Local ablative therapies for colorectal liver metastases and the immune system

Duijnhoven, Frederieke van

Chapter 6

Photodynamic therapy with mTHPBC for colorectal liver metastases

F.H. van Duijnhoven, J.P. Rovers, K. Engelmann, Z. Krajina, F.A.N. Zoetmulder, T.J. Vogl and O.T. Terpstra. Photodynamic therapy with mTHPBC for colorectal liver metastases is safe and feasible: results from a phase I study. Annals of Surgical Oncology 2005, in press.

Introduction

Photodynamic therapy (PDT) is a method for local tumour treatment, which is currently applied in several cancers. In PDT, a photosensitising agent is administered systemically and will, with varying specificity, localize in tumour tissue[1,2]. Upon subsequent tumour illumination by light of an appropriate wavelength, the photosensitiser is excited by photons to an unstable higher energy level. When returning to its ground state energy level, the absorbed energy is transferred to oxygen, which leads to the formation of reactive oxygen species. These reactive oxygen species are cytotoxic and cause direct tumour cell damage, vascular damage[3] and possibly activation of the immune system[4,5]. The efficacy of PDT is dependent on various parameters, such as the interval between sensitiser administration and tumour illumination, doses of photosensitiser and light, and pharmacological properties of the photosensitiser. As exposure of photosensitiser to light is essential for activation, the pharmacological effect without illumination is absent. Unfortunately, the reverse is also true: as the photosensitiser is also present in skin tissue, patients remain photosensitive for several weeks after sensitiser administration and should avoid bright (sun)light in this period to avoid skin phototoxicity[6,7].

The first and still most commonly used photosensitisers are porphyrin-based compounds, such as Haematoporphyrin derivative (HpD) and its purified version Photofrin. These sensitisers are activated by light of approximately 630 nm wavelength, which only penetrates tissue for several millimetres. PDT with these sensitisers is very effective against superficial and luminal tumours such as cholangiocarcinoma, basal cell, bladder and oesophagus carcinoma[8-11]. Photofrin was also the photosensitiser used in the first study with PDT of experimental liver metastases in a rat model for adenocarcinoma[12]. Along with tumour tissue, surrounding normal tissue was extensively damaged, due to the poor tumour selectivity of Photofrin. Shortly after, Purkiss *et al.* further developed this technique by using multiple optical fibres interstitially[13]. Application of this technique in HpD based PDT of colorectal liver metastases in patients resulted in tumour destruction, which was, however, incomplete and did not affect patient survival[14]. This was partly due to the limited depth of tissue penetration by light of 630 nm wavelength and the poor tumour selectivity of Photofrin in liver tissue, which required high drug and light dose to induce sufficient effect[15,16].

Further pharmacological developments resulted in production of second-generation sensitisers that are activated by wavelengths of over 700 nm, allowing deeper tissue penetration depth of up to 1 cm. Consequently, these sensitisers are more suitable for interstitial treatment of solid tumours, in which light is delivered directly in the tumour by the insertion of optical fibres. One of these sensitisers is the hydrophobic tetrahydroporphyrin 5,10,15,20-tetrakis(*m*-hydroxyphenyl)bacterio-chlorin (mTHPBC), a bacteriochlorin of

temoporfin (Foscan®), which is used successfully in clinical treatment of head and neck cancer[17]. mTHPBC is activated by 740 nm light and has already shown to be highly effective when compared to conventional photosensitisers in colon tumour cell lines and animal models[18,19]. A study in white pigs showed that it was feasible to get clinically relevant lesions with mTHPBC-based PDT using interstitial optical fibres. Preclinical work indicated that the effect of PDT with temoporfin increases with a shorter drug-light interval and with increased drug or light doses, with optimal anti-tumour effect of illumination at 24–48 hours after drug administration. For mTHPBC, similar results were found in rat studies[20-22].

The combination of this effective sensitiser, activation by deeply penetrating light and interstitial treatment should increase the efficacy of PDT for deeply seated, solid tumours like liver metastases. As PDT is a minimally invasive technique that can be applied under ultrasound and CT guidance, it could be a valuable addition to the range of treatment options available to patients with nonresectable colorectal liver metastases. In this study, we report the results of a multicentre phase I trial into the safety and technical feasibility of mTHPBC based PDT for colorectal liver metastases.

Patients and methods

Study objectives

The aim of this phase I study was to assess the feasibility and safety of PDT for nonresectable colorectal liver metastases with several treatment regimens.

Patient characteristics

Between April 2000 and May 2001, 24 patients were included in this multicentre trial, with participating centres in Germany, Croatia, UK and The Netherlands. Only patients with nonresectable liver metastases of previously resected primary colorectal carcinoma without evidence of local disease or other distant metastases were included. Additional inclusion criteria were a Karnofsky status of at least 60%, age over 18 years and accessibility of the liver metastases for adequate percutaneous fibre placement. Exclusion criteria were metastases larger than 7 cm in diameter, abnormal blood coagulation (prothrombin time $> 1.3 \times N$, platelet count $< 100 \times 10^9$/l), grade 3 or 4 alanine transaminase (ALT) or total bilirubin toxicity (Common Toxicity Criteria of the National Cancer Institute, CTC of the NCI)[23], chronic liver impairment, ascites, treatment with either chemotherapy, radiotherapy, other photosensitising or experimental drugs 30 days before inclusion and presence of disease which is caused or exacerbated by light. Local medical ethics committees approved the trial and written informed consent was obtained from all patients.

Study design
To minimize risk of damage to normally functioning liver tissue, the drug-light interval in the first group of patients was set at 120 hours, as the ratio of photosensitiser in tumour tissue vs. normal liver tissue is high at this time point. The first 12 patients (group A) were treated at 120 hours after administration of 0.6 mg/kg mTHPBC. As this drug dose proved to be highly effective but was associated with systemic drug-related adverse events like skin phototoxicity, drug dose was lowered to 0.3 mg/kg for the next 6 patients (group B). Since no adverse events occurred with this drug dose and drug-light interval and to ensure maximum efficacy with this lower systemic drug dose, drug-light interval was shortened to 48 hours in the last 6 patients (group C).

Administration of mTHPBC
The photosensitiser mTHPBC was administered by slow intravenous injections over a minimum of 15 minutes. After photosensitiser administration, patients were kept in a room with subdued lighting. They received a Lux light meter with light exposure instructions to avoid skin phototoxicity (table 1).

Days	Advice	Maximum light meter reading
day 1	remain in darkened room, may watch television, use light bulbs of 60W or less (60W equals 100 Lux)	100 Lux
days 2-7	remain indoors during daylight, avoid direct sunlight, get out of light if skin feels prickly or burning	from 200 Lux (day 2) to 700 Lux (day 7)
days 8-21	remain in shaded areas when outdoors, avoid strong indoor lighting, wear dark close weave clothes	increase by 100 Lux each day
> 22 days	return to normal activities, test sensitivity by holding back of hand in sunlight for 5 minutes	> 2200 Lux

Table 1. Summary of the guidelines regarding light exposure and corresponding maximum Lux doses after administration of mTHPBC

To monitor cardiovascular effects of drug administration, ECGs were made directly before and 1 hour after drug administration. Vital signs (heart rate, temperature, blood pressure and saturation) were assessed before and 4, 8, 12 and 24 hours after drug administration. To assess pharmacokinetics of mTHPBC, blood samples were taken 1, 4, 6, 8, 24 hours and 3, 4, 5, 7, 14 and 28 days after drug administration.

Tumour illumination

For tumour illumination, 18 G needles were placed in the tumours under local or general anaesthesia under CT guidance (figure 1). Optical fibres (CeramOptec, Bonn, Germany) with cylindrical diffuser lengths varying between 1 and 6 cm were inserted through the needles. Continuous wave diode lasers from CeramOptec produced laser light of 740 nm. Tumours were illuminated with 60 J/cm diffuser per single fibre application. To shorten treatment time when treating large tumours, a fibre beam splitter with 1 to 4 ratio (ATI Optique, Courcouronnes, France) was used to ensure simultaneous illumination with 4 fibres. Control spiral CT scans were performed according to local protocol and assessed by both the local treating radiologist and an independent radiologist 9 days and 1 month after mTHPBC administration and treated tumours were qualified as being locally progressive or stable, according to the reporting criteria proposed by the International Working Group on Image-Guided Tumour ablation[24]. Blood samples for haematological and biochemical parameters (total bilirubin, alkalic phosphatase (AP), gamma-glutamyltransferase (γ-GT), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and coagulation parameters) were obtained before drug administration and 1, 2, 3, 4, 5, 6, 7 and 8 days and 1 and 3 months after drug administration. Toxicity of serum enzyme levels was graded according to CTC of the NCI[23].

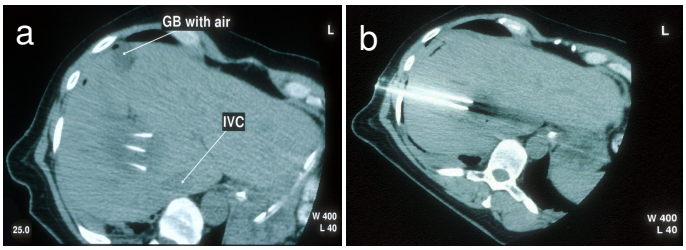

Figure 1. Simultaneous placement of optic fibres into central tumour under CT guidance. a. Second pass of fibres 1 to 3, centrally in the tumour and b. Fourth pass of needles 1 and 2, caudally in the tumour

Statistics
Occurrence of hepatotoxicity, mTHPBC related adverse events and local tumour progression was compared between different groups by using Pearsons' Chi-Square test, with a p-value ≤ 0.05 considered statistically significant.

Results

Patient and treatment characteristics
Of 24 included patients, 23 were treated with laser illumination. Laser treatment was cancelled in one patient due to the detection of extrahepatic disease after mTHPBC administration. Mean age of patients was 60 years (range 35–78), with 13 females and 11 males. The median interval between diagnosis of primary colorectal carcinoma and PDT was 14.5 months (range 1.5–122).

A total of 31 liver metastases (1–4 lesions per patient), with an average diameter of 3.2 cm (median 3.0 cm, range 1.2–6.8 cm) were treated by PDT. As a single optic fibre causes a cylinder of necrotic tissue measuring 2 cm in diameter and 1–6 cm in length (depending on diffuser length), multiple applications were in general necessary to adequately treat the entire tumour. A total of 124 fibre applications were used (median 4 fibre applications per lesion, range 1–12) to treat these 31 metastases. A light dose of 60 J/cm diffuser length was delivered at an intensity of 143–200 mW/cm diffuser length. Treatment time per application varied between 300 and 460 seconds (table 2). According to varying local hospital protocols, 14 patients received prophylactic antibiotics. One-month follow-up for assessment of feasibility and safety was completed by 21 out of 23 evaluable patients. Two patients were lost in follow-up.

	n lesions	tumour size (mm)	treatment time (sec)	energy (J)	output power (mW)
group A	15	36.5	327	186	655
group B	10	28.7	300	234	780
group C	6	28.7	300	130	433

Table 2. Average tumour size, treatment time, total delivered energy and output power for tumours in groups A, B and C

Pharmacokinetics of mTHPBC

After initial increase of mTHPBC plasma concentration in the first 24 hours, mTHPBC levels in both dose groups decreased to 50% of maximum value at 2 days after administration (figure 2). At 14 days after administration, mTHPBC was hardly present in plasma anymore. Both in the 0.6 mg/kg and in the 0.3 mg/kg dose group, concentration of mTHPBC was highest at 6-8 hours after drug administration (figure 3). mTHPBC plasma concentration was higher in the 0.6 mg/kg dose group than in the 0.3 mg/kg dose group, a difference that was significant at all time points within 6 days after drug administration ($p < 0.01$). At 7, 14 and 28 days after administration, there was no significant difference between both dose groups anymore.

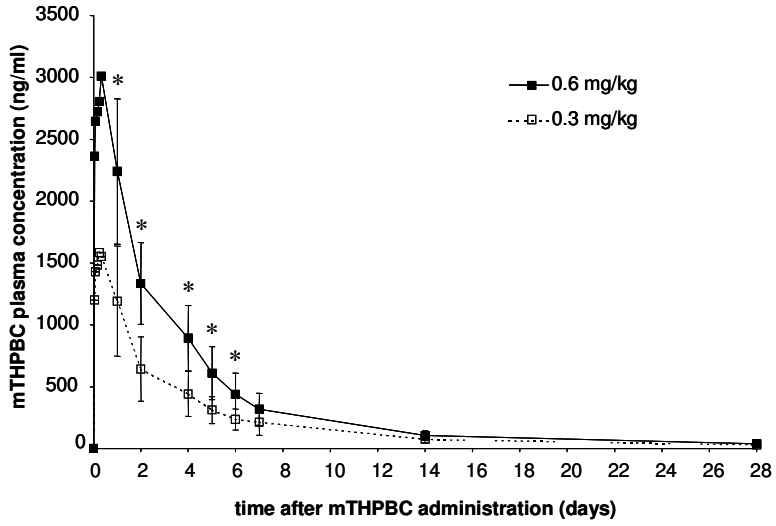

*Figure 2. mTHPBC concentration (ng/ml) in plasma from patients in group A (0.6 mg/kg, n=12) and in groups B and C (0.3 mg/kg, n=12) from 0 to 24 hours after mTHPBC administration, * $p < 0.01$ for 0.6 mg/kg mTHPBC group versus 0.3 mg/kg mTHPBC group*

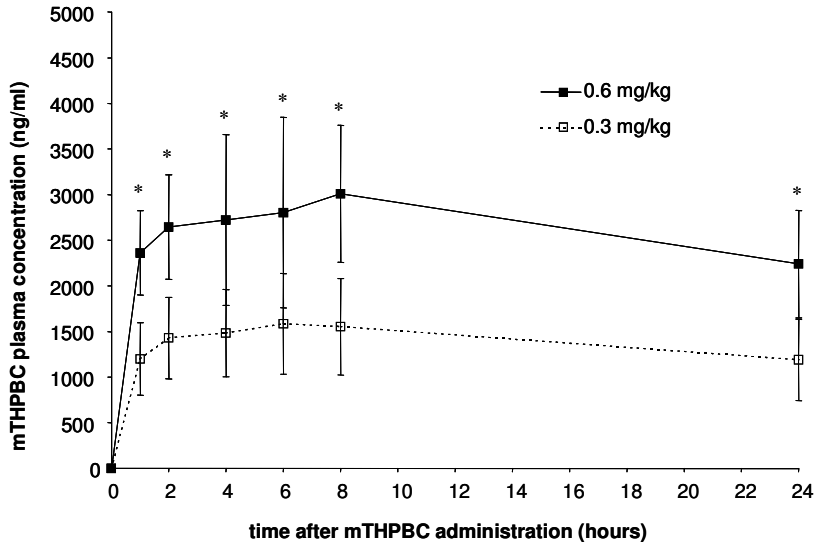

*Figure 3. mTHPBC concentration (ng/ml) in plasma from patients in group A (0.6 mg/kg, n=12) and in groups B and C (0.3 mg/kg, n=12) from 0 to 28 days after mTHPBC administration, * p < 0.01 for 0.6 mg/kg mTHPBC group versus 0.3 mg/kg mTHPBC group and B*

Toxicity of mTHPBC

Intravenous administration of mTHPBC was accompanied by pain and tingling in 7 patients (29%), which was independent of injection duration (table 3). In the days following mTHPBC administration, 10 patients (42%) developed a transient phlebitis of the injection vein. Hyperpigmentation of the injection arm was seen in 4 patients, in two of whom residual hyperpigmentation remained. Occurrence of these mild (n=18) to moderate (n=5) adverse events was mTHPBC dose-related, as they were observed significantly ($p \leq 0.05$) more often in the 0.6 mg/kg group than in the 0.3 mg/kg groups (table 3).

Due to skin photosensitivity, moderate hyperpigmentation, other than of the injection arm, occurred in two patients. Three patients suffered mild sunburn after excess light exposure in the first week (n=2) or in the third week (n=1) due to insufficient adherence to light instructions. One of these patients also suffered

two mild oxymeter burns during laser treatment. No hepatotoxicity occurred following mTHPBC administration, nor was there an effect on vital signs. ECG readings remained stable after mTHPBC administration.

		0.6 mg/kg	*0.3 mg/kg*	*total (%)*
mTHPBC administration	pain or tingling	7 (29%)*	2 (8%)	9 (38%)
	phlebitis of injection vein	8 (33%)*	1 (4%)	9 (38%)
	hyperpigmentation arm	5 (21%)	0 (0%)	5 (21%)
Skin photosensitivity	sunburn	3 (13%)	0 (0%)	3 (13%)
	hyperpigmentation	2 (8%)	0 (0%)	2 (8%)
	oxymeter burn	1 (4%)	0 (0%)	1 (4%)

*Table 3. Clinical adverse events following administration of mTHPBC in 24 patients, with * $p \leq 0.05$ for 0.6 mg/kg patient group compared to 0.3 mg/kg group*

Safety and feasibility of tumour illumination

In all patients, lesions could be treated with interstitial tumour illumination. A severe adverse event occurred in one patient: bleeding of the treated liver metastasis during treatment, that recovered fully and without lasting effects. Two other patients suffered moderate adverse events from tumour illumination: in one patient, a lesion of the skin surrounding the fibre insertion site (∅ 5 cm) was seen after treatment, that recovered over the course of several months and in an other patient damage of the pancreas was caused while illuminating an adjacent tumour, without clinical symptoms or biochemical abnormalities.

Other adverse events related to tumour illumination were (1) experience of abdominal and/or fibre insertion site pain (n=8) (2) appearance of pleural fluid (n=2) and (3) pyrexia (n=3) (table 4). All these events were graded as mild (n=9) or moderate (n=4). Karnofsky performance status remained stable throughout treatment except in one patient, who experienced pain, elevated temperature and a skin lesion at fibre insertion site after treatment.

Within 48 hours after tumour illumination, one or more liver enzymes were increased in 21 patients. Hepatotoxicity was not clinically significant and transient, as at 1 month all values had returned to pre-PDT levels. Occurrence of hepatotoxicity was not related to drug-light interval or mTHPBC drug dose (table 5) as

there were no significant statistical differences between groups regarding hepatotoxic levels of γ-GT, bilirubin, AP, AST and ALT. Tumour illumination had no effect on coagulation parameters.

	adverse event	*n (%)*
Laser treatment	pain	8 (35%)
	pyrexia	3 (13%)
	pleural fluid	2 (7%)
	bleeding into liver metastasis	1 (4%)
	PDT lesion insertion site	1 (4%)

Table 4. Clinical adverse events following laser therapy in 23 patients

	group A (n=12)				group B (n=6)				group C (n=6)			
grade	1	2	3	4	1	2	3	4	1	2	3	4
γ-GT	5*(4)*	0	0*(1)*	0	1	1	0*(1)*	0	2*(1)*	0*(2)*	0	0*(1)*
bilirubin	3	0	0	0	1	1	0	0	0	0	1	0
AP	4	0	0	0	3	0	0	0	0*(1)*	0*(1)*	0*(1)*	0
ALT	3	3	4	0	1	0	2	0	4	0	0*(1)*	0
AST	2	4	4	0	0	1	2	0	4	1	1	0

Table 5. Hepatotoxicity graded according to CTC of the NCI in groups A, B and C within 48 hours of laser illumination. Italic numbers in brackets indicate patients with pre-existing hepatotoxicity

Anti tumour efficacy

Adequate necrosis was induced in 30 of 31 treated lesions, as shown by the absence of contrast enhancement in treated lesions on CT scans performed at 4 days after PDT (figure 4; table 6). At 1 month after PDT, all lesions in patient group B were stable. In group A, 13 out of 15 treated lesions were stable (87%), 2 lesions in 2 different patients were locally progressive. In group C, 4 of 6 treated lesions were stable (66%) and 2 lesions showed local tumour progression (33%) (table 6). Although there was a trend indicating that lesions in group C did worse than those in groups A and B, these differences were not significant.

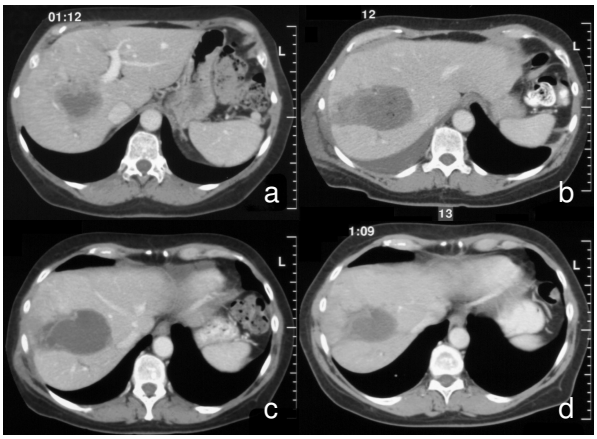

Figure 4. CT scans showing large central tumour at 6 days before PDT (a), 5 days post-PDT (b), 3 weeks post-PDT (c) and 3 months post-PDT (d). Initially, a large area of necrosis at the site of the tumour is induced, that gradually decreases in time, with no viable tumour tissue left at 3 months after PDT

group	n lesions	necrosis	lesion status 1 month post PDT	
			stable	progressive
A	15	14 (93%)	13	2
B	10	10 (100%)	10	0
C	6	6 (100%)	4	2

Table 6. Induction of tumour necrosis and lesion status at 1 month after PDT

Discussion

Our study showed interstitial PDT with mTHPBC to be a safe and feasible treatment of deeply seated liver metastases. Adequate tumour necrosis was induced in 30 of 31 treated lesions, with 84% of lesions showing no signs of local tumour progression at 1 month after PDT. No severe complications occurred. Two patients in our study inadvertently suffered PDT damage of pancreas respectively fibre insertion site. Visual confirmation of correct fibre placement and adequate control of laser treatment may help to avoid this damage to adjacent organs and tissues. Although skin photosensitivity reactions were reduced to a minimum by decreasing the sensitiser dose to 0.3 mg/kg, patients should be made well aware of the risk of light exposure as skin remains photosensitive for several weeks after intravenous administration[6,25,26]. The disadvantage of skin photosensitivity has a pronounced impact on patients' daily routine, especially in sunny seasons.

A recent study by Lustig *et al.* also showed PDT for solid tumours to be feasible and safe[27]. In this phase I study, talaporfin sodium was used for PDT of 21 solid tumours from various origins. Talaporfin sodium is activated by 664 nm wavelength light and therefore does not penetrate tissue quite as good as mTHPBC, but it has the advantage of a very short drug-light interval, as tumours can be treated at 1 hour after i.v. administration. In this study, tumours were not illuminated by laser but by using newly developed light-emitting diodes that can be percutaneously inserted in the tumour, enabling tumour illumination for a prolonged time period (up till 664 minutes in this study)[28]. Although these new sensitisers and techniques greatly facilitate treatment of solid tumours, PDT should at present not be used for tumours > 7 cm. As the diameter of necrosis induced by one fibre does not extend beyond 2 cm, several fibre insertions are necessary to treat large tumours, with an increased risk of inadequate placement. An associate problem is the lack of real-time visualization during PDT as the necrosis caused by PDT is not immediately present, but develops in time till after 48 hours the maximum effect is reached[18]. As the efficacy of PDT is largely dependent on the induction of a necrotic lesion that exceeds the tumour in size, the correct placement of fibres is essential. To do so without real life imaging requires skills and experience and, as there is limited earlier experience with this technique, a learning curve element in this study must not be underestimated.

Other local ablative techniques used in treatment for colorectal liver metastases include radiofrequency ablation (RFA) and laser-induced thermotherapy (LITT). In RFA, needle electrodes deliver a high frequency alternating current to the tissue, causing hyperthermia of the tissue and thus inducing coagulative necrosis. Currently, RFA is an established therapy and has resulted in complete response rates of 52-95%[29,30]. It can offer palliation by prolongation of disease-free and overall survival to respectively 50% and 94% at 1 year[31] and possibly even curation, although at present the limited follow-up time in most studies does not allow a

meaningful determination of survival rates. In LITT, a laser applicator delivers light energy through optical fibres, resulting in coagulative necrosis. Studies have shown tumour responses up to 97% after 6 months, with median survival ranging from 32 to 39 months[32-34].

The main advantage of PDT, RFA and LITT is their feasibility in patients that are not eligible for resection, which is still considered the gold standard therapy for colorectal liver metastases. In addition, they can be applied percutaneously and may be repeated if necessary. PDT may however be specifically suitable for those patients with liver metastases in the vicinity of large vessels. Blood flow in vessels near the treated tumour has a cooling effect on thermal-energy based treatments like RFA and LITT, preventing tissue from reaching a sufficiently high temperature (>60°C) to be irreversibly destructed[35]. Photodynamic therapy however is not compromised by this heat-sink effect, as the effectiveness of this minimally invasive local technique is not dependent on the generation of heat, but on the generation of reactive oxygen species. If an open procedure, in which a Pringle manoeuvre could be performed to prevent this effect, is contra-indicated or otherwise undesirable, percutaneous PDT could be a very useful therapeutic option.

References

1. Boyle RW, Dolphin D. Structure and biodistribution relationships of photodynamic sensitisers. *Photochem Photobiol* 1996; 64: 469-85.

2. Bugelski PJ, Porter CW, Dougherty TJ. Autoradiographic distribution of hematoporphyrin derivative in normal and tumour tissue of the mouse. *Cancer Res* 1981; 41: 4606-12.

3. Fingar VH. Vascular effects of photodynamic therapy. *J Clin Laser Med Surg* 1996; 14: 323-8.

4. Chen WR, Zhu WG, Dynlacht JR, Liu H, Nordquist RE. Long-term tumour resistance induced by laser photo-immunotherapy. *Int J Cancer* 1999; 81: 808-12.

5. Korbelik M. Induction of tumour immunity by photodynamic therapy. *J Clin Laser Med Surg* 1996; 14: 329-34.

6. Dougherty TJ, Cooper MT, Mang TS. Cutaneous phototoxic occurrences in patients receiving Photofrin. *Lasers Surg Med* 1990; 10: 485-8.

7. Razum N, Balchum OJ, Profio AE, Carstens F. Skin photosensitivity: duration and intensity following intravenous hematoporphyrin derivates, HpD and DHE. *Photochem Photobiol* 1987; 46: 925-8.

8. Ackroyd R et al. Eradication of Dysplastic Barrett's Oesophagus Using Photodynamic Therapy: Long-Term Follow-Up. *Endoscopy* 2003; 35: 496-501.

9. Moghissi K, Dixon K, Thorpe JA, Stringer M, Moore PJ. The role of photodynamic therapy (PDT) in inoperable oesophageal cancer. *Eur J Cardiothorac Surg* 2000; 17: 95-100.

10. Ortner M. Photodynamic therapy for cholangiocarcinoma. *J Hepatobiliary Pancreat Surg* 2001; 8: 137-9.

11. Waidelich R et al. Clinical experience with 5-aminolevulinic acid and photodynamic therapy for refractory superficial bladder cancer. *J Urol* 2001; 165: 1904-7.

12. van Hillegersberg R et al. Interstitial photodynamic therapy in a rat liver metastasis model. *Br J Cancer* 1992; 66: 1005-14.

13. Purkiss SF, Dean R, Allardice JT, Grahn MF, Williams NS. An interstitial light delivery system for photodynamic therapy within the liver. *Lasers Med Sci* 1993; 8: 253-7.

14. Purkiss SF et al. Multiple fiber interstitial photodynamic therapy of patients with colorectal liver metastases. *Lasers Med Sci* 1994; 9: 27-35.

15. Bellnier DA, Ho YK, Pandey RK, Missert JR, Dougherty TJ. Distribution and elimination of Photofrin II in mice. *Photochem Photobiol* 1989; 50: 221-8.

16. Hara N, Ichinose Y, Motohiro A, Ohta M, Yagawa K. Distribution of hematoporphyrin derivative in normal and malignant tissue. *Jpn J Exp Med* 1988; 58: 139-43.

17. Copper MP, Tan IB, Oppelaar H, Ruevekamp MC, Stewart FA. Meta-tetra(hydroxyphenyl)chlorin photodynamic therapy in early-stage squamous cell carcinoma of the head and neck. *Arch Otolaryngol Head Neck Surg* 2003; 129: 709-11.

18. Rovers JP, de Jode ML, Grahn MF. Significantly increased lesion size by using the near-infrared photosensitiser 5,10,15,20-tetrakis (m-hydroxyphenyl)bacteriochlorin in interstitial photodynamic therapy of normal rat liver tissue. *Lasers Surg Med* 2000; 27: 235-40.

19. Yow CM, Chen JY, Mak NK, Cheung NH, Leung AW. Cellular uptake, subcellular localization and photodamaging effect of temoporfin (mTHPC) in nasopharyngeal carcinoma cells: comparison with hematoporphyrin derivative. *Cancer Lett* 2000; 157: 123-31.

20. Rovers JP et al. Effective treatment of liver metastases with photodynamic therapy, using the second-generation photosensitiser meta- tetra(hydroxyphenyl)chlorin (mTHPC), in a rat model. *Br J Cancer* 1999; 81: 600-8.

21. Rovers JP, de Jode ML, Rezzoug H, Grahn MF. In vivo photodynamic characteristics of the near-infrared photosensitiser 5,10,15,20-tetrakis(M-hydroxyphenyl) bacteriochlorin. *Photochem Photobiol* 2000; 72: 358-64.

22. Rovers JP et al. Biodistribution and bioactivity of tetra-pegylated meta-tetra(hydroxyphenyl)chlorin compared to native meta-tetra(hydroxyphenyl)chlorin in a rat liver tumour model. *Photochem Photobiol* 2000; 71: 211-7.

23. Green S, Weiss GR. Southwest Oncology Group standard response criteria, endpoint definitions and toxicity criteria. *Invest New Drugs* 1992; 10: 239-53.

24. Goldberg SN et al. Image-guided tumour ablation: proposal for standardization of terms and reporting criteria. *Radiology* 2003; 228: 335-45.

25. Wagnieres G et al. Clinical evaluation of the cutaneous phototoxicity of 5,10,15,20-tetra(m-hydroxyphenyl)chlorin. *Photochem Photobiol* 1998; 68: 382-7.

26. Wooten RS, Smith KC, Ahlquist DA, Muller SA, Balm RK. Prospective study of cutaneous phototoxicity after systemic hematoporphyrin derivative. *Lasers Surg Med* 1988; 8: 294-300.

27. Lustig RA et al. A multicenter Phase I safety study of intratumoural photoactivation of talaporfin sodium in patients with refractory solid tumours. *Cancer* 2003; 98: 1767-71.

28. Chen J et al. New technology for deep light distribution in tissue for phototherapy. *Cancer J* 2002; 8: 154-63.

29. Parikh AA, Curley SA, Fornage BD, Ellis LM. Radiofrequency ablation of hepatic metastases. *Semin Oncol* 2002; 29: 168-82.

30. Curley SA. Radiofrequency ablation of malignant liver tumours. *Ann Surg Oncol* 2003; 10: 338-47.

31. Solbiati L et al. Hepatic metastases: percutaneous radio-frequency ablation with cooled-tip electrodes. *Radiology* 1997; 205: 367-73.

32. Gillams AR, Lees WR. Survival after percutaneous, image-guided, thermal ablation of hepatic metastases from colorectal cancer. *Dis Colon Rectum* 2000; 43: 656-61.

33. Mack MG et al. Percutaneous MR imaging-guided laser-induced thermotherapy of hepatic metastases. *Abdom Imaging* 2001; 26: 369-74.

34. Vogl TJ, Mack MG, Straub R, Roggan A, Felix R. Magnetic resonance imaging--guided abdominal interventional radiology: laser-induced thermotherapy of liver metastases. *Endoscopy* 1997; 29: 577-83.

35. Lu DS et al. Effect of vessel size on creation of hepatic radiofrequency lesions in pigs: assessment of the "heat sink" effect. *AJR Am J Roentgenol* 2002; 178: 47-51.