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Photodynamic therapy as a tool for suppressing the haematogenous dissemination of tumour cells

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Abstract

The chance of most cancer patients surviving their disease is to a high degree dependent on the status of the metastatic processes. One general route of cancer-cell dissemination is passive transport in the blood stream, i.e., haematogenous dissemination. In this study we try to find an answer to the following question: is it possible to use photodynamic therapy for suppressing the haematogenous dissemination of cancer cells? In first in vitro experiments we incubated CX1 cells (colon carcinoma cells) with two photosensitizers, Photofrin II and *meso*-tetra(hydroxyphenyl)chlorin (*m*THPC). We added the cells to fresh whole blood and irradiated the blood with suitable laser light in a flow-through irradiation system. The tumour-cell survival fraction (SF) was determined with plating efficiency. Using Photofrin II we observed a minimal tumour-cell survival in blood of SF = 3.5% and using *m*THPC we measured SF = 0.02%. These results encourage further investigations concerning the use of photodynamic therapy for suppressing haematogenous dissemination.

Keywords: Blood; Haematogenous metastasis; Photodynamic therapy; Photofrin II; *m*THPC

1. Introduction

In the past few decades intensive international research has led to visible progress in the fight against cancer. In particular, the treatment of the primary tumour is often successful. On the other hand, there is seldom the possibility of suppressing the metastatic process of cancer. Furthermore, the status of the metastatic process has an enormous influence on the survival ability of the cancer patient. For example, for colorectal cancer the survival rate (five years after treatment) is around 90%, if the tumour is limited to the colon or rectum, respectively [1]. After regional tumour spread (involvement of nearby organs and lymph nodes), the survival rate decreases to about 55% [1]. After the development of first metastatic tumour growth in distant body sites (distant metastasis), only 7% of the patients survive [1].

As well as direct and lymphatic spread of tumour cells in the host organism, they can also disseminate via the blood. The rate of this haematogenous dissemination can be very high. For example, for colorectal tumours the rate of haematogenous dissemination of tumour cells from the colon or rectum, respectively, to the liver could be determined from autopsy data to be around 72% [2].

In the present study we investigated photodynamic therapy (PDT) as a tool for the eradication of tumour cells within flowing blood. PDT is a promising oncological therapy [3–6]. A photosensitizer (PS) is injected intravenously. After a suitable time the PS shows higher concentrations in tumour tissue than in normal tissue. On activation with suitable laser light, the PS produces toxic substances and eventually damages the PS-containing tissue compartments without destroying the surrounding tissue [5].

The laser fluence rate used to activate the PS in PDT has to be lower than the damage threshold of the surrounding tissue with lower PS content to avoid damage of this normal tissue. In the case of purging blood from tumour cells with PDT, this means that the fluence rate of laser light should not be higher than the threshold of first blood damage.

The most common PS in clinical PDT is Photofrin II [3,4], a haematoporphyrin derivative. To activate Photofrin II, laser light of 630 nm is used in clinical practice [3,4,7].

A recently developed PS is *meso*-tetra(hydroxyphenyl)-chlorin (*m*THPC). *m*THPC shows a higher optical density

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than Photofrin II at an irradiation wavelength of 653 nm [8]. This wavelength allows higher penetration depths in most tissues and especially in blood.

In the past few few years, numerous applications of PDT in blood have been developed [9–15], including the purification of blood products from viruses or bacteriophages for blood-banking applications [16–21]. The absorption properties of blood suggest the use of a PS with a PDT activation maximum in the wavelength range 650–750 nm. Furthermore, the scattering properties of blood may influence the light dosimetry during PDT in blood.

The idea leading to the investigations described in this paper is to kill haematogenous metastasizing tumour cells in the blood which were sensitized at the site of the primary tumour before migrating into the blood stream. The application of this technique could be suppressing or even preventing haematogenous dissemination of tumour cells in cancer patients, for example, while undergoing a tumour resection. We decided to investigate first the principle possibility of killing sensitized tumour cells in blood by PDT. Furthermore, we wanted to know whether the resulting cell death is only an effect of PDT and not of PS alone or laser light alone.

2. Materials and methods

2.1. Irradiation system

A dye laser (Spectra Physics, Darmstadt, Germany), pumped by an Ar^+ laser (Spectra Physics, Darmstadt, Germany) was used as irradiation source. The laser dye was DCM (4-dicyanomethylene-2-methyl-6-*p*-dimethylaminostyrol-4h-pyran, Radiant Dyes Laser Accessories, Wermelskirchen, Germany). The dye laser was adjusted to the desired wavelength maximum of (630 ± 1) nm for Photofrin II or (653 ± 1) nm for *m*THPC with a width of (3 ± 1) nm.

This laser light was coupled into a microlens fibre (QLT, Phototherapeutics, New York, USA) which was adjusted to a flow-through suprasil cell (QS 170, Hellma, Müllheim, Germany) containing the blood or cell suspension. The whole aperture of the suprasil cell was covered by the laser light from the microlens fibre. The cell aperture was 17.5×3.5 mm and the light path was 1 mm.

Blood was pumped through the flow-through cell to prevent settling of blood or tumour cells. The blood flow was realized by a peristaltic pump (Gilson Minipuls 2, Abimed, Langenfeld, Germany) using platinum silicone tubes (Cole-Parmer Instrument Co., Chicago, USA). Various flow velocities (v_{Fl}) were possible. The use of platinum tubes prevented blood damage by the tubing material. This was tested by blood counts.

The light transmission of the blood layer was measured using an integrating sphere (Aesculap Meditec, Heroldsberg, Germany). Furthermore, the integrating sphere was used to control a proper blood flow, without air bubbles or aggregation of blood in the flow-through cell. The blood or cell suspension flowing through the suprasil cell was irradiated with various radiant powers, producing various fluence rates in the blood layer.

The radiant power was measured closely behind the tip of the microlens fibre using a power meter (Spectra Physics, Darmstadt, Germany). Fluence rates were calculated as the ratio between the measured radiant power of the laser light and the area of the laser beam at the blood layer front. This area was equal to the suprasil cell aperture.

The irradiation time t can be calculated from the irradiated blood volume of the suprasil cell V_B and from the flow velocity v_{Fl} by $t = V_B / v_{Fl}$. For example, a flow velocity of $v_{Fl} = 1.15$ ml/h resulted in an irradiation time of 6 min. A flow velocity of 15.72 ml/h resulted in an irradiation time of 26 s.

To avoid damage of the blood during irradiation, a cooling device was installed. This consisted of a temperature sensor (Conrad Electronic, Hirschau, Germany) and a blower directed at the suprasil cell. The sensor was connected to the outer wall of the suprasil cell. If the measured temperature rose above $T = 30°C$, the sensor switched on the blower. When the temperature fell below $T = 30°C$ the blower was switched off.

2.2. Photosensitizers

Photofrin II was provided by QLT (Vancouver, BC, Canada). It was dissolved in 5% dextrose at a concentration of 2 mg/ml for further use. *m*THPC was supplied by Scotia Pharmaceuticals (Guildford, UK) and was dissolved in 35% polyethylene glycol 250, 20% ethanol and 45% tris buffer (pH 7.5) at a concentration of 1 mg/ml for further use.

2.3. Cells and culture conditions

CX1 colon carcinoma cells were obtained from the Tumorbank of the Deutsches Krebsforschungszentrum (Heidelberg, Germany). These cells were chosen because colon carcinomas show a high degree of haematogenous metastasis in the liver. Furthermore, the inherent properties of these cell lines allowed the application of the plating-efficiency-in-blood method, described below.

The CX1 cells were maintained as monolayers in exponential growth by subculturing them twice weekly in FCS-M, i.e., Ham's F12 medium (Sigma Chemie, Deisenhofen, Germany) supplemented with 10% foetal calf serum, 1% Penicillin/Streptomycin and 20–25 mM Hepes buffer (all from Gibco BRL, Eggenstein, Germany). Cells were inoculated in 25 cm^2 plastic tissue-culture flasks (Nunclon, Roskilde, Denmark) at 37°C. For this purpose cells were treated with trypsin–EDTA for 10 min and were counted with a cell analyser and counter (CASY 1, Schärfe Systems, Reutlingen, Germany). The cells were diluted in FCS-M to 1×10^5 cells/ml and put into a new culture flask. Vitality was tested by a dye-exclusion test using trypan blue. During incubation of cells with PS (24 h) and after irradiation (0.5–2 h), the cells were cultured or rested in serum-free medium (SF-M). In

this medium, foetal calf serum was replaced by 2% Ultroser G (Gibco BRL, Eggenstein, Germany).

All steps involving photosensitizers were conducted in a nearly dark room. After incubation with PS (24 h), cells were washed three times with phosphate-buffered saline (PBS, Dulbecco's buffer saline without Ca and Mg from Gibco BRL, Eggenstein, Germany).

Dark toxicity measurements were conducted three times using the method of plating efficiency. The survival fraction of CX1 cells was determined after a 24 h incubation of cells at 37°C without any light irradiation for various concentrations of Photofrin II and *m*THPC in SF-M. Cells without PS served as controls. After incubation with PS, the cells were rinsed for 30 min with fresh SF-M. After washing twice with PBS, the cells were treated with trypsin–EDTA, counted and diluted in FCS-M to the concentration necessary for plating-efficiency measurements. For every plating-efficiency measurement three flasks were prepared.

Before the irradiation experiments, cells were treated with trypsin–EDTA for 10 min. The reaction was stopped with FCS-M and cells were washed twice (600 rpm, 10 min). Afterwards the cells were suspended in SF-M or blood at defined concentrations. The cell number was determined with a haemocytometer or a cell counter (CASY 1, Schärfe Systems, Reutlingen, Germany).

2.4. Plating efficiency (PE) and plating efficiency in blood (PEIB)

The survival of irradiated CX1 cells was measured by the plating efficiency (PE). The PE of cells irradiated in SF-M was determined as described in Ref. [22]. The survival fraction (SF) was the PE of treated cells divided by the PE of untreated control cells kept in the same medium as the treated cells (SF-M or blood).

For determination of the SF of cells treated in blood, the procedure of PE measurement was changed. Cells incubated with PS after the washing procedure (600 rpm, 10 min) were counted and suspended in blood. After irradiation with laser light, the blood–CX1 cell suspension was diluted in SF-M to a CX1 cell number (determined from cell counts before treatment) suitable for PE determination. A minimum dilution of 1:1000 was necessary to avoid agglutination of blood. The diluted cell suspension was incubated for 24 h. This time was long enough for CX1 cells to adhere to the bottom of the cell flask. For CX1 cells, the time required for total adherence to the cell-flask bottom was determined to be 7 h. After 24 h the blood cells were taken out of the cell flask by washing with PBS (three times). The CX1 cells left in the flask were incubated with FCS-M until colonies could be counted (cf. PE procedure without blood). If the dilution of the blood–CX1 cell suspension was too low, not all of the agglutinated blood cells could be taken out of the flask and determination of PE was impossible. Neither FCS-M nor SF-M contained progenitor factors. Therefore, adherent blood cells did not show colony formation. This was confirmed by microscopic observations.

In order to determine the changes in the PE of the CX1 cells during experiments due to their resting in suspension, this study included three kinds of control experiments. The PE was determined for

1. untreated cells,
2. cells incubated with PS, but not irradiated or led through the irradiation device, and
3. cells incubated with PS and led through the irradiation device with the same flow rate as the corresponding irradiation experiment, but not irradiated.

These control experiments were conducted within every series of irradiation experiments directly after the corresponding irradiation experiment.

For all irradiation experiments in this study, the SF shown is the PE of irradiated cells divided by the PE of cells which were incubated with the PS and led through the irradiation system but not irradiated (control 3).

All controls show no significant change in SF during the experiments described in this paper. Therefore, they are not given in the corresponding Figures.

2.5. Blood and blood-damage determination

Blood was obtained from a healthy 33 year old male volunteer. It was kept on a shaking device at room temperature until use. This was done for a maximum of 8 h in total. For determining the blood damage, we prepared blood films, measured the osmotic fragility and performed blood counts. The fresh blood was anticoagulated with sodium heparin (Vacutainer, Becton Dickinson Vacutainer Systems Europe, Meylan Cedex, France) for the preparation of blood films and osmotic-fragility measurements. For automated blood counts blood was anticoagulated with sodium citrate at puncture (Seditainer, Becton Dickinson Vacutainer Systems Europe, Meylan Cedex, France) and with EDTA/KE (Sarstedt, Nürnbrecht, Germany) after irradiation. The mixture of two anticoagulating substances was necessary to avoid changes in blood components caused by the irradiation device or by the time before irradiation or blood counts took place (sodium citrate) and to allow correct measurements of blood counts (EDTA/KE). After addition of EDTA/KE, blood samples were allowed to stand at room temperature for at least 30 min before blood counting.

Automated blood counts were carried out with a Technicon H3 (Bayer Diagnostic, Munich, Germany). The counter was able to measure white blood cells (WBC), neutrophils (NEUT), lymphocytes (LYMPH), monocytes (MONO), eosinophils (EOS), basophils (BASO), red blood cells (RBC), haemoglobin content (HGB), haematocrit (HCT), mean (red) cell volume (MCV), mean content of haemoglobin (MCH), mean content of haemoglobin per cell (MCHC), red-cell distribution weight (RDW), haemoglobin distribution weight (HDW), platelets (PLT), mean platelet volume (MPV), platelet distribution weight (PDW)

and plateletcrit (PCT) of a blood sample. For more explanations about blood counting with the Technicon H3, see Ref. [23].

For dark toxicity measurements blood was incubated with PS at different PS concentrations (0, 0.1, 0.5, 1, 5, 10, 50 and 100 μg/ml PS). Incubation was conducted at room temperature. The blood was kept on the shaking device between blood counts. Blood counts were conducted 1, 2.5, 5, 12, 15.5, 18 and 24.5 h after addition of PS.

To examine the effect of laser irradiation on blood without using any sensitizer, blood was irradiated with different fluence rates ($0 < E < 300$ mW/cm^2 for $\lambda = 630$ nm and $0 < E < 1800$ mW/cm^2 for $\lambda = 653$ nm). Changes in blood due to irradiation were measured using blood counts, osmotic-fragility measurements and blood films. For details see Ref. [24].

3. Results

3.1. Dark toxicity of Photofrin II and mTHPC in CX1 cells and in blood

The cytotoxicity of Photofrin II and *m*THPC to the CX1 cells in the dark was measured in FCS-M. The results are shown in Fig. 1. The D_{50} dose of the drugs in these cells was determined to be D_{50}(PII) = 50 μg/ml for Photofrin II and D_{50}(*m*THPC) = 3.8 μg/ml for *m*THPC (see Table 1). The D_{50} dose is the drug dose that leads to a survival fraction of 50% without using irradiation.

The dark toxicity of Photofrin II and *m*THPC in blood was determined using blood counts. The incubation of blood with Photofrin II produced the first changes 5 h after the beginning of incubation for Photofrin II concentrations [PII] > 10 μg/ml. A significant change in white blood cells (WBC) and platelet count (PLT and PDW) was observed (see Table 1).

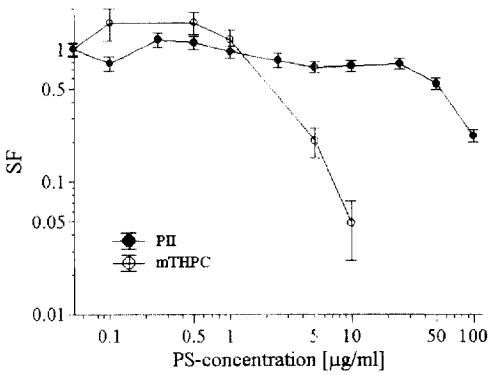

Fig. 1. Dose–response curve of CX1 cells incubated in SF-M ($T = 37$°C) with different concentrations of Photofrin II and *m*THPC (logarithmic scale) in the absence of light for 24 h.

The incubation of blood with *m*THPC showed the first changes a few minutes after the beginning of incubation. For *m*THPC concentrations [*m*THPC] > 50 μg/ml the white and red blood-count parameters (WBC, RBC, HGB, HCT, MCV, MCHC, HDW) changed. Additionally, after 2 h of incubation the platelet count (PLT) decreased significantly for [*m*THPC] > 50 μg/ml and after 4.5 h the mean platelet volume (MPV) changed for [*m*THPC] > 10 μg/ml (see Table 1).

To avoid changes in CX1 cells due to the PS alone, the PS concentrations used in incubation in the remainder of the study are 10 μg/ml Photofrin II and 1 μg/ml *m*THPC. Due to the dark toxicity measurements in blood, it is obvious that the small amount of PS which might diffuse out of the CX1 cells into the blood when mixing CX1 cells and blood cannot lead to any blood damage in the dark during the time of the experiment.

Table 1
Dark toxicity of PII and *m*THPC in CX1-cells and in whole human blood. The dark toxicity of both photosensitizers in CX1 is given in terms of D_{50} dose values. The dark toxicity in blood is given by the photosensitizer concentrations and incubation times leading to first changes in blood counts. There were no changes for both photosensitizers in the differential blood counts (NEUT, LYMPH, MONO, BASO, EOS), in MCH and in RDW

	Photofrin II		*m*THPC	
D_{50} dose for CX1 cells	50 μg/ml		3.8 μg/ml	
Blood counts	Concentration [μg/ml]	Incub. time [h]	Concentration [μg/ml]	Incub. time [h]
WBC	10	15.5	100	0.5
RBC	n.c.[a]	n.c.	100	0.5
HGB	n.c.	n.c.	100	0.5
HCT	n.c.	n.c.	100	0.5
MCV	n.c.	n.c.	100	0.5
MCHC	n.c.	n.c.	100	0.5
HDW	n.c.	n.c.	100	0.5
PLT	10	5	50	2
MPV	100	2.5	10	4.5
PDW	10	5	100	0.5
PCT	n.u.[b]	n.u.	100	11.5

[a] n.c. = no changes.
[b] n.u. = values not usable.

Table 2
Fluence-rate thresholds for irradiating whole human blood with two different wavelengths. There was no photosensitizer given to the blood. Blood was irradiated both with and without controlling the temperature. In parentheses are given the site of first changes in whole human blood occurring at the given fluence rate

	$\lambda = 630$ nm	$\lambda = 653$ nm
Without cooling	130 mW/cm^2 (WBC)	900 mW/cm^2 (PDW and OF [a])
With cooling	270 mW/cm^2 (WBC)	1500 mW/cm^2 (WBC)

[a] OF = osmotic fragility.

3.2. Effect of laser irradiation without PS

The irradiation of CX1 cells in SF-M suspension for fluence rates $0 < E < 250$ mW/cm^2 (630 nm, 10 ml/h flow velocity) produced no significant change in survival fraction.

Irradiation of blood with $\lambda = 630$ nm produced the first changes in blood (change in white blood cell count, WBC) at fluence rates $E = 130$ mW/cm^2. Controlling the temperature increases this threshold to $E = 270$ mW/cm^2 (changes in WBC, see Table 2). Each series of measurements included all the fluence rates used ($0 < E < 300$ mW/cm^2 for $\lambda = 630$ nm and $0 < E < 1800$ mW/cm^2 for $\lambda = 653$ nm). Each series of measurements was carried out at least twice.

Irradiation of blood using the wavelength $\lambda = 653$ nm produced first changes in osmotic fragility at $E = 900$ mW/cm^2 without a cooling device. With a cooling device we determined a fluence-rate threshold of $E = 1500$ mW/cm^2 (changes in WBC, see Table 2).

Further irradiation experiments were conducted using the cooling device and fluence-rate ranges of $0 < E < 250$ mW/cm^2 for both wavelengths.

3.3. PDT of CX1 cells in blood and in medium

In Fig. 2 the survival curves of CX1 cells incubated with Photofrin II and irradiated in SF-M and in blood are shown. For irradiation in SF-M a flow velocity of $v_{Fl} = 15.72$ ml/h was maintained, while in blood it was $v_{Fl} = 15.72$ ml/h and $v_{Fl} = 1.15$ ml/h, respectively.

The control experiments 1 and 2 show a slight decrease in SF with increasing time in all series of measurements. Control experiment 3 shows a slight increase in SF.

The course of survival curves measured in SF-M (Fig. 2) shows a decrease of the SF for $0 < E < 101$ mW/cm^2. For $101 < E < 253$ mW/cm^2 the SF oscillates around a constant value of SF = 0.035.

The lowest SF measured in SF-M is SF = (0.016 ± 0.006) at $E = 101$ mW/cm^2.

In blood the SF decreases for $0 < E < 190$ mW/cm^2 to a constant value of SF = 0.0035. The lowest measured value is SF = (0.002 ± 0.001) at $E = 190$ mW/cm^2.

The PDT in blood with *m*THPC was conducted with the same irradiation parameters (power or fluence rate, flow velocity) for better comparison (see Fig. 3), although higher fluence rates could be used. Only the wavelength ($\lambda = 653$ nm) and concentration of PS ([*m*THPC] = 1 μg/ml) were changed.

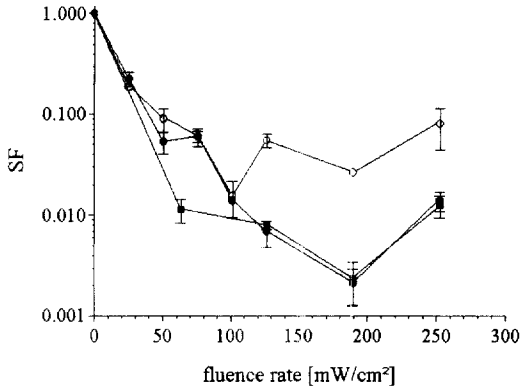

Fig. 2. Survival fraction of CX1 cells after incubation ($T = 37$°C) with 10 μg/ml Photofrin II and following irradiation with laser light vs. fluence rate: ○ = irradiation in SF-M, $v_{Fl} = 15.72$ ml/h, $\lambda = 630$ nm; ● = irradiation in blood, $v_{Fl} = 15.72$ ml/h, $\lambda = 630$ nm; ■ = irradiation in blood, $v_{Fl} = 1.15$ ml/h, $\lambda = 630$ nm. Different fluence rates were obtained by different settings of the laser power. Irradiations were carried out at controlled temperature ($T \leq 30$°C).

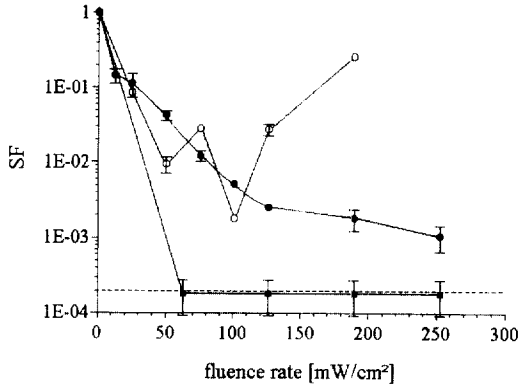

Fig. 3. Survival fraction of CX1 cells after incubation with 1 μg/ml *m*THPC and following irradiation with laser light vs. fluence rate: ○ = irradiation in SF-M, $v_{Fl} = 15.72$ ml/h, $\lambda = 653$ nm; ● = irradiation in blood, $v_{Fl} = 15.72$ ml/h, $\lambda = 653$ nm; ■ = irradiation in blood, $v_{Fl} = 1.15$ ml/h, $\lambda = 653$ nm. Different fluence rates were obtained by different settings of the laser power. Irradiations were carried out at controlled temperature ($T \leq 30$°C).

The survival characteristics of the *m*THPC-incubated cells in SF-M after laser light irradiation are shown in Fig. 3. There is a decrease in SF for $0 < E < 120$ mW/cm^2. For fluence rates $E > 126$ mW/cm^2 the SF curve rises very high. At $E = 190$ mW/cm^2 we measured SF = (0.26 ± 0.02). The SF at $E = 252$ mW/cm^2 could not be determined, because the colonies grown were too numerous to be counted. That means the SF at $E = 252$ mW/cm^2 is higher than SF = 0.26.

The irradiation in SF-M shows a minimum value of SF = (0.0018 ± 0.0002) at $E = 101$ mW/cm^2.

In blood the survival curves determined after *m*THPC PDT show a decrease in SF down to a measured value of SF = 0.0013 at a flow velocity $v_{Fl} = 15.72$ ml/h. The survival

curve shows a small shoulder at $E \approx 10$–20 mW/cm^2 with SF = 0.25–0.2.

The survival fractions of the *m*THPC PDT in blood at $v_{Fl} = 1.15$ ml/h are all lower than the detection limit of the PEIB method. The detection limit was due to agglutination of blood (see Section 2) and could be determined to be SF = 0.0002 for the unirradiated control sample. The detection limit could be lowered by increasing the CX1 cell concentration in blood during preparation (before irradiation) to more than 1×10^7 cells/ml. As far as we know, such high concentrations of tumour cells have never been found in haematogenous dissemination processes. Therefore, in comparison to the unirradiated control, more than 99.98% of the CX1 cells could be eradicated in blood by the *m*THPC PDT.

4. Discussion

At high fluence rates the survival curves show a constant survival fraction. This course of curves could not be found in other studies (e.g., [25]), where classical dose–response curves [26] are seen. In other studies the gas exchange in the irradiated suspension was better (open dishes) [25,27,28]. In the present study there was no gas exchange in the irradiation system. We assume that the oxygen necessary for the photodynamic reaction had been consumed after some irradiation time. Therefore, some of the irradiated cells were spared toxic reaction products of the photodynamic reaction or could repair cell damage.

Using an irradiation device that allows gas exchange, the lowest SF for both types of PS will probably be lower than the values measured in this paper. However, we found it interesting to see that tumour-cell killing in blood is possible at a good rate using PDT without additional gas exchange. With a suitable optical fibre we now have the opportunity to irradiate blood within the blood vessel of a patient. Inside the blood vessel no gas exchange is possible.

Furthermore, in the present study higher fluence rates were used than in other in vitro PDT studies (i.e., [27,29]). A higher bleaching rate of PS might hence cause a higher survival fraction at high fluence rates. This is particularly evident for the survival curves of *m*THPC PDT in SF-M (Fig. 3).

A comparison between the two media (SF-M and blood) during irradiation shows a lower survival fraction of CX1 cells in blood after PDT. An influence of the irradiation system or the relevant irradiation parameters on this difference can be ruled out, because all survival curves were normalized to an unirradiated control. Furthermore, the same range of irradiation parameters was used for both media.

We assume that

1. the oxygen bound in the erythrocytes may cause a higher eradication of CX1 cells after PDT,
2. the different light propagation in both media may cause different energy radiances within each medium,
3. differences in the molecular compounds may cause different quenching or intensified reaction in each compound.

However, it can be seen that within a wide range of fluence rates (about 120–150 mW/cm^2 for PII and 60–250 mW/cm^2 for *m*THPC) the killing of sensitized tumour cells in blood is possible without destroying any blood components.

With *m*THPC, cell killing in blood was significantly better than with Photofrin II. We explain this as follows:

1. The PS *m*THPC is better enriched in the cells ([27,28]).
2. *m*THPC has better absorption or activation properties ([8,27]).
3. The activation wavelength of *m*THPC (653 nm) shows more favourable properties due to light propagation in blood.

Other photosensitizers might lead to an even better tumour-cell killing in blood than *m*THPC.

All in all, the best rate of tumour-cell killing under the conditions given in our experiments could be determined to be more than 99.98% using *m*THPC PDT. Bearing in mind that we only optimized a few parameters of this new method, this seems to be a promising result. Other parameters like the oxygen concentration in blood and the chosen sensitizer might lead to even higher tumour-cell killing rates. This encourages future investigations in this new method of cancer therapy. Additionally, these investigations should include first in vivo studies and the development of suitable irradiation devices for different types of tumours, leading to different routes of tumour-cell dissemination in the human body. We think that PDT applied for blood-purging purposes can have a chance of suppressing haematogenous dissemination of tumour cells in the human body.

5. Conclusions

A multitude of parameters influence the PDT effect in blood. In addition to the optimized parameters used in this study (path length of blood layer, flow velocity, tubing of irradiation system, fluence rates, PS concentration), measurement of the oxygen concentration and/or oxygen enrichment of the cell–blood suspension during PDT seems useful.

However, the parameters used in this first in vitro study already produced a survival fraction SF < 0.02%. We think this value is low enough to continue investigating the application of PDT as a tool for suppressing the haematogenous dissemination of cancer.

Acknowledgements

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