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Interstitial photodynamic therapy – diagnostic measurements and treatment in rat malignant experimental tumours

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ABSTRACT

A recently developed multiple fibre system for treating malignant tumours with interstitial photodynamic therapy was used in studies on rats with colon adenocarcinoma inoculated into the muscles of the hind legs. The animals were intraperitonially administrated δ-aminolevulinic acid (ALA), which is metabolised to protoporphyrin IX (PpIX) in the tissue. The treatment system consists of a laser light source, a beam-splitting system dividing the light into three or six output fibres and a dosimetry programme calculating the optimal fibre position within the tumour as well as the treatment time needed to obtain a given threshold value of the light dose. One aim of the study was to compare the treatment outcome with the modelled dosimetry predictions. Tumour reduction was examined three days *post* treatment. A volume decrease was found in 85% of the treated tumours. The mean volume reduction was 44%, with one tumour completely disappearing. Histopathological examination three days *post* treatment showed substantial necrotic parts which, however, to a smaller extent were present also for non-treated tumours. These results indicated that the tumours have been under treated and the light dose has to be increased. Measurements of the build-up and photo-induced bleaching of PpIX using laser-induced fluorescence were also performed during the experiments.

Keywords: interstitial photodynamic therapy; laser-induced fluorescence; ALA; PpIX

1. INTRODUCTION

Cancer is today a fairly common disease. Recent reports show that the cases of malignant tumours are increasing. In the search for new treatment modalities, photodynamic therapy (PDT) seems to be a conceivable alternative.[1,2] A photosenzitising drug is administrated intravenous, orally or topically and is accumulated in the tumour to a higher degree than the surrounding normal tissue. When the tissue is irradiated with light, a photochemical reaction, which involves the excitation of triplet oxygen to its singlet state, is induced. Singlet oxygen is very toxic and will thus induce tumour cell death. The method has several advantages. It offers a safe and efficient treatment with a short healing period and no or minor cosmetic damages in the treatment of e.g. skin malignancies.[3,4] The treatment can be performed with minimal side effects and it can easily be performed on an outpatient basis. PDT has so far mainly been used for superficial lesions. This is due to poor light penetration through the tissue. To enhance the method new photosensitizers are under development and evaluation. A new report where esterified δ-aminolevulinic acid (ALA-me) was used shows promising results.[5] Another alternative would be to use sensitizers with a high absorption in the near IR-wavelength range since the penetration of light in tissue is better for this wavelength region.[6] Still the treatment depth will not be more than 3-7 mm. To be able to treat thicker and/or deeper lying tumours interstitial PDT can be used.[7,8] This is performed by guiding the light into the tumour mass via a number of optical fibres inserted in the tumour. This gives a selective local treatment, which minimizes the effect on the surrounding healthy tissue.

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In this study a system for treating malignant tumours interstitially was used to treat colon adenocarcinoma inoculated into the muscle of the hind leg of Wistar/Furth rats. One aim of the present experimental study was to compare the treatment outcome with predicted dosimetry parameters given by the dosimetry programme.

2. MATERIALS AND METHODS

Briefly, the system consists of a diode laser light source, a beam-splitting unit dividing the light into three or six pathways and focusing the light down to optical fibres that are inserted into the tumour. Further, a software unit automatically controls the beam-splitting unit, and a dosimetry programme calculates the optimal fibre positions and optimal treatment time given a threshold light dose. As a photosensitizer δ-aminolevulinic acid (ALA), which in converted to protoporphyrin IX (PpIX) in the cells via the heam cycle, was used. During the treatment the build-up and photo-induced bleaching of PpIX were measured using laser-induced fluorescence (LIF). Volume reduction was examined three days *post* the interstitial PDT treatment. Histopathological changes were then also examined.

2.1. Animals and drug

In total 13 Wistar/Furth rats, each weighting approximately 250 g were treated (three more rats were included but treatment cloud not be successfully completed for these). The original cell line of the adenocarcinoma was induced in each of the hind legs of the rats by injection of cell suspension. After seven to eight days, the rats had developed a tumour with a volume of 19.1±5.9 cm^3 on each hind leg. One of the tumours was treated while the other served as a control tumour for the histopathological and statistical tumour examination. The rats were intraperitonially administrated with δ-aminolevulinic acid (Porphyrin Products, Logan UT, USA; Lot no.101598) at a dose of 100 mg/kg body weight 60 minutes before treatment. All animals were under general anaesthesia during the procedure. They were put asleep using chloral hydrate at a dose of 5 ml/kg body weight. A dose of 0.1-0.2 ml Temgesic was used for pain relieving.

2.2. Interstitial photodynamic system

Interstitial PDT treatment was performed using a multiple-fibre system recently developed at the Department of Physic, Lund Institute of Technology.[9] The system consists of a light source, which is a CW InGaAsP diode laser designed for medical use (CeramOptec Ceralas PDT 635). The laser emits light at a wavelength of 635 nm and has a variable output power in the range of 0-2 W. The light is guided into an optical fibre with a core diameter of 600 μm (KP-600L, ANDA, Latvia) leading to the beam-splitting system, which divides and focuses the light into three or six individual output fibres. It also enables measurements of the light fluence rate within the tumours via the same fibres using six separate photo diodes mounted on flip-in stages at the optical fibre connectors. When the detector is flipped in the laser light into the fibre is blocked. The photo diodes are connected to a computer board with a multiplexed 10 bit ADC. These signals thus enable feedback light dosimetry and treatment control. A computer with a dosimetry software is used to control and automatically operate the beam-splitting unit. The programme also performs necessary dosimetry calculations. It calculates the optimal fibre positions and minimum treatment time using given tissue optical parameters such as the absorption coefficient, scattering coefficient and anisotropy factor of the tumour and surrounding tissue, as well as the tumour size, shape, light dose for successful treatment and output powers from the fibres.

2.3. Interstitial photodynamic therapy procedure

After 60 minutes of build-up time of PpIX following intraperitonially injection of ALA the animals were treated with interstitial PDT. The time interval was based on kinetic studies.[10,11] The skin covering the tumours was shaved to avoid hair in the operating field. The tumours were then exposed by removing the skin. The treatment fibres were carefully placed in the calculated positions as accurately as possible. Also a fibre for fluorescence measurements was inserted in a position in the tumour far from the source fibres. The fibres had a core diameter of 600 μm (KP-600L, ANDA, Latvia). Figure 1 shows a situation where a tumour is treated with three fibres inserted in the tumour mass. The fibres delivered an output power in the range of 50-160 mW each. The treatment time was 5 to 40 minutes depending on the given light threshold dose, size and shape of the tumours and the output from the fibres. The treatment was interrupted in time intervals of 60 seconds to measure the light fluence rate and the photo bleaching of PpIX within the tumour. The animals were sacrificed three days *post* treatment and both control and treated tumour were removed for histopathological examination. The animals were divided into three groups with different treatment parameters; see Table 1.

Table 1 The different groups of the animals.

Group number	*Number of rats*	*Number of fibres*	*Total estimated absorbed light dose in the tumour region farthest from the light source (J/cm^3)*
1	3	6	15
2	4	3	7.5
3	6	6	15

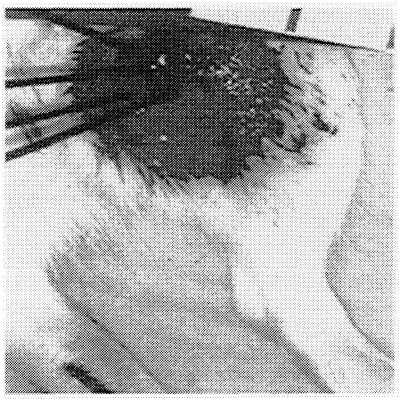

Figure 1 A treatment situation where a tumour is treated with three optical fibres inserted in the tumour mass. The fourth fibre is the one measuring fluorescence.

2.4. Laser-induced fluorescence (LIF)

The PpIX build-up and the PDT-induced bleaching of the photosensitizer during treatment were measured with an optical fibre-based fluorescence system described in detail by af Klinteberg *et al.*[12] The excitation source was a nitrogen-laser pumped dye laser with an excitation wavelength of 405 nm (Laser Science Inc, Cambridge, MA, model VSL-337 and DML-110, respectively) and a pulse repetition rate of about 15 Hz. The excitation light was focused into a fused silica optical fibre with a core diameter of 600 μm (Fiberguide Ind., Stirling, NJ, model SFS600N). The distal end of this fibre was positioned in the tumour as described above. The fluorescence light from the tissue was guided back to the instrument through the same fibre and focused on the entrance slit of a spectrometer (Oriel Corp., Stratford, CT, model MS125). An image-intensified diode array detector (Andor Technology, Belfast, Northern Ireland model DH501-25U-01) was used to record the fluorescence spectrum ranging from 400 to 800 nm. The fluorescence from twenty laser pulses was integrated to obtain spectra with high signal-to-noise ratios. The recorded spectra were displayed on a screen and stored in a computer for later evaluation. Fluorescence spectra were measured just before and during the treatment. A cuvette with Rhodamine 6G was used as an intensity standard and was measured before and after each treatment of the animals.

2.5. Volume reduction

The size of the tumours was measured with sliding callipers just before the treatment and at the time when the animals were sacrificed and the tumours were removed. The tumour volumes were calculated with the formula $(a \times b^2) \times \pi \times 4/3$, where a is the maximum diameter and b is the minimum diameter of the tumour. The growth rate of the individual tumours was calculated by forming the ratio between tumour volume *post* treatment divided by the tumour volume three days *pre* treatment. This value was used as an estimation, together with the histopathological examination, of the therapeutic efficiency.

2.6. Histopathlogical examination

Both treated and control tumours were histopathologically examined. The tumours were fixed in 4 % formaldehyde immediately following removal and embedded in paraffin. Heamatoxylin-eosin was used to stain sections, which were then examined under a microscope by a pathologist. The pathologist used lattice ocular to obtain the fraction of necrosis in the tumours.

3. RESULTS

3.1. Dosimetry predictions

To test the stability of the dosimetry programme some data simulations were made. The programme was used to calculate the treatment time for a homogenous tumour with a shape of a sphere. Six fibres with an output power of 100 mW each were used. The fibres were placed in the following position (x, y, z); fibre 1 ($r/\sqrt{2}$, 0, 0), fibre 2 (0, $r/\sqrt{2}$, 0), fibre 3 ($-r/\sqrt{2}$, 0,

0), fibre 4 (0, -r/√2, 0), fibre 5 (0, 0, r/√2) and fibre 6 (0, 0, -r/√2), where r is the tumour radius. This was assumed to be close to the optimal fibre positions for this geometry. The tissue optical parameters used were; μ_s=15.00 mm^{-1}, μ_a=0.07 mm^{-1} and g=0.9 for the tumour and μ_s=15.00 mm^{-1}, μ_a=0.05 mm^{-1} and g=0.9 for the surrounding tissue, where μ_s is the scattering coefficient, μ_a the absorption coefficient and g is the anisotropy factor. The treatment time was calculated for different tumour radius for an absorbed threshold light dose of 15 J/cm^3. The results obtained from the dosimetry programme were compared with theoretical calculations of the treatment time obtained from the fluence rate, $\phi(r)$, using the analytical solution to diffusion theory in an infinite medium with the same optical properties as the tumour. Figure 2 shows the treatment time as a function of the tumour radius calculated with the dosimetry programme and the results using diffusion theory. This shows a good agreement and thus a good stability of the programme.

As an example of the dosimetry calculation obtained from the dosimetry programme, the fibre positions in one treated tumour are shown in Figure 3. The tumour had a shape of an ellipse with the size 19×15.5 mm, maximum and minimum diameter, respectively. The calculated fibre positions (x, y, z) were; fibre 1 (-0.7, 6.0, 0.7), fibre 2 (4.5, -2.2, 0.0.), fibre 3 (-3.0, -4.5, 0.7), fibre 4 (6.0, 2.2, -0.7), fibre 5 (-6.0, 0.7, -0.7) and fibre 6 (-0.7, -1.5, 0.0). The values are given in mm and are calculated from the centre of the tumour. The last co-ordinate indicates the depth in the tumour. Output powers from each fibre were 130, 120, 160, 110, 130 and 140 mW, respectively. The threshold light dose was set to 15 J/cm^3, which gave a resulting treatment time of 15 minutes. Figure 4 shows the evolution of delivered light dose, which can be followed during the treatment.

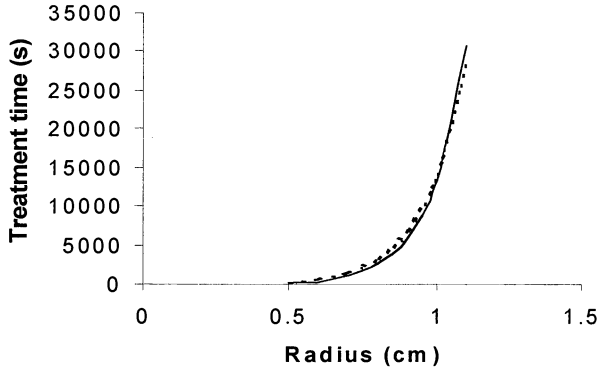

Figure 2 The treatment time as a function of tumour radius calculated using the dosimetry programme (solid line) and theoretic calculations using diffusion theory (dotted line).

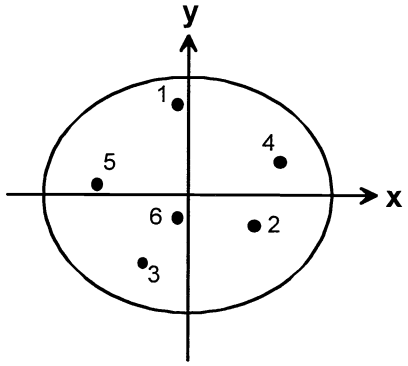

Figure 3 The optimal fibre positions given by the dosimetry programme.

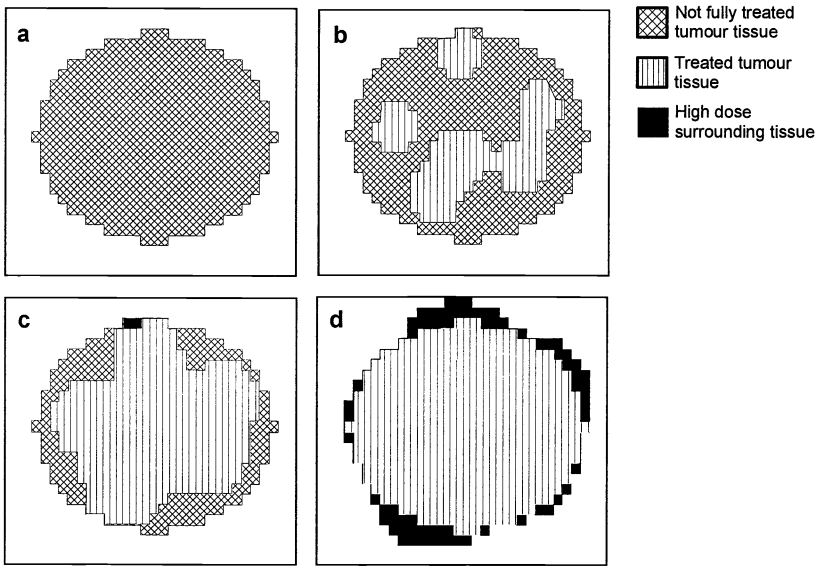

Figure 4 The evolution (a-d) of delivered light dose.

3.2. Photodynamic effects

The histopathological result showed that on an average 70% of the treated tumour mass was necrotic. As a reaction to the treatment a large amount of polymorphonuclear leucocytes were found in the necrotic area. The examination also showed that the area around the tumours was well supported with blood vessels, supplying the tumour with oxygen. Possible channels produced by inserting the optical fibres in connection with the treatment from the fibres were found in some samples. As an example a histopathological section of one treated tumour is shown in Figure 5. Necrotic parts were also present to a smaller extent (on an average 38% of the tumour mass) in the control tumours (spontaneously).

The evaluated relative tumour volumes three days *post* treatment are given in Figure 6. The results from 13 rats are shown. Among the treated tumours 85% decreased in volume, with one tumour completely disappearing. The mean volume reduction of all treated tumours was 44%. Two tumours increased their volume. Volumes of the control tumours were on an average unchanged.

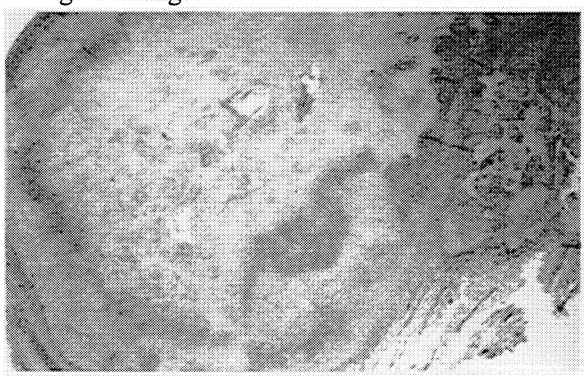

Figure 5 The treated tumour showing approximately 90% necrosis (light grey region in the centre of the picture) and bands of polymorphonuclear leucocytes. The right top of the picture shows vital tumour.

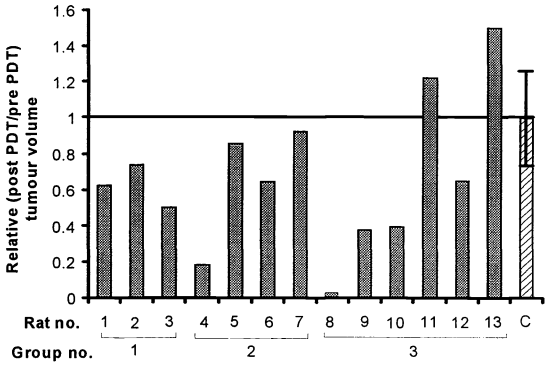

Figure 6 The relative tumour volume. 85% of the tumours decreased their volume. The over-all tumour reduction was on an average 44%. C indicates the average of the control tumours ± standard deviation.

3.3. Photo bleaching

Measurements with laser-induced fluorescence of photo bleaching of PpIX indicated that the sensitizer was in all cases completely bleached away as a result of the PDT treatment. Figure 7 shows the photo bleaching of the 635 nm peak of PpIX and in Figure 8 the whole spectra are shown.

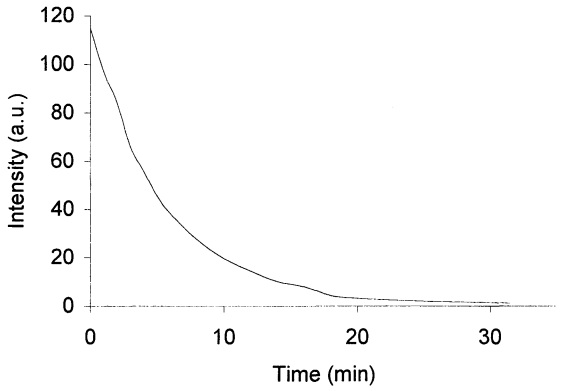

Figure 7 Photo bleaching of the PpIX peak at 635 nm measured with LIF during the interstitial PDT treatment.

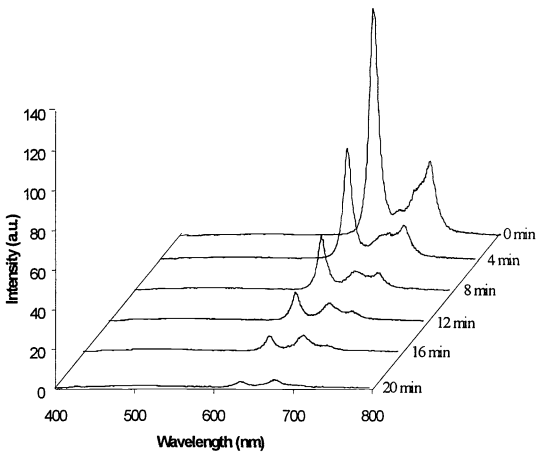

Figure 8 LIF spectra for PpIX bleaching measured during treatment.

4. DISCUSSION

Interstitial photodynamic therapy is a fairly new method of treating large tumours with very good treatment efficiency. The method has the potential of providing good selectivity with large damage to the target tissue while the effects on the surrounding tissue are minimized. The method offers a possibility to treat tumours that are large and difficult to reach with superficial illumination. Several reports in the literature, including this one, show that interstitial PDT is a promising method. [8,13]

The system used in these experiments has several unique features. It calculates the optimal positions of the fibres to obtain an effective treatment. These calculations are based on the size and shape of the tumour and the output effect from each fibre. The dosimetry programme also calculates the treatment time for a given threshold light dose. The treatment time is sufficiently long to obtain tumour necrosis but short enough so that the effect on the surrounding tissue is minimized.

The light dose delivered to the tissue is graphically illustrated on the computer screen and can be followed during the treatment. When a high dose is reached in one part of the tissue the light delivered in this part can be switched off by closing the output gate for the corresponding optical fibre. Measuring the fluence rate inside the tumour as well as the photo bleaching offers a feedback into the dosimetry and a better treatment control. When the fluorescence from the photosensitizer is too low it is no longer useful to continue and the treatment can therefore be interrupted.

An aim of this study was to compare the predicted light dose with the treatment outcome. The results from the histopathological examination showed that in average 70 % of the treated tumour mass was necrotic. This indicates that the threshold light dose has to be increased to make the treatment more efficient. Necrotic areas were also found in the control tumours. This is probably due to the fact, that tumours had grown too large, which for this tumour model induces necrosis. To be able to better correlate the necrotic tumour volumes with the treatment parameters, a better tumour model would be desirable. Another possible improvement would be to increase the accuracy with which the optical fibres were positioned. For this purpose an ultra-sound imaging system can be used, which will also give better measurements of the size and shape of the tumour.

Further improvements of the system are planned. Optical fibres with a smaller core diameter are preferable, causing less mechanical damage on the tissue. Then an improved light source with ability to effectively couple the light into smaller optical fibres is also needed. Sensitizers absorbing light in the near IR wavelength range will allow an increase in the penetration depth of the light and larger tumours could thus be treated. New experiments along these lines are planned for the future.

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