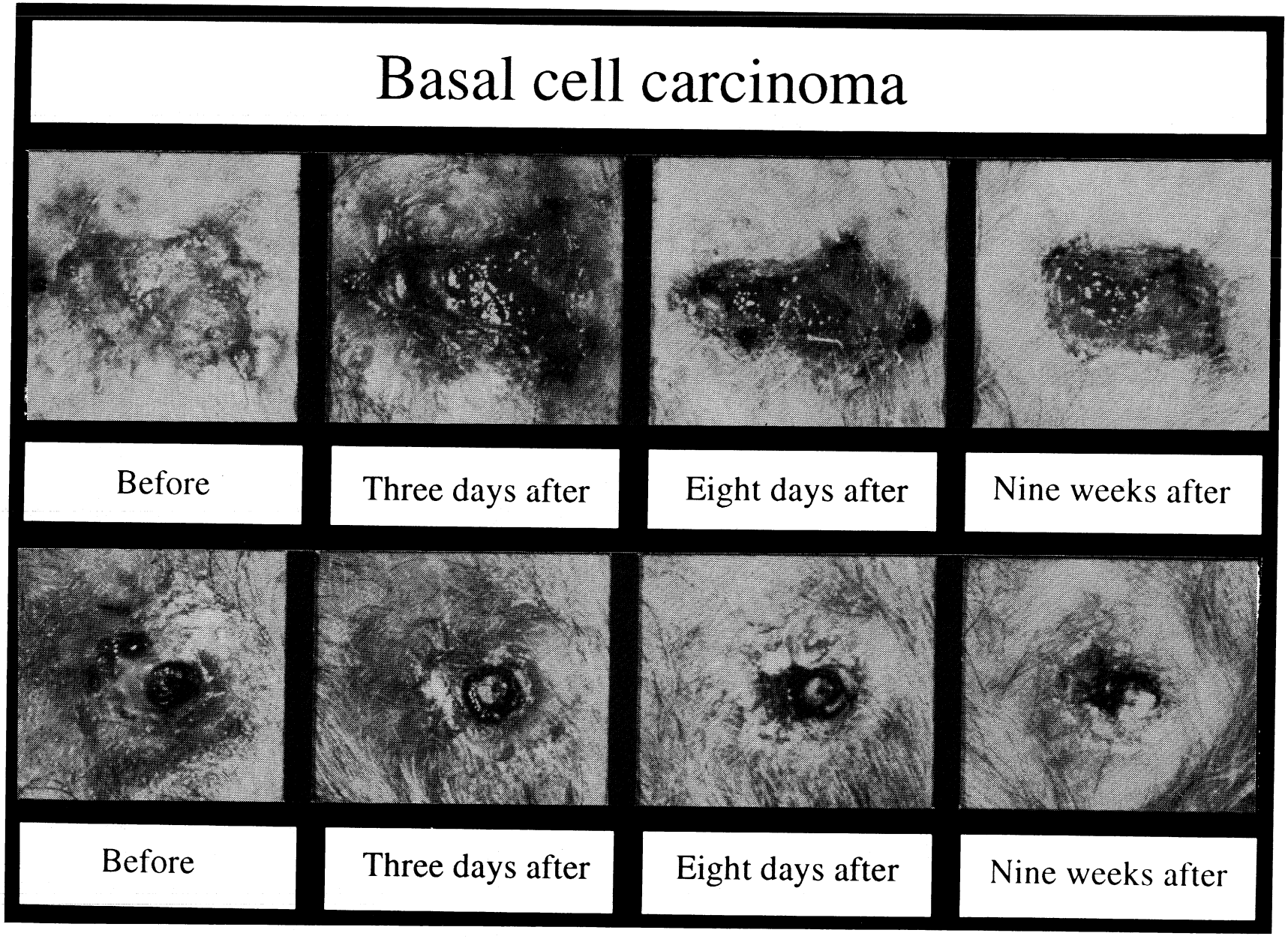

Fig 1. Photographs of basal cell carcinoma before and 3 days, 8 days and 9 weeks after treatment with PDT/ALA

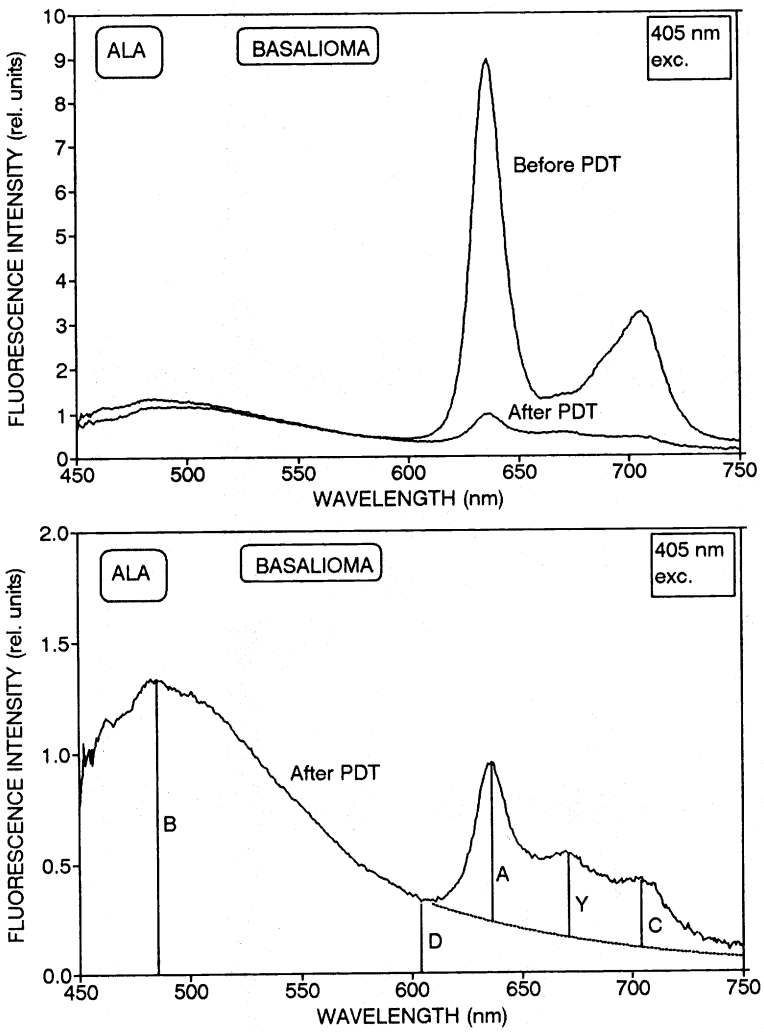

Fig. 2. Two typical fluorescence spectra (top) for a basal cell carcinoma taken before and after treatment at a dose of 40 J/cm^2. The tumor was exposed to ALA cream for 6 hours before the treatment. Note the bleaching of the porphyrin signal and the occurrence of a new intermediate peak (Y) at about 670 nm. Below, five times magnified, the same fluorescence spectrum recorded after PDT is shown with the fluorescence intensities A, B, C, D and Y indicated.

The various aspects of the photobleaching of the porphyrins are shown in Fig. 3 for case 1 (Photofrin injection). To the upper left the fluorescence intensity A is plotted in a scan through a tumor region. To the upper right and the lower left the fluorescence ratios A/B and Y/A, respectively, are shown. The bleaching of the porphyrins can be seen as a decrease of the fluorescence intensity A but also as an increase of the fluorescence ratio Y/A. Furthermore, in the points where the decrease of A was strongest, the increase of Y/A was most obvious. The fluorescence spectra for a tumor and for normal skin are given to the lower right. In Fig. 4 the same set of scans are shown for a basal cell carcinoma treated with ALA (case 2). Also in this case the bleaching was prominent. In the scan of A/B an excellent tumor demarcation can be seen. This is also clear in the two spectra shown in this figure. It should be noted, that the points representing normal areas were exposed to the same amount of ALA cream as the tumor. Nevertheless, no conversion to protoporphyrin has occurred in these areas. For case 3, two sets of scans are shown, one for Photofrin (Fig. 5a) and one with ALA sensitization (Fig. 5b). Interestingly, only the tumors exposed to ALA showed strong bleaching. Also the tumor demarcation was better for the case with ALA.

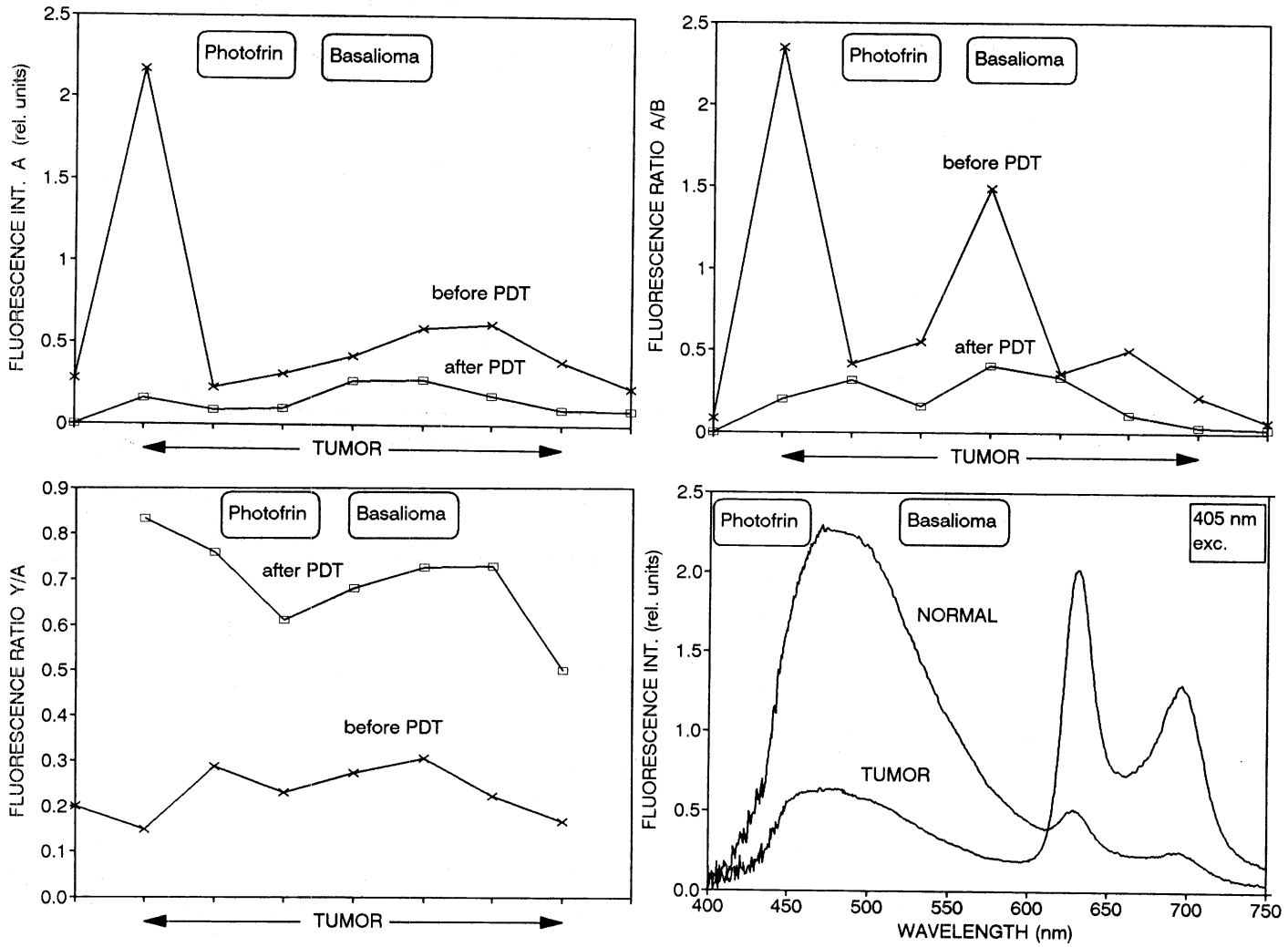

Fig. 3. Fluorescence scan through a superficial basal cell carcinoma before and after PDT showing the fluorescence intensity A, the fluorescence ratios A/B and Y/A. To the lower right two examples of fluorescence spectra from a tumor site in a basalioma and a normal skin area in the scan are shown. The patient was injected with 2 mg/kg bodyweight of Photofrin 48 hours before the treatment.

In Fig. 6 the light dose dependence of the bleaching is shown for the A intensity as well as for the Y/A ratio. The former values are normalized on the initial values prior to the treatment. In accordance with what one would expect, the bleaching increases with the light dose. We find a light dose of about 40 J/cm^2 for reduction of the A intensity to about one tenth of the initial value, corresponding to about $\Delta = 20 J/cm^2$ in an $I = Ioe^{-D/\Delta}$ description of the intensity I as a function of dose D.

The tumor selectivity of ALA and Photofrin is shown in Fig. 7 for breast cancer metastases and basal cell carcinoma. The ratios A(tumor)/A(normal) and [A/B](tumor)/[A/B](normal) are formed. The statistical material consists of 19 tumor sites and 5 normal sites for 3 breast cancer lesions (case 3) and 19 tumor and 27 normal sites for 5 basaliomas (case 2) for the ALA measurements. For the Photofrin studies the corresponding numbers are 30 tumor and 20 normal sites for 7 breast cancer

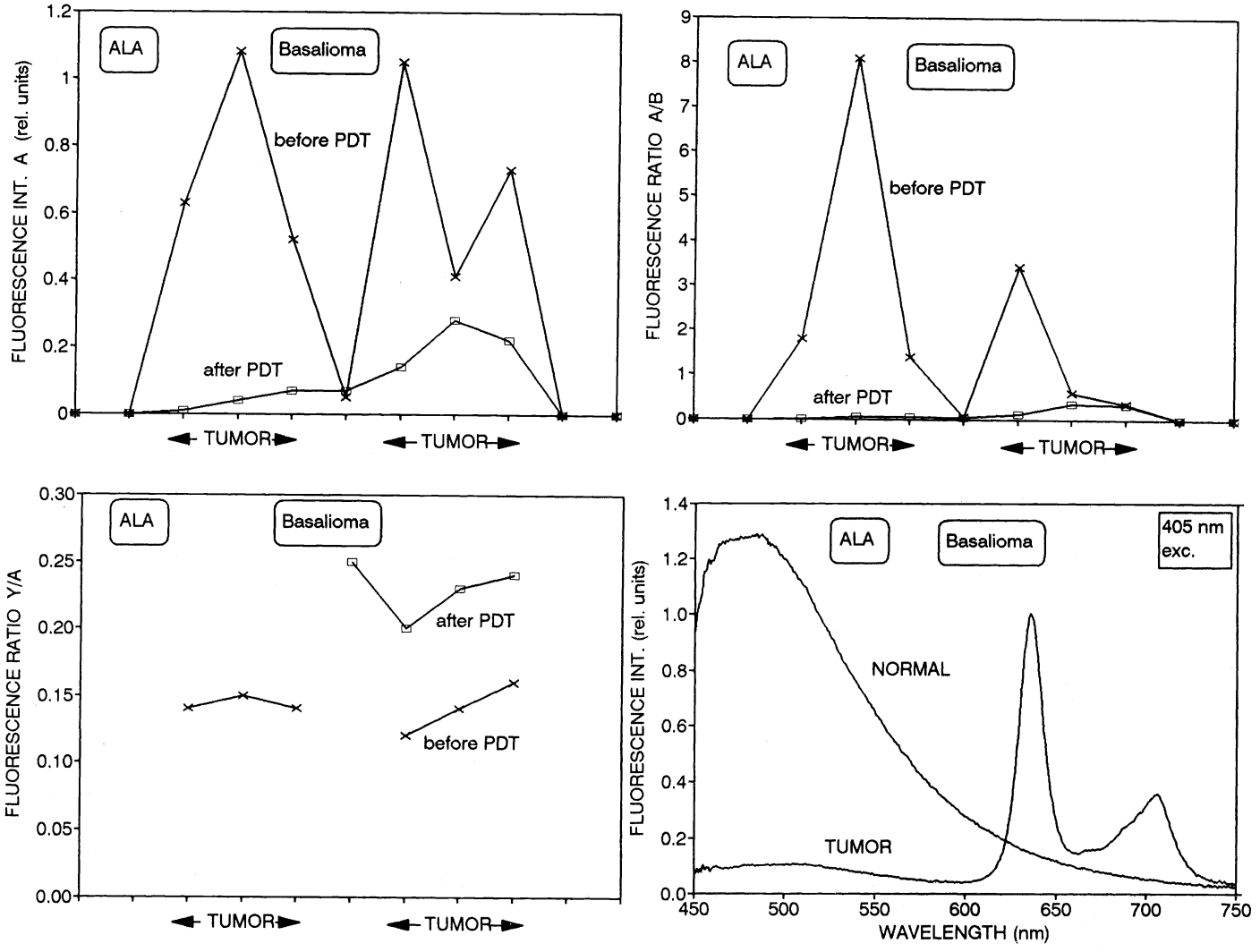

Fig. 4. Fluorescence scan through a superficial basal cell carcinoma before and after PDT showing the fluorescence intensity A, the fluorescence ratios A/B and Y/A. To the lower right two examples of fluorescence spectra from the scan are shown. The tumor and the surrounding boarder zone had been locally exposed to an ALA cream 6 hours before the treatment.

lesion (case 3) and 9 tumor and 6 normal sites for 2 basaliomas (case 1). Claerly, ALA has an extremely good selectivity and is very suited for tumor demarcation.

Fig. 7 also shows that the autofluorescence (B) gives a substantial contribution to the fluorescence demarcation. Thus, if the A/B criterion is used, the tumor to normal ratio is a factor of 2-5 higher than if the A intensity alone is used.

Discussion

PDT can be a valuable complement to other treatment modalities. In some kinds of tumors, PDT may also be used as the primary therapy. In cases with single lesions or wide spread basal cell carcinoma, PDT following topical administration of ALA maybe is the most favorable treatment procedure for the patients, as the response rate is very

PHOTOFRIN AND ALA IN SUPERFICIAL BREAST CANCER METASTASES

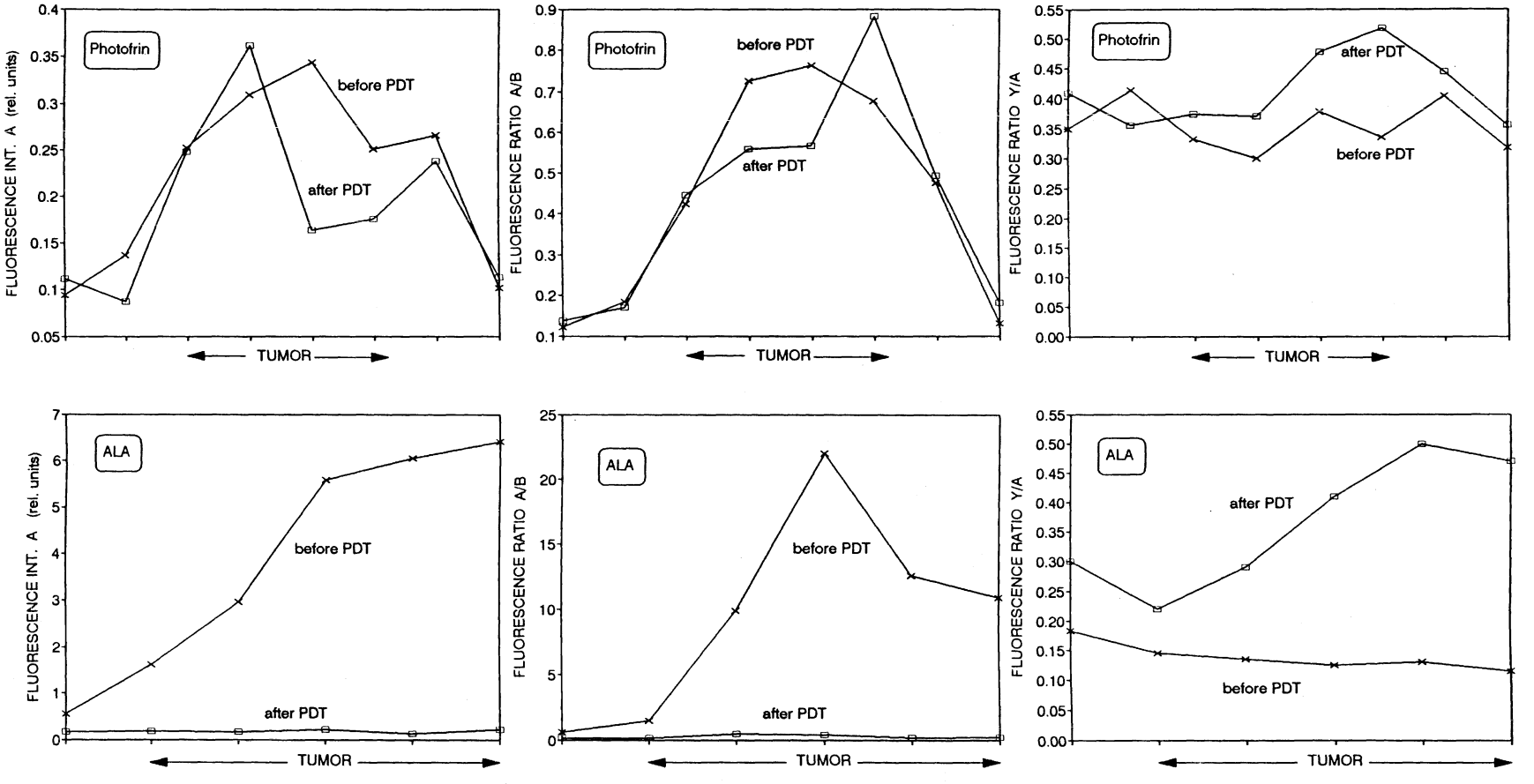

Fig. 5. Fluorescence scan through cutaneous breast cancer metastases before and after PDT showing the fluorescence intensity A, the fluorescence ratios A/B and Y/A. a) The patient had been injected with 1 mg/kg bodyweight of Photofrin 48 hours before the treatment. b) The tumor and the surrounding boarder zone had been locally exposed to an ALA cream 6 hours before the treatment.

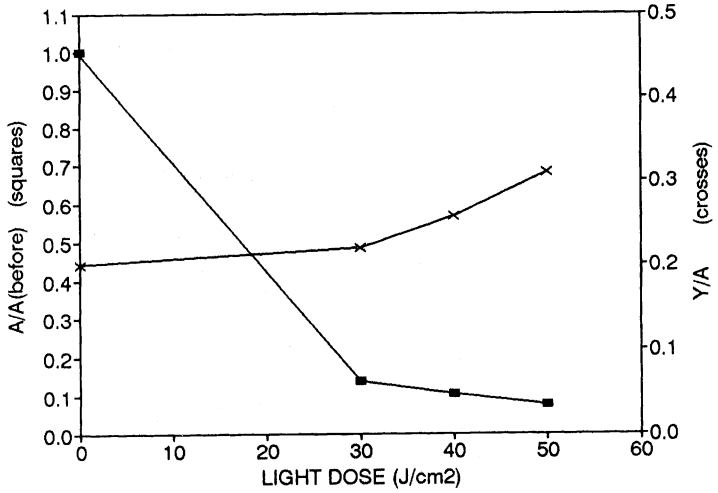

Fig. 6. Diagram showing the ratio of A recorded after certain amount of light doses and A before treatment (squares). The diagram also shows the ratio for the fluorescence intensity at about 670 nm (Y) and A, both as a function of different light doses (crosses).

high as shown in Ref. [5] and confirmed in the present work. The treatment procedure does not include any side effects and can easily be performed on an out-patient basis. In cases with local recurrences of breast cancer, PDT following intravenous administration of Photofrin is a promising complementary treatment modality, as the patients often have gone through many other treatment modalities including full radiation therapy and nothing else can be offered. PDT_2 utilizing ALA in locally recurrent breast cancer may require light doses of 100 J/cm^2 and also longer time for the ALA to penetrate the skin more efficiently as the local metastases of breast cancer are located subcutaneously as well as cutaneously. With optimal laser light dosimetry and efficient ALA administration PDT following topical administration sensitizer may also be valuable for this kind of skin tumors.

When porphyrin-containing tissue is irradiated to achieve photodynamic action, a fluorescence peak at about 670 nm, not detectable prior to irradiation, will normally

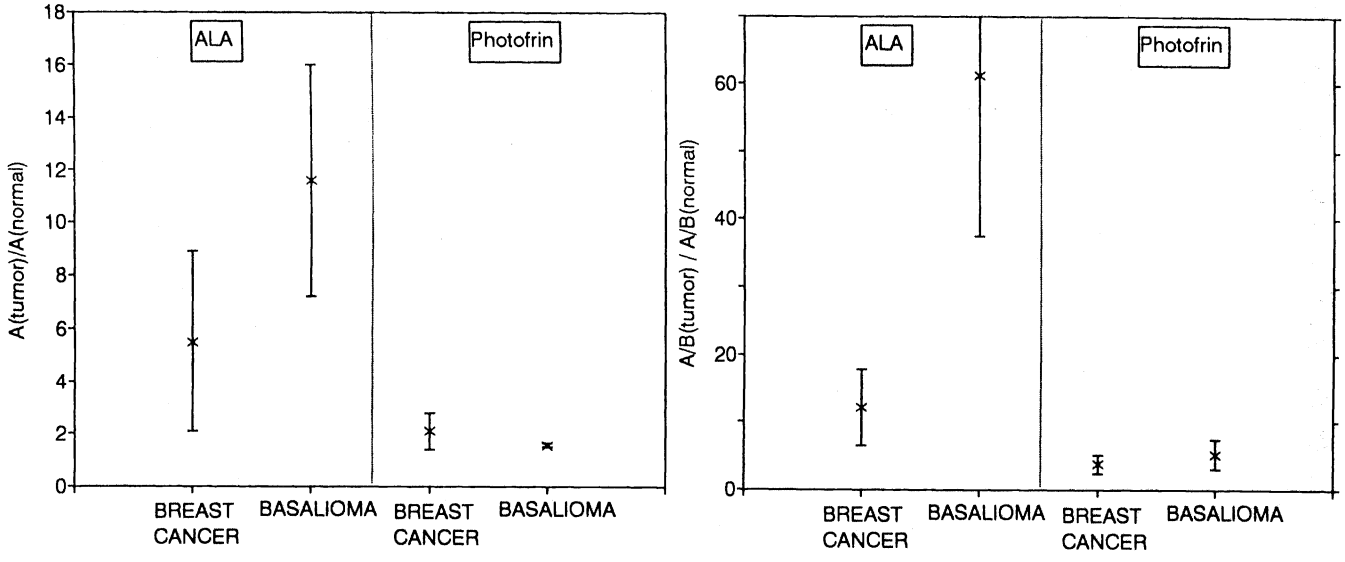

Fig. 7. Diagram showing the tumor selectivity for ALA and Photofrin expressed as the ratio of the fluorescence intensity A for tumor to A for normal (left) as well as the tumor/normal ratio for A/B (right).

appear, as we have shown *in vivo* in the work presented in this paper. This extra peak may be due to a photoproduct arising as a result of the irradiation of the red light, converting the primary porphyrins into a new fraction, or may reflect a displacement of the porphyrins to other binding sites in the tissue cells. Photoinduced bleaching with the appearance of a new fluorescence emission peak at about 660-670 nm has been shown *in vitro* by Moan *et al.* [9]. The new peak has also been studied by Yamashita *et al.* [10] using picosecond fluorescence techniques.

In view of Fig. 2 the Y peak may, on the other hand have been there all the time, although not distinguishable prior to the photobleaching since the two major peaks at about 635 and 700 nm tend to hide the signal in between the two peaks. If this is the case, the constituents that represent the fluorescence emission at about 675 nm do not bleach as much as the ones contributing to the two major fluorescence emission peaks. However, in both cases the appearance of the spectrum changes substantially and the fluorescence emission at 675 nm appears clearly after light exposure and the ratio of the Y and A intensities is light dose dependent as shown in Fig. 6. In some crucial dosimetry situations in PDT, such as in the treatment of the bronchs with difficult geometries, the appearance of the fluorescence peak at 675 nm and the decrease of the fluorescence intensity at about 630 and 690 nm may be used to guide the light delivery. Areas in a tumor that are expected to show bleaching behavior but which do not show these changes in the fluorescence may require further illumination for the achievement of the proper dose and the desired tumor treatment effects.

In principle, it should be possible to obtain iso-dose images using the multi-color imaging technique developed in our group [11,12]. Here, up to four images can be recorded simultaneously, each displaying the intensity distribution in a well-defined wave-length interval selected by a proper filter. By measuring the spatial light distribution at 675, 635, 700, and 470 nm (Fig. 2b) it is possible to calculate an image in the function Y/A, which according to Fig. 6, has a dose-dependent intensity. The intensities D and B are useful for evaluating the sloping autofluorescence intensity needed to calculate the background-free A and Y intensities. It also allows the formation of an A/B image, which we have shown produces a strong tumor demarcation. The intensity loss according to Fig. 6 in the A/B image as the treatment proceeds can also be used for dose assessment.

Our PDT of basal cell carcinoma with Photofrin and ALA shows complete response in all tumors treated. The longest tumor free follow-up time is 4 years in a patient injected with Photofrin. In the case of ALA we have only about three months of follow-up period. The cosmetic results are very good. Especially nice healing of the skin has been achieved in the face, where almost no scar formation appeared in the patients. The treatment procedure utilizing ALA as photosensitizer in basal cell carcinoma is very attractive, since no side-effects occur and the patients can be treated on an out-patient basis in half a day. PDT applying ALA is especially valuable in basalioma located in areas with low blood supply, such as on the front of the lower limb of elderly people, since the treated areas heal without any open wounds. PDT can also be used in lesions that cannot be treated with radiation therapy, e.g. lesions on the hands or feet. PDT is also interesting for patients with some specific syndroma, such as Multiple Basal Cell Nevoid Syndrom, connected with the appearance of multiple basalioma lesions. PDT of local recurrences of breast carcinoma employing Photofrin can be efficient and is especially interesting if a drug dose reduction is used to reduce the skin sensitivity, as we have presented in this work.

The fact that we did not observe any bleaching of the sensitizer for the breast cancer patient employing Photofrin application is a puzzle. A substantial light dose of 50

J/cm^2 was applied to the irradiated areas and good tumor response was observed. The absence of bleaching for some porphyrin fractions has been observed *in vitro* [13]. It may be that the biological surrounding influences the porphyrin molecules and their response to the laser light. The pH value may play an important role. The tumor areas in which we could not observe any bleaching were low differentiated cancer with very fast growth and probably low pH value and minimal oxygen content. When employing ALA for the same kind of tumor we observed a substantial bleaching in the fluorescence but a very weak tumor effect. I may be that the ALA molecules entering the cancer cells via the heme cycle find binding sites where they are not phototoxically active although influenced by the light. The strongly fluorescent molecules formed may not be able to transform triplet oxygen into singlet oxygen to induce cell death. ALA in the treatment of local recurrences of breast cancer has to be investigated further. The light dosimetry and the drug application must be improved. The possibility for the ALA creme to penetrate more efficiently may be improved by stripping off the covering *stratum corneum*. Another possibility would be to repeat the treatment procedure several times to penetrate deeper into the subcutaneous tissue.

The present work indicates, that fluorescence monitoring in interplay with PDT can provide information of value for optimizing the laser treatment procedure and the light dose applied. If these phenomena are well understood, fluorescence measurements should help considerably in order to avoid under- as well as overtreatment. Obviously, it is necessary to perform careful studies for different types of tumors to gain the insight needed to take full advantage of the diagnostic information provided by a clinical fluorosensor.

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