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Plasma pharmacokinetics and tissue distribution in CD_2F_1 mice of Pc4 (NSC 676418), a silicone phthalocyanine photodynamic sensitizing agent

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Abstract *Purpose*: Pc4 is a silicone phthalocyanine photosensitizing agent that is entering clinical trials. Studies were undertaken in mice to develop a suitable formulation and analytical methodology for use in pharmacokinetic studies and to define the plasma pharmacokinetics, tissue distribution, and urinary excretion of Pc4 after i.v. delivery. *Methods*: An HPLC method suitable for separation and quantification of Pc4 was developed and validated for use in mouse plasma, tissues, and urine. The stability of Pc4 was characterized in a variety of formulations as well as in mouse plasma. Before pursuing pharmacokinetic studies, preliminary toxicity studies were undertaken. These studies utilized Pc4 formulated in diluent 12:0.154 *M* NaCl (1:3, v:v). Pharmacokinetic studies involved Pc4 doses of 40 mg/kg, 10 mg/kg and 2 mg/kg administered as i.v. boluses to female, CD_2F_1 mice . Doses of 40 mg/kg, 10 mg/kg, and 2 mg/kg were studied with drug formulated in diluent 12:0.154 *M* NaCl (1:3, v:v). Doses of 10 mg/kg and 2 mg/kg were also studied with drug formulated in a vehicle consisting of polyethylene glycol:Tween 80:0.01 *M* sodium phosphate buffer, pH 7.0 (40:0.2:59.8, v:v:v). Compartmental and non-compartmental analyses were applied to the plasma concentration-versus-time data. Concentrations of Pc4 were also determined in a variety of tissues, including brain, lung, liver, kidney, skeletal muscle, skin, heart, spleen, and abdominal fat. Urine was collected from animals treated with each of the doses of Pc4 mentioned above, and daily, as well as cumulative drug excretion was calculated until 168 h after treatment. *Results*: At a dose of 80 mg/kg, two of five male and two of five female mice were dead by 24 h after injection. Pathologic examination revealed gross findings of blue discoloration affecting many tissues, with lungs that were grossly hemorrhagic and very blue-black. Microscopic examination of the lungs revealed mild acute interstitial pneumonia, with perivascular edema and inflammation, and a detectable margination of neutrophils around larger pulmonary blood vessels. Animals sacrificed 14 days after treatment showed mild granulomatous pneumonia, characterized by clusters of multi-nucleated giant cells, with fewer macrophages and neutrophils. The giant cells frequently contained phagocytized particles, which were clear and relatively fusiform. All mice treated with 40 mg/kg or 20 mg/kg survived and returned to pretreatment weight during the 14 days after treatment. Intravenous bolus delivery of Pc4, at a dose of 40 mg/kg, produced "peak" plasma Pc4 concentrations between 7.81 and 8.92 μg/ml in mice killed at 5 min after injection (the earliest time studied after drug delivery). Sequential reduction of the Pc4 dose to 10 mg/kg in diluent 12:0.154 *M* NaCl (1:3, v:v), 10 mg/kg in polyethylene glycol:Tween 80:sodium phosphate buffer (40:0.2:59.8, v:v:v), 2 mg/kg in diluent 12:0.154 *M* NaCl (1:3, v:v), and, finally, 2 mg/kg in polyethylene glycol:Tween 80:sodium phosphate buffer

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(40:0.2:59.8, v:v:v) resulted in "peak" plasma Pc4 concentrations between 2.07 and 3.24, 0.68 and 0.98 μg/ml, and 0.29 and 0.41 μg/ml, respectively. Pc4 persisted in plasma for prolonged periods of time (72–168 h). Noncompartmental analysis of plasma Pc4 concentration-versus-time data showed an increase in area under the plasma Pc4 concentration-versus-time curve (AUC) when the dose of Pc4 increased from 2 mg/kg to 40 mg/kg. Across the 20-fold range of doses studied, total body clearance (CL_{tb}) varied from 376 to 1106 ml h^{-1} kg^{-1}. Compartmental modeling of plasma Pc4 concentration versus time data showed the data to be fit best by a two-compartment, open, linear model. Minimal amounts of Pc4 were detected in the urine of mice. After i.v. bolus delivery to mice, Pc4 distributed rapidly to all tissues and persisted in most tissues for the duration of each pharmacokinetic study. Tissue exposure, as measured by AUC, increased in a dose-dependent fashion. *Conclusions*: The HPLC method developed for quantification of Pc4 in plasma, urine, and tissues should be suitable for clinical studies of the drug. Pc4 is widely distributed and persists in plasma and tissues of mice for prolonged periods of time. These data are relevant to the design of forthcoming clinical trials of Pc4.

Key words Pc4 · Phthalocyanines · Photodynamic therapy · Pharmacokinetics

Introduction

Photodynamic therapy (PDT) is a bimodal therapy that initially involves the uptake and retention in malignant tissue of an i.v. administered sensitizing drug, and subsequently the activation of that drug by light of the appropriate wavelength [17, 21, 23, 40, 46, 51, 54]. The activated drug is highly reactive, generates singlet oxygen, interacts with cellular targets, and results in cell death.

Hematoporphyrin derivatives (HpD) [15, 16, 22, 25, 30] and Photofrin [54] are the most frequently used clinical photosensitizers, although several other compounds are currently in clinical trials [2, 7, 14, 17, 18, 38, 41, 42]. None of these photosensitizers are ideal for treatment. HpD is composed of an unpredictable mixture of porphyrins [30, 31, 37] and has only low absorption in the red part of the light spectrum [9, 17, 25], which contains the wavelengths of activating light optimal for tissue penetration [17, 24, 25]. Both Photofrin and HpD also produce cutaneous photosensitivity in patients, and this photosensitivity can last for several weeks [17].

As a result of the previously mentioned problems with available photosensitizing agents, alternative compounds are being investigated for their suitability as PDT photosensitizers. The phthalocyanine group of dyes are compounds that appear promising because they, like HpD, have a maximal absorbance peak between 300 and 400 nm; but, in addition, they also exhibit strong absorption bands between 600 and 700 nm and can be synthesized in a purer form than can HpD [8, 10, 35, 36].

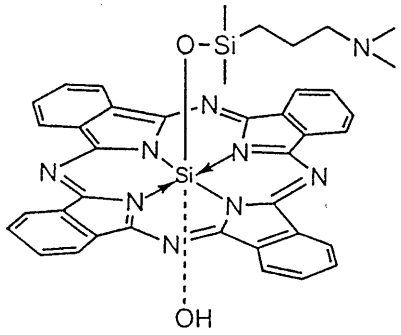

Fig. 1 Structure of the silicone phthalocyanine Pc4 (NSC 676418)

A number of phthalocyanines have been synthesized and evaluated for their use in PDT [3, 4, 12, 27, 32, 39, 45, 49, 50, 52, 55, 59]. Silicone phthalocyanine (Pc4; Fig. 1) is a highly efficient photosensitizer in vitro of a variety of hamster, mouse, and human cells and has been found more suitable for this use than aluminum-tetrasulfonated phthalocyanine [3, 20, 29, 47, 56, 59]. Initial in vivo tests of Pc4 delivered sufficient photosensitizer to the tumor to provide good responses upon irradiation with 675 nm light [3]. In fact, Pc4 has proven superior to Photofrin in PDT against several mouse and rat tumors [3, 20, 29, 47], yet Pc4 causes less cutaneous photosensitivity than does Photofrin [3]. Furthermore, Pc4 and Photofrin are claimed to differ in their cell-killing mechanisms [20, 47, 56]. Pc4 is claimed to work primarily by a nonvascular, direct, cell-killing mechanism and this has raised the possibility that Pc4 may be effective for a different range of tumors than is Photofrin. In view of these data, Pc4 has been developed for clinical use, and initial clinical trials are beginning.

In addition to its potential antineoplastic use, Pc4 has been selected by the New York Blood Center for development as an agent for photodynamic purging of viruses from red cell concentrate [5, 33, 34], because Pc4 has proven particularly effective in killing human immunodeficiency virus (HIV) and other lipid envelope viruses [6, 28, 62] as well as blood-borne parasites [19, 26, 60]. Based on each of these considerations, we undertook definition of the plasma pharmacokinetics and tisssue distribution of Pc4. Our goals were to develop an analytical methodology that would be applicable to biological matrices and to develop information in a preclinical model that would be useful in planning and interpretation of clinical trials with Pc4. Those studies form the basis for this report.

Materials and methods

Reagents

Pc4 was obtained from the Developmental Therapeutics Program, National Cancer Institute (Bethesda, Md., USA) both as bulk compound and as lyophylized preparations (Pc4, 1 mg/vial, lot

#NSC001,12-20-95, Pharmacia and Pc4, 0.5 mg/vial, lot #96B14FY, 3/8/96, Pharmacia and Upjohn, Bridgewater, NJ) developed as potential formulations for clinical testing. Diluent 12, a mixture of cremophor:ethanol (1:1, v:v), and an additional vehicle, consisting of polyethylene glycol:Tween 80:0.01 *M* sodium phosphate buffer, pH 7.0 (40:0.2:59.8, v:v:v; Pc4 vehicle, lot #2256-52, 2/8/96, Pharmacia), were also obtained from the Developmental Therapeutics Program. Nile Blue HCl (internal standard) was obtained from Aldrich Chemicals (Milwaukee, Wis., USA). Methanol was obtained from Fisher Scientific (Pittsburgh, Pa., USA). Ethyl acetate and glacial acetic acid (Baker Analyzed) were obtained from J.T. Baker (Phillipsburg, N.J., USA).

Mice

Specific-pathogen-free, male and female, adult CD_2F_1 mice (5–6 weeks of age) were obtained from the Animal Program administered by the Animal Genetics and Production Branch of the National Cancer Institute. Mice were allowed to acclimate to the University of Maryland at Baltimore Animal Facility for at least 1 week before studies were initiated. To minimize exogenous infection, mice were maintained in conventional cages in a separate room and handled in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH No. 85-23, 1985). Ventilation and air flow in the Animal Facility were set to 12 changes/h. Room temperatures were regulated at 72 ± 2 °F (22 ± 1 °C), and the rooms were on automatic 12-h light/dark cycles. Mice received Teklad LM-484 sterilizable mouse/rat diet 7012 (Harlan Teklad Diets, Madison, Wis., USA) and water ad libitum except on the evening prior to dosing, when all food was removed and withheld until 4 h after dosing. Sentinel mice (CD-1 mice housed in cages containing one fifth bedding removed from study mice cages at cage change) were maintained in the animal room and assayed at monthly intervals for specific murine pathogens by murine antibody profile (MAP) test (Litton Bionetics, Charleston, S.C., USA). These mice remained free of specific pathogens throughout the study period, indicating that study mice were free of specific pathogens. In preliminary toxicology studies, groups of five mice of each sex were treated with specified doses of Pc4. In pharmacokinetic studies, three mice were studied at each time point after delivery of specified doses and formulations of Pc4.

Pc4 administration

For studies in which Pc4 was given i.v. at doses of 80 or 40 mg/kg, drug was initially dissolved to a concentration of 16 mg/ml in diluent 12 and then diluted further with sterile 0.154 *M* NaCl to a final concentration of 4 mg/ml. Using this solution, Pc4 was delivered i.v. in volumes of 20 or 10 ml/kg of body weight so as to deliver doses of 80 or 40 mg/kg. For studies in which Pc4 was given i.v. at doses of 10 mg/kg or 2 mg/kg, drug was dissolved respectively at 1 mg/ml or 0.2 mg/ml, either in diluent 12:0.154 *M* NaCl (1:3, v:v) or polyethylene glycol:Tween 80: sodium phosphate buffer (40:0.2:59.8, v:v:v), so that the desired dose could be delivered in a volume of 10 ml/kg of body weight.

All i.v. doses of Pc4 were administered as boluses through a tail vein. Due to the potential for Pc4 to photosensitize skin, all animals were dosed, handled, and housed after dosing in rooms that had their fluorescent lights covered with sheets of Lee filter #124 (Baltimore Stage Lighting, Baltimore, Md., USA). This filter transmits light almost exclusively between 450 and 600 nm, which is not absorbed by Pc4.

Sampling

In all studies, blood was sampled at 5, 10, 15, 30 min, and 45 min and 1, 1.5, 2, 3, 4, 6, 8, 16, 24, 48, 72, 96 h, and 120 h after dosing. In both studies using the polyethylene glycol:Tween 80:sodium phosphate buffer vehicle and the study using a 40-mg/kg dose of Pc4 in diluent 12:0.154 *M* NaCl, additional groups of mice were sampled at 144 h and 168 h after dosing. In all studies except the one in which Pc4 was delivered at 2 mg/kg in diluent 12:0.154 *M* NaCl (1:1, v:v), brains, hearts, lungs, livers, kidneys, spleens, abdominal fat, skeletal muscles, skin, and gastrointestinal tracts were collected at the same times noted for blood samples. In each study, blood and tissues from mice killed 5 min after delivery of the appropriate vehicle served as controls. Blood was collected by cardiac puncture into heparinized syringes, transferred to Eppendorf microcentrifuge tubes and stored on ice until centrifuged at 13 000 *g* for 5 min to obtain plasma. Tissues were rapidly dissected, placed on ice until weighed, and then snap frozen in liquid nitrogen. Sets of animals to be sampled at 120, 144, or 168 h after dosing were gang-housed in metabolism cages, and daily urine output was collected on ice until animals were killed for blood and tissue sampling. Plasma, tissues, urine, and dosing solutions were stored frozen at −70 °C until analysis.

Analysis of Pc4

Plasma and tissue concentrations of Pc4 were determined by HPLC. Due to the light-absorbing properties of Pc4, all sample preparation was performed in darkened rooms, the HPLC system used for these assays was housed in a darkened room, and the window on the autosampler that handled samples was covered with an opaque shield. Briefly, 200-µl samples of plasma were placed into Eppendorf microcentrifuge tubes, and 5 µl of internal standard was added to each tube. The internal standard consisted of a 10-µg/ml solution of Nile Blue HCl. The tubes were vortexed, 1 ml of ethyl acetate was added to each tube, and the tubes were then shaken on a Vortex Genie 2 (Model G-560, Scientific Industries, Bohemia, N.Y., USA), set at position 5, for 10 min. After shaking, tubes were centrifuged at 13 000 *g* for 10 min, and the resulting upper, organic layers were transferred, with glass Pasteur pipets, into 12 × 75-mm glass tubes. Each remaining aqueous residue was then extracted in a similar fashion with two additional 1-ml aliquots of ethyl acetate, with the 3 ethyl acetate fractions from each sample being pooled. The ethyl acetate extracts were evaporated to dryness under N_2, resuspended in 250 µl of methanol, and the resuspended material was transferred, with glass Pasteur pipets, into glass microvial inserts. Two hundred microliters of each sample was injected by autosampler onto the HPLC system described below.

Tissue samples were thawed, immediately transferred to 17 × 100-mm polypropylene tubes that were held in an ice bath, and homogenized, using a Polytron (Brinkman Instruments, Westbury, N.Y., USA), in 3–9 parts (weight to volume) of phosphate-buffered saline (1.2 m*M* KH_2PO_4, 2.9 m*M* Na_2HPO_4, 154 m*M* NaCl, pH 7.4, Biofluids, Rockville, Md., USA). Two hundred microliters of each homogenate was placed into Eppendorf microcentrifuge tubes, mixed with 5 µl of internal standard, extracted with ethyl acetate and prepared for injection onto the HPLC as described for plasma samples. Analyses of tissue samples containing very high concentrations of Pc4 were modified by using 10 µl of a 25-µg/ml solution of Nile Blue internal standard and injecting only 25 µl of resuspended sample onto the HPLC.

The HPLC system employed consisted of a Waters WISP 710B autosampler (Waters Associates, Milford, Mass., USA) and a Waters M-45 pump fitted with a µ Bondapak C_{18} column (3.9 × 300 mm, Waters Associates) that was protected with a 1.5-cm Brownlee RP_{18} NewGuard cartridge (Applied Biosystems, San Jose, Calif., USA). The isocratic mobile phase, consisting of methanol:distilled, deionized water:glacial acetic acid (80:20:1, v:v:v), was pumped at 1 ml/min. Column eluent was monitored with a Waters 440 absorbance detector fitted with a 658-nm filter and slit kit. The detector signal was processed with a Waters 740 Data Module so as to integrate the area under each peak. Under these conditions, the retention times of Pc4 and internal standard were approximately 4.5 min and 5.3 min, respectively (Fig. 2). Pc4 concentration in each sample was calculated by determining the ratio of Pc4 peak area to that of the corresponding internal standard peak and comparing that ratio with a concomitantly performed standard curve prepared in the appropriate matrix.

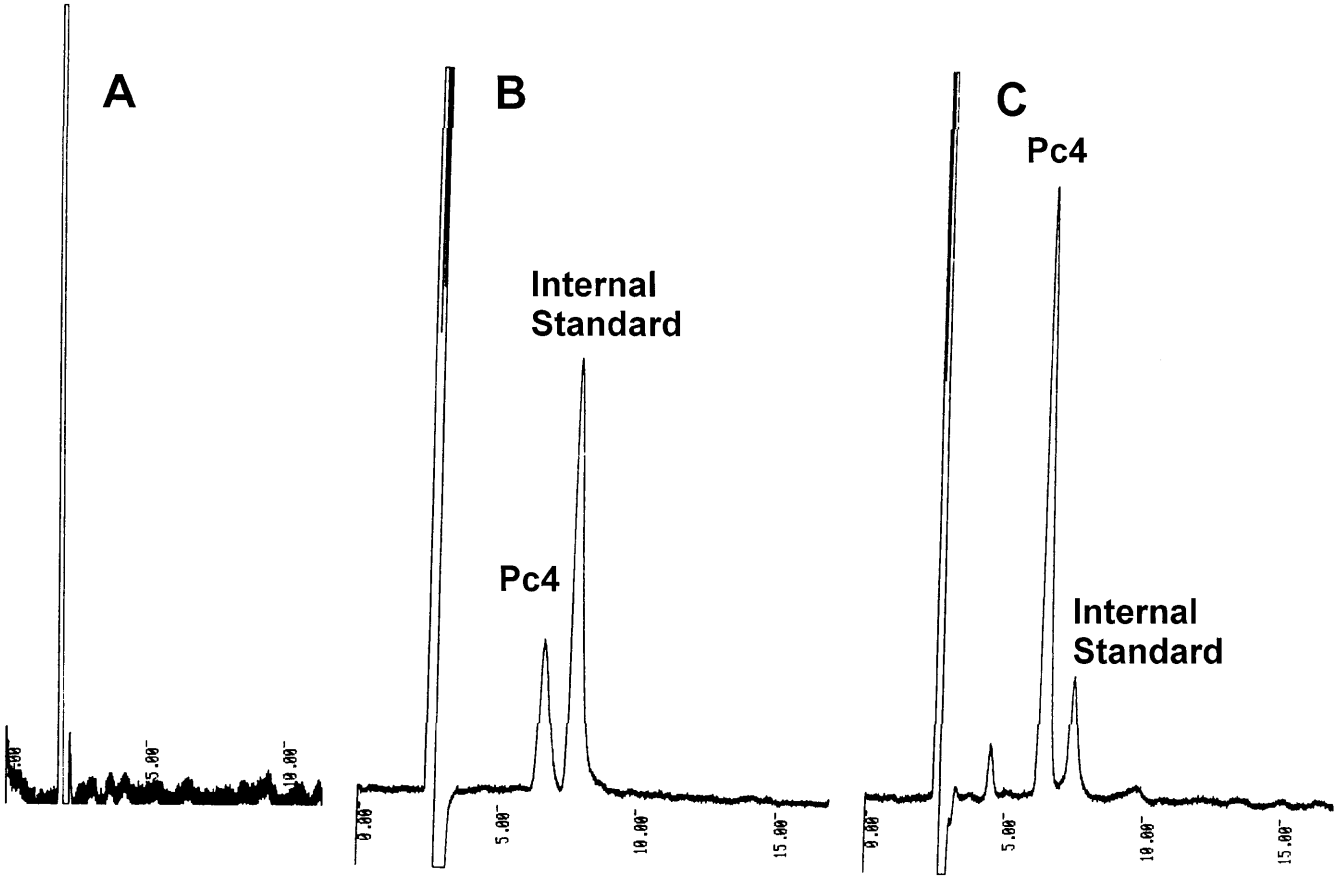

Fig. 2 HPLC tracings of **A** control mouse plasma, **B** control mouse plasma to which Pc4 and internal standard had been added, and **C** plasma obtained from a mouse 60 min after i.v. injection of Pc4 at a dose of 10 mg/kg and formulated in diluent 12:0.154 *M* NaCl (1:3, v:v)

Standard curves were performed in triplicate and included Pc4 concentrations of 2.5, 5, 10, 30, 100, 300, 1000, 3000 ng/ml, and 10 000 ng/ml. There was $<10\%$ decomposition of a 4-mg/ml solution of Pc4 prepared in diluent 12:0.154 *M* NaCl (1:3, v:v) and stored for up to 48 h at 22 °C and in the dark. There was minimal decomposition observed in 50-ng/ml solutions of Pc4 prepared in mouse plasma and stored at 4 or 22 °C for 1, 2, 4, or 24 h. Similarly, there was no decomposition of a 50-ng/ml solution of Pc4 in ethyl acetate stored at 22 °C for 2 h. This time was chosen because it was felt to be the maximum time that the Pc4 would be in ethyl acetate before being evaporated to dryness and resuspended in methanol. Finally, no decomposition was observed over 10.5 h in a 50-ng/ml solution of Pc4 that was prepared in methanol and repeatedly injected by autosampler onto the HPLC system used in these studies. There were no endogenous materials in mouse plasma, mouse tissues, or dosing vehicles that interfered with the determination of Pc4 or internal standard. In plasma, the limit of quantification was 5 ng/ml (48), and the assay was linear between 5 ng/ml and 10 000 ng/ml. In tissues, the limit of quantification was 30 ng/g, and the assay was linear to 10 000 ng/g. Recovery from spiked samples of plasma containing 50 ng/ml of Pc4 ($n = 3$) was 81%. The coefficient of variability for the analysis in plasma was $<15\%$ with regard to both intraday analysis of any concentration on the linear portion of the standard curve or interday comparison of standard curves.

An attempt was made to determine the binding of Pc4 to plasma proteins. In these studies, solutions of Pc4 (50 ng/ml) were prepared in 0.154 *M* NaCl or mouse plasma and placed into Amicon Centrifree ultrafiltration devices (Amicon Division of W.R. Grace, Beverly, Mass., USA). Ultrafiltrates were prepared by centrifugation at 2000 *g* for 20 min. Concentrations of Pc4 in 200-µl aliquots of both the original sample and the ultrafiltrate were determined with the HPLC assay described above.

In another approach, 250-, 1000-ng/ml, and 10 000-ng/ml solutions of Pc4 were prepared in either plasma or phosphate-buffered saline, and 0.9 ml of each solution was placed into one half of lucite chambers (Bel-Art Products, Pequannock, N.J., USA) that were separated by 25 000 mw cutoff Spectra/Por 7 Molecularporous Dialysis Membrane (Spectrum, Houston, Tex., USA). Phosphate-buffered saline (0.9 ml) was placed into the other half of the dialysis chambers, and the equilibrium dialysis apparatus were allowed to incubate in the dark for 24 h at 37 °C. At the end of this incubation, solutions from each half of each dialysis chamber were aspirated and 200-µl aliquots were assayed for Pc4 with the HPLC assay described above.

Pharmacokinetic analysis

Time courses of plasma concentrations of Pc4 were analyzed by both non-compartmental and compartmental methods. Area under the curve from zero to infinity AUC_{0-inf} and terminal half-life ($t_{1/2}$) were estimated by non-compartmental analysis with the LaGrange function [58] as implemented by the LAGRAN computer program [44]. CL_{tb} was calculated from the definition:

$$CL_{tb} = \frac{\text{Dose}}{\text{AUC}}$$

and steady-state volume of distribution (Vd_{SS}) was calculated from the formula:

$$Vd_{SS} = CL_{tb}/k_{el}$$

where k_{el} is the elimination constant.

In addition, two- and three-compartment, open, linear models were fit to individual concentrations of Pc4 in plasma versus time. Compartmental modeling used the program ADAPT II [11] with generalized least squares estimation. Model discrimination was based on Akaike's Information Criteria (AIC) [1], defined as: AIC = 2p + n(lnWSSR), where p represents the number of parameters in the model, n represents the number of observations, and WSSR represents the weighted sum of squares residuals.

Results

Plasma pharmacokinetics

Because no information was available with regard to the toxicity of Pc4 after i.v. administration to mice, preliminary efforts were directed at defining a suitably high, but not lethal, i.v. dose. Pc4 was dissolved in diluent 12:0.154 *M* NaCl (1:3, v:v). Doses of 80, 40, or 20 mg/kg were delivered in a maximum volume of 10 ml/kg to groups of five female and five male CD_2F_1 mice. With a dose of 80 mg/kg of Pc4, two of five female and two of five male mice were dead or moribund by 24 h after injection, but all of the control mice injected only with vehicle survived with no adverse effects. Mice that died or were killed because they were moribund after receiving 80 mg/kg were necropsied. Gross examination revealed a blue discoloration affecting many tissues, although this discoloration was not evident at microscopic examination. The most significant changes were present in the lungs, which were noted to be hemorrhagic and very blue-black. Microscopically there was a mild, acute interstitial pneumonia characterized by an increase of neutrophils within the alveolar septae, with occasional neutrophils and fibrin within the alveolar spaces. Gross and microscopic changes in mice that received 80 mg/kg and survived for 14 days before being killed for pathologic evaluation were markedly different from those observed in the animals that died or were killed because they were moribund 24 h after dosing. In the lungs of these animals, there was a mild, granulomatous pneumonia characterized by clusters of multi-nucleated giant cells that frequently contained phagocytized particles, which were clear and relatively fusiform. All mice injected with a Pc4 dose of 40 mg/kg survived and returned to their pretreatment weight, as did all mice injected with a dose of 20 mg/kg. Mice receiving 40 mg/kg retained blue discoloration of skin, which was especially noticeable in the ears and tails. At 14 days after dosing, kidneys and lungs from mice given 40-mg/kg doses of Pc4 were blue-gray. In contrast, tissues of mice killed 14 days after receiving 20 mg/kg of Pc4 appeared normal to gross inspection.

On the basis of the above-mentioned observations, 40 mg/kg was selected as the highest dose to be used in studies investigating the pharmacokinetics and tissue distribution of Pc4 after i.v. administration. Subsequent i.v. studies using 10- or 2-mg/kg doses of Pc4 were undertaken to evaluate the linearity of Pc4 pharmacokinetics over a reasonably broad, yet relevant, range of doses. The plan for sequential pharmacokinetic studies involved 40 mg/kg, 10 mg/kg, and 2 mg/kg in diluent 12:0.154 *M* NaCl and then 10 mg/kg and 2 mg/kg in polyethylene glycol:Tween 80:sodium phosphate buffer. It was felt that this scheme would allow at least preliminary investigation of any potential differences in Pc4 pharmacokinetics due to formulation.

Intravenous bolus delivery of Pc4, at a dose of 40 mg/kg, produced "peak" plasma Pc4 concentrations

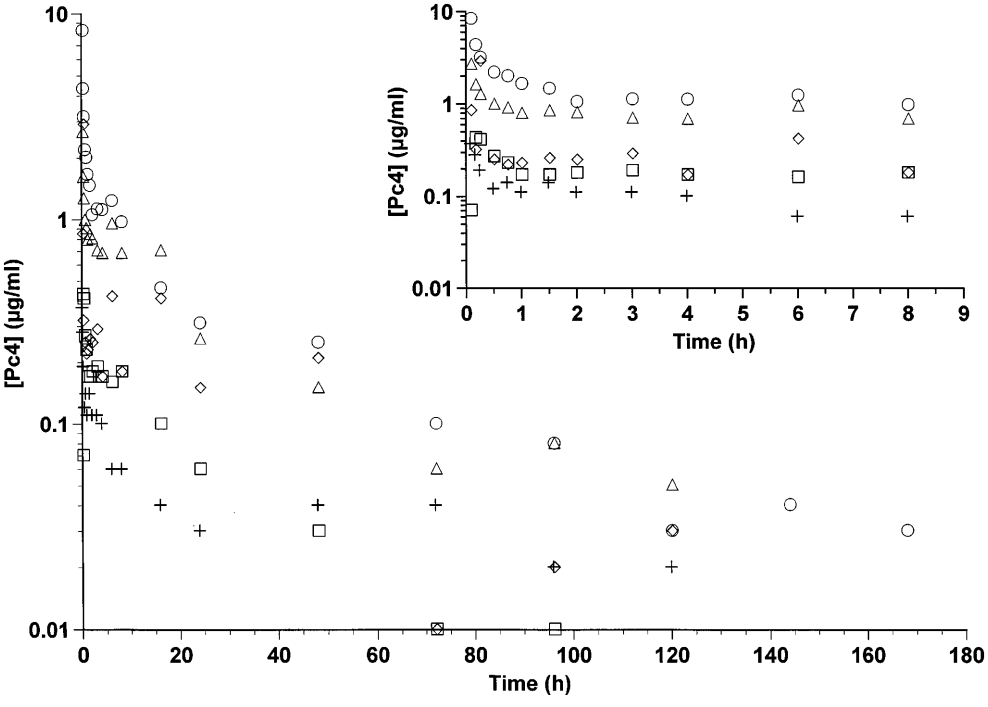

Fig. 3 Concentrations of Pc4 detected in plasma of female CD_2F_1 mice given Pc4 i.v. at various doses and in different formulations. (○) 40 mg/kg in diluent 12:0.154 *M* NaCl; (△) 10 mg/kg in diluent 12:0.154 *M* NaCl; (◇) 10 mg/kg in polyethylene glycol:Tween 80:sodium phosphate buffer; (+) 2 mg/kg in diluent 12:0.154 *M* NaCl; and (□) 2 mg/kg in polyethylene glycol:Tween 80:sodium phosphate buffer. Symbols represent the means of three mice at each time point. The inset displays data for the first 8 h after injection on an expanded scale

between 7.81 and 8.92 μg/ml in mice killed at 5 min after injection (Fig. 3). Sequential reduction of the Pc4 dose to 10 mg/kg in diluent 12:0.154 *M* NaCl (1:3, v:v), 10 mg/kg in polyethylene glycol:Tween 80:sodium phosphate buffer (40:0.2:59.8, v:v:v), 2 mg/kg in diluent 12:0.154 *M* NaCl (1:3, v:v), and, finally, 2 mg/kg in polyethylene glycol:Tween 80:sodium phosphate buffer (40:0.2:59.8, v:v:v) resulted in "peak" plasma Pc4 concentrations between 2.07 and 3.24, 0.68 and 0.98, and 0.29 and 0.41 μg/ml, respectively (Fig. 3, Table 1). Pc4 persisted in plasma for prolonged periods of time (Fig. 3, Table 1). In mice injected with a Pc4 dose of 40 mg/kg, plasma concentrations of Pc4 remained above the lower limit of quantification of the assay for at least 168 h. In mice injected with doses of 10 mg/kg in either vehicle employed or with a dose of 2 mg/kg in polyethylene glycol:Tween 80:sodium phosphate buffer vehicle, plasma concentrations of Pc4 remained above the lower limit of quantification of the assay for at least 120 h. When a Pc4 dose of 2 mg/kg was delivered in diluent 12:0.154 *M* NaCl (1:3, v:v), plasma concentrations of Pc4 remained above the lower limit of quantification for at least 96 h.

Non-compartmental analysis of plasma Pc4 concentration-versus-time data showed an increase in AUC when the dose of Pc4 increased from 2 mg/kg to 40 mg/kg (Table 2). The AUC associated with 2-mg/kg doses delivered in the two vehicles employed were very similar (Table 2). The increased AUC associated with a 10-mg/kg dose of Pc4 delivered in diluent 12:0.154 *M* NaCl (1:3, v:v) was proportionally greater than the AUC associated with a 2-mg/kg dose in the same vehicle (Table 2). In contrast, the AUC associated with the 10-mg/kg Pc4 dose delivered in the polyethylene glycol:Tween 80:sodium phosphate buffer was only 2.5 times greater than the AUC associated with a 2-mg/kg dose of Pc4 formulated in that vehicle (Table 2). Furthermore, when the dose of Pc4 was increased to 40 mg/kg, there was a less than twofold increase in AUC above that produced by the 10-mg/kg dose formulated in diluent 12:0.154 *M* NaCl and a less than eightfold increase in AUC above that produced by the 2-mg/kg doses formulated in either vehicle (Table 2). Across the 20-fold range of doses studied, CL_{tb} varied from 376 to 1106 ml/h/kg.

Compartmental modeling of plasma Pc4 concentration-versus-time data showed the data to be fit best by a two-compartment, open, linear model. The parameters in this model were the volume of the central compartment (V), the elimination constant (ke), and the transfer constants between central and peripheral compartment (kcp), and between peripheral and central compartment (kpc). The individual model parameters resulting from fitting of a two-compartment, open, linear model to the plasma Pc4 concentration-versus-time data from each study are displayed in Table 3, as are the values for $t_{1/2\alpha}$, $t_{1/2\beta}$, CL_{tb}, and Vd_{ss} that were derived from these parameters.

Unfortunately, Pc4, at the concentrations used to study plasma protein binding, bound quantitatively to

Table 1 Concentrations of Pc4 in plasma of mice injected with various i.v. doses and formulations. *SD* standard deviation, *ND* not done

Time (h)	Dose (mg/kg) and Formulation				
	40 Dil 12:NaCl[a]	10 Dil 12:NaCl	10 PEG:Tween:PO_4[b]	2 Dil 12:NaCl	2 PEG:Tween:PO_4
0.083	8.25 ± 0.59[c,d,e]	2.65 ± 0.59	0.85 ± 0.15	0.37 ± 0.07	0.07 ± 0.21
0.167	4.31 ± 0.44	1.6 ± 0.16	0.32 ± 0.02	0.28 ± 0.04	0.43 ± 0.06
0.25	3.15 ± 0.17	1.26 ± 0.08	0.29 ± 0.03	0.19 ± 0.01	0.41 ± 0.06
0.5	2.18 ± 0.62	0.99 ± 0.13	0.25 ± 0.04	0.12 ± 0.03	0.27 ± 0.03
0.75	2.0 ± 0.28	0.9 ± 0.07	0.22 ± 0.01	0.14 ± 0.01	0.23 ± 0.03
1	1.65 ± 0.04	0.79 ± 0.18	0.23 ± 0.40	0.11 ± 0.03	0.17 ± 0.04
1.5	1.46 ± 0.09	0.84 ± 0.1	0.26 ± 0.07	0.14 ± 0.02	0.17 ± 0.03
2	1.05 ± 0.09	0.8 ± 0.05	0.25[f]	0.11 ± 0.02	0.18 ± 0.02
3	1.12 ± 0.09	0.7 ± 0.12	0.29 ± 0.05	0.11 ± 0.01	0.19 ± 0.01
4	1.11 ± 0.14	0.68 ± 0.03	0.17 ± 0.13	0.1 ± 0.01	0.17 ± 0
6	1.23 ± 0.18	0.95 ± 0.15	0.42 ± 0.11	0.06 ± 0	0.16 ± 0.02
8	0.97 ± 0.29	0.68 ± 0.34	0.18 ± 0.1	0.06 ± 0.01	0.18 ± 0.04
16	0.46 ± 0.48	0.7 ± 0.06	0.41 ± 0.01	0.04 ± 0.01	0.1 ± 0.01
24	0.31 ± 0.04	0.26 ± 0.12	0.15 ± 0.01	0.03 ± 0	0.06 ± 0.02
48	0.25 ± 0.06	0.15 ± 0.7	0.12 ± 0.08	0.04 ± 0	0.03 ± 0.01
72	0.1 ± 0.04	0.06 ± 0.04	0.01 ± 0.01	0.04 ± 0.02	0.01 ± 0
96	0.08 ± 0.06	0.08 ± 0.1	0.02 ± 0.01	0.02 ± 0.02	0.01 ± 0
120	0.03 ± 0.05	0.05 ± 0.02	0.03 ± 0.03	0.02 ± 0	0
144	0.04 ± 0.02	ND	0	0	0
168	0.03 ± 0.02	ND	0	0	0

[a] Diluent 12:0.154 *M* NaCl (1:3, v:v)
[b] Polyethylene glycol:Tween 80:sodium phosphate buffer (40:0.2:59.8, v:v:v)
[c] Mean ± SD
[d] μg/ml
[e] $n = 3$
[f] $n = 1$

Table 2 Non-compartmental pharmacokinetic analyses of Pc4 plasma concentration-versus-time curves. AUC_{0-inf} area under the curve from time zero to infinity, $t_{1/2}$ terminal half-life, VD_{SS} steady-state volume of distribution, CL_{tb} total body clearance

	Dose (mg/kg)				
	40	10	10	2	2
Vehicle	dil 12:NaCl[a]	dil 12:NaCl	PEG:Tween:PO_4[b]	dil 12:NaCl	PEG:Tween:PO_4
AUC_{0-inf}	36.2	22.8	12	5.3	4.8
$t_{1/2}$ (h)	39	24.5	31.8	24.1	46.6
VD_{SS} (ml/kg)	46 900	15 900	41 500	10 700	25 500
CL_{tb} (ml/h/kg)	1106	439	831	376	414

[a] Diluent 12:0.154 *M* NaCl (1:3, v:v)
[b] Polyethylene glycol:Tween 80:sodium phosphate buffer (40:0.2:59.8, v:v:v)

Table 3 Pharmacokinetic parameters resulting from fitting of a two–compartment, open, linear model to plasma Pc4 concentration-versus-time data. *V* volume of the central compartment, *ke* elimination constant, *kcp* transfer constants between central and peripheral compartment and between peripheral compartment (*kpc*), $t_{1/2\alpha}$ □, $t_{1/2\beta}$ □, CL_{tb} total body clearance, VD_{SS} steady-state volume of distribution

	Dose (mg/kg)				
	40	10	10	2	2
Vehicle	dil 12:NaCl[a]	dil 12:NaCl	PEG:Tween:PO_4[b]	dil 12:NaCl	PEG:Tween:PO_4
Parameter					
V (ml/kg)	4396	2049	397	2232	5284
ke (h)	0.1713	0.1475	1.526	0.1578	0.0647
kcp (h)	3.321	7.863	43.77	3.42	2.265
kpc (h)	0.5653	1.812	0.585	1.041	0.7944
$t_{1/2\alpha}$ (h)	0.018	0.07	0.02	0.1512	0.22
$t_{1/2\beta}$ (h)	28.06	25.4	35.6	19.4	41.9
CL_{tb} (ml/h/kg)	753	302	606	352	342
Vd_{SS} (ml/kg)	29360	10940	30100	9567	20350

[a] Diluent 12:0.154 *M* NaCl (1:3, v:v)
[b] Polyethylene glycol:Tween 80:sodium phosphate buffer (40:0.2:59.8, v:v:v)

the membranes of Amicon Centrifree devices and to the membrane separating the 2 halves of the equilibrium dialysis chambers so that none of the drug present in the 0.154 *M* NaCl or phosphate-buffered saline solutions was detectable in the ultrafiltrates or dialysates prepared from those solutions. Therefore, no data describing the extent of binding of Pc4 to mouse plasma proteins could be obtained.

Urinary excretion of Pc4

Minimal amounts of Pc4 were detected in the urine of mice (Table 4). In groups of mice injected i.v. with 40 mg/kg, between 0.014 and 0.093% of the administered dose was accounted for by urinary excretion of parent compound in the first 24 h after injection, and the cumulative excretion over 144 h and 168 h was 0.107% and 0.248%, respectively. In mice injected i.v. with 10 mg/kg of Pc4 in diluent 12:0.154 *M* NaCl (1:3, v:v), urinary excretion of parent compound accounted for 0.05% of the administered dose in the first 24 h after injection, and the cumulative excretion over 120 h was 0.116%. In mice injected i.v. with Pc4 at 2 mg/kg in polyethylene glycol:Tween 80:sodium phosphate buffer (40:0.2:59.8, v:v:v), between 0.042% and 0.073% of the administered dose was accounted for by urinary excretion in the first 24 h after injection, and the cumulative excretion over 144 h and 168 h was 0.27% and 0.41%, respectively.

Tissue Pc4 concentrations following i.v. administration

After i.v. bolus delivery to mice, Pc4 distributed rapidly to all tissues and persisted in most tissues for the duration of each pharmacokinetic study (Tables 5–8). In mice receiving 40-mg/kg and 10-mg/kg doses of Pc4 formulated in diluent 12:0.154 *M* NaCl (1:3, v:v), tissue concentrations of Pc4 were ≥ those in concomitant plasma samples by 10 min after injection of drug (Tables 5 and 6). When Pc4 was delivered in polyethylene glycol:Tween 80:sodium phosphate buffer (40:0.2:59.8, v:v:v), this pattern was altered in that concentrations of Pc4 in brain were less than concomitant plasma concentrations until 8–24 h after injection of drug. Furthermore, delivery of Pc4 in the polyethylene glycol:Tween 80:sodium phosphate buffer produced much lower concentrations of drug in the brain than did delivery of Pc4 formulated in diluent 12:0.154 *M* NaCl (1:3, v:v) (Tables 5–8). Tissue exposure, as measured by AUC, increased in a dose-dependent fashion (Table 9).

Table 4 Urinary excretion of Pc4 by mice. *ND* not done

Dose (mg/kg)	Vehicle	Excretion 0–24 h (% of dose)	Excretion 0–168 h (% of dose)
40	dil 12:NaCl[a]	0.014–0.93	0.248
10	dil 12:NaCl	0.05	0.116[c]
10	PEG:Tween:PO_4[b]	ND	ND
2	PEG:Tween:PO_4	0.042–0.073	0.41
2	dil 12:NaCl	ND	ND

[a] Diluent 12: 0.154 *M* NaCl (1:3, v:v)
[b] Polyethylene glycol:Tween80:sodium phosphate buffer (40:0.2:59.8, v:v:v)
[c] 0–120 h

Discussion

Although it is too early to define the ultimate role of PDT in cancer treatment, there continues to be much research activity in this area [17, 21, 40, 46, 51, 54]. Studies are ongoing to define basic cellular and molecular mechanisms underlying the effects produced by PDT. Similarly, there are ongoing efforts to develop new PDT-sensitizing agents with superior or more desirable properties than are available in currently employed agents [2, 7, 14, 17, 41, 42], and to develop better instrumentation with which to deliver the light required to activate those agents [13, 17, 43, 53, 57, 61].

The studies presented in the current paper were undertaken to develop information on the new PDT-sensitizing agent, Pc4, with the intent that such information would assist in its clinical evaluation and development. Relevant results from those studies include both methodological and pharmacological information related to Pc4. The HPLC method developed to quantify Pc4 should be directly applicable to clinical trials. It is facile, specific, and sensitive enough for measurement of Pc4 in tissue as well as plasma samples from patients receiving Pc4. Such measurements should facilitate integration of the pharmacokinetics of the drug with the clinical effects it produces. Obviously, the HPLC method should be applicable in additional pre-clinical studies that might be undertaken before, in concert with, or subsequent to the initiation of clinical trials of Pc4. Although the utility of the HPLC method in studies evaluating Pc4 as a means to remove pathogens from red blood cell concentrates [19, 26, 28, 60] may be less obvious, its availability could conceivably enhance such work.

The demonstration that a vehicle containing diluent 12 and 0.154 *M* NaCl allows i.v. delivery of the relatively hydrophobic Pc4 molecule has obvious clinical impact. In fact, clinical formulation of Pc4 will utilize a vehicle containing diluent 12 and 0.154 *M* NaCl.

The pharmacokinetic and preliminary toxicology data generated by the current studies also have direct clinical implications. Pc4 is widely distributed to tissues and persists in them for a prolonged period of time. Furthermore, the data demonstrate that increased doses of Pc4 produce increased, although not necessarily proportional increases, in tissue concentrations and exposures of Pc4. The pulmonary toxicity observed with the 80-mg/kg dose of Pc4 is likely secondary to precipitation of the agent in plasma with subsequent pulmonary sequestration and inflammatory reaction. Fortunately, this reaction was not observed with the 10-mg/kg and 2-mg/kg doses of Pc4, which are much more reflective of the doses used in preclinical in vivo studies

Table 5 Concentrations of Pc4 in plasma and tissue of mice injected with Pc4 dose of 40 mg/kg formulated in diluent 12:0.154 *M* NaCl (1:3,v:v)

Time (h)	Plasma (μg/ml)	Brain (μg/g)	Heart (μg/g)	Lung (μg/g)	Liver (μg/g)	Kidney (μg/g)	Spleen (μg/g)	Skeletal muscle (μg/g)	Fat (μg/g)	Skin (μg/g)
0.083	8.25[a]	5.98	105.02	586.13	92.19	49.17	57.17	6.37	1.85	3.49
0.167	4.31	5.11	59.61	328.54	77.48	40.12	43.62	6.27	5.36	4.29
0.25	3.15	6.07	53.00	236.41	85.67	46.07	57.87	5.19	4.05	3.75
0.5	2.18	4.53	39.41	176.95	95.40	41.45	74.35	6.35	5.72	3.15
0.75	2.00	3.54	51.13	174.31	89.34	48.58	71.06	6.82	5.41	3.74
1	1.65	3.58	49.07	210.82	81.97	51.17	76.90	7.31	9.61	6.77
1.5	1.46	3.19	42.07	119.96	85.35	52.80	79.76	4.29	8.83	5.45
2	1.05	3.52	48.89	101.44	84.87	54.35	70.85	6.10	10.56	5.02
3	1.12	3.72	44.79	117.56	89.80	57.41	96.09	8.05	12.36	4.01
4	1.11	3.11	33.55	112.20	96.20	58.64	114.46	7.27	14.92	6.32
6	1.23	2.71	27.22	156.70	83.57	57.78	116.53	4.64	9.26	5.59
8	0.97	2.68	23.41	122.46	76.54	53.39	145.31	5.69	11.99	5.82
16	0.46	2.07	19.23	127.66	80.30	50.41	125.94	3.40	4.60	4.02
24	0.31	1.78	15.72	75.64	75.42	45.83	97.41	2.05	2.78	3.59
48	0.25	4.65	19.58	84.46	52.36	35.81	77.63	1.87	1.61	2.65
72	0.10	0.69	9.01	96.62	37.32	26.39	72.27	1.67	0.60	2.41
96	0.08	0.99	46.36	89.42	35.95	24.40	37.19	1.43	1.21	1.63
120	0.03	1.33	9.31	47.95	31.56	22.25	42.78	1.36	0.54	1.41
144	0.04	0.51	8.13	5.24	26.51	25.27	70.93	1.00	1.03	1.63
168	0.03	0.30	6.46	3.48	27.02	29.83	39.26	1.08	0.88	1.12

[a] Mean of samples from three mice at each time

Table 6 Concentrations of Pc4 in plasma and tissues of mice injected with a Pc4 dose of 10 mg/kg formulated in diluent 12:0.154 *M* NaCl (1:3, v:v)

Time (h)	Plasma (μg/ml)	Brain (μg/g)	Heart (μg/g)	Lung (μg/g)	Liver (μg/g)	Kidney (μg/g)	Spleen (μg/g)	Skeletal muscle (μg/g)	Fat (μg/g)	Skin (μg/g)
0.083	2.65[a]	1.55	41.64	72.49	41.54	18.15	31.58	2.93	2.20	1.15
0.167	1.60	1.74	44.92	103.52	34.51	16.93	24.60	2.88	3.25	1.48
0.25	1.26	1.30	33.96	110.71	37.09	19.49	29.37	3.12	2.76	1.29
0.5	0.99	0.96	12.34	60.92	35.70	19.98	27.52	3.18	6.29	1.69
0.75	0.90	1.12	28.34	86.12	37.48	24.25	32.35	7.35	5.86	2.09
1	0.79	0.61	23.17	56.13	37.47	24.13	31.11	5.46	8.30	1.85
1.5	0.84	0.94	31.17	56.37	34.97	25.54	44.05	2.93	6.20	2.10
2	0.80	1.37	20.91	58.62	30.72	26.03	63.24	3.11	18.60	2.19
3	0.70	0.82	19.24	53.08	38.27	28.24	46.20	5.91	5.75	1.95
4	0.68	0.76	10.71	44.84	33.71	24.51	35.45	5.38	2.98	2.02
6	0.95	0.89	5.19	39.48	28.98	25.97	34.60	3.78	11.36	2.24
8	0.68	0.00	14.62	35.67	25.01	21.38	29.18	2.55	8.85	1.93
16	0.70	1.27	13.91	25.44	24.15	19.31	37.74	1.98	6.31	2.35
24	0.26	0.91	10.79	29.00	20.54	15.30	27.88	1.08	3.49	0.95
48	0.15	0.33	11.37	39.64	10.03	7.30	22.66	0.40	2.24	0.85
72	0.06	0.18	7.25	17.18	7.29	6.15	14.27	0.40	1.90	0.47
96	0.08	0.29	4.97	30.79	6.87	6.25	10.36	0.44	0.57	0.39
120	0.05	0.21	7.54	14.36	7.52	7.54	7.60	0.46	1.09	0.40

[a] Mean of samples from three mice at each time

Table 7 Concentrations of Pc4 in plasma and tissues of mice injected with a Pc4 dose of 10 mg/kg formulated in polyethylene glycol:Tween 80:sodium phosphate buffer (40:0.2:59.8, v:v:v). *SL* sample lost

Time (h)	Plasma (μg/ml)	Brain (μg/g)	Heart (μg/g)	Lung (μg/g)	Liver (μg/g)	Kidney (μg/g)	Spleen (μg/g)	Skeletal muscle (μg/g)	Skin (μg/g)
0.083	0.85[a]	0.25	9.86	44.16	S.L.[b]	9.18	0.45	2.02	1.29
0.167	0.32	0.18	6.03	28.79	1.51	7.52	0.76	1.16	1.12
0.25	0.29	0.10	8.41	23.48	2.10	9.54	0.41	2.64	1.31
0.5	0.25	0.12	8.02	14.12	2.01	13.68	0.82	4.22	1.53
0.75	0.22	0.10	7.73	13.76	1.93	14.17	0.61	3.28	1.63
1	0.23	0.11	9.21	24.18	2.30	14.25	0.72	3.31	2.77
1.5	0.26	0.09	4.85	18.15	1.21	16.96	0.77	3.26	1.96
2	0.25	0.08	5.05	31.80	1.26	15.56	0.77	3.08	1.80
3	0.29	0.16	4.17	30.26	1.04	17.75	1.32	3.79	2.46
4	0.17	0.21	3.77	22.93	0.94	19.85	1.84	4.10	2.47
6	0.42	0.14	5.12	30.30	1.28	21.80	1.01	4.75	3.18
8	0.18	0.18	2.76	17.13	0.69	16.44	1.11	2.92	2.55
16	0.41	0.21	1.80	19.55	0.45	18.79	0.54	2.55	3.20
24	0.15	0.32	1.70	12.48	0.42	16.54	1.10	2.27	2.86
48	0.12	0.14	1.51	9.25	0.38	8.03	0.38	1.16	2.18
72	0.01	0.09	1.01	5.63	0.25	4.37	0.29	0.68	1.26
96	0.02	0.08	1.23	3.85	0.31	4.31	0.15	0.66	0.66
120	0.03	0.01	0.72	2.08	0.18	2.95	0.17	0.56	0.57
144	0.00	0.00	1.06	1.82	0.26	2.69	0.09	0.30	0.36
168	0.01	0.00	0.03	1.95	0.18	2.51	0.08	0.23	0.31

[a] Mean of samples from three mice of each time

of the drug and of the doses proposed for clinical use. The concentrations of Pc4 documented in skin have obvious clinical implications in view of the proposed use of Pc4 to treat cutaneous malignancies and the potential for Pc4-related cutaneous photosensitivity. The very small amount of Pc4 excreted in the urine argues against renal excretion being a major route of clearance of the drug and implies that Pc4 might be given to patients with renal impairment. We did not observe any obvious metabolites of Pc4 in plasma or tissue of mice given the drug; however, it is possible that our HPLC method, which monitored high wavelengths of light, was not suitable for detection of such chemical entities, especially if their structures included major alterations of the light-absorbing phthalocyanine moiety.

In summary, we have developed a facile and sensitive HPLC technique for quantifying Pc4 in biological matrices and have applied that method to a series of pharmacokinetic studies wherein we have characterized the plasma pharmacokinetics and tissue concentrations

Table 8 Concentrations of Pc4 in plasma and tissues of mice injected with a Pc4 dose of 2 mg/kg formulated in polyethylene glycol: Tween 80:sodium phosphate buffer (40:0.2:59.8, v:v:v)

Time (h)	Plasma (μg/ml)	Brain (μg/g)	Heart (μg/g)	Lung (μg/g)	Liver (μg/g)	Kidney (μg/g)	Spleen (μg/g)	Skeletal muscle (μg/g)	Skin (μg/g)
0.083	0.37[a]	0.00	0.00	12.16	8.14	5.07	0.60	0.77	0.84
0.167	0.28	0.24	0.96	7.97	8.59	4.74	0.26	0.84	1.54
0.25	0.19	0.26	1.06	11.69	9.17	3.83	0.40	0.77	0.79
0.5	0.12	0.25	1.01	9.69	9.79	5.90	0.81	0.61	1.45
0.75	0.14	0.18	0.73	8.81	34.28	6.18	0.70	0.57	1.23
1	0.11	0.16	0.63	6.13	8.88	6.28	0.75	0.77	1.59
1.5	0.14	0.20	0.79	5.97	7.36	7.63	1.01	0.80	1.98
2	0.11	0.15	0.60	5.94	8.31	8.46	0.97	0.84	1.85
3	0.11	0.20	0.81	5.65	8.04	7.05	0.82	0.84	0.10
4	0.10	0.12	0.47	5.11	7.69	7.22	0.82	0.68	1.20
6	0.06	0.10	0.42	7.82	4.50	6.98	1.01	0.68	1.09
8	0.06	0.07	0.28	8.61	4.89	6.90	1.10	0.59	1.84
16	0.04	0.08	0.33	5.68	3.78	4.44	1.04	0.18	0.77
24	0.03	0.08	0.33	4.32	3.88	4.61	0.67	0.19	1.81
48	0.04	0.04	0.11	1.68	2.09	2.59	0.35	0.18	1.01
72	0.04	0.04	0.16	1.32	1.56	1.65	0.24	0.12	0.65
96	0.02	0.02	0.10	0.65	1.22	1.67	0.19	0.09	0.23
120	0.02	0.00	0.00	1.00	0.84	1.30	0.13	0.04	0.09
144	0.01	0.00	0.00	0.62	0.87	1.38	0.14	0.07	0.20
168	0.00	0.00	0.00	0.46	0.67	1.06	0.09	0.03	0.06

[a] Mean of samples from three mice of each time

Table 9 Areas under the curve of Pc4 concentrations in plasma and tissues of mice injected with various i.v. doses and formulations

Dose (mg/kg)	Vehicle	Plasma (μg/ml · h)	Brain (μg/g · h)	Heart (μg/g · h)	Lung (μg/g · h)	Liver (μg/g · h)	Kidney (μg/g · h)	Spleen (μg/g · h)	Skeletal muscle (μg/g · h)	Skin (μg/g · h)
40	dil 12:NaCl[a]	33	247	3058	10663	6207	4099	9081	263	332
10	dil 12:NaCl	22	61	1117	3544	1614	1210	496	104	45
10	PEG:Tween: PO_4[b]	11	17	222	1031	1668	1104	66	176	222
2	PEG:Tween: PO_4	4	4	22	300	295	351	56	22	106

[a] Diluent 12:0.154 M NaCl (1:3, v:v)
[b] Polyethylene glycol:Tween 80:sodium phosphate buffer (40:0.2:59.8, v:v:v)

associated with a variety of doses of Pc4. These data have assisted in the design of clinical trials of Pc4 that are being initiated and which are being planned. Furthermore, the HPLC method is being used, or planned for use, in those studies.

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References

1. Akaike H (1979) A Bayesian extension of the minimal AIC procedures of autoregressive model fitting. Biometrika 66: 237
2. Allen RP, Kessel D, Tharratt RS, Volz W (1992) Photodynamic therapy of superficial malignancies with NPe6 in man. In: Spinelli P, Dal Fante M, Marchisini R (eds) Photodynamic therapy and biomedical lasers. Elsevier Science, Amsterdam, p 441
3. Anderson CY, Freye K, Tubesing KA, Li YS, Kenney ME, Mukhtar H, Elmets CA (1998) A comparative analysis of silicon phthalocyanine photosensitizers for in vivo photodynamic therapy of RIF-1 tumors in C3H mice. Photochem Photobiol 67: 332
4. Ben-Hur E, Rosenthal I (1985) The phthalocyanines: a new class of mammalian cell photosensitizers with a potential for cancer phototherapy. Int J Radiat Biol 47: 145
5. Ben-Hur E, Hoeben RC, Van Ormondt H, Dubbelman TM, Van Steveninck J (1992) Photodynamic inactivation of retroviruses by phthalocyanines: the effects of sulphonation, metal ligand and fluoride. J Photochem Photobiol B 13: 145
6. Ben-Hur E, Oetjen J, Horowitz B (1997) Silicon phthalocyanine Pc 4 and red light causes apoptosis in HIV-infected cells. Photochem Photobiol 65: 456
7. Berenbaum M, Bonnett R, Cheorettan E (1995) Selectivity of meso-tetra-(hydroxyphenyl) porphyrins and chlorins and Photofrin in causing photodamage in tumor, skin, muscle, and bladder. Lasers Med Sci 8: 235
8. Boyle RW, Dolphin D (1996) Structure and biodistribution relationships of photodynamic sensitizers. Photochem Photobiol 64: 469

9. Cubeddu R, Canti G, Musolino M, Pifferi A, Taroni P, Valentini G (1994) Absorption spectrum of hematoporphyrin derivative in vivo in a murine tumor model. Photochem Photobiol 60: 582
10. Cubeddu R, Canti G, Musolino M, Pifferi A, Taroni P, Valentini G (1996) In vivo absorption of disulphonated aluminium phthalocyanine in a murine tumour model. J Photochem Photobiol B 34: 229
11. D'Argenio DZ, Schumitzky A (1979) A program package for simulation and parameter estimation in pharmacokinetic systems. Comput Methods Programs Biomed 9: 115
12. Daziano JP, Steenken S, Chabannon C, Mannoni P, Chanon M, Julliard M (1996) Photophysical and redox properties of a series of phthalocyanines: relation with their photodynamic activities on TF-1 and Daudi leukemic cells. Photochem Photobiol 64: 712
13. De Jode ML, Mcgilligan JA, Dilkes MG, Cameron I, Hart PB, Grahn MF (1997) A comparison of novel light sources for photodynamic therapy. Lasers Med Sci 12: 260
14. Dilkes MG, DeJode ML, Rowntree-Taylor A (1997) m-THPC photodynamic therapy for head and neck cancers. Lasers Med Sci 11: 23
15. Dougherty TJ (1996) A brief history of clinical photodynamic therapy development at Roswell Park Cancer Institute. J Clin Laser Med 14: 219
16. Dougherty TJ, Henderson B (1992) Historical perspective: Schwartz S, Winkelman JW, Lipson RL. In: Henderson BW, Dougherty TJ (eds) Photodynamic therapy. Dekker, New York, p 1
17. Dougherty TJ, Gomer CJ, Henderson BW, Jori G, Kessel D, Korbelik M, Moan J, Peng Q (1998) Photodynamic therapy. J Natl Cancer Inst 90: 889
18. Gomer CJ (1991) Preclinical examination of first and second generation photosensitizers used in photodynamic therapy. Photochem Photobiol 54: 1093
19. Gottlieb P, Shen LG, Chimezie E, Bahng S, Kenney ME, Horowitz B, Ben-Hur E (1995) Inactivation of Trypanosoma cruzi trypomastigote forms in blood components by photodynamic treatment with phthalocyanines. Photochem Photobiol 62: 869
20. Gupta S, Ahmad N, Mukhtar H (1998) Involvement of nitric oxide during phthalocyanine (Pc4) photodynamic therapy-mediated apoptosis. Cancer Res 58: 1785
21. Hampton JA, Goldblatt PJ, Selman SH (1994) Photodynamic therapy: a new modality for the treatment of cancer. Ann Clin Lab Sci 24: 203
22. Kaye AH, Hill JS (1992) Photodynamic therapy of cerebral tumors. Neurosurg Quarterly 1: 233
23. Keller SM (1995) Photodynamic therapy. Biology and clinical application. Chest Surg Clin N Am 5: 121
24. Lee LK, Whitehurst C, Pantelides ML, Moore JV (1995) In situ comparison of 665 nm and 633 nm wavelength light penetration in the human prostate gland. Photochem Photobiol 62: 882
25. Levy JG (1994) Photosensitizers in photodynamic therapy. Sem Oncol 21 [Suppl 15]: 4
26. Lustigman S, Ben-Hur E (1996) Photosensitized inactivation of *Plasmodium falciparum* in human red cells by phthalocyanines. Transfusion 36: 543
27. Margaron P, Gregoire MJ, Scasnar V, Ali H, van Lier JE (1996) Structure-photodynamic activity relationships of a series of 4-substituted zinc phthalocyanines. Photochem Photobiol 63: 217
28. Margolis-Nunno H, Ben-Hur E, Gottlieb P, Robinson R, Oetjen J, Horowitz B (1996) Inactivation by phthalocyanine photosensitization of multiple forms of human immunodeficiency virus in red cell concentrates. Transfusion 36: 743
29. Megerian CA, Saidi SI, Sprecher RC, Setrakian S, Stepnick DW, Oleinick NL, Mukhtar H (1993) Photodynamic therapy of human squamous cell carcinoma in vitro and in xenografts in nude mice. Laryngoscope 103: 967
30. Moan J (1986) Porphyrin photosensitization and phototherapy. Photochem Photobiol 43: 681
31. Moan J, Rimington C, Western A (1985) The binding of dihematoporphyrin ether (Photofrin II) to human serum albumin. Clin Chim Acta 145: 227
32. Moan J, Berg K, Bommer JC, Western A (1992) Action spectra of phthalocyanines with respect to photosensitization of cells. Photochem Photobiol 56: 171
33. Mohr H, Lambrecht B, Selz A (1995) Photodynamic virus inactivation of blood components. Immunol Invest 24: 73
34. Moor AC, van der Veen A, Wagenaars-van Gompel AE, Dubbelman TM, Van Steveninck J, Brand A (1997) Shelf-life of photodynamically sterilized red cell concentrates with various numbers of white cells. Transfusion 37: 592
35. Ochsner M (1996) Light scattering of human skin: a comparison between zinc (II)-phthalocyanine and Photofrin II. J Photochem Photobiol B 32: 3
36. Ochsner M (1997) Photophysical and photobiological processes in the photodynamic therapy of tumours. J Photochem Photobiol B 39: 1
37. Owens JW, Yang L, Adeola G, Robins M, Smith R, Robinson R, Elayan N, McMahon L (1995) Isolation and photodynamic effects of hematoporphyrin derivative components: a chromatographic analysis of the starting materials. J Chromatogr B Biomed Sci Appl 669: 295
38. Pass HI (1993) Photodynamic therapy in oncology: mechanisms and clinical use. J Natl Cancer Inst 85: 443
39. Peng Q, Moan J (1995) Correlation of distribution of sulphonated aluminium phthalocyanines with their photodynamic effect in tumour and skin of mice bearing CaD2 mammary carcinoma. Br J Cancer 72: 565
40. Penning LC, Dubbelman TM (1994) Fundamentals of photodynamic therapy: cellular and biochemical aspects. Anticancer Drugs 5: 139
41. Razum N, Snyder A, Doiron D (1996) SnET2: clinical update. Proc SPIE 2675: 43
42. Renschler M, Yuen A, Panella T (1997) Photodynamic therapy trials with lutetium texaphyrin. Photochem Photobiol 65: 475
43. Ripley PM (1996) The physics of diode lasers. Lasers Med Sci 11: 71
44. Rocci ML, Jusko WJ (1983) LAGRAN program for area and moments in pharmacokinetic analysis. Comput Methods Programs Biomed 16: 203
45. Rosenthal I (1991) Phthalocyanines as photodynamic sensitizers. Photochem Photobiol 53: 859
46. Rosenthal DI, Glatstein E (1994) Clinical applications of photodynamic therapy. Ann Med 26: 405
47. Separovic D, He J, Oleinick NL (1997) Ceramide generation in response to photodynamic treatment of L5178Y mouse lymphoma cells. Cancer Res 57: 1717
48. Shah VP, Midha KK, Dighe S, McGilvery IJ, Skelly, JP, Yacobi A, Layloff T, Viswanathan CE, Cook CE, McDowall RD, Pittman KA, Spector S (1991) Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies. Eur J Drug Metab Pharmacokinet 16: 249
49. Soncin M, Polo L, Reddi E, Jori G, Kenney ME, Cheng G, Rodgers MA (1995) Effect of axial ligation and delivery system on the tumour-localising and -photosensitising properties of Ge(IV)-octabutoxy-phthalocyanines. Br J Cancer 71: 727
50. Spikes JD (1986) Phthalocyanines as photosensitizers in biological systems and for the photodynamic therapy of tumors. Photochem Photobiol 43: 691
51. Stables GI, Ash DV (1995) Photodynamic therapy. Cancer Treat Rev 21: 311
52. Van Lier JE, Spikes JD (1989) The chemistry, photophysics and photosensitizing properties of phthalocyanines. Ciba Found Symp 53: 17
53. Whitehurst C, Byrne K, Moore JV (1993) Development of an alternative light source to lasers for photodynamic therapy: I. comparative in vitro dose response characteristics. Lasers Med Sci 8: 8259
54. Wilson BC, Jeeves WP (1987) Photodynamic therapy in cancer. In: Ben-Hur E (ed) Photomedicine, volume 2. CRC Press, Boca Raton, Flo., USA, p 127

55. Wohrle D, Iskander N, Graschew G, Sinn H, Friedrich EA, Maier-Borst W, Stern J, Schlag P (1990) Synthesis of positively charged phthalocyanines and their activity in the photodynamic therapy of cancer cells. Photochem Photobiol 51: 351
56. Xue LY, He J, Oleinick NL (1997) Rapid tyrosine phosphorylation of HS1 in the response of mouse lymphoma L5178y-R cells to photodynamic treatment sensitized by the phthalocyanine Pc4. Photochem Photobiol 66: 105
57. Yariv A (1989) Quantum electronics, Chapter 17. Wiley, New York
58. Yeh KC, Kwan KC (1978) A comparison of numerical integrating algorithms by trapezoidal, LaGrange and spline approximation. J Pharmacokinet Biopharm 6: 79
59. Zaidi SI, Agarwal R, Eichler G, Rihter BD, Kenney ME, Mukhtar H (1993) 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
60. Zhao XJ, Lustigman S, Li YS, Kenney ME, Ben-Hur E (1997) Structure-function and mechanism studies on silicon phthalocyanines with *Plasmodium falciparum* in the dark and under red light. Photochem Photobiol 66: 282
61. Zhou JX, Hou X, Tsai SJ, Yang KX, Michel RG (1997) Characterization of a tunable optical parametric oscillator laser system for multielement flame laser excited atomic fluorescence spectrometry of cobalt, copper, lead, manganese, and thallium in buffalo river sediment. Anal Chem 69: 490
62. Zmudzka BZ, Strickland AG, Beer JZ, Ben-Hur E (1997) Photosensitized decontamination of blood with the silicon phthalocyanine Pc4: no activation of the human immunodeficiency virus promoter. Photochem Photobiol 65: 461