Photodynamic therapy for malignant pleural mesothelioma
Schouwink, J.H.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
PHOTODYNAMIC THERAPY FOR MALIGNANT MESOTHELIOMA: PRECLINICAL STUDIES FOR OPTIMIZATION OF TREATMENT PROTOCOLS

Hugo Schouwink¹ ², Marjan Ruevekamp¹, Hugo Oppelaar¹, Robert van Veen³, Paul Baas¹ ², Fiona A. Stewart¹

¹Division of Experimental Therapy, ²Department of Thoracic Oncology, The Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital, Plesmanlaan 121, 1066 CX Amsterdam, ³University Hospital, Rotterdam, The Netherlands.

ABSTRACT

Effective photodynamic therapy (PDT) depends on optimization of factors such as drug dose, drug-light interval, fluence rate and total light dose (or fluence). In addition, sufficient oxygen has to be present for the photochemical reaction to occur. Oxygen deficits may arise during PDT if the photochemical reaction consumes oxygen more rapidly than it can be replenished and this could limit the efficacy of PDT. In this study we investigated the influence of drug-light interval, illumination fluence rate and total fluence on PDT efficacy, for the photosensitizer meta-tetrahydroxyphenylchlorin (mTHPC). The influence of increasing the oxygenation status of tumors during PDT was also investigated. PDT response was assessed from tumor growth delay and cures for human malignant mesothelioma xenografts (H-MES01) grown in nude mice. Tumor bearing mice were injected i.v. with 0.15 or 0.3 mg.kg^{-1} mTHPC and, after intervals of 24 to 120 h, the s.c. tumors were illuminated with laser light (652 nm) at fluence rates of 20, 100 or 200 mW.cm^{-2}. Tumor response was strongly dependent on drug-light interval. Illumination at 24 h after photosensitization was always significantly more effective than illumination at 72 or 120 h. For a drug-light interval of 24 h, tumor response increased with total fluence but for longer drug-light intervals, even high total fluences failed to produce a significant delay in tumor regrowth. No fluence rate dependence of PDT response was demonstrated in these studies. Nicotinamide injection and carbogen breathing significantly increased tumor oxygenation and increased the tumor response for PDT schedules with illumination at 24 h after photosensitizer injection.

INTRODUCTION

Photodynamic therapy is increasingly used as a primary treatment for small localized cancers, e.g. head and neck (1-6), or as an adjuvant to debulking surgery for more widespread disease, e.g. malignant mesothelioma (7-10). One of the most potent photosensitizers currently in clinical use is meta-tetrahydroxyphenylchlorin (mTHPC). Clinical protocols for mTHPC-PDT prescribe a drug dose of 0.1 or 0.15 mg.kg^{-1} and illumination of the tumor area with a total fluence of 10 to 20 J.cm^{-2} red light (652 nm) after an interval of 4 to 6 days. This interval is based on times at which maximum drug concentration differentials have been measured between tumor and surrounding normal tissues (11-16). The relationship between drug dose, light dose, illumination interval and PDT effect is, however, complex and there are many published reports of a dissociation between tumor photosensitizer levels and PDT effect, both for mTHPC (17-19) and for other drugs (20,21). The question which drug-light interval is optimal for PDT in different situations deserves further investigation.

Other factors, which may be important in determining the efficacy of PDT are the fluence rate used for illumination and levels of tissue oxygenation. Oxygen is a necessary substrate for the photochemical reaction and in the absence of oxygen, PDT-mediated cell killing does not occur. In vitro experiments with Photofrin sensitization demonstrated that 5% oxygen is sufficient for a full effect and half-maximum photosensitization is achieved at 0.5 to 1% oxygen (22,23). Tumors often contain some hypoxic regions prior to treatment but oxygen deficits can also arise during the photochemical reaction if oxygen
consumption occurs at a faster rate than oxygen replenishment. Mathematical modeling indicates that such effects will be more pronounced for high fluence rates (24-27). Reduction of the fluence rate, while maintaining the same desired total fluence, results in increased overall treatment times, which might be impractical. An alternative approach to overcome PDT induced oxygen deficits is to intervene by increasing the oxygen status of tumors during PDT. There are a few published reports of attempts to improve PDT efficacy using hyperbaric oxygen (28) or carbogen breathing combined with a perfluorocarbon emulsion (29). Another effective method for improving tumor oxygenation, which has been used in combination with ionizing radiation, is nicotinamide and carbogen (30-32). The rationale for this combination is that carbogen overcomes diffusion limited hypoxia, whereas nicotinamide prevents cyclic closing down of tumor vessels and therefore overcomes acute, perfusion limited hypoxia (33,34).

The aims of the present study were to investigate the following aspects of PDT treatment in a human malignant mesothelioma xenograft model: 1) the relationship between drug-light interval and tumor effect for mTHPC-mediated PDT; 2) the influence of total fluence on PDT response; 3) the influence of fluence rate on PDT response; 4) the possible benefits of combining PDT with carbogen and nicotinamide.

MATERIALS AND METHODS

Animals and tumor model.

All experiments were carried out in accordance with protocols approved by the local experimental animal welfare committee and conformed to national and European regulations. Female balb/c nude mice were used for tumor implantation when they were 10 to 20 weeks old (25 ± 5 g). Mice were bred and maintained in autoclaved filter top micro isolators in our animal house facility and were fed standard laboratory pellets and acidified water ad libitum.

Human malignant mesothelioma cells (H-MESO1, from R.F. Camalier, National Cancer Institute, Maryland, U.S.A.) were taken from frozen stock and grown in vitro, in RPMI medium supplemented with 10% FCS, prior to implantation in nude mice. Cells were kept in culture for a maximum of 12 passages before returning to the frozen stock. To propagate tumors in vivo, 5 x 10^6 cells were injected s.c. in the flanks of nude donor mice. The tumors were allowed to grow to 8 to 10 mm diameter before excision and transplantation of small fragments (diameter about 2 mm) of the donor tumor to recipient experimental mice. Tumor fragments were transplanted s.c. on the lower dorsum (using a trochar) under anesthesia. Tumor bearing mice were used for PDT studies when the mean tumor diameter reached 5 ± 1 mm; this was typically 30 to 50 days after transplantation. Tumor size was then measured in 3 orthogonal diameters 2 or 3 times per week and mice were sacrificed when the mean tumor diameter reached 8 mm, or earlier if they became sick. Tumor regrowth times were calculated as the time taken to regrow from treatment size "T" to a mean geometric diameter "T + 2 mm" (i.e. a 2 to 3 fold increase in tumor volume). Cures were defined as no palpable tumor at 120 days after treatment.
Photodynamic therapy.

Tumor bearing mice were injected i.v. with 0.15 or 0.3mg.kg⁻¹ mTHPC (supplied by Scotia Pharmaceuticals, Stirling Scotland), dissolved in 25% polyethylene glycol 400 (PEG), 25% ethanol and 50% water, at a final concentration of 0.03 mg.ml⁻¹. Mice were kept in subdued lighting for a period of 2 weeks after injecting the sensitizer. Tumors were illuminated at intervals of 24, 72 or 120h after photosensitization. Illumination was performed with monochromatic light of 652 nm, produced by a medical 6 watt diode laser (Applied Optronics, South Plainfield, U.S.A.) and delivered to the tumor site by a microlens (Patrick Thilen Microtechnique, Geneva, Switzerland), using a spot size of 12 mm in diameter. Fluence rates of 20, 100 or 200 mW.cm⁻² were used and total fluences of 15 to 90 J.cm⁻² were delivered to the surface of the tumors. During illumination the mice were held in restraining jigs without anesthesia. Each treatment group contained 8 to 10 mice and separate light alone (60 J.cm⁻²) or mTHPC alone (0.15 mg.kg⁻¹) groups, as well as untreated controls, were included in the analyses.

Carbogen and nicotinamide.

The oxygen status (pO₂) of tumors of specific groups of mice was measured before and 30 minutes after injecting nicotinamide (300 mg.kg⁻¹; Sigma, St. Louis, USA). The mice were then given carbogen to breathe (95% oxygen with 5% carbodioxide) and tumor pO₂ was re-measured after 5 and 10 minutes carbogen. Gassing was done via nose cones attached to the restraining jigs, using a flow rate of 2 L.min⁻¹.

Tumor pO₂ values were measured using the Eppendorf polarographic oxygen electrode system, as previously described (35). These measurements were made in mice anesthetised with Hypnorm and Dormicum (12.5 mg.kg⁻¹ fluanisol + 0.4 mg.kg⁻¹ fentanyl citrate and 6.25 mg.kg⁻¹ midazolam hydrochloride). Prior to and between measurements, the cathode probe was calibrated in physiological saline, gassed alternately with 100% and 0% oxygen. The anode probe was then attached to the ventral surface of the mouse and ambient and tumor temperatures were taken for pO₂ measurement calibrations. The calibrated cathode probe was inserted 1 mm into the tumor and automatically advanced by 1.0 mm steps with 0.3 mm retraction. For each tumor, a total of 32 pO₂ measurements was made from 4 separate tracks, under each experimental condition. A separate group of control animals was used to investigate the effect of repeated insertion of the probe to a maximum of 16 times (tracks only). Animals were killed immediately after the last pO₂ measurements.

For studies investigating the effects of oxygenation status on PDT response, photosensitized animals (0.15 mg.kg⁻¹ mTHPC) were given nicotinamide 30 minutes before illumination, or carbogen starting 5 minutes before and during illumination (total gassing time of 10 to 20 minutes, depending on the fluence delivered) or both nicotinamide and carbogen. A fluence rate of 100 mW.cm⁻², total fluences of 30 to 90 J.cm⁻² and drug light intervals of 24 and 72 h were used for this study.
Chapter 2

Photobleaching experiments

Fluorescence spectra of tumor bearing mice were recorded before and after illumination (30, 60 and 90 J.cm$^{-2}$; 100 mW.cm$^{-2}$), applied 24 h after 0.15 mg.kg$^{-1}$ mTHPC. Measurements were done at three positions: subcutaneously near the base of the tumor, at the tumor skin surface, and normal skin outside the tumor area. Fluorescence was induced with a band pass filtered 100 W Hg-lamp (Oriel 56541), using the 405 nm emission line. The 405 nm excitation wavelength was delivered through a Y shaped fiber for simultaneous light delivery and detection. An optical multi-channel analyzer (Oriel MS257) in combination with a 256-1024 pixel CCD camera (Oriel Instaspec IV) recorded the fluorescence spectra from 530 to 812 nm, with a 3 nm resolution, and the excitation wavelength was blocked by a OG 530 nm long pass filter. Three spectra were taken at each position. After dark current subtraction the spectra were averaged. The mTHPC photobleaching after illumination is defined as the percentage of maximum mTHPC fluorescence intensity at the 650-680 nm wavelength prior to PDT. An estimation of the autofluorescence contribution to the mTHPC peak (650-680 nm) was made by interpolation of the autofluorescence intensities from 625 nm to 780 nm (36) and subtracted from the mTHPC fluorescence.

Statistical analysis

Mean time to regrowth of tumor (or last follow-up at 120 days) and associated variance were calculated by the Kaplan-Meier actuarial technique, with cures as censored observations. Differences in tumor regrowth times between groups were tested by censored rank tests, giving slightly more weight to early differences ($r=0.5$, Harrington and Fleming). No assumptions were made on the ordering (trend) within dose, fluence rate or drug light interval.

Tumor oxygenation data were analyzed for differences per tumor as experimental conditions varied (i.e. number of measurements made or the addition of nicotinamide and carbogen) and for differences between tumors (controls versus oxygenation modified). The adjusted means of median tumor $pO_2$ and percentage of values $<2.5$ mm Hg were first logarithmically transformed to obtain a normal distribution of residuals. Covariance structure to be used in the repeated measurements ANOVA (RM-ANOVA) was then selected, based on the Bayesian Information Criterion, resulting in the choice of AR(I) model. RM-ANOVA was used to calculate group means and standard errors; $p$-values were calculated from approximate type III F-tests.

RESULTS

The mean time from transplant to reach a size of 5 mm mean diameter (time "T") was $41.9 \pm 8.5$, SD) days for a group of 30 control H-MESO1 tumors (range 27 to 58 d). The mean regrowth time for established tumors to increase by 2 mm mean diameter ("T+ 2 mm") was $16.6 \pm 3.9$ days (range 10 to 23 d). Neither mTHPC alone ($0.3$ mg.kg$^{-1}$) nor light alone ($60$ J.cm$^{-2}$; $100$ mW.cm$^{-2}$) induced any delay in regrowth compared with untreated controls ($17.4 \pm 4.8$ days and $16.9 \pm 3.8$ days, respectively).

The PDT effect was strongly dependent on drug-light interval for each drug dose
Table 1. Influence of drug-light interval on tumor response to PDT

<table>
<thead>
<tr>
<th>Drug-light interval (hours)</th>
<th>0 mg.kg⁻¹</th>
<th>0.15 mg.kg⁻¹ mTHPC</th>
<th>0.3 mg.kg⁻¹ mTHPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mg.kg⁻¹</td>
<td>16.6±0.1</td>
<td>30.5±5.4</td>
<td>44.2±6.2</td>
</tr>
<tr>
<td>0.15 mg.kg⁻¹ mTHPC</td>
<td></td>
<td>57.4±12.7</td>
<td>72.2±12.4</td>
</tr>
<tr>
<td>24 h</td>
<td>64.3±12.5</td>
<td>84.0±12.5</td>
<td>(91.2±19.9)</td>
</tr>
<tr>
<td>72 h</td>
<td>61.1±11.2</td>
<td>63.5±15.4</td>
<td>0.06</td>
</tr>
<tr>
<td>120 h</td>
<td>0.0009</td>
<td>0.0001</td>
<td>0.0002</td>
</tr>
<tr>
<td>P value¹</td>
<td>0.0001</td>
<td>0.0007</td>
<td>0.0008</td>
</tr>
<tr>
<td>0.3 mg.kg⁻¹ mTHPC</td>
<td></td>
<td>22.4±2.3</td>
<td>23.1±2.6</td>
</tr>
<tr>
<td>24 h</td>
<td>19.3±1.6</td>
<td>22.9±2.3</td>
<td>0.076</td>
</tr>
<tr>
<td>72 h</td>
<td>0.0002</td>
<td>0.0004</td>
<td>0.681</td>
</tr>
<tr>
<td>120 h</td>
<td>0.0008</td>
<td>0.0003</td>
<td></td>
</tr>
</tbody>
</table>

* Means based on actuarial calculations with cures as censored observations. Each group mean is for 8 to 10 mice except group + in which 3 mice died of early toxicity.

P value¹ = significance of the influence of drug-light interval

P value² = significance of the influence of total fluence

and total fluence tested (at a fluence rate of 100 mW/cm²) (Table 1). Tumor response was always greatest for a drug-light interval of 24 h and longer intervals resulted in very little regrowth delay, relative to control values, in most treatment groups.

For drug-light intervals of 24h, long delays in tumor regrowth and some cures were seen. These data are shown as Kaplan-Meier plots for recurrence free survival in Fig. 1. Times to recurrence were generally longer for higher light doses but this was only significant for a drug dose of 0.15 mg.kg⁻¹ (Table 1), and the increased response tended to plateau at light doses >30 J.cm⁻².

The influence of fluence rate on PDT response was investigated for mTHPC doses of 0.15 mg.kg⁻¹ given 24 h before illumination. There were no significant differences in mean tumor regrowth times for illumination with fluence rates of 20, 100 or 200 mW/cm² (p=0.459, p=0.085 and p=0.364 for 15, 30, 60 J/cm², respectively) (Fig. 2). Eppendorf electrode measurements in untreated control tumors demonstrated a large variability in median pO₂ values (range 4.7-32.9 mmHg. However, in 10 of the 12 tumors tested, the addition of nicotinamide shifted the distribution histograms to higher pO₂ values and carbogen breathing further improved oxygenation.

Cumulative distribution histograms for pO₂ values for a group of 12 tumors measured before nicotinamide, after nicotinamide and after 10 minutes carbogen breathing are shown in Figure 3. The mean median pO₂ increased from a control value of 12.6 mm Hg, to 17.6 mm Hg after nicotinamide and 24.8 mm Hg after carbogen (Table 2).
Kaplan Meier plots for recurrence free survival times in mice with untreated tumors or after PDT with total fluences of 15 to 90 J.cm\(^{-2}\) (100 mW.cm\(^{-2}\)) at 24 h after mTHPC doses of 0.15 mg.kg\(^{-1}\) (top) and 0.3 mg.kg\(^{-1}\) (bottom). Each dose group comprises 8 to 10 mice.

A separate group of 12 control animals was included to investigate the effects of repeated insertion of the probe (to a maximum of 16 times). These results show that increasing number of tracks had no significant influence on tumor pO\(_2\) (p=0.93). There was, however, a significant increase in oxygenation, considering median pO\(_2\) values, in the group of animals given nicotinamide and carbogen, compared with both the baseline oxygenation level (p=0.0004) and with the tracks alone control group (p=0.015). The increase in median pO\(_2\) for nicotinamide alone (comparison of values at T1 and T2) did not reach significance (p=0.14).

The influence of increased tumor oxygenation during PDT was subsequently investigated. PDT parameters of mTHPC dose and fluence rate were kept constant (0.15 mg.kg\(^{-1}\) and 100 mW.cm\(^{-2}\)). Data for the PDT response using a drug-light interval of 24 hours are shown as Kaplan-Meier plots for recurrence free survival in Fig. 4. The tumor response to PDT was significantly increased by nicotinamide, carbogen or their combination for a total fluence of 60 J.cm\(^{2}\) (p=0.0067) (Fig. 4 B). There was no significant
Figure 2
Tumor regrowth times for fluence rates of 20, 100 and 200 mW.cm\(^{-2}\). Experiments were performed with 0.15 mg.kg\(^{-1}\) mTHPC, a drug light interval of 24 h, and total fluences of 15, 30 and 60 J.cm\(^{-2}\). Values are expressed as estimated mean regrowth times + SEM (n=8-10 per group) with censoring to include cures. Cures/number of animals per experimental group are indicated.

difference in responses for fluences of 30 J.cm\(^{-2}\) (p=0.261), although there was a trend for increased response for PDT and carbogen, with or without nicotinamide (Fig. 4 A). The long-term recurrence free survival for a fluence of 60 J.cm\(^{-2}\) increased from 11% (1/9) for PDT alone to 58% (14/24) for PDT plus carbogen with or without nicotinamide. Carbogen and/or nicotinamide in combination with a sub-optimal PDT regime (illumination 72 h after mTHPC) did not lead to an improvement in PDT response and no long-term cures were obtained, even for a fluence of 90 J.cm\(^{-2}\) (data not shown).

Fluorescence measurements in the tumor (near the base), on the skin overlying the tumor and on normal skin all demonstrated marked photobleaching (Fig. 5). It was difficult to place the measurement probes in exactly the same position at the base of the different tumors, which resulted in a large inter-tumor variation on these measurements. Mean tumor fluorescence, however, showed some dependence on total fluence and was reduced to 62% of the pre-illumination value after 30 J.cm\(^{-2}\), to 40% after 60 J.cm\(^{-2}\) and to 28% after 90 J.cm\(^{-2}\). Photobleaching in normal skin and skin over the tumor was already almost maximum after 30 J.cm\(^{-2}\). There were, however, differences between mice in the maximum extent of photobleaching (5% to 30% of pre-illumination fluorescence).
Figure 3
Cumulative histograms for pO$_2$ values measured in a group of 12 H-MESO1 tumors before and 30 minutes after nicotinamide (300 mg.kg$^{-1}$) and after 10 minutes gassing with carbogen. The mean median pO$_2$ increased after nicotinamide injection and further increased after carbogen (Table 2).
Table 2 Influence of nicotinamide and carbogen breathing on tumor oxygenation

<table>
<thead>
<tr>
<th>Time of measurement</th>
<th>Mean median pO₂ (±1SD)</th>
<th>% &lt; 2.5 mmHg (±1SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tracks only</td>
<td>Nicotinamide Carbogen</td>
</tr>
<tr>
<td>1) before nic</td>
<td>8.4 ± 6.0</td>
<td>12.6 ± 8.7</td>
</tr>
<tr>
<td>2) after nic</td>
<td>10.1 ± 9.8</td>
<td>17.6 ± 7.4</td>
</tr>
<tr>
<td>3) nic + 5' carb</td>
<td>9.7 ± 8.9</td>
<td>23.1 ± 9.2</td>
</tr>
<tr>
<td>4) nic + 10' carb</td>
<td>10.4 ± 6.2</td>
<td>24.8 ± 12.6</td>
</tr>
<tr>
<td>Change with time (P value)</td>
<td><strong>0.93</strong></td>
<td><strong>0.0004</strong></td>
</tr>
<tr>
<td>Difference between tracks alone and nic + carb (P value)*</td>
<td>0.015</td>
<td>0.034</td>
</tr>
</tbody>
</table>

* Comparison of slopes of linear increase in pO₂ or decrease % < 2.5 mm Hg with time for the two groups.

DISCUSSION

These studies dealt with the influence of drug-light interval, illumination fluence rate, total fluence and oxygenation status on the efficacy of PDT in a human malignant mesothelioma xenograft model. The results demonstrated a pronounced decrease in tumor response, assessed from growth delay and tumor control, as the drug-light interval increased beyond 24 hours. Earlier publications on the histological assessment of response of H-MESO1 xenografts to mTHPC-PDT showed that a light dose of 20 J.cm⁻² at 24 to 96 h after 0.1 mg.kg⁻¹ mTHPC induced substantial tumor necrosis without significant differences between the time intervals (38). Histological assessment of tumor response in the current study also indicated substantial tumor necrosis for drug light intervals of 24 to 120 h (results not shown). However, this did not translate into equivalent growth delays for the different treatment schedules. Extent of tumor necrosis after PDT is an efficient method for demonstrating the biological activity of different PDT schedules and results are obtained much faster than by regrowth delay or cure assays. However, the final goal of PDT is long term tumor control and it is clear that rapid tumor regrowth can still
occur after PDT treatments which produce extensive tumor necrosis. The survival of only a few tumor cells is sufficient to lead to treatment failure. For this reason, long term assays of tumor response are required in addition to short term assessment of necrosis for a proper evaluation of treatment efficacy.

The marked decrease in tumor response with increasing drug-light interval observed in this study was surprising, since other studies had demonstrated that high concentrations of mTHPC in H-MES01 tumors were maintained between 24 and 120 hours after injection of 14C labeled drug (39). The tumor drug levels increased from 0.35% injected dose per gram at 5 minutes to a maximum of 1.5% injected dose per gram at 72 hours after injection. This contrasted with the rapid decrease in plasma drug levels over this time. Plasma clearance was biexponential with $T_1$ values of 1.4 and 34.2 hours. There is a very poor correspondence between tumor drug levels and degree of photosensitization for H-MES01, as has been previously demonstrated in several other tumor models for mTHPC-mediated PDT (17-19) and for other sensitizers (20,21). This could indicate that factors other than direct tumor cell phototoxicity, possibly vascular mediated responses, are important in determining tumor response to PDT. In some cases, plasma drug levels have been found to be more important than tumor drug levels in predicting response (18,20).

It is difficult to extrapolate results obtained using animal models to the clinical situation. The H-MES01 xenografts used in these studies were grown subcutaneously in a mouse host and some necrosis was evident as the tumors grew. This is obviously different from the clinical situation, where pleural malignant mesothelioma grows as a thin sheet, usually without obvious necrosis. However, it is not possible to investigate a wide range of treatment parameters within the context of a clinical trial. Pre-clinical studies can provide useful information for further testing in a clinical setting, although caution must be used in interpretation and application of the results.

Few published clinical data are available concerning tumor response to PDT using less than 4 days between mTHPC administration and illumination. The only study in which this parameter was systemically investigated, for constant drug and light doses, also demonstrated a significant decrease in tumor response for basal cell skin carcinomas illuminated at 3 to 4 days after mTHPC compared with intervals of 1 to 2 days (40).

The improved tumor responses seen with shorter drug-light intervals do not necessarily imply that these time intervals offer the best therapeutic advantage, since this takes no account of normal tissue damage. For PDT used as additional treatment of large surfaces, such as is generally used for malignant mesothelioma, it is important to achieve selective tumor destruction. Several preclinical (38,41-43) and clinical studies (44,45) have demonstrated a more selective mTHPC-PDT response for drug-light intervals longer than 2 days.(1,44). For large surface PDT it seems prudent to continue to use these long time intervals to minimize the risk of excessive normal tissue damage. In our view, however, the use of shorter drug-light intervals with reduced drug doses has been insufficiently explored. For PDT of small localized tumors this could well be a feasible approach to maximize long term tumor response in situations where only a small margin of normal tissue is to be illuminated.

In these experiments the influence of drug-light interval was greater than the influence of total fluence. In general, there was little further increase in tumor response for fluences of $> 30 \text{ J/cm}^2$. This implies that the photochemical reaction was relatively inefficient with large total fluences. The plateauing of tumor response with large total fluences was much more pronounced for the low sensitizer dose, which suggests that
photobleaching may be involved. mTHPC has been shown to be highly susceptible to photobleaching both in vitro (46,47) and in vivo (48). Our own studies confirmed that a fluence of 60 J.cm⁻², applied 24 h after 0.15 mg.kg⁻¹ leads to > 60% bleaching of the drug in tumor and normal tissue in this model. The photobleaching of mTHPC may have reduced the drug levels below the threshold required for a photochemical reaction to occur, rendering further illumination ineffective. Increasing the light dose was therefore not able to compensate for sub-optimal drug dosing schedules.

Another possible explanation for the lack of additional tumor response with large total fluences could be the development of PDT induced oxygen deficits. Experimental studies have demonstrated that oxygen depletion can occur within 1 minute of tumor illumination after photosensitization with hematoporphyrin derivatives or Photofrin, (26,27,49). This phenomenon of oxygen depletion can be more pronounced at high fluence rates (26,50). No significant fluence rate effect was seen, in our tumor response.
Figure 5
Photobleaching on 3 different locations (normal skin, tumor skin and at the base of the tumor) Values are expressed as the percentage of fluorescence measured before illumination. Each bar represents the mean ± standard deviation of 4-6 measurements. PDT was performed with 0.15 mg.kg⁻¹ mTHPC given 24 h before illumination with 100 mW.cm⁻² and total fluences of 30, 60 or 90 J.cm⁻².

Despite the lack of evidence for a PDT induced oxygen deficit in the current studies, tumor response was significantly improved by providing external carbogen and or nicotinamide at the time of PDT. Other studies using the porphyrin sensitizer meta-tetrahydroyxophenylporphyrin (m-THPP) also demonstrated that the response of intracranial mouse gliomas to PDT increased when mice were given 100% oxygen during treatment (51). Another small pre-clinical study demonstrated improved tumor response for human rectal carcinoma xenografts treated with PDT under hyperbaric oxygen (28). Recently published results of a clinical pilot study also show an advantage for this combination in treatment of advanced esophageal cancer (52). In this clinical study the 12 month survival of 17 patients treated with a hematoporphyrin derivative PDT plus hyperbaric oxygen was 41% compared with 29% in a group of 14 patients treated with PDT alone (p=0.06).
In conclusion, these studies have demonstrated that the response of H-MESO1 xenografts to mTHPC was strongly influenced by the drug-light interval but was not influenced by fluence rate over the range 20 to 200 mW.cm$^2$. Despite the lack of evidence for PDT induced oxygen depletion, the combination of carbogen and nicotinamide did improve PDT tumour response.

Acknowledgements

The authors are grateful to Scotia Pharmaceuticals for supplying the mTHPC and Harm van Tinteren and Guus Hart for statistical analysis. The work was supported by a grant from the Dutch Cancer Foundation (Project NKI 97-1446).
REFERENCES