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 mesotetra(hydroxyphenyl)chlorin (mTHPC). We added the cells to fresh whole blood and irradiated the blood with suitable laser light in a flowthrough 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 mTHPC we measured SF = 0.017%. These results encourage further investigations concerning the use of photodynamic therapy for suppressing haematogenous dissemination.

Keywords: Blood; Haematogenous metastasis; Photodynamic therapy; Photofrin II; mTHPC

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 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 mesotetra(hydroxyphenyl)-chlorin (mTHPC). mTHPC shows a higher optical density
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 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 Ar¹ laser (Spectra Physics, Darmstadt, Germany) was used as irradiation source. The laser dye was DCM (4-dicyanomethylene-2-methyl-6-­p-dimethylaminostyryl-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 mTHPC 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 (v₁) 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, Heroldsborg, 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 Vₛ and from the flow velocity v₁ by t = Vₛ/v₁. For example, a flow velocity of v₁ = 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 20 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. mTHPC was supplied by Scotia Pharmaceuticals (Guildford, UK) and was dissolved in 35% polyethylene glycol 750, 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 cm² 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 × 10⁵ 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, t,oe blood serum was replaced by 2% Cibaro- 
G (Gibco BRL, Eggenstein, Germany).

All steps involving photosensitizers were conducted in a 
neatly dark room. After incubation with PS (24 h), cells were 
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 concentra-
tions of Photofrin II and mTHPC 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 meas-
urement 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 I, Schärte 
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 (PF). The PE of cells irradiated in SF-M 
was determined as described in Ref. [22]. The survival frac-
tion (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 treat-
ment) 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 colonies 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
d. cells incubated with PS and led through the irradiation 
device with the same flow rate as the corresponding irra-
diation experiment, but not irradiated.

These control experiments were conducted within every 
series of irradiation experiments directly after the correspond-
ing irradiation experiment.

For all irradiation experiments in this study, 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 vol-
unteer. 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 punc-
ture (Sedrainer, Becton Dickinson Vacutainer Systems 
Europe, Meylan Cedex, France) and with EDTA/KE (Sur-
stedt, 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 
II ( Bayer Diagnostic, Munich, Germany). The counter was 
able to measure white blood cells (WBC), neutrophils 
(NEUT), lymphocytes (LYMPH), monocytes (MONO), 
esinophils (EOS), basophils (BASO), red blood cells 
(RBC), haemoglobin content (Hb), haematocrit (HCT), 
mean (red) cell volume (MCV), mean content of haemo-
globin (MCH), mean content of haemoglobin per cell 
(MCHC), red-cell distribution weight (RDW), haemoglo-in 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/cm² for λ = 630 nm and 0 < E < 1800 mW/cm² for λ = 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 mTHPC to the CX1 cells in the dark was measured in FCS-M. The results are shown in Fig. 1. The D₅₀ dose of the drugs in these cells was determined to be D₅₀ (Photofrin II) = 50 μg/ml for Photofrin II and D₅₀ (mTHPC) = 3.8 μg/ml for mTHPC (see Table 1). The D₅₀ dose is the drug dose that leads to a survival fraction of 50% without using irradiation.

The dark toxicity of Photofrin II and mTHPC 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 [PH₁] > 10 μg/ml. A significant change in white blood cells (WBC) and platelet count (PLT and PDW) was observed (see Table 1). The incubation of blood with mTHPC showed the first changes a few minutes after the beginning of incubation. For mTHPC concentrations [mTHPC] > 50 μg/ml the white and red blood-count parameters (WBC, RBC, HGB, HCT, MCV, MCHC, HDW) changed. Additionally, after 4.5 h of incubation the mean platelet volume (MPV) changed for [mTHPC] > 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 mTHPC. 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

<table>
<thead>
<tr>
<th>D₅₀ dose for CX1 cells</th>
<th>Photofrin II</th>
<th>mTHPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood counts</td>
<td>Concentration [μg/ml]</td>
<td>Incub. time [h]</td>
</tr>
<tr>
<td>WBC</td>
<td>10</td>
<td>15.5</td>
</tr>
<tr>
<td>RBC</td>
<td>n.c.</td>
<td>n.c.</td>
</tr>
<tr>
<td>HGB</td>
<td>n.c.</td>
<td>n.c.</td>
</tr>
<tr>
<td>HCT</td>
<td>n.c.</td>
<td>n.c.</td>
</tr>
<tr>
<td>MCV</td>
<td>n.c.</td>
<td>n.c.</td>
</tr>
<tr>
<td>MCHC</td>
<td>n.c.</td>
<td>n.c.</td>
</tr>
<tr>
<td>HDW</td>
<td>n.c.</td>
<td>n.c.</td>
</tr>
<tr>
<td>MPV</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>PDW</td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td>PLT</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>PCT</td>
<td>n.u.</td>
<td>n.u.</td>
</tr>
</tbody>
</table>

n.c. = no changes.

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.

<table>
<thead>
<tr>
<th></th>
<th>λ = 630 nm</th>
<th>λ = 653 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without cooling</td>
<td>130 mW/cm²</td>
<td>900 mW/cm²</td>
</tr>
<tr>
<td>(WBC)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With cooling</td>
<td>270 mW/cm²</td>
<td>1500 mW/cm²</td>
</tr>
<tr>
<td>(WBC)</td>
<td></td>
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</table>

*OF = osmotic fragility.

3.2. Effect of laser irradiation without PS

The irradiation of CXI cells in SF-M suspension for fluence rates $0 < E < 250$ mW/cm² (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/cm². Controlling the temperature increases this threshold to $E = 270$ mW/cm² (changes in WBC, see Table 2). Each series of measurements included all the fluence rates used ($0 < E < 300$ mW/cm² for $\lambda = 630$ nm and $0 < E < 1800$ mW/cm² 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/cm² without a cooling device. With a cooling device we determined a fluence-rate threshold of $E = 1500$ mW/cm² (changes in WBC, see Table 2). Further irradiation experiments were conducted using the cooling device and fluence-rate ranges of $0 < E < 250$ mW/cm² for both wavelengths.

3.3. PDT of CXI cells in blood and in medium

In Fig. 2 the survival curves of CXI cells incubated with Photofrin II and irradiated in SF-M and in blood are shown. For irradiation in SF-M a flow velocity of $v_{31} = 15.72$ ml/h was maintained, while in blood it was $v_{11} = 15.72$ ml/h and $v_{11} = 1.15$ 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/cm². For $101 < E < 253$ mW/cm² 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/cm².

In blood the SF decreases for $0 < E < 150$ mW/cm² to a constant value of SF = 0.0035. The lowest measured value is SF = (0.002 ± 0.001) at $E = 190$ mW/cm².

The PDT in blood with mTHPC 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 ([mTHPC] = 1 μg/ml) were changed.

The survival characteristics of the mTHPC-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/cm². For fluence rates $E > 126$ mW/cm² the SF curve rises very high. At $E = 190$ mW/cm² we measured SF = (0.26 ± 0.02). The SF at $E = 252$ mW/cm² could not be determined, because the colonies grown were too numerous to be counted. That means the SF at $E = 252$ mW/cm² 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/cm².

In blood the survival curves determined after mTHPC PDT show a decrease in SF down to a measured value of SF = 0.0013 at a flow velocity $v_{31} = 15.72$ ml/h. The survival
The survival fractions of the mTHPC PDT in blood at v_3 = 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 CX I cell concentration in blood during preparation (before irradiation) to more than 1 x 10^5 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 CX I cells could be eradicated in blood by the mTHPC 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 mTHPC PDT in SF-M (Fig. 3). A comparison between the two media (SF-M and blood) during irradiation shows a lower survival fraction of CX I 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 CX I 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/cm² for PII and 60–250 mW/cm² for mTHPC) the killing of sensitised tumour cells in blood is possible without destroying any blood components.

With mTHPC, cell killing in blood was significantly better than with Photofrin II. We explain this as follows:
1. The PS mTHPC is better enriched in the cells ([27, 28]).
2. mTHPC has better absorption or activation properties ([18, 27]).
3. The activation wavelength of mTHPC (635 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 mTHPC.

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 mTHPC 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.

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