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# PHOTODYMAMIC THERAPY OF TUMOURS WITH HEXADECAFLUORO ZINC PHTHALOCYANINE FORMULATED IN PEG-COATED POLY(LACTIC ACID) NANOPARTICLES

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**Hexadecafluoro zinc phthalocyanine ($ZnPcF_{16}$), a second-generation sensitizer for the photodynamic therapy (PDT) of cancer, was formulated in polyethylene-glycol-coated poly(lactic acid) nanoparticles (PEG-coated PLA-NP) and tested in EMT-6 tumour-bearing mice for its photodynamic activity. The tumour response was compared to that induced by the same dye formulated as a Cremophor EL (CRM) emulsion. Formulation in the biodegradable NP improved PDT response of the tumour while providing prolonged tumour sensitivity towards PDT.**


The treatment of solid tumours with red light after systemic administration of a mixture of porphyrins (Photofrin), known as photodynamic therapy (PDT) has recently been accepted in several countries for the treatment of selected, light-accessible cancers (Henderson and Dougherty, 1992). However, the use of Photofrin in a clinical setting has several shortcomings and "second-generation" photosensitizers of known composition and increased photodynamic activities are currently being developed to provide an improved drug. Phthalocyanines (Pc) have been put forward in view of their advantageous physicochemical properties (Rosenthal, 1991; van Lier, 1990) and we have shown that the fluorinated analogue of ZnPc, *i.e.*, $ZnPcF_{16}$, exhibits sufficient lipid solubility to allow its formulation in biodegradable nanoparticles (Allémann *et al.*, 1995). $ZnPcF_{16}$ can be conveniently synthesized as a single, pure product (Boyle *et al.*, 1996), and its increased solubility provides a distinct advantage over ZnPc which has been studied extensively as a sensitizer for PDT (Isele *et al.*, 1995, 1994; van Leengoed *et al.*, 1994; Ginevra *et al.*, 1990; Reddi *et al.*, 1990). As a Cremophor EL (CRM) emulsion, $ZnPcF_{16}$-PDT was shown to induce tumour responses up to 72 hr following drug administration (Boyle *et al.*, 1996).

Biodegradable nanoparticles (NP) have received considerable attention as a possible means of targeting drugs (Davis *et al.*, 1993; Leroux *et al.*, 1996*a,b*). Commonly used NP suffer a major drawback in that they are massively taken up by the cells of the mononuclear phagocyte system (MPS) (Leroux *et al.*, 1994). Coating of poly(D,L-lactic acid) (PLA) NP with polyethylene glycol (PEG) 20,000 has been shown to significantly reduce this unwanted side-effect (Allémann *et al.*, 1995). Incorporation of $ZnPcF_{16}$ in PLA-NP resulted in rapid uptake of the dye by the reticuloendothelial system (RES), whereas PEG-coating of the particles resulted in reduced RES uptake and enhanced tumour retention (Allémann *et al.*, 1995). Based on these findings, the objective of this study was to compare the photodynamic activity of a $ZnPcF_{16}$ incorporated in PEG-coated PLA NP with the conventional formulation of the dye in a CRM-based o/w emulsion.

## MATERIAL AND METHODS

### *Materials*

$ZnPcF_{16}$ was synthesized by condensation of tetrafluorophthalonitrile (Aldrich Milwaukee, WI) with zinc acetate dehydrate (1:1) (Boyle *et al.*, 1996). CRM was obtained from BASF (Toronto, Canada). PLA was a gift from Medisorb (Cincinnati, OH). Poly(vinyl alcohol) (PVAL) was chosen as stabilizing colloid (Mowiol 4-88, Hoechst, Frankfurt/M, Germany). Polyethylene glycol 20,000 (PEG) (Sigma, St. Louis, MO) was used as hydrophilic coating agent for the NP.

### *$ZnPcF_{16}$ formulations*

NP were prepared by the salting-out technique (Allémann *et al.*, 1992). An aqueous gel (25 g) containing 11% of PVAL and 35% of magnesium acetate tetrahydrate was added under mechanical stirring (5,500 rpm) to an acetone solution (10 g) of 5% (w/w) PLA and 0.05% (w/w) $ZnPcF_{16}$, leading to the formation of an oil-in-water emulsion. Then, 12.5 g of PEG 5% and 12.5 g of pure water were successively added to allow the complete diffusion of acetone into the aqueous phase, leading to the formation of PEG-coated NP (Leroux *et al.*, 1994). The nanoparticulate suspension was purified by cross-flow filtration and freeze-dried as previously described (Allémann *et al.*, 1993). NP had a mean size of 988 nm and a drug load of 0.61%. The PEG content of the coated particles represented 2% (w/w) of the nanoparticles.

For the CRM emulsion, the dye was first dissolved in 1-methyl-2-pyrrolidinone, CRM (10% final) was added under sonication, and the solvent was removed by means of a 24-hr dialysis. 1,2-propanediol (3% final) was then added to the solution which was diluted with PBS (pH 7.4) and filtered (0.2 μm) (Boyle *et al.*, 1996). The final concentration of $ZnPcF_{16}$ (100 mmol/l) was determined spectroscopically in pyridine.

### *Animal experiments*

All experiments were performed on male BALB/c mice (19 to 23 g) (Charles River, Montreal, Canada). The protocol was approved by the Canadian Council on Animal Care and an in-house ethics committee. The animals were allowed free access to water and food throughout the course of the experiment. A tumour was implanted into each thigh by intradermal injection of $2 \times 10^5$ EMT-6 cells suspended in 0.05 ml of Waymouth's medium (GIBCO, Grand Island, NY) (Brasseur *et al.*, 1993). After 7 days, tumours had reached a volume of 17.8 $mm^3$ (SEM ± 7.1) and were large enough to be measured, while the therapeutic response could be measured without interference of spontaneous necrosis (Margaron *et al.*, 1996). At this time the animals were injected i.v. with 1 μmol/kg of $ZnPcF_{16}$ formulated in CRM or NP.

After 24 or 48 hr, one tumour from each mouse was exposed to 400 J $cm^{-2}$ of red light (650–700 nm) delivered by a 1,000 W Xenon lamp, equipped with a 10-cm water filter, and LL-650 and LS-700 filters (Corion, Holliston, MA) cooled by air. The mean fluence rate was 200 mW $cm^{-2}$. Light was focused on the tumour with lenses to give a final beam of 8 mm in diameter. Tumour response was assessed for a period of 21 days following PDT.

In a second set of experiments, administered doses of dye were increased to 2 and 5 μmol/kg. Light treatment was given as above at 24, 48 or 72 hr post dye injection. The tumour response was rated according to Figure 1, which depicts the

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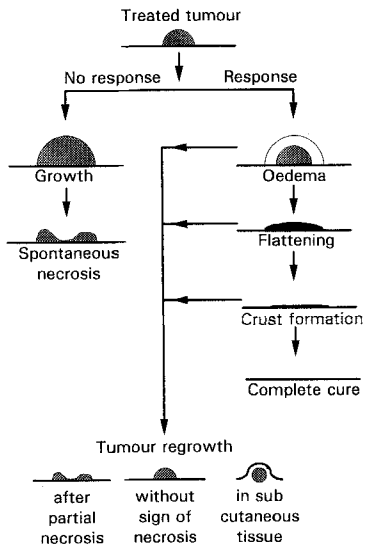


**FIGURE 1** – Sequence of events leading to tumour cure following PDT with $ZnPcF_{16}$.

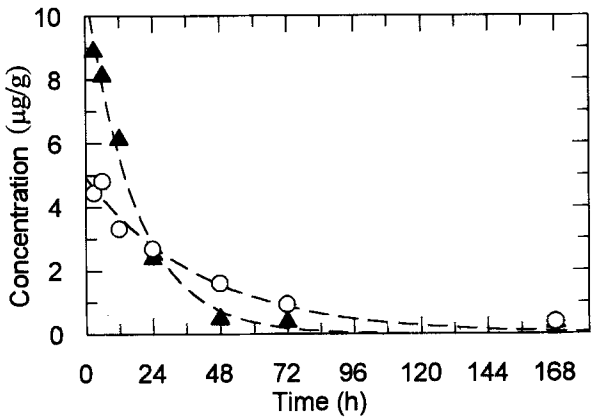


**FIGURE 2** – $ZnPcF_{16}$ blood concentration after i.v. injection of 1 μmol/kg in EMT-6 tumour-bearing mice (○, PEG-coated PLA NP; ▲, CRM emulsion) (mean, SEM smaller than symbol, n = 5) (adapted from Allémann *et al.,* 1995).

sequence of events leading to tumour cure following $ZnPcF_{16}$ PDT.

## RESULTS AND DISCUSSION

$ZnPcF_{16}$ loaded in PEG-coated NP exhibited a delayed blood clearance (Fig. 2), reflecting an extended circulation of the dye (Allémann *et al.,* 1995). During the course of the experiment, blood clearance followed first-order kinetics with a half-life ($t_{1/2}$) of 28.8 hr, compared to 12.3 hr with the CRM emulsion. Tumour-to-skin and tumour-to-muscle uptake ratios are important in predicting the risk of damage to tumour-adjacent tissues during the PDT. Both CRM and PEG-coated NP formulations gave rise to high tumour-to-skin ratios (Fig. 3), although the ratios for CRM were slightly lower throughout the whole experiment (Boyle *et al.,* 1996).

Considering these tumour-uptake and tumour-to-organ ratios, photoirradiation was performed at 24, 48 and 72 hr post-injection (p.i.) of the dye preparations. Tumour-response data are summarized in Table I. It can be seen, from the first set of experiments, that the best tumour response was ob-

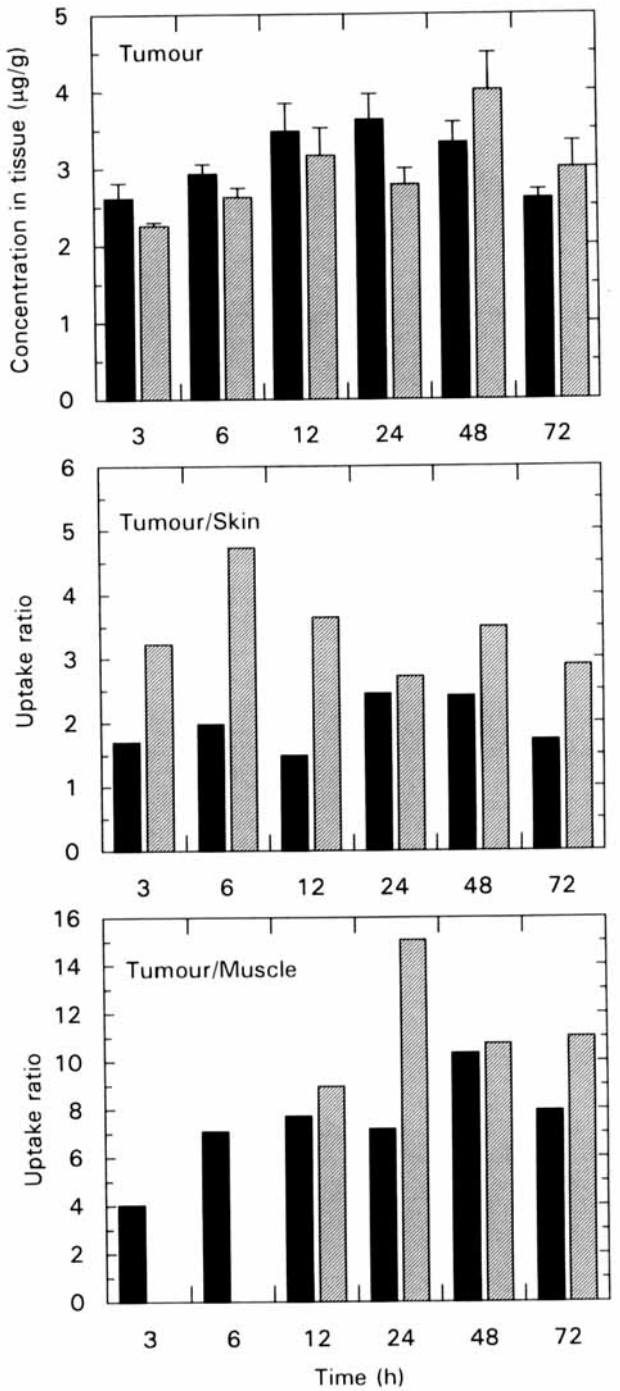


**FIGURE 3** – Tumour uptake (±SEM) and tumour-to-organ uptake ratios for $ZnPcF_{16}$ incorporated into PEG-coated PLA NP (▨) and CRM (■) after i.v. injection of 1 μmol/kg (n = 5) (adapted from Allémann *et al.,* 1995).

**TABLE I** – TUMOUR RESPONSE AFTER $ZnPcF_{16}$-PDT

| Dose (μmol/kg) | Time interval p.i. (hr) | Formulation | Tumour response[1] (% of mice) | | N |
|---|---|---|---|---|---|
| | | | Oedema | Cured | |
| 1 | 24 | NP | 63 | 63 | 8 |
| 1 | 24 | CRM | 14 | 14 | 7 |
| 1 | 48 | NP | 71 | 14 | 8 |
| 1 | 48 | CRM | 33 | 17 | 6 |

[1]Tumour response: oedema post-PDT, cure at 3 weeks post-PDT.

TABLE II – TUMOUR RESPONSE AFTER $ZnPcF_{16}$-PDT

| Dose (μmol/kg) | Time interval p.i. (hr) | Formulation | Tumour response[1] (% of mice) | | | | N |
|---|---|---|---|---|---|---|---|
| | | | Oedema | Flat | Crust | Cured | |
| 2 | 24 | CRM | 100 | 43 | 43 | 0 | 7 |
| 2 | 48 | CRM | 100 | 62 | 62 | 25 | 8 |
| 2 | 72 | CRM | 100 | 100 | 78 | 22 | 9 |
| 2 | 24 | NP | 100 | 90 | 90 | 40 | 10 |
| 2 | 48 | NP | 100 | 100 | 70 | 40 | 10 |
| 2 | 72 | NP | 100 | 80 | 60 | 40 | 10 |
| 5 | 24 | CRM | 100 | 80 | 80 | 60 | 5 |
| 5 | 24 | NP | 100 | 100 | 100 | 100 | 5 |

[1]Tumour response as depicted in Figure 1. Oedema post-PDT, flat/crust post-PDT, cured at 3 weeks post-PDT.

served following treatment with the NP formulation, achieving PDT 24 hr after dye administration. One week after treatment, 63% of the mice showed no macroscopic signs of tumour regrowth and at 3 weeks post-PDT they exhibited complete healing. The surrounding healthy tissues were only slightly affected. In contrast, with the CRM formulation using an identical protocol, only 14% tumour regression was observed. These differences in tumour response probably reflect differences in the interstitial distribution patterns of the photosensitizer, since overall tumour concentrations were similar at the selected time point. The prolonged circulation of NP at 24 hr p.i. also might play a role in the vascular shut-down. The particle size (988 nm) may favour trapping in the capillaries, followed by micro-embolism and anoxia in the tumour tissues. This hypothesis should, however, be confirmed by further histological investigations.

PDT was also achieved 48 hr p.i. of the dye. In this case, absence of palpable tumours after 21 days was only observed in one mouse of each group. In animals treated with 1 μmol/kg of $ZnPcF_{16}$, PDT often failed to induce early tumour response (*e.g.*, oedema). This led us to increase the administered dose of dye in the second set of experiments. The tumour responses shown in Table II are rated according to the sequence of events leading to tumour cure (Fig. 1). For doses of 2 and 5 μmol/kg, early tumour response (oedema) was observed for all treated mice (Table II). When $ZnPcF_{16}$ was administered as a CRM emulsion, the PDT response was most persistent when the illumination was carried out after 48 or 72 hr p.i. instead of the usual 24 hr. The improved PDT response at these time intervals most likely reflects the higher tumour-to-blood ratio (Boyle *et al.*, 1996; Allémann *et al.*, 1995). The use of PEG-coated PLA-NP as a vehicle for $ZnPcF_{16}$ led to further improvement of tumour response for all time delays and concentrations tested in this study. Furthermore, no effect of delay between dye administration and tumour illumination was observed. At a $ZnPcF_{16}$ dose of 2 μmol/kg, 40% of the mice were cured after PDT at 24, 48 or 72 hr p.i. of the dye. Finally, at 5 μmol/kg, 100% of the mice were cured (only 60% in the corresponding CRM group).

In summary, our data show that $ZnPcF_{16}$ is a promising photosensitizer for PDT. The improved solubility characteristics of this dye, as compared to plain ZnPc, allow it to be formulated as emulsions and nanoparticulate suspensions. The PEG-coated PLA-NP preparation provided improved tumour response as compared to conventional CRM emulsions. Moreover, the NP preparation made it possible to prolong the delay between dye injection and light treatment, while maintaining the same tumour response.

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## REFERENCES

ALLÉMANN, E., BRASSEUR, N., BENREZZAK, O., ROUSSEAU, J., KUDREVICH, S.V., BOYLE, R.W., LEROUX, J.C., GURNY, R. and VAN LIER, J.E., PEG-coated poly(lactic acid) nanoparticles for the delivery of hexadecafluoro zinc phthalocyanine to EMT-6 mouse mammary tumours. *J. Pharm. Pharm.*, **47**, 382–387 (1995).

ALLÉMANN, E., DOELKER, E. and GURNY, R., Drug loaded poly(lactic acid) nanoparticles produced by a reversible salting-out process: purification of an injectable dosage form. *Europ. J. Pharma. Biopharm.*, **39**, 13–18 (1993).

ALLÉMANN, E., GURNY, R. and DOELKER, E., Preparation of aqueous polymeric nanodispersions by a reversible salting-out process, influence of process parameters on particle size. *Int. J. Pharm.*, **87**, 247–253 (1992).

BOYLE, R.W., ROUSSEAU, J., KUDREVICH, S.V., OBOCHI, M.O.K. and VAN LIER, J.E., Hexadecafluorinated zinc phthalocyanine: photodynamic properties against the EMT-6 tumour in mice and pharmacokinetics using 65Zn as a radiotracer. *Br. J. Cancer*, **73**, 49–53 (1996).

BRASSEUR, N., NGUYEN, T.L., LANGLOIS, R., OUELLET, R., MARENGO, S., HOUDE, D. and VAN LIER, J.E., Synthesis and photodynamic activities of silicon 2,3-naphthalocyanine derivatives. *J. med. Chem.*, **37**, 415–420 (1993).

DAVIS, S.S., ILLUM, L., MOGHIMI, S.M., DAVIES, M.C., PORTER, C.J.H., MUIR, I.S., BRINDLEY, A., CHRISTY, N.M., NORMAN, M.E., WILLIAMS, P. and DUNN, S.E., Microspheres for targeting drugs to specific body sites. *J. contr. Release*, **24**, 157–163 (1993).

GINEVRA, F., BIFFANTI, S., PAGNAN, A., BIOLO, R., REDDI, E. and JORI, G., Delivery of the tumour photosensitizer zinc(II)-phthalocyanine to serum proteins by different liposomes: studies *in vitro* and *in vivo*. *Cancer Lett.*, **49**, 59–65 (1990).

HENDERSON, B.W. and DOUGHERTY, T.J., How does photodynamic therapy work? *Photochem. Photobiol.*, **55**, 145–157 (1992).

ISELE, U., SCHIEWECK, K., KESSLER, R., VAN HOOGEVEST, P. and CAPRARO, H.G., Pharmacokinetics and body distribution of liposomal zinc phthalocyanine in tumor-bearing mice: influence of aggregation state, particle size, and composition. *J. pharm. Sci.*, **84**, 166–173 (1995).

ISELE, U., VAN HOOGEVEST, P., HILFIKER, R., CAPRARO, H.G., SCHIEWECK, K. and LEUENBERGER, H., Large-scale production of liposomes containing monomeric zinc phthalocyanine by controlled dilution of organic solvents. *J. pharm. Sci.*, **83**, 1608–1616 (1994).

LEROUX, J.C., ALLÉMANN, E., DE JAEGHERE, F., DOELKER, E. and GURNY, R., Biodegradable nanoparticles—from sustained release formulations to improved site-specific drug delivery. *Advanc. Drug Deliv. Syst.*, (1996*a*). (In press).

LEROUX, J.C., DOELKER, E. and GURNY, R., The use of drug-loaded nanoparticles in cancer chemotherapy. *In:* S. Benita (ed.), *Microencapsulation methods and industrial applications*, (1996*b*). (In press).

LEROUX, J.C., GRAVEL, P., BALANT, L., VOLET, B., ANNER, B.M., ALLÉMANN, E., DOELKER, E. and GURNY, R., Internalization of poly(D,L-lactic acid) nanoparticles by isolated human leukocytes and analysis of plasma proteins adsorbed onto the particles. *J. biomed. Mat. Res.,* **28,** 471–481 (1994).

MARGARON, P., MADARNAS, P., OUELLET, R. and VAN LIER, J.E., Biological activities of phthalocyanines. XVII. Histopathologic evidence for different mechanisms of EMT-6 tumor necrosis induced by photodynamic therapy with disulfonated aluminum phthalocyanine and Photofrin. *Anticancer Res.,* (1996). (In press).

REDDI, E., ZHOU, C., BIOLO, R., MENEGALDO, E. and JORI, G., Liposome- or LDL-administered Zn (II)-phthalocyanine as a photodynamic agent for tumours. I. Pharmacokinetic properties and photodynamic therapeutic efficiency. *Br. J. Cancer,* **61,** 407–411 (1990).

ROSENTHAL, I., Phthalocyanines as photodynamic sensitizers. *Photochem. Photobiol.,* **53,** 859–870 (1991).

VAN LEENGOED, H.L.L.M., CUOMO, V., VERSTEEG, A.A.C., VAN DER VEEN, N., JORI, G. and STAR, W.M., *In vivo* fluorescence and photodynamic activity of zinc phthalocyanine administered in liposomes. *Br J. Cancer,* **69,** 840–845 (1994).

VAN LIER, J.E., Phthalocyanines as sensitizers for the photodynamic therapy of cancer. *In:* D. Kessel (ed.), *Photodynamic therapy of cancers,* Vol. **I,** pp. 279–291, CRC Press, Boca Raton (1990).