Evaluation of Human Erythrocytes as Model Cells in Photodynamic Therapy

L. Kaestner[1,2]

[1] *Norwegian Radiation Protection Authority, Department of Radiation Medicine, P.O.Box 55, 1332 Oslo, Norway*
[2] *Gentian AS, Belsetsvingen 39, 1348 Oslo, Norway*

Abstract. The role of erythrocytes as targets in photodynamic therapy is a controversially discussed topic in the literature. Therefore five different, but well known photosensitisers (three zinc phthalocyanines, tetrabenzoporphine and pheophorbide *a* delivered in liposomes were used for photodynamic treatment of human erythrocytes. The phototoxic effect on these cells showed pronounced differences. It was in the range: zinc phthalocyanine = pheophorbide *a* > tetrabenzoporphine ≫ zinc octa-n-alkyl phthalocyanines. Data from the zinc octa-n-alkyl phthalocyanines were compared with photodynamic effects within cutaneous cell lines, treated under the same experimental conditions. The results show that erythrocytes are unlikely to make good models for predicting the efficiency of the photosensitiser in general, and the same applies to cells other than erythrocytes and *in vivo*. Possible reasons could be differences in dye accumulation. However, erythrocytes may well serve as model cells to explore the cellular and molecular mechanisms of photodynamic treatment.

Key words: Photodynamic therapy — Red blood cells — Phthalocyanines — Tetrabenzoporphine — Pheophorbide *a*

Introduction

Photodynamic therapy (PDT) is based on the application of a tumour-localising and photosensitising drug followed by exposure of the tumour area to light. Photoinduced singlet oxygen is believed to be the toxic agent responsible for the destruction of the tumour cells (Dougherty et al. 1998). Erythrocytes are closely connected to PDT as they are the most readily obtained living human cells. They served and are still serving as model cells in many studies as either: i) erythrocyte ghosts (e.g. Kaestner 1997) or ii) intact red blood cells (e.g. Moor et al. 1997).

Correspondence to: Dr. Lars Kaestner, Institute for Molecular Cell Biology, Medical Faculty, Saarland University, Building 61, 66421 Homburg, Germany
E-mail: biophysics@web.de

In contrast immense studies on PDT of erythrocyte concentrates for virus inactivation, e.g. malaria, HIV, etc. have been performed (Ben-Hur et al. 1999a,b). In these studies the authors claim that erythrocytes survive PDT without any substantial damage. This contradiction is reflected in the introduction of the state of the art monograph "Photodynamic Tumor Therapy" by Moser (1998): "Another chapter concerned with the description of biophysical parameters governing PDT effects on erythrocytes will be written in about 10 years when the different authors come to an agreement ..." This highlights the need for further research. The aim of this paper is to provide an evaluation as to whether erythrocytes are "proper" model cells for testing the photosensitising activity of dyes and determine under which conditions this is the case. To this end, five differing photosensitisers were tested: three zinc phthalocyanines, zinc tetrabenzoporphine and pheophorbide *a*. Previously, zinc phthalocyanines have been proofed to be efficient photosensitisers in cell cultures (Fabris et al. 2001; Kaestner et al. 2003) as well as in transplanted tumours *in vivo* (Ometto et al. 1996; Fabris et al. 1997). For benzoporphines in general a photosensitising potency has been shown in differing biological systems such as red blood cells, rat mitochondria and stomatitis viruses (Richter et al. 1996). Pheophorbide *a* associated studies also show its efficiency as a photosensitiser, e.g. in lung fibroblasts (Inanami et al. 1999), jurcat cells (Paul et al. 2002) and colonic cancer cells (Hajri et al. 2002).

Materials and Methods

Dyes and chemicals

The following photosensitisers were used: 1,4,8,11,15,18,22,25-octakis(decyl)phthalocyaninato zinc(II) (ZnODPc) and 1,4,8,11,15,18,22,25-octakis(pentyl)phthalocyaninato zinc(II) (ZnOPPc), unsubstituted zinc(II) phthalocyanine (ZnPc), zinc 29,31-tetrabenzoporphine (ZnTBP) and pheophorbide *a* (pheo).

The octa-n-alkyl zinc phthalocyanines were prepared as described elsewhere (Cook et al. 1995) under a licence agreement between Gentian AS (Rykkinn, Norway) and QinetiQ Ltd. (Farnborough, UK). Unsubstituted zinc phthalocyanine and zinc tetrabenzoporphine were bought from Aldrich Chemical Co. (Milwaukee, USA). Pheophorbide *a* was extracted from young leaves of *Urtica urens* according to Willstätter and Stoll (1913).

In Fig. 1 the structural formulae (part A) and the absorption spectra (part B) of all dyes under investigation are shown.

All chemicals used were of analytical grade. Tetrahydrofuran (THF) was supplied by the Aldrich Chemical Co. (Milwaukee, USA), and the Chloroform by Merck Chemicals (Darmstadt, Germany). 1,2-dioleoyl-sn-glycero-3-phosphocholin was supplied by Avanti Polar Lipids Inc. (Alabaster, USA) and the phosphate buffered solution (PBS) without $CaCl_2$ and $MgCl_2$ was purchased from Life Technologies Ltd. (Paisley, Scotland). It contained: 150 mmol/l NaCl, 5.8 mmol/l NaH_2PO_4-Na_2HPO_4, (pH 7.4).

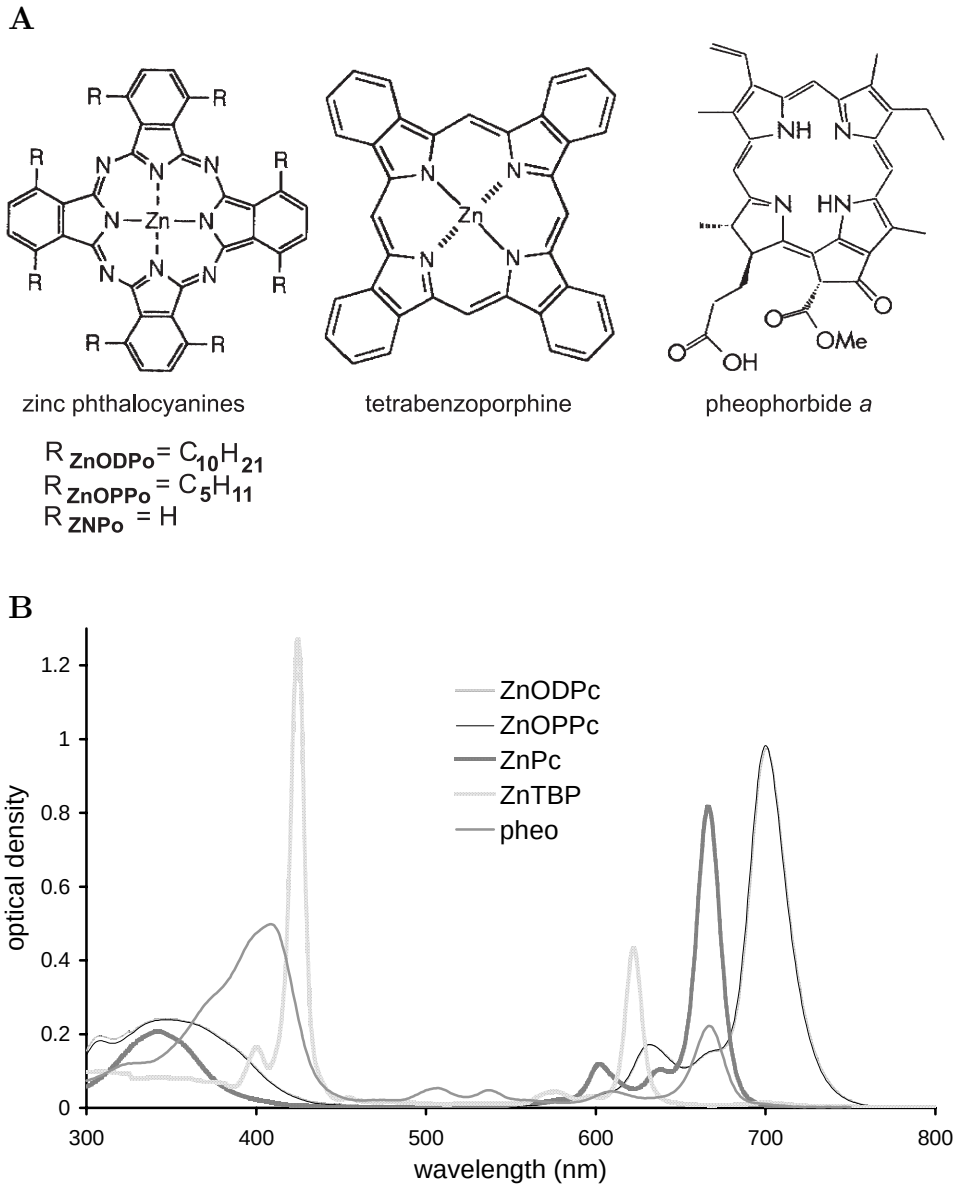

Figure 1. A. Structural formulae of the synthesised zinc phthalocyanines including the appropriate substitutens (Cook et al. 1995), tetrabenzoporphine and the natural occurring pheophorbide *a* (Röder 1986); **B.** Absorption spectra of all the photosensitisers presented in A with the following abbreviations: ZnODPc for 1,4,8,11,15,18,22,25-octakis(decyl)phthalocyaninato zinc(II); ZnOPPc for 1,4,8,11,15,18,22,25-octakis (pentyl)phthalocyaninato zinc(II); ZnPc for unsubtituted zinc(II) phthalocyanine; ZnTBP for zinc 29,31-tetrabenzoporphine and pheo for pheophorbide *a*. The dyes are solved in tetrahydrofuran at a concentration of 5 μmol/l.

Optical equipment

Spectrometers used throughout the experiments were a Perkin Elmer Spectrophotometer Lambda 2 for absorptions and a Perkin Elmer Luminescence Spectrometer LS50B for fluorescence measurements.

For irradiation of the cells, the light source was a slide projector (Kodak Ektopro 3010), containing a 300 Watt Xenon bulb, with a red filter (B+W, Schneider Kreuznach) attached. The resulting spectra showed a maximum at about 627 nm and was close to zero below 580 nm. For monitoring the power of this light source an optical power meter (S371 with sensor head model 247; United Detector Technology, San Diego, USA) attached with a 3.9% transmittance grey filter was used. A mirror was placed directly beneath the radiated cells to achieve a "double-radiation", thereby increasing the radiation power.

Liposomes

The preparation of the liposomes was performed as described by Valduga et al. 1996. The 1,2-dioleoyl-sn-glycero-3-phosphocholin supplied in form of a solution containing 50 mg/ml dissolved in chloroform. The ratio of lipids to dye was chosen to have a molar ratio of 200 : 1. The chloroform dissolved dye was mixed with the lipid solution before the chloroform was evaporated off using a rotation-evaporator. All handling of the lipids was done under N_2-gas. The solid lipid-dye mixture was "dissolved" in PBS, pH 7.4 (15 ml *per* 200 mg lipids). The solution was then sonicated (KONTES Micro Ultrasonic Cell Disruptor KT50) three times, each time of 5 min duration with 10 min breaks in between under N_2-gas flow at about 10 Hz. The mixture was cooled by ice during the whole procedure. After 24 h the solution was filtered using a disc-filter of 0.2 μm filtration value.

Red blood cells

Freshly drawn blood (1–3 days old) from healthy human donors was used for the experiments. The blood was washed three times by centrifugation ($1500 \times g$, 8 min) at room temperature in PBS, pH 7.4. Plasma and buffy coat were removed by aspiration. A 10% erythrocyte suspension was then prepared by dilution in PBS. For dye incubation approximately 14.5×10^6 erythrocytes were placed in a solution, containing a known amount of dye, delivered by liposomes. The concentration of the dye was chosen in such a way, that the product of the integrals of the light emission curve, and the lipid corrected absorption spectra of the liposomes (dyes in solvent – Fig. 1B) gave the same value for all dyes. This was done in order to create comparable irradiation conditions even when using a non-monochromatic light source.

Cells were dye-incubated for two hours at 37°C before being washed three times in 4°C PBS using an Eppendorf centrifuge. For irradiation purposes, the same number of erythrocytes was kept in each well of a 6-well cell culture plate, giving an unilamilar cell layer within the wells. Radiation was carried out using the slide projector at distances that gave fluence rates of between 6 and 30 mW/cm^2.

After irradiation, the whole suspension was centrifuged and the supernatant was investigated by absorbance at 414 nm. 100 % haemolysis was taken as the value obtained when the given number of erythrocytes were placed directly in distilled water.

Cultured cell treatment

The cultured cells were fibrosarcoma cells (HT1080) and keratinocytes (HaCaT). All cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10 % foetal bovine serum. The culture medium was completed by the addition of 100 units/ml of penicillin and 100 μg/ml of streptomycin as well as by 2.5 μg/ml of amphotericin B. The cells were kept in a humid atmosphere containing 5 % CO_2 (95 % humidity) at a constant temperature of 37 ℃. The cells were sown approximately 1 : 5 every two or three days. The following procedure was adopted: the culture medium was removed and the cells were washed twice with PBS containing Ca^{2+} and Mg^{2+}, the HT1080 cells were then incubated for 1 to 2 min with a 0.05 % trypsin / 0.02 % EDTA solution, whilst for HaCaT cells 0.1 % trypsin and 0.04 % EDTA solution was used. The action of trypsin was stopped by the addition of serum. The cells were then sedimented by centrifugation for 10 min, and the resultant cell pellet, which formed the basis of a new suspension in DMEM, before beeing seeded in 12-well cell culture plates. After 24 hours the cells were washed twice with PBS, and the cells incubated in a liposome/medium mixture containing dye of a 10 μmol/l concentration. The dye concentration was determined using a fluorometric calibration curve. Again, incubated cells were kept in a humid atmosphere containing 5 % CO_2 (95 % humidity) at a constant temperature of 37 ℃.

During the radiation experiments the cells were suspended in DMEM. Two wells were used as a control and two wells for each dye were incubated on the plate for irradiation. A duplicate dark control plate was prepared, containing two wells with cells only and four wells with two dyes. Immediately after incubation, the cells were irradiated as previously described for red blood cells. 24 h after irradiation, the cells were washed twice, before being trypsinised and stained trypan blue, prior to surviving cells being counted in a Bürker chamber. The survival percentage was calculated by relating the dye-incubated and irradiated counts to the not incubated but irradiated cells. However, there was no significant difference between i) non-incubated but irradiated, ii) dye-incubated but not irradiated nor iii) non-incubated and non-irradiated cells.

Results

As it has previously been shown the photodynamic treatment of erythrocytes causes the release of potassium ions, on an identical, but time shifted behaviour, when compared with haemolysis (Ball et al. 1998). The latter has been taken to define the cell death of erythrocytes.

Fig. 2 shows the cell survival of erythrocytes after two hours of incubation with the indicated dye and irradiation with red light at a fluence rate of 15 mW/cm^2 and

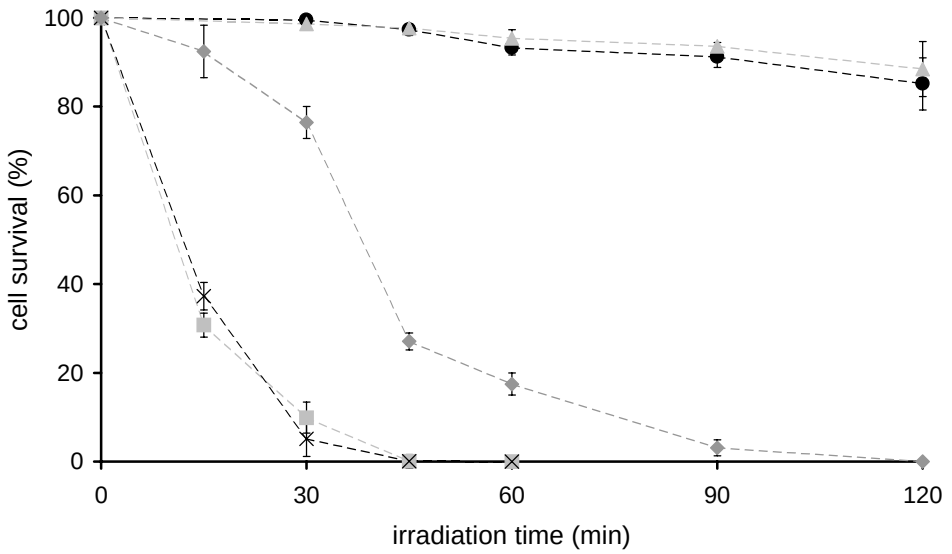

Figure 2. Cell survival curves after incubation with the photosensitisers under investigation after irradiation with red light at a fluence rate of 15 mW/cm^2 and the indicated irradiation time; ●, ZnODPc; ▲, ZnOPPc; ■, ZnPc; ◆, ZnTBP; **x**, pheo. Each point is the mean of at least three independent experiments and the error bars represent the standard deviation. The dashed lines are printed to allow a better overview.

the indicated irradiation time. It was found that the efficiency of the dyes tested was quite different. While for ZnPc and pheo, the cell survival is down to zero after 45 min of irradiation, whilst for ZnTBP, 120 min of irradiation is required to achieve the same effect. The behaviour of erythrocytes incubated with zinc octa-n-alkyl phthalocyanines was found to be totally different, and even after 120 min of irradiation, cell survival for ZnODPc and ZnOPPc was as high as 85 % and 88 %, respectively.

This is, as Fig. 3 shows, in contradiction to the phototoxic action of the zinc octa-n-alkyl phthalocyanines on cutaneous cells. In Fig. 3 a comparison of the cell survival of erythrocytes, HT1080 and HaCaT incubated with ZnODPc and ZnOPPc after application of two different irradiation energies are seen. The phototoxicity shows a pronounced effect in fibrosarcoma cells and an even higher efficiency in keratinocytes whilst in erythrocytes the effect is rather small for both zinc octa-n-alkyl phthalocyanines. The higher efficiency in HaCaT cells compared to HT1080 cells is due to a higher percentage of dye accumulation in HaCaT (Kaestner et al. 2003). This protocol of measurements could not be applied for erythrocytes, due to overlapping spectra of the photosensitisers (Fig. 1B) and haemoglobin, plus the quantitative predominance of the latter.

Erythrocyte survival dependence on the irradiation fluence rate for zinc

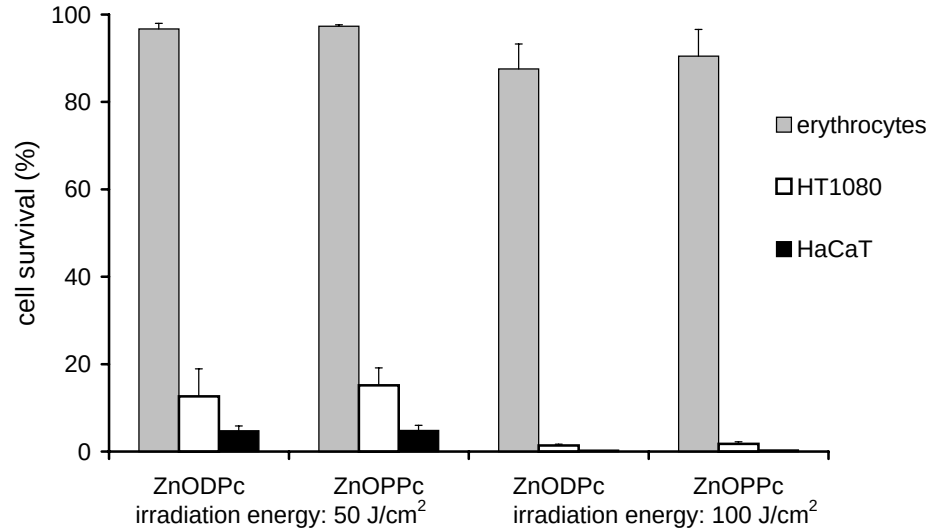

Figure 3. Cell survival of erythrocytes, fibrosarcoma cells (HT1080) and keratinocytes (HaCaT) after liposome delivered ZnODPc and ZnOPPc at a concentration of 10 μmol/l and irradiation of red light with energies of 50 and 100 J/cm^2. The value of each column is the mean of three independent experiments and the error bars represent the standard deviation.

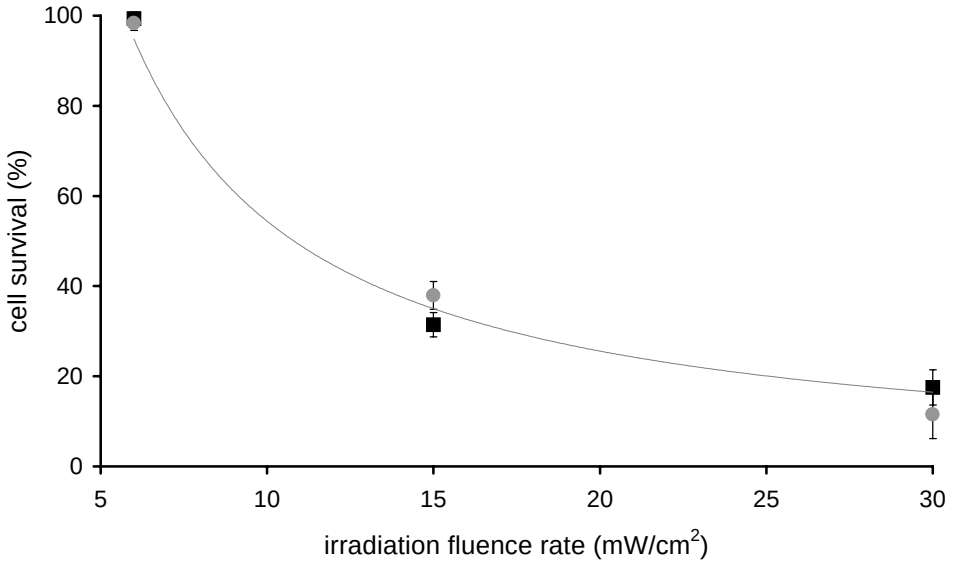

Figure 4. Cell survival after incubation with ZnPc (■) and pheo (●) after 15 min irradiation with red light at a fluence rate like indicated. Each point is the mean of at least four independent experiments and the error bars represent the standard derivation. A regression curve shows an exponential dependence.

phthalocyanine and pheophorbide *a* after 15 min irradiation is shown in Fig. 4. An exponential regression line shows the superlinear increase in cell death with an increasing fluence rate for both substances under test.

Discussion

A decrease of cell survival with increase in irradiation time (Fig. 2) is an expected result, especially because the dyes under investigation have historically proved to be good photosensitisers in various biological systems (see Introduction). Furthermore, a variation in the efficiencies is already indicated by different structures and different photophysical properties (Fig. 1) of the dyes. However, the range of differences with ZnPc and pheo on the one hand, and ZnODPc and ZnOPPc on the other, is surprising, especially the low phototoxicity of the latter in erythrocytes. This is especially so, since they are efficient photosensitiser in other cell lines (Fig. 3 and Kaestner et al. 2003).

A similar effect has been achieved previously for a silicon based phthalocyanine (Pc 4) (Oleinick et al. 1993). Pc 4 could be proved to be a highly efficient photosensitiser in many different cell cultures and tumours, e.g. in rat hepatic microsomes, transplanted tumours in mice (Zaidi et al. 1993a,b), Chinese hamster lung fibroblasts and murine and human leukemic lymphoblasts (He et al. 1997, 1998). In contrast, the photodamage of erythrocytes after photodynamic treatment with Pc 4 is minor (Ben-Hur et al. 1997, 1999a). In those reports, a very low irradiation fluence of some 10 J/cm^2 was applied. Therefore, the cell survival was measured in dependence of the light fluence rate (Fig. 4), indicating that with a constant irradiation dose the cell survival is higher for low irradiation fluence rates. So, the value for the light dose where 50 % of the cells survive (LD_{50}), is not a constant; e.g. for ZnPc, as well as for pheo, LD_{50} could be derived to be 9.9 J/cm^2 for 11 mW/cm^2 (15 min irradiation) and 10.8 J/cm^2 for 15 mW/cm^2 (12 min irradiation).

However, reasons for the differences in dye actions within erythrocytes (Fig. 2), and the differences of a single dye in differing cells (Fig. 3) cannot be explained within the remit of experiments presented here. Possible reasons for the differences may be linked to the differing substituents on the aromatic rings of the tetrapyrol core (Fig. 1A), giving the molecules different chemical properties. Differences in the π-electron system of the tetrapyrroles, and hence differences in the singlet oxygen quantum yield, if any, may explain a fractional part of the distinct phototoxic behaviour of the dyes. The singlet oxygen quantum yields in organic solvents are 0.51 ± 0.07 for pheophorbide *a* in ethanol (Roeder et al. 1990), 0.53 ± 0.01 and 0.47 ± 0.02 for ZnPc and ZnODPc, respectively in tetrahydrofuran (Kaestner et al. 2003). Tetrabenzoporphine and ZnOPPc data are not available for comparative purposes. However, a difference of roughly 12 % in the oxygen quantum yields between ZnODPc and ZnPc (the latter one was chosen for comparison to stay within the same solvent) cannot explain the different phototoxic action in erythrocytes as shown in Fig. 2.

The differing degree of action of a particular photosensitier within various cells is potentially connected to the amount of dye accumulated within cells, as differences could be detected between HaCaT and HT1080 cells. Another potential mechanism could be the release of the photosensitiser from erythrocytes triggered by light irradiation as described for protoporphyrin from erythrocytes of erythropoitic protoporpyria patients (Brun et al. 1990).

Erythrocytes cannot therefore be generally regarded as good model cells for photodynamic therapy, especially for the comparison of quantitative phototoxic action of differing compounds within cells. The comparison between treated cultured cells and erythrocytes as shown in Fig. 3, supports this conclusion. Further support for this is shown in Kaestner et al. 2003, where ZnODPc and ZnPc were evaluated, showing similar photosensitising action (even with a slight advantage of ZnODPc) in mouse skin after topical application and irradiation. The erythrocyte model with the results expressed in Fig. 2 would give an incorrect prediction. This evaluation of human erythrocytes as model cells in photodynamic therapy is a new approach, as erythrocytes have previously been believed to be a convenient model system for screening new dyes (Spikes 1983).

In light of these findings, erythrocytes may well serve as model cells in order to further elucidate the fundamental principles in photodynamic therapy, as was done previously to prove the involvement of singlet oxygen (Sonoda et al. 1987), or to describe the mechanism leading to photodynamic induced haemolysis (Zavodnik et al. 2002).

Acknowledgements. I wish to thank Prof. Terje Christensen (Norwegian Radiation Protection Authority) for inspiring discussions and Dr. Martin Southwell (University of the West of England) for proof-reading the manuscript.

References

Ball D. J., Wood S. R., Vernon D. I., Griffiths J., Dubbelman T. M. A., Brown S. B. (1998): The Characterisation of three substituted zinc phthalocyanines of differing charge for use in photodynamic therapy. A comparative study of their aggregation and photosensitising ability in relation to *m*THPC and polyhaematoporphyrin. J. Photochem. Photobiol., B **45**, 28—35

Ben-Hur E., Barshtein G., Chen S., Yedgar S. (1997): Photodynamic treatment of red blood cell concentrates for virus inactivation enhances red blood cell aggregation: protection with antioxidants. Photochem. Photobiol. **66**, 509—512

Ben-Hur E., Zuk M. M., Oetjen J., Chan W. S., Lenny L., Horowitz B. (1999a): Photochemical decontamination of red cell cancentrates with the silicon phthalocyanine Pc 4 and red light. J. Biomed. Optics **4**, 292—297

Ben-Hur E., Chan W. S., Yim Z., Zuk M. M., Dayal V., Roth N., Heldman E., Lazo, A., Valeri C. R., Horowitz B. (1999b): Photochemical decontamination of red cell cancentrates with the silicon phthalocyanine Pc 4 and red light. In: Advances in Transfusion Safety102. (Eds. F. Brown and G. Vyas), pp. 149—155, Dev. Biol. Stand., Basel

Brun A., Western A., Malik Z., Sandberg S. (1990): Erythropoietic protoporphyria: Photodynamic transfer of protoporphyrin from intact erythrocytes to other cells. Photochem. Photobiol. **51**, 573—577

Cook M. J., Chambrier I., Cracknell S. J., Mayes D. A., Russell D. A. (1995): Octaalkyl zinc phthalocyanines: potential photosensitizers for use in the photodynamic therapy of cancer. Photochem. Photobiol. **62**, 542—554

Dougherty T. J., Gomer C. J., Henderson B. W., Jori G., Kessel D., Korbelik M., Moan J., Peng Q. (1998): Photodynamic therapy. J. Nat. Cancer Inst. **90**, 889—905

Fabris C., Ometto C., Milanesi C., Jori G., Cook M. J., Russell D. A. (1997): Tumourlocalizing and tumour-photosensitizing properties of zinc(II)-octapentyl-phthalocyanine. J. Photochem. Photobiol., B **39**, 279—284

Fabris C., Valduga G., Miotto G., Borsetto L., Jori G., Garbisa S., Reddi E. (2001): Photosensitization with zinc (II) phthalocyanine as a switch in the decision between apoptosis and necrosis. Cancer Res. **61**, 7495—7500

Hajri A., Wack S., Meyer C., Smith M. K., Leberquier C., Kedinger M., Aprahamian M. (2002): *In vitro* and *in vivo* efficacy of photofrin and pheophorbide a, a bacteriochlorin, in photodynamic therapy of colonic cancer cells. Photochem. Photobiol. **75**, 140—148

He J., Larkin H. E., Li Y. S., Rihter D., Zaidi S. I. A., Rodgers M. A. J., Mukhtar H., Kenney M. E., Oleinick N. L. (1997): The synthesis, photophysical and photobiological properties and *in vitro* structure-activity relationships of a set of silicon phthalocyanine PDT photosensitizers. Photochem. Photobiol. **65**, 581—586

He J., Horng M. F., Deahl J. T., Oleinick N. L., Evans H. H. (1998): Variation in photodynamic efficiency during the cellular uptake of two phthalocyanine photosensitizers. Photochem. Photobiol. **67**, 720—728

Inanami O., Yoshito A., Takahashi K., Hiraoka W., Kuwabara M. (1999): Effects of BAPTA-AM and forskolin on apoptosis and cytochrome c release in photosensitized Chinese hamster V79 cells. Photochem. Photobiol. **70**, 650—655

Kaestner L. (1997): Tetraphenylporphyrine – Farbstoffe für die Photodynamische Therapie. Logos Verlag, Berlin

Kaestner L., Cesson M., Kassab K., Christensen T., Edminson P. D., Cook M. J., Chambrier, I., Jori G. (2003): Zinc octa-n-alkyl phthalocyanines in photodynamic therapy: photophysical properties, accumulation and apoptosis in cell cultures, studies in erythrocytes and topical application to Balb/c mice skin. Photochem. Photobiol. Sci. **2**, 660—667

Moor A. C. E., Lagerberg J. W. M., Tijssen K., Foley S., Truscott T. G., Kochevar I. E., Brand A., Dubbelman T. M. A., VanSteveninck J. (1997): *In vitro* fluence rate effects in photodynamic reactions with AlPcS4 as sensitizer. Photochem. Photobiol. **66**, 860—865

Moser J. G. (1998): Photodynamic Tumor Therapy. 2nd and 3rd Generation Photosensitizers (Ed. J. G. Moser), Harwood Academic Publishers, Amsterdam

Oleinick N. L., Antunez A. R., Clay M. E., Rihter B. D., Kenney M. E. (1993): New phthalocyanine photosensitizers for photodynamic therapie. Photochem. Photobiol. **57**, 242—247

Ometto C., Fabris C., Milanesi C., Jori G., Cook M. J., Russell D. A. (1996): Tumourlocalising and -photosensitising properties of a novel zinc(II) octadecylphthalocyanine. Br. J. Cancer **74**, 1891—1899

Paul A., Hackbarth S., Mölich A., Luban C., Oelckers S., Böhm F., Röder B. (2002): Comparative study on the photosensitization of jurkat cells *in vitro* by pheophorbide-a and a pheophorbide-a diaminobutane poly-propylene-imine dendrimer complex. Laser Phys. **13**, 22—29

Richter A. M., Yib S., Meadows H., Jain A. K., Neyndorff H., Moreno G., Salet C., Levy J. G. (1996): Photosensitizing potencies of the structural analogues of benzoporphyrin derivative in different biological test systems. J. Clin. Laser Med. Surg. **14**, 335—341

Röder B. (1986): Pheophorbide a – a new photosensitizer for PDT. Stud. Biophys. **114**, 183—186

Roeder B., Naether D., Lewald T., Braune M., Nowak C., Freyer W. (1990): Photophysical properties and photodynamic activity *in vivo* of some tetrapyrroles. Biophys. Chem. **35**, 303—312

Sonoda M., Krishna C. M., Riesz P. (1987): The role of singlet oxygen in the photohemolysis of red blood cells sensitized by phthalocyanine sulfonates. Photochem. Photobiol. **46**, 625—631

Spikes J. D. (1983): Photosensitation in mammalian cells. In: Photoimmunology (Eds. J. A. Parrish, M. L. Kripke and W. Morison), pp. 23—49, Plenum Press, New York

Valduga G., Bianco G., Csik G., Reddi E., Masiero L., Garbisa S., Jori, G. (1996): Interaction of hydro- or lipophilic phthalocyanines with cells of different metastatic potential. Biochem. Pharmacol. **51**, 585—590

Willstätter A., Stoll R. (1913): Untersuchungen über Chlorophyll, Methoden und Ergebnisse. Vol. 3, Springer, Berlin

Zaidi S. I. A., Agarwal R., Eichler G., Rihter B. D., Kenney M. E., Mukhtar H. (1993a): Photodynamic effects of new silicon phthalocyanines: *In vitro* studies utilizing rat hepatic microsomes and human erythrocyte ghosts as model membrane sources. Photochem. Photobiol. **58**, 204—210

Zaidi S. I. A., Oleinick N. L., Zaim M. T., Mukhtar H. (1993b): Apoptosis during photodynamic therapy-induced ablation of RIF-1 tumors in C3H mice: Electron microscopic, histopathologic and biochemical evidence. Photochem. Photobiol. **58**, 771—776

Zavodnik I. B., Zavodnik L. B., Bryszewska M. J. (2002): The mechanism of Zn-phthalocyanine photosensitized lysis of human erythrocytes. J. Photochem. Photobiol., B **67**, 1—10

Final version accepted: May 29, 2003