Biological activities of phthalocyanines XIV. Effect of hydrophobic phthalimidomethyl groups on the in vivo phototoxicity and mechanism of photodynamic action of sulphonated aluminium phthalocyanines

R.W. Boyle, B. Paquette & J.E. van Lier

MRC Group in the Radiation Sciences, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Québec J1H 5N4, Canada.

Summary
Aluminium phthalocyanines substituted to different degrees with hydrophilic sulphonic acid and hydrophobic phthalimidomethyl groups were investigated in vivo as new agents for the photodynamic therapy of malignant tumours. Parameters studied included the photodynamic action on EMT-6 mammary tumours in BALB/c mice, the therapeutic window and the potential for direct cell killing, assayed via an in vivo/in vitro test. Although the efficiency of photoactivation of the EMT-6 tumour increases by a factor of ten with reduction of the number of sulphonic acid groups from four to two, no further effect was seen with the addition of the hydrophobic phthalimidomethyl groups. Addition of the latter groups however increased the potential for direct cell killing by a factor of two and expanded the therapeutic window by a factor of four, thus improving the usefulness of the dye as a photosensitiser for the photodynamic therapy of cancer.

Photodynamic therapy of cancer is a treatment which exploits the ability of certain dyes to absorb light and, via an excited state, interact with and cause damage to substrate molecules. If the dye, through differential rates of uptake or release by neoplastic tissue, as compared to normal tissue, concentrates in the tumour, activation with light of appropriate wavelengths can induce selective destruction of the malignant tumour (Kessel, 1984; Moan, 1986; Dougherty, 1987). Porphyrin based photosensitisers and in particular Photofrin II™ (P-II), a commercially available preparation of haematoporphyrin derivatives, have been extensively investigated; however, although these compounds absorb visible light efficiently at short wavelengths (ε405 ≈ 1010M⁻¹cm⁻¹), they have much less intense absorbance maxima at useful therapeutic wavelengths (ε630 ≈ 105M⁻¹cm⁻¹). Phthalocyanines (Pc) have been proposed as sensitisers to supersede P-II (for reviews see van Lier & Spikes, 1989; van Lier, 1990; Rosenthal, 1991). This is due to the high molar absorptivity of these compounds at wavelengths permitting greater penetration of light in normal tissues (typically ε ≈ 1010M⁻¹cm⁻¹ at 670–680 nm when fully monomerised). Due to the extreme insolubility of unsubstituted Pc in most common solvents, attention has focused mainly on sulphonated derivatives, which are water soluble.

A correlation has been found to exist between hydrophobicity of the sulphonated Pc and photodynamic potency (Brasseur et al., 1978a), the activity increasing as the number of sulphonic acid groups is decreased from four to two. It has been postulated (Paquette et al., 1988) that this relationship is due to the increasing amphiphilic nature of the lower sulphonates, which leads to greater membrane penetration. We recently tested this hypothesis, in vivo, using sulphonated phthalimidomethyl aluminium phthalocyanines (AlPcSP) (Figure 1), a novel photosensitiser substituted with both hydrophilic and hydrophobic groups (Paquette et al., 1991a). It was found that the lower sulphonated fractions, with added phthalimidomethyl groups, were more effective with regard to photodynamic inactivation of V79 Chinese hamster fibroblasts, compared to the corresponding sulphonates without phthalimidomethyl substituents. This enhanced photoactivity was directly related to cell uptake of these dyes and correlated to an increase in the hydrophobic character due to the addition of the phthalimidomethyl groups.

We now present in vivo photodynamic tumour response results, and evaluate the potential for direct tumour cell killing for these substituted sulphonated phthalocyanines. In addition, it has been reported that PDT can result in vascular stasis and traumatic shock reactions (Ferrario & Gomer, 1990), mediated by release of eicosanoids from endothelial cells (Finger et al., 1991), and histamine from degranulation of mast cells (Lim et al., 1986; Kerdel, 1987). Thus, to establish the therapeutic window of these drugs, we have investigated the drug dose at which inflammatory response following PDT becomes deleterious, or fatal.

Materials and methods

Photosensitisers

The synthesis and purification of sulphonated aluminium phthalocyanine (AlPcS) and sulphonated phthalimidomethyl aluminium phthalocyanine (AlPcSP) have been described in detail previously (Ali et al., 1988; Paquette et al., 1991a). Briefly: AlPcSP was synthesised in a 'one pot' reaction which introduced both sulpho and phthalimidomethyl groups on to the AlPc molecule in one step. The crude reaction product was a mixture of AlPcSs substituted to different degrees with sulpho and phthalimidomethyl groups which was subsequently purified by reverse phase chromatography. Eluted fractions were analysed to determine the degree of substitution which was then assigned e.g. AlPcS₂P₁₄ was substituted to an average level of 2.4 sulphonic acid groups and 1.4 phthalimidomethyl groups. Retention times (Rt) for reverse phase HPLC, which reflect polarity of the compounds, were AlPcS₂P₁₄, Rₜ = 28–33 min; AlPcS₄P₄, Rₜ = 40 min. Photofrin II™ (P-II) was obtained from Quadralogic Technologies Inc., Vancouver, B.C. Spectral characteristics include: AlPcS, ε₆₂₃ = 1.9 x 10⁶ M⁻¹ cm⁻¹, λₘₐₓ = 674 nm; AlPcSP, ε₆₇₄ = 1.5 x 10⁶ M⁻¹ cm⁻¹, λₘₐₓ = 676 nm; P-II, ε₆₉₀ ≈ 10⁵ M⁻¹ cm⁻¹, λₘₐₓ = 623 nm.

Photodynamic therapy

Animal experiments were conducted following the recommendations of the Canadian Council on Animal Care and of an in-house ethics committee. The animals were allowed free access to water and food throughout the experiments. Male BALB/c mice had one tumour transplanted into the right hind thigh by intradermal injection of 2 x 10⁵ EMT-6 mammary tumour cells suspended in 0.05 ml of Waymouth's medium (Gibco). Mice were injected intravenously, via the tail vein, with Pc or P-II in a solution of Cremophor EL (Sigma).
propane-1,2-diol, and saline (10:3:87) 6–9 days post-inoculation when tumours had reached a diameter of 3–5 mm. After 24 h the tumour was irradiated with 650–700 nm light (400 J cm⁻² at a fluence rate of 180 mW cm⁻²) delivered by a 1000 W Xenon lamp fitted with 10 cm water filter, and LS-700 (Corion) and 2-58 (Corning) filters. In the case of P-II a band of 600–650 nm was used at the same fluence, and fluence rate, using LS-600 (Corion) and 650-FL07-50 (Ealing) filters. Light was focused on the tumour with lenses to give a final beam 8 mm in diameter. Tumour temperature was measured (Brasseur et al., 1987b) and rose to 35°C (externally) and 32°C (internally) after 10 min, in both cases the temperature remained constant for the remainder of the irradiation time. Tumour response was assessed qualitatively and followed from initial necrosis (within 48 h), to cure (within 15–30 days). Cure was defined as complete eradication of tumour mass and regrowth of non-neoplastic tissue in its place. Nine mice were used to confirm the minimal dose of dye needed to reach the cure.

**In vivo/in vitro assay**

BALB/c mice were implanted with two EMT-6 tumours in the hind thighs. When the tumours reached a diameter of 3–5 mm (6–9 days) mice were injected with 10 μmol kg⁻¹ of Pc or 10 μmol kg⁻¹ of P-II in saline containing 10% Cremophor EL and 3% propane-1,2-diol. 24 h post-injection of drug animals were sacrificed and the tumours were excised, minced, and enzymatically digested for 30 min in 10 ml Hank’s buffer saline solution, containing 10 mM CaCl₂, 6.5 U protease K (Sigma), 3 U nuclease micrococal (Sigma) and 17 U collagenase (Sigma). The digested preparation was then filtered through a 200 mesh sieve and centrifuged at 600 g for 5 min. Two hundred cells were placed in 60 mm Petri dishes and incubated for 3 h at 37°C in 5% CO₂ in Waymouth’s culture medium to allow adhesion to the support. Cells were illuminated with red light from two 500 W tungsten/halogen lamps (Sylvania) fitted with a circulating, refrigerated filter containing aqueous Rhodamine (OD₅₀ = 1.25), and a red filter (Kodak, no. 23A). Cells were illuminated with a fluence from 1 to 40 J cm⁻²-calculated for a window of 40 nm centred on the maximum absorption wavelength of each dye.

**Therapeutic window**

Tumour bearing animals were prepared and irradiated in a procedure identical to that used for photodynamic therapy (see above); however, in this experiment drug doses were increased sequentially, until the lethal dose for PDT with that compound was found. Any sub-lethal deleterious effects were also assessed qualitatively.

**Results**

The potential of photosensitisers to affect tumours upon PDT via a direct cell killing mechanism can be deduced using an in vivo/in vitro cell survival assay (Henderson, 1990). Photosensitisers were injected intravenously at a dose of 10 μmol kg⁻¹ or 10 mg kg⁻¹ for P-II in BALB/c mouse bearing an EMT-6 tumour on the hind thigh. 24 h later neoplastic cells were isolated, plated in dishes, and illuminated at a dose ranging from 1 to 40 J cm⁻². The cell survival curves for two differently substituted fractions, AlPcS₂P₄₄ and AlPcS₂P₄₈, relative to standards AlPcS₂ and P-II, are shown in Figure 2. This assay confirmed the poor direct cell killing potential of P-II as only about 20% EMT-6 cells were photoinactivated at the maximum fluence of 40 J cm⁻². On the other hand, the intrinsic character of sulphonated fractions of phthalocyanine to provide direct cell killing increased with addition of phthalimidomethyl groups. LD₅₀ values approximately half of that observed with AlPcS₂ (15.7 J cm⁻²) were obtained with AlPcS₂P₄₁₂ and AlPcS₂P₄₈ (5.2 and 6.4 J cm⁻²).

The potential of these dyes to cure the EMT-6 tumour implanted on BALB/c mice has also been tested. At a fluence of 400 J cm⁻², tumour response results for AlPcS₂P₄₄, AlPcS₂P₄₁₂ and AlPcS₂ indicate that all three compounds gave an initial necrosis within 48 h, followed by a 100% tumour cure, at similar injected doses (0.5 μmol kg⁻¹) without apparent discomfort, and gave partial response at lower

![Chemical structure of sulphonated phthalimidomethyl aluminium phthalocyanine (AlPcSF).](image)

**Figure 1** Chemical structure of sulphonated phthalimidomethyl aluminium phthalocyanine (AlPcSF). R = H, SO₃⁻ or CH₃-phthalimide, depending on the degree of substitution and sulphonation.
doses. AlPcS₄ and P-II attained the same tumour response only at concentrations about 10 and 20 times higher, in mg kg⁻¹ unit, respectively. Tumour response results for all compounds tested are summarised in Table I.

The therapeutic window of these dyes was determined by increasing progressively the injected dose. Results in Table II indicate that AlPcS₂ and AlPcS₃P₄₄ can be used safely in this model under our experimental conditions, up to a limit of 1 μmol kg⁻¹. Above this dose, deleterious effect ranging from inflammation to complete necrosis of the leg and death result. Thus, these two dyes showed a poor therapeutic window as EMT-6 tumour could not be cured at a dose lower than 0.5 μmol kg⁻¹. On the other hand, increasing the number of phthalimidomethyl groups, as in fraction AlPcS₃P₁₄, increased the therapeutic window by a factor of four as compared to AlPcS₂.

Discussion

The use of Photofrin II™ in PDT protocols induces necrosis mainly by tumour microcirculation stasis (Fingar & Henderson, 1987; Selman et al., 1985; Reed et al., 1989). The resulting indirect cell killing is usually incomplete allowing tumour regrowth unless blood vessels surrounding the tumour are also destroyed (Star et al., 1986). In the clinic this limits efficient treatment not only of single, well-defined tumours where red light could be easily focused, but also when more diffuse illumination must be used, as in the case of multiple tumours in bladder cancer (Jocham et al., 1989). Furthermore, severe damage to normal tissue could arise and this deleterious effect greatly compromises successful cure of malignant tumour. Accordingly there is a need for a new PDT photosensitiser acting more directly on neoplastic cells and sulphonated phthalocyanines have shown promise in this regard.

Apart from distribution and PDT effect in normal rat colon with AlPcS purified with respect to sulphonation (Chatlani et al., 1991), most in vivo studies with sulphonated aluminium phthalocyanines reported to date were conducted with mixtures of differently sulphonated products (Barr et al., 1990; Chan et al., 1986; Tralau et al., 1987). Resolution of sulphonated metallo phthalocyanines into homogeneous components requires tedious chromatographic procedures (Ali et al., 1988) and mainly in vitro biological testing on these individual compounds has been reported (Brasseur et al., 1987a; Paquette et al., 1988; Peng et al., 1991). Although such procedures allow for the comparison of the phototoxicity of dyes after cell uptake, the procedure does not take into account the many important in vivo parameters which govern dye distribution and phototoxicity, including interactions with blood components (albumin or lipoproteins), capillary permeability in tumour, or distribution in interstitial tumour space (for a recent review see Paquette and van Lier, 1991b).

Using the in vivo/in vitro cell survival assay described in this report, actual in vivo distribution and cell uptake of dye are respected, while an in vitro illumination allows accurate quantification of direct cell killing potential. EMT-6 cell survival curves (Figure 2) after in vivo administration of 10 mg kg⁻¹ P-II revealed that at the maximum fluence of 40 J cm⁻² only 20% of the EMT-6 cells were inactivated. However, to elicit a complete in vivo tumour response with the same dye dose, a 10-fold higher fluence of 400 J cm⁻² was required, suggesting that at least at the top layer of the tumour, direct cell killing may contribute to tumour necrosis. Overall tumour cell survival after in vivo PDT with P-II has previously been shown to be high, this suggests a predominantly indirect action mechanism (Fingar & Henderson, 1987) which is in agreement with the high cell survival observed in our in vivo/in vitro test.

Unlike P-II, all three phthalocyanines tested in this study exhibited high potential for direct cell killing in PDT. In vitro photoactivation of EMT-6 cells after in vivo administration of 10 μmol kg⁻¹ dye gave LD₅₀ varying from 15.7 J cm⁻² for AlPcS₂ through 5.2 and 6.4 J cm⁻² for AlPcS₃P₁₄ and AlPcS₃P₄₄ respectively (Figure 2). The phthalimidomethylated AlPcSP LD₅₀ values are approximately half that of AlPcS₂ which parallels their relative photoactivities under in vitro conditions with Chinese hamster fibroblast V-79 cells (Paquette et al., 1991a). In addition to passive diffusion, dye uptake by neoplastic cells in vivo can be mediated by endocytosis (Ben-Hur et al., 1987; Roberts and Berns, 1989). These processes are modulated by dye interaction with constituents in interstitial liquid such as albumin, collagen, and low density lipoproteins, which compete with dye uptake by neoplastic cells. On the other hand, binding of hydrophilic dye on low density lipoprotein is believed to favour specific endocytosis by neoplastic cells (Kessel et al., 1987). Our data suggest that addition of phthalimidomethyl groups to AlPcS favours their localisation in neoplastic cells, resulting in a two fold increase in direct cell killing and a 4-fold increase in the therapeutic window.

Comparison of the in vivo and in vitro photoactivities (Paquette et al., 1991a) of the AlPcS and AlPcSP suggest that the hydrophobic/amphiphilic properties of the dyes have less impact on the in vivo as compared to the in vitro activities. In the V-79 cell survival assay, the more hydrophobic and amphiphilic AlPcS₃P₄₄ fraction was over 3-fold more photoactive than the hydrophobic AlPcS₃P₄₄ fraction. In the in vivo/in vitro EMT-6 cell survival assay, both dye preparations exhibited similar direct cell killing potentials. This suggests the futility of further attempts to increase the hydrophobic/amphiphilic character of these photosensitisers in order to improve their direct cell killing potential during in vivo PDT.

The degree of sulfonation of AlPcS strongly effects the
minimal drug dose required for tumour response. Thus, AlPcS is ten times more photoactive than the corresponding AlPcS3 (0.5 μmol kg−1 and 5 μmol kg−1, respectively), confirming that the increase in hydrophobic/amphiphilic character of AlPcS improves the effective overall distribution of the dye in the tumour. This assumption is in agreement with recent observations by Peng et al. (1990a,b) showing that AlPcS2 at 24 h post-injection, mainly localised in the neoplastic cells of LOX tumour bearing mice. In contrast, the AlPcS remained mainly in the stroma, even at 48 h post-injection. It is likely that the highly hydrophobic character of AlPcS contributes to strong binding to the protein component in the stroma, while the amphiphilic nature of AlPcS2 promotes interaction with the plasma membrane of neoplastic cells. Adding lipophilic phthalimidomethyl substituents to AlPcS did not significantly improve the required dye dose for 100% tumour cure. Both AlPcS2-P14 and AlPcS2-P4a fractions required, in our EMT-6 model, the same minimal dye dose for tumour cure as AlPcS2 (0.5 μmol kg−1). It thus appears that the inclusion of hydrophobic phthalimidomethyl groups in AlPcS has a beneficial PDT effect in that it outweighs the expected decrease in activity resulting from higher sulphonation levels (Brasseur et al., 1987a).

Extrapolation of the high direct cell killing potential of the AlPcS2 to the mechanisms of the PDT response of the EMT-6 tumour, should be done with caution. Although the minimal injected dye dose for tumour response was 20-fold lower than the dye dose employed in the in vivo/in vitro cell survival studies (0.5 and 10 μmol kg−1, respectively), the fluorescence in the in vivo PDT assay was at 60-fold higher than that required for a 90% cell inactivation in the in vivo/in vitro assay. Furthermore, absorption of red light by the tumour tissue allows for only about 20% of incident light to reach the lower part of tumour (Henderson, 1989). These factors combined suggest that the deeper seated part of the EMT-6 tumour will receive somewhat lower combined light/dye dose than the LD50 values obtained in the in vitro cell killing assay. Thus it is likely that in the deeper seated parts of the tumour, and at the lower effective dye doses, tumour cure will involve indirect mechanism for cell killing. Indirect tumour cell killing after PDT has been related to the release of vasoactive agents including histamine and prostaglandins, and this, in addition to local effects, could trigger also substantial systemic toxicity (Fingar et al., 1991; Kerdel et al., 1987; Lim et al., 1986). Accordingly, we evaluated the therapeutic window in our tumour model, i.e. the range of the minimal dye doses required for 100% tumour cure and the dose which induced systemic toxicity (Table II). Under our experimental conditions both AlPcS2 and AlPcS3-P6a proved highly phototoxic at 5.0 μmol kg−1 suggesting that, although these compounds have a good potential for direct cell killing, they also localise in sites responsible for the release of inflammatory agents. Among the three sensitisers which exhibit photodynamic action at 0.5 μmol kg−1, the AlPcS2-P12 has the largest therapeutic window. This suggests that the addition of the phthalimidomethyl group on amphiphilic AlPcS reduces unwanted dye interaction with cells capable of releasing vasoactive agents (e.g. mast cells).

In conclusion, these results imply a subtle balance between the hydrophobic and the amphiphilic characteristics of phthalocyanine sensitisers and their action mechanisms, with important implications in terms of both photodynamic potency and deleterious effects. The addition of the hydrophobic phthalimidomethyl groups on lower sulphonated AlPcS should improve the PDT outcome by increasing direct tumour cell killing while limiting deleterious effects on normal surrounding tissue. In the EMT-6 tumour model, the current class of phthalocyanines induces a higher direct cell killing and tumour response as compared to P-II. Further chemical modifications should be aimed at improving the therapeutic window, which would allow the administration of higher doses of dye and red light, resulting in augmented direct cell killing and cure of malignant tumours.

References

ALI, H., LANGLOIS, R., WAGNER, J.R., BRASSEUR, N., PAQUETTE, B. & VAN LIER, J.E. (1988). Biological activities of phthalocyanines X. Syntheses and analyses of sulphonated phthalocyanines. Photochem. Photobiol., 44, 713.

BARR, H., TRALAU, C.J., BOULES, P.B. & 4 others (1990). Selective necrosis in dimethylhydrazine-induced rat colon tumours using phthalocyanine photodynamic therapy. Gastroenterology, 98, 1532.

BEN-MERED, E., SIWECKI, J.A., NEWMAN, H.C., CRANE, S.W. & ROSENTHAL, I. (1987). Mechanism of uptake of sulphonated metallophthalocyanines by cultured mammalian cells. Cancer Let., 38, 215.

BRASSEUR, N., ALI, H., LANGLOIS, R. & VAN LIER, J.E. (1987a). Biological activities of phthalocyanines – VII. Photoactivation of V-79 Chinese hamster cells by selectively sulphonated gallium phthalocyanines. Photochem. Photobiol., 46, 739.

BRASSEUR, N., ALI, H., LANGLOIS, R., WAGNER, J.R. & VAN LIER, J.E. (1987b). Biological activities of phthalocyanines – V. Photodynamic therapy of EMT-6 mammary tumours in mice with sulphonated phthalocyanines. Photochem. Photobiol., 45, 581.

CHAN, W.S., SVENSEN, R., PHILLIPS, D. & HART, I.R. (1986). Cell uptake, distribution, and response to aluminium chloro sulphonated phthalocyanine, a potential anti-tumour photosensitizer. Br. J. Cancer, 53, 255.

CHATTIANI, P.T., BEDWELL, J., MACROBERT, A.J. & 5 others (1991). Comparison of distribution and photodynamic effects of di- and tetra-sulphonated aluminium phthalocyanines in normal rat colon. Photochem. Photobiol., 53, 745.

DOUGHERTY, T.J. (1987). Photosensitizers: therapy and detection of malignant tumors. Photochem. Photobiol., 45, 897.

FERRARIO, A. & GOMER, C.J. (1990). Systemic toxicity in mice induced by localized porphyrin photodynamic therapy. Cancer Res., 50, 539.

FINNER, V.H. & HENDERSON, B.W. (1987). Drug and light dose dependence of photodynamic therapy: a study of tumour and normal tissue response. Photochem. Photobiol., 46, 837.

FINGER, V.H., WIEMAN, T.J. & WEBER DOAK, K. (1991). Changes in tumor interstitial pressure induced by photodynamic therapy. Photochem. Photobiol., 53, 763.

HENDERSON, B.W. & BELLNIE, D.A. (1989). Tissue localization of photosensitizers and the mechanism of photodynamic tissue destruction. In Photosensitizing Compounds: Their Chemistry, Biology and Clinical Use (Ciba Foundation Symposium; Vol. 146). John Wiley & Sons: New York.

HENDERSON, B.W. (1990). The significance of vascular photosensitization in photodynamic therapy. In Future Directions and Applications in Photodynamic Therapy, Gomer, C.J. (ed.), p. 153. SPIE Institutes for Advanced Optical Technologies. Vol. IS 6. Bellingham: Washington.

JOCHAM, D., BEER, M., BAUMGARTNER, R., STAEBLER, G. & UNSOLD, E. (1989). Long-term experience with integral photodynamic therapy of TIS bladder carcinoma. In Photosensitizing Compounds: Their Chemistry, Biology and Clinical Use. Bock, G. & Harrell (eds), P. 198, Ciba Foundation Symposium, 146, Wiley: New York.

KERDEL, F.A., SOTER, N.A. & LIM, H.W. (1987). In vivo mediator release and degradation of mast cells in hematoporphyrin derivative-induced phototoxicity in mice. J. Invest. Dermatol., 88, 277.

KESSEL, D. (1984). Hematoporphyrin and HPD: photophysics, photochemistry and phototherapy. Photochem. Photobiol., 39, 851.

KESSEL, D., THOMPSON, P., SATIOT, K. & NANTWI, K.D. (1987). Tumor localization and photosensitization by sulphonated derivatives of tetraphenylporphine. Photochem. Photobiol., 45, 787.

LIM, H.W., PARKER, D. & MARCUS, A.J. (1986). Generation of eicosanoids from mast cells exposed to protoporphyrin and hematoporphyrin. Clin. Res., 34, 769.

MOAN, J. (1986). Porphyrin photosensitization and phototherapy. Photochem. Photobiol., 43, 681.

This work was supported by the Medical Research Council of Canada. The authors thank Huguette Savoie and Gloria Cheal for expert technical assistance.
PAQUETTE, B., ALI, H., LANGLOIS, R. & VAN LIER, J.E. (1988). Biological activities of phthalocyanines VIII. Cellular distribution in V-79 Chinese hamster cells and phototoxicity of selectively sulfonated aluminium phthalocyanines. Photochem. Photobiol., 47, 215.

PAQUETTE, B., BOYLE, R.W., ALI, H., MCLENAN, A.M., TRUSCOTT, T.G. & VAN LIER, J.E. (1991a). Sulfonated phthalimidomethyl aluminium phthalocyanine: The effect of hydrophobic substituents on the in vitro phototoxicity of phthalocyanines. Photochem. Photobiol., 53, 323.

PAQUETTE, B. & VAN LIER, J.E. (1991b). Phthalocyanines and related compounds: structure-activity relationships. In Photodynamic Therapy: Basic Principles and Clinical Aspects, Dougherty, T.J. & Henderson, B.W. (eds), Marcel Dekker (in press).

PENG, Q., MOAN, J., FARRANTS, G., DANIELSEN, H.E. & RIMINGTON, C. (1991). Localization of potent photosensitizers in human tumor LOX by means of laser scanning microscopy. Cancer Lett., 58, 17.

PENG, Q., MOAN, J., NESLAND, J.M. & RIMINGTON, C. (1990a). Aluminium phthalocyanines with asymmetrical lower sulfonation and with symmetrical higher sulfonation: a comparison of localizing and photosensitizing mechanism in human tumor LOX xenografts. Int J. Cancer, 46, 719.

PENG, Q., NESLAND, J.M., MOAN, J., EVENSEN, J.F., KONGSHANG, M. & RIMINGTON, C. (1990b). Localization of fluorescent Photofrin II and aluminium phthalocyanine tetrasulfonate in transplanted human malignant tumor LOX and normal tissues of nude mice using highly light-sensitive video intensification microscopy. Int. J. Cancer, 45, 972.

REED, M.W.R., WIEMAN, T.J., SCHUSCHKE, D.A., TSENG, M.T. & MILLER, F.N. (1989). A comparison of the effects of photodynamic therapy on normal and tumor blood vessels in the rat microcirculation. Radiation Res., 119, 542.

ROBERTS, W.G. & BERNS, M.W. (1989). In vitro photosensitization 1: Cellular uptake and subcellular localization of mono-L-aspartyl chlorin e₆, chloro-aluminium sulfonated phthalocyanine, and Photofrin II. Lasers in Surg. & Med., 9, 90.

ROSENTHAL, I. (1991). Phthalocyanines as photodynamic sensitizers. Photochem. Photobiol., 53, 859.

SELMAN, S.H., KREIJER-BIRNBAUM, M., KLAUNIG, J.E., GOLD-BLATT, P.J., KECK, R.W. & BRITTON, S.L. (1985). Blood flow in transplantable tumors treated with hematoporphrin derivative and light. Cancer Res., 45, 1924.

STAR, W.M., MARUNISSEN, H.P.A., VAN DEN BERG BLOK, A.E., VERSTEEG, J.A.C., FRANKEN, K.A.P. & REINHOLD, H.S. (1986). Destruction of rat mammary tumor and normal tissue microcirculation by hematoporphyrin derivative photoradiation observed in vivo in sandwich observation chambers. Cancer Res., 46, 2532.

TRALAU, C.J., BARR, H., SANDERMAN, D.R., BARTON, T., LEWIN, M.R. & BOWN, S.G. (1987). Aluminium sulfonated phthalocyanine distribution in rodent tumors of the colon, brain and pancreas. Photochem. Photobiol., 46, 777.

VAN LIER, J.E. & SPIKES, J.D. (1989). The chemistry, photophysics and photosensitizing properties of phthalocyanines. In Photosensitizing Compounds: Their Chemistry, Biology and Clinical Use, Bock, G. & Harnell (eds), P. 17, Ciba Foundation Symposium, 146, Wiley: Chichester.

VAN LIER, J.E. (1990). Phthalocyanines as sensitizers for PDT of cancer. In Photodynamic Therapy of Neoplastic Disease. Vol. 1, Kessel, D. (ed.), p. 279, CRC Press: Boca Raton, FL.