Evaluation of fluorescence measurement techniques for tumour detection in vivo
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CHAPTER 7

Effective treatment of liver metastases with photodynamic therapy, using the second generation photosensitizer meta-tetra(hydroxyphenyl)chlorin (mTHPC), in a rat model.

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submitted to Br J Cancer
Abstract

The only curative treatment for patients with liver metastases to date is surgery, but few patients are suitable candidates for hepatic resection. The majority of patients will have to rely on other treatment modalities for palliation. Photodynamic therapy (PDT) could be a selective, minimally-invasive treatment for patients with liver metastases. We studied PDT in an implanted colon carcinoma in the liver of Wag/Rij rats, using the photosensitizer meta-tetra(hydroxyphenyl)chlorin (mTHPC). mTHPC tissue kinetics was studied using ex vivo extractions and in vivo fluorescence measurements. Both methods showed that mTHPC kinetics were different for liver and tumour tissue. After initial high levels at 4 hours after administration (0.1 and 0.3 mg kg$^{-1}$) mTHPC in liver tissue decreased rapidly in time. In tumour tissue no decrease in photosensitizer levels occurred, with mTHPC remained high up to 48 hours after administration. Both concentration data and fluorescence data showed an increase in tumour to liver ratios of up to 6.3 and 5.0, respectively. Illumination with 652 nm (15 J) resulted in extensive damage to tumour tissue, with necrosis of up to 13 mm in diameter. Damage to normal liver tissue was mild and transient as serum ASAT and ALAT levels normalized within a week after PDT treatment. Long-term effects of mTHPC-PDT were studied on day 28 after treatment. Regardless of drug dose and drug-light interval, PDT with mTHPC resulted in complete tumour remission in 27 out of 31 treated animals (87%), with only four animals in which tumour regrowth was observed. Non-responding tumours proved to be significantly larger (p<0.001) in size before PDT treatment. This study demonstrates that mTHPC is retained in an intrahepatic tumour and that mTHPC-PDT is capable of inducing complete tumour remission of liver tumours.
Introduction

Colorectal cancer is the third leading cause of cancer death in western communities. At the time of death approximately two-thirds of patients with colorectal carcinoma will have liver metastases (Welch and Donaldson, 1979). Median survival of untreated patients with liver metastases ranges from 6 to 10 months, mostly depending on the number and size of the metastases (Cady, 1983). Resection of colorectal liver metastases, the only curative treatment to date, is applicable in 10% of all patients, only (Ballantyne and Quin 1993). The majority of patients will have to rely on other, mainly palliative treatment modalities, of which none of them have proven to be of real benefit to the patient with irresectable liver metastases (Bush and Kemeny 1995). Interstitial photodynamic therapy could be an effective, minimally-invasive treatment for patients with a few liver metastases. Photodynamic therapy (PDT) is a treatment modality for cancer, in which a photosensitizing drug (photosensitizer) is administered and subsequently illuminated with light of a specific wavelength, matching an absorption peak of the drug. Upon illumination the photosensitizer becomes activated and reacts with available oxygen, causing the production of reactive oxygen species, leading to vascular damage and direct cellular damage (Star et al. 1986, Henderson and Dougherty, 1992). Light used in PDT treatment can be delivered selectively to target tissue via optical fibres placed in the tissue; a treatment called interstitial therapy (Marijnissen et al. 1992). Next to a photochemical reaction, the activated photosensitizer can emit light useful for detection of sensitized tissue (photodiagnostics). In vivo fluorescence measurements can be used to study photosensitizer kinetics non-invasively (Braichotte et al. 1995a).

Clinically, PDT is mainly used for treatment of superficially located malignancies, such as lung, skin, bladder, oesophagus and head and neck cancer (Schuitmaker et al. 1996). It has rarely been used to treat deep-seated malignancies, like liver metastases. The use of PDT for liver neoplasms has been limited as most photosensitizers are efficiently accumulated in normal liver tissue, not leading to selective uptake into malignant tissue. Also, liver tissue, being a highly pigmented tissue, limits deep penetration of light and thus treatment volumes. Experimental studies, using first generation photosensitizers Haematoporphyrin derivative (HpD) and Photofrin, have shown PDT to be capable of inducing tumour destruction within the liver (Holt et al. 1985, van Hillegersberg et al. 1992), despite limitations like non-selective uptake and limited light penetration. New, second generation photosensitizers could possibly establish a more selective accumulation in tumour tissue and, when absorbing at longer wavelength (> 650 nm), could result in larger volumes of necrosis. In a previously performed study we used the photosensitizer bacteriochlorin a (BCA), which has an absorption maximum at a wavelength of 760 nm (Rovers et al. 1998). Due to deeper penetration of 760 nm light, we were able to induce lesions of up to 16 mm in diameter with a single, plain cut fibre (diameter 0.4 mm). Although extensive tumour necrosis was induced by BCA-PDT, islands of viable tumour cells remained, leading to tumour regrowth in due time. Because of this we decided to use a more potent photosensitizer, which is meta-tetra(hydroxyphenyl)chlorin (mTHPC).

mTHPC is a single and pure substance with a high absorption peak at a wavelength of 652 nm. Recently, mTHPC has shown to be a very effective photosensitizer in various tumour models and clinical trials (Mlkvy et al. 1997, Peng et al. 1995, Lofgren et al. 1994, Ris et al. 1991, Dilkes et al. 1996, Grosjean et al. 1996), with possible preferential uptake in a colon carcinoma in mice compared to liver concentrations (Whelpton et al. 1995). Furthermore, mTHPC drug and light doses needed to induce tumour necrosis are much lower than that of HpD (Berenbaum et al. 1986).
Aim of this study is to determine mTHPC distribution in tumour and adjacent liver tissue, via tissue extractions and in vivo fluorescence measurements, and to assess short-term and long-term effects of mTHPC-PDT treatment in a rat liver tumour model.

Material and methods

Animals and Tumour model
A total of 66 male Wag/Rij rats (Charles River, Sulzfeld, Germany), weighing 200 - 240 gram, were used in these experiments. The animals had free access to food and water. The experiments were approved by the Animal Welfare Committee of the Leiden University Medical Centre and the animals received care in accordance with established guidelines.

We used the CC531 cell line, which is a chemically-induced adenocarcinoma of the rat colon, moderately differentiated, syngeneic and transplantable to Wag/Rij rats, for tumour induction in the liver (Marquet et al. 1984). Tumour cells were cultured on RPMI 1640 (Dutch modification) supplemented with 2 mM L-glutamine (Gibco, Grand Island, NY, USA), 10% heat inactivated fetal calf serum, 100 U ml⁻¹ penicillin and 0.1 mg ml⁻¹ streptomycin sulfate. At laparotomy, 5 x 10⁵ tumour cells were injected subcapsulary into the liver. For the distribution study three tumours per rat were induced (left lateral lobe, upper right lobe and lower right lobe), whereas for the PDT efficacy studies one tumour per rat was induced (left lateral lobe). Animals were treated ten days after tumour cell injection, when tumours had reached a diameter of 5 to 7 mm.

Experimental design
In the first part of the study we investigated mTHPC distribution in tumour and liver tissue at different time intervals after intravenous administration. All rats (n=20) were administered 0.3 mg kg⁻¹ bodyweight mTHPC via the femoral vein and they were randomly assigned to four groups. Animals were killed 4, 24, 48 or 72 hours after mTHPC administration, after which the liver was removed and tumours were dissected. Tissue samples were immediately frozen in liquid nitrogen and stored at -20° C until mTHPC analysis was performed.

In the second part of the study we measured in vivo fluorescence levels in tumour and liver tissue after mTHPC administration and, subsequently, determined the effect of interstitial illumination. All animals (n=46) were treated 9 ± 1 days after tumour inoculation. They were randomly assigned to four treatment groups (n=10 per group) and one control group (n=6). Illumination was performed at 4, 24, 48 or 72 hours after mTHPC administration. In each treatment group animals received either a dose of 0.1 mg kg⁻¹ or 0.3 mg kg⁻¹ bodyweight mTHPC and animals in the control group received either light illumination only or mTHPC administration (0.3 mg kg⁻¹) only. At laparotomy, prior to light illumination, in vivo fluorescence measurements were performed on liver and tumour tissue. To measure photosensitizer bleaching, immediately after illumination fluorescence of tumour tissue was determined. Before treatment tumour sizes were measured using sliding callipers and calculated using the formula: ¼ π R₁ R₂, where R₁ and R₂ are diameters perpendicular to each other.

To assess short term effects of PDT treatment, in each treatment group two animals, one of each mTHPC dose, were killed 48 hours after illumination (n=8). Sizes of induced damage were measured and livers were sectioned for histological examination. All other animals (n=32) were allowed to survive for 28 days after PDT treatment, to assess long term effects of PDT treatment. 28 days after PDT treatment, animals were killed and the livers were removed. Macroscopically tumour sizes were determined and microscopically the presence of viable tumour cells was
examined to assess tumour response: no viable tumour cells present was considered to be a complete remission (CR), whereas presence of viable tumour cells and tumour growth was considered to be no response (NR) to PDT treatment.

To determine serum levels of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) as a parameter of liver damage, blood samples (0.5 ml) were taken by orbital puncture immediately before and 1, 3, 7, 14, 21 and 28 days after PDT treatment.

Photosensitizer and Light delivery
Meta-tetra(hydroxyphenyl)chlorin (mTHPC) was kindly donated by Scotia Pharmaceuticals Ltd. (Guildford, UK). mTHPC (dry, purple crystals) was dissolved in 20% ethanol (96%), 30% polyethylene glycol (PEG) and 50% water. Animals were kept in subdued light after mTHPC administration to avoid possible side effects. For light illumination, an argon pumped dye laser (Spectra Physics Lasers, Mountain View, USA), with sulphorodamine B as dye, was tuned to emit light of 652 nm. Laser light was coupled into two quartz fibres with a core diameter of 0.6 mm, allowing simultaneous illumination of two animals. At laparotomy the liver was mobilized, tumours were exposed and a plain cut fibre was positioned on the tumour surface. Light illumination, with a power output of 100 mW per fibre, was performed for a period of 150 seconds, delivering an energy of 15 J to the surface of each tumour.

Fluorescence measurements in vivo
Fluorescence was measured with a setup previously described by Sterenborg et al. (1996, Chapter 2). In short, a Hg-lamp was used as light source and the excitation wavelength was 405 nm, selected through an interference filter (Oriel 56541). Excitation light and fluorescence were delivered to and from tissue through a bundle of optical fibres (200 μm) put in contact with the tissue. Fluorescence was detected at two wavelength ranges: red fluorescence (630 - 750 nm) was detected with a long-pass filter (Schott RG 630) and a red-sensitive photomultiplier tube (Hamamatsu R 636-10), and autofluorescence (550 - 600 nm) with a 600 nm cut-off glass filter, a long-pass filter (Schott KV 550) and a green-sensitive photomultiplier tube (Hamamatsu IP 128). A standard lock-in technique was used. A fluorescence ratio (FR) was calculated between the two detected fluorescence intensities to correct measurements for changes in excitation light intensity and measurement geometry. Five measurements were performed per tissue, repositioning the fibre between each measurement, of which the mean ± standard error of the mean (sem) FR’s was calculated.

mTHPC concentration determination
mTHPC concentrations were determined using standard extraction techniques. Briefly, frozen tissue samples were weighed and mechanically homogenized in three ml dimethyl sulfoxide (DMSO). The homogenate was centrifuged (5000 RPM for 10 minutes) and fluorescence in the supernatant was determined (excitation 420 nm, emission 650 ± 10 nm) using a standard spectrofluorometer (Aminco SPF 500) and converted into concentration by interpolation in a standard curve constructed with known mTHPC concentrations. After correction for sample weight, mTHPC concentrations were expressed as μg mg⁻¹ wet tissue. For each animal the T/L concentration ratio was calculated and per treatment group the mean (± sem) T/L-ratio was calculated.

Histological examination
Livers were fixated in a 3.6% buffered formalin solution, sliced through the largest diameter of the tumours, embedded in paraffin wax and sectioned (4 μm). Sections were stained with haematoxylin-
eosin (HE) and examined by a pathologist to detect presence of viable looking tumour cells and to examine induced damage to tumour and surrounding liver tissue.

Statistical analysis

All values were expressed as mean ± sem. The unpaired Student’s t-test was used to evaluate differences in mTHPC concentration, fluorescence levels and T/L-ratio’s between the different time intervals after mTHPC administration and between the two doses of mTHPC used in the experiments. A P-value of < 0.05 was considered to be statistically significant.

Results

mTHPC concentration and in vivo fluorescence measurements

Figure 1 shows that mTHPC concentrations in liver tissue were highest 4 hours after administration, with no significant difference between liver and tumour tissue concentrations, as illustrated by a T/L-ratio of 0.9 (Table 1). Concentrations in tumour tissue were highest 24 and 48 hours after mTHPC administration. mTHPC concentrations in liver tissue decreased rapidly in time, whereas mTHPC concentrations in tumour tissue declined slowly, resulting in a significant difference between liver and tumour mTHPC concentrations at 24 (p=0.04) and 48 (p=0.006) hours after administration.

![Figure 1: mTHPC tissue concentrations determined using ex vivo extractions. The graph represents the mean (± sem) mTHPC concentration in liver and tumour tissue at 4, 24, 48 and 72 hours after intravenous administration of mTHPC. Significantly higher mTHPC concentrations were detected in tumour tissue than in liver tissue at 24 (p=0.04) and 48 (p=0.006) hours after drug administration. Difference in tissue concentrations at 72 hours after administration was not significant (p=0.06). All values are the mean of 5 animals, with at least two measurements per tissue per animal.](image)

The mean T/L-ratio increased up to 6.3 at 72 hours after mTHPC administration.

In vivo fluorescence measurements showed comparable results to extraction data, as illustrated in Figure 2; highest fluorescence ratios (FR) in liver tissue were found 4 hours after mTHPC administration with a rapid decrease in time. From 24 hours on, FR’s were significantly higher (p<0.01) in tumour tissue than in liver tissue. The FR’s in tumour tissue remained high, whereas in liver tissue they decreased rapidly, as clearly indicated by retention of fluorescence (Figure 3).
Retention represents the FR as a percentage of FR measured at 4 hours after mTHPC administration. Retention in tumour tissue was 93% and 109%, for 0.1 and 0.3 mg kg\(^{-1}\) mTHPC respectively, 24 hours after administration and decreases to 52% and 68% at 72 hours, whereas retention in liver tissue was 25% and 40% at 24 hours and 12% for both drug doses at 72 hours after administration. The T/L-ratio's of fluorescence data were comparable to concentration ratios, as shown in Table 1.

**Figure 2a**

![Graph of fluorescence ratio in liver tissue](image)

**Figure 2b**

![Graph of fluorescence ratio in tumour tissue](image)

*Figure 2: Fluorescence ratio's in liver and tumour tissue. Fluorescence ratio (FR) is the ratio of red fluorescence (630 - 750 nm) over the autofluorescence (550 - 600 nm), to compensate for tissue optical properties. The graphs represent the mean (± sem) FR in (a) liver and (b) tumour tissue 4, 24, 48, and 72 hours after administration of 0.1 or 0.3 mg kg\(^{-1}\) mTHPC. In liver tissue there is a significant decline in the FR in time (4 to 24 hours; \(p=0.007\) and \(p<0.001\), 24 to 48 hours; \(p=0.003\) and \(p=0.006\), 48 to 72 hours; \(p=0.006\) and \(p=0.007\), for 0.1 and 0.3 mg kg\(^{-1}\) mTHPC respectively). In tumour tissue there was no significant decline in the FR, except for the FR 48 hours after 0.3 mg kg\(^{-1}\) of mTHPC (\(p=0.005\)). FR values are the mean of 5 animals per treatment group.*
Table 1: Tumour to liver ratio's at different time intervals after mTHPC administration. The mean (± sem) T/L-ratio's were calculated using ex vivo extraction data and in vivo fluorescence data (FR). For in vivo fluorescence measurements 0.1 and 0.3 mg kg⁻¹ mTHPC was administered, whereas for concentration determinations only 0.3 mg kg⁻¹ mTHPC was given. All values are the mean of 5 animals.

<table>
<thead>
<tr>
<th>T/L-ratio</th>
<th>Drug-light interval</th>
<th>Fluorescence measurements</th>
<th>Concentration measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 mg kg⁻¹</td>
<td>0.3 mg kg⁻¹</td>
<td>0.1 mg kg⁻¹</td>
</tr>
<tr>
<td>4 hours</td>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>-</td>
</tr>
<tr>
<td>24 h</td>
<td>2.5 ± 0.5</td>
<td>2.5 ± 0.3</td>
<td>-</td>
</tr>
<tr>
<td>48 h</td>
<td>2.1 ± 0.1</td>
<td>2.9 ± 0.2</td>
<td>-</td>
</tr>
<tr>
<td>72 h</td>
<td>3.1 ± 0.5</td>
<td>5.0 ± 0.5</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 3: Retention of fluorescence in liver and tumour tissue. This graph represents FR at 24, 48 and 72 hours after mTHPC administration as percentage of the initial FR at 4 hours after administration of 0.1 or 0.3 mg kg⁻¹. Values represent the mean (± sem) of 5 animals per treatment group. In liver tissue the FR’s drops rapidly to 25 to 40% and eventually 12% at 72 hours, whereas tumour FR’s remain high with 52 to 68% remaining at 72 hours after administration.

In all treatment groups a decrease in tumour FR was seen immediately after illumination (bleaching), which was largest at illumination 4 hours after mTHPC administration (Figure 4). The overall percentage of bleaching was between 60% and 75% and there was no significant difference in percentage of bleaching between the different treatment groups, except for animals illuminated 72 hours after injection of 0.1 mg kg⁻¹ mTHPC, where bleaching was only 41%.

**Short term PDT effect**

As shown in Table 2, largest PDT-induced necrotic areas were found upon illumination 4 hours after mTHPC administration. Diameters of necrosis were 13.4 mm and 10.1 mm, with a drug dose of 0.3 mg kg⁻¹ and 0.1 mg kg⁻¹ respectively. Necrotic areas at later time intervals were more comparable to tumour sizes before treatment and it seems that damage at these time intervals was more restricted to the tumour area.
On histological examination sharply demarcated lesions were seen with extensive necrosis of tumour tissue and surrounding liver tissue, but in some cases islands of viable looking tumour cells could be identified (Figure 5.1). Invasion of granulocytes and macrophages was seen in all sections, indicating the occurrence of an acute inflammatory response.

![Graph](image)

**Figure 4:** Photosensitizer bleaching in tumour tissue. This graph shows the mean (± sem) percentage of FR decrease in tumour tissue after illumination with 652 nm, representing photobleaching of mTHPC. There is no significant difference in bleaching between treatment groups, with values ranging from 60 to 72%, except for 72 hours after administration of 0.1 mg kg⁻¹, where bleaching is only 41%. Values are the mean of 5 animals per treatment group.

<table>
<thead>
<tr>
<th>Time §</th>
<th>Area of PDT damage (mm²) ††</th>
<th>Max. diameter of PDT damage (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 mg kg⁻¹ ††</td>
<td>0.3 mg kg⁻¹ ††</td>
</tr>
<tr>
<td>4 h</td>
<td>79.3</td>
<td>39.4</td>
</tr>
<tr>
<td>24 h</td>
<td>28.3</td>
<td>30.1</td>
</tr>
<tr>
<td>48 h</td>
<td>27.6</td>
<td>15.9</td>
</tr>
<tr>
<td>72 h</td>
<td>41.0</td>
<td>31.0</td>
</tr>
</tbody>
</table>

†† Figures in italics represent tumour sizes (mm²) before PDT treatment.
§ Time between drug administration and light delivery (hours).
† Administered dose of mTHPC via the femoral vein.
Figure 5.1: Histological section of a PDT treated tumour, 48 hours after illumination. Extensive tumour necrosis (Tn) and necrosis of a rim of normal liver tissue (Ln) is seen in the liver (L). Islands of viable looking tumour cells (Tv) can be identified within the treated area.

Figure 5.2a
Directly after PDT treatment both serum ASAT and ALAT levels rose, as represented in Figure 6. Rise in serum enzyme levels was more profound upon illumination 4 hours after mTHPC administration than upon illumination 48 or 72 hours after administration, indicating that the extent of PDT induced liver damage was highest at earlier time points between drug administration and illumination. Serum ASAT and ALAT levels normalized within a week after PDT treatment.

**Long term PDT effect**

Assessment of tumour response, 28 days after PDT treatment, showed complete remissions in 27 out of 31 treated animals (87%), with only 4 animals in which PDT treatment had no effect, as shown in Table 3. Tumour sizes of non-responding animals were comparable to tumour sizes of control animals. Although there was no significant difference in tumour size before PDT treatment between different treatment groups, non-responding tumours proved to be significantly larger in size before PDT treatment than responding tumours, with mean tumour sizes of $37.0 \pm 8.5$ mm$^2$ and $24.7 \pm 9.6$ mm$^2$ respectively (p<0.001).
Table 3: Tumour response 28 days after PDT treatment. Illumination (λ=652 nm, 15 J) was performed 4, 24, 48 and 72 hours after iv administration of either 0.1 or 0.3 mg kg\(^{-1}\) mTHPC. No viable tumour cells upon histological examination was considered to be a complete remission (CR), whereas tumour growth was considered to be no remission (NR).

<table>
<thead>
<tr>
<th>Complete remission §</th>
<th>No remission §</th>
</tr>
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<tbody>
<tr>
<td>0.1 mg kg(^{-1})</td>
<td>0.3 mg kg(^{-1})</td>
</tr>
<tr>
<td>4 hours</td>
<td>3/4</td>
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<tr>
<td>24 h</td>
<td>4/4</td>
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<tr>
<td>48 h</td>
<td>4/4</td>
</tr>
<tr>
<td>72 h</td>
<td>4/4</td>
</tr>
<tr>
<td>control ±</td>
<td>-</td>
</tr>
</tbody>
</table>

± Control group received mTHPC administration only.
§ No. of tumours in complete remission / no. of animals.

On histological examination, in case of a complete remission, only a small fibrotic lesion was visible on the site were the tumour had been (Figure 5.2). There was a sharp demarcation between healthy and PDT damaged tissue, with the occurrence of liver regeneration at the border; a proliferation of bile ducts was seen as well as proliferation of hepatocytes. In fibrotic lesions different zones could be identified: in the centre necrotic tissue, surrounded by a rim of granulocytes and lymphocytes, which was surrounded by a rim of macrophages (Figure 5.2). Non-responding tumours did not show a difference in morphology compared to non-treated tumours.

Figure 6a

![Graph showing ALAT levels before and after PDT treatment](image)
Figure 6b

Discussion

Photodynamic therapy has the potential of selectively destroying malignant tissue with minimal damage to healthy tissue. Selectivity of PDT depends on both photosensitizer localization in tissue and light administration, which makes it important to determine photosensitizer distribution in target tissue and its surrounding tissue. In case of a tumour in a highly vascularized organ as the liver, it will be difficult to reach selective drug uptake, as most photosensitizers are efficiently accumulated in liver tissue (Bellnier et al. 1989, Bown et al. 1986). Only for endogenously generated protoporphyrin-IX, after aminolaevulinic acid (ALA) administration, tumour selectivity has been reported, with tumour to liver ratio's of 4:1 (Hillegersberg et al. 1992).

We studied the mTHPC distribution in a transplanted colon adenocarcinoma in a rat liver at different times after intravenous administration, using ex vivo tissue extractions and in vivo fluorescence measurements. Both methods showed different mTHPC pharmacokinetics in liver and tumour tissue. In time mTHPC concentrations in liver tissue decreased rapidly, whereas mTHPC in tumour tissue remained high up to 48 hours after injection. As previously reported by Whelpton (1995, 1996), mTHPC showed a biexponential decline in liver tissue: an initial rapid decline in the first hours after administration, followed by a slow decline. We observed similar kinetics in liver tissue for bacteriochlorin a (Rovers et al. 1998).

In vivo fluorescence measurements have been used as a minimally-invasive method to study photosensitizer pharmacokinetics in animals and humans (Braichotte et al. 1995b, Alian et al. 1994). However, a problem associated with fluorescence measurements is the difficulty of obtaining quantitative fluorophore concentrations, due to varying optical properties of tissues. This makes
comparison of fluorescence intensities between tissue types difficult, especially between dark red liver tissue and pale tumour tissue; as absorption in liver tissue is higher than in tumour tissue, less light is transmitted back for fluorescence measurements, possibly leading to underestimation of fluorophores in liver tissue compared to those in tumour tissue. Use of the FR corrects partially for differences in optical properties as fluorescence values are divided by the autofluorescence, making comparison of the FR between two tissue types more reliable. Comparison of fluorescence levels within the same organ is not hindered by difference in optical properties and thus seems a reliable method to study in vivo photosensitizer kinetics.

In vivo fluorescence measurements showed similar mTHPC tissue kinetics as concentration data; FR in liver tissue rapidly declined in time, to only 12% of the initial value measured at 4 hours after administration, confirming findings of Alian et al. (1994). While liver tissue FR decreased in time, tumour tissue showed no significant decrease in FR’s, leading to significantly higher FR levels in tumour tissue with a mean T/L-ratio of up to 5.0 ± 0.5 at 72 hours after mTHPC administration. A similar increase in T/L-ratio’s, up to 6.3 ± 2.7 at 72 hours after administration, was seen using concentration data. In vivo fluorescence measurement showed to be a useful, non-invasive technique to study drug pharmacokinetics and the use of the FR allowed tissue comparisons. Both ex vivo extractions and in vivo fluorescence measurements showed a selective retention of mTHPC in tumour tissue, highest 3 days after drug administration.

FR’s in tumour tissue dropped to 25-40% of the initial value after illumination, which is caused by photosensitizer bleaching. Providing mTHPC does not produce toxic products on bleaching, strong bleaching of mTHPC could be advantageous at drug threshold levels, at which sensitizer levels in normal tissue are low enough to be totally bleached before inducing toxicity. Some even propose that precise dosimetry is not essential, when using a highly bleachable photosensitizer (Potter et al. 1987). Photobleaching could be used to provide a real-time indication of the PDT effect upon treatment (Wilson et al. 1997).

PDT with mTHPC was capable of inducing complete tumour destruction of transplanted tumours within the liver. Although a zone of liver tissue is damaged around the illuminated tumour, liver damage is minimal and transient as serum enzyme levels of ASAT and ALAT normalize within a week after treatment. Normal tissue damage is limited by 1] local light administration using optical fibres, 2] strong absorption of light in liver tissue, limiting light penetration, and 3] strong bleaching of mTHPC at threshold levels, which will be the case at longer drug-light intervals. mTHPC-PDT of liver tumours resulted in an overall CR rate of 87%, with only four out of the 31 treated animals in which tumour regrowth occurred. Tumour regrowth seemed to be the result of insufficient tumour illumination, as tumour sizes before PDT treatment were significantly larger in these animals. Using a single, plain cut fibre we were able to reach a 100% CR of all tumours less than 30 mm² in size. Optimizing tumour illumination, by using cylindrical diffusers and multiple fibres, will insure a more homogenous light administration over larger areas, enabling effective treatment of larger tumour volumes (Mizeret et al. 1996).

A drug dose of 0.1 mg kg⁻¹ mTHPC and a light dose of 15 J were sufficient to effectively treat liver tumours in the rat model, stating mTHPC’s potency. Although PDT has proven to be effective in tumour destruction within the liver using Haematoporphyrin Derivative (Holt et al. 1985), Photofrin (van Hillegersberg et al. 1992), pheophorbide a (Nishiwaki et al. 1989) and ALA (Svanberg et al.
1996), much higher light and drug doses were needed. This is illustrated for Photofrin in a liver tumour model in rats: best results were obtained at a light dose of 800 J cm\(^{-1}\), with complete remission of 4 out of 6 tumours (van Hillegersberg et al. 1992). Our study clearly indicates that mTHPC is much more potent than Photofrin, resulting in complete remissions at lower light doses and, consequently, short treatment times. This makes mTHPC one of the most potent photosensitizer currently available for treatment of intrahepatic tumours.

Based on our results, we were not able to determine an optimal drug-light interval for mTHPC, as treatment at each time point resulted in complete remissions. Illumination shortly after mTHPC administration is feasible, as liver damage is minimal and drug levels in tissue are high. PDT in this case will mainly rely on vascular damage and less on direct cellular damage. However, as drug levels in tumour surrounding liver tissue are high, light delivery, and thus fibre placement, needs to be accurate. A practical advantage would be that drug injection and light illumination could be performed the same day, limiting hospitalization times. On the other hand, treatment at later time intervals will limit damage to surrounding liver tissue even further, based on drug induced tumour selectivity, making accurate fibre placement less important. We believe drug selectivity is less important, as damage to a rim of normal liver tissue is tolerated and even preferred in treatment of cancerous tissue. Of utmost importance is presence of enough photosensitizer in tumour tissue to effectively eradicate tumour cells. Illumination should thus be performed when tumour tissue concentrations are highest, which is at later time points after mTHPC administration.

Like many others tissues, liver tissue heals mainly by regeneration after PDT treatment, which is apparent by bile duct proliferation and hepatocyte proliferation. In case of complete remission, only a fibrotic lesion remained at the site where a tumour had been, with liver regeneration at its border. Different zones could be identified within the lesion with a (1) central necrotic part, surrounded by a (2) zone of granulocytes and a (3) zone of macrophages. The presence of these cells confirms the occurrence of a non-specific immune response upon PDT treatment, with activation and accumulation of host immune cells (Korbelik and Krosl 1994, de Vree et al. 1996). We observed the presence of some viable looking tumour cells at histological examination two days after PDT treatment, though in the same treatment group all animals had complete remission, 28 days after PDT treatment. An explanation for this could well be effective destruction of remaining tumour cells by the PDT elicited immune response.

In conclusion, mTHPC was retained in tumour tissue, leading to tumour selectivity in time. Illumination of sensitized tumours resulted in complete remission of all tumours less than 30 mm\(^2\) in size, without inducing severe liver damage. Drug doses and light doses used for mTHPC-PDT were far less than needed with other photosensitizers, making mTHPC the most potent photosensitizer currently available for treatment of intrahepatic tumours. In patients, light delivery can be performed percutaneously using laser fibres positioned in the tumour under ultrasound or computerized tomography (CT), as is being done in laser photocoagulation (Amin et al. 1993). Feasibility of interstitial photodynamic therapy (IPDT) has been demonstrated by Purkiss et al. (1993). The aim of IPDT for liver metastases will at first be palliative treatment of patients with few irresectable metastases. A clinical study is in progress to assess safety and effect of IPDT with mTHPC in treatment of colorectal liver metastases.
Chapter 7

Acknowledgements

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References


Ballantyne GH, Quin J, Surgical treatment of liver metastases in patients with colorectal cancer, Cancer, 71:4252-4266, 1993

Bellnier DA, Ho YK, Pandey RK, Missert JR, Dougherty TJ, Distribution and elimination of Photofrin II in mice, Photochem Photobiol, 50: 221-228, 1989


Braichotte D, Wagnieres GA, Bays R, Monnier P, van den Bergh HE, Clinical pharmacokinetic studies of photofrin by fluorescence spectroscopy in the oral cavity, the esophagus, and the bronchi, Cancer, 75:2768-2778, 1995b


Cady B, Natural history of primary and secondary tumors of the liver, Semin Oncol, 10:127-133, 1983


Fingar VH, Wieman TJ, Doak KW, Role of thromboxane and prostacyclin release on photodynamic therapy-induced tumor destruction, Cancer Res, 50:2599-2603, 1990


Chapter 7
