Phase I clinical trial of the use of zinc phthalocyanine tetrasulfonate as a photosensitizer for photodynamic therapy in dogs

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Objective—To determine the threshold for acute toxicosis of parenterally administered zinc phthalocyanine tetrasulfonate ($ZnPcS_4$), a candidate second-generation photosensitizer, in mice and evaluate the compound's safety in a phase I clinical trial of $ZnPcS_4$-based photodynamic therapy (PDT) in pet dogs with naturally occurring tumors.

Animals—Male Swiss-Webster mice and client-owned dogs with naturally occurring neoplasms.

Procedures—For the study of acute toxicosis, mice were given graded doses of $ZnPcS_4$. To determine safety, a rapid-titration phase I clinical trial of $ZnPcS_4$-based PDT in tumor-bearing dogs was conducted.

Results—In mice, administration of $\geq$ 100 mg of $ZnPcS_4$/kg resulted in renal tubular necrosis 24 hours after IP injection. In tumor-bearing dogs, $ZnPcS_4$ doses $\leq$ 4 mg/kg induced no signs of toxicosis and resulted in partial to complete tumor responses in 10 of 12 dogs 4 weeks after PDT. Tumor remission was observed with $ZnPcS_4$ doses as low as 0.25 mg/kg.

Conclusions and Clinical Relevance—A conservative starting dose of $ZnPcS_4$ was arrived at on the basis of mouse toxicosis findings. Zinc phthalocyanine tetrasulfonate–based PDT was tolerated well by all dogs and warrants further study. The identification of the maximum tolerated dose through traditional phase I clinical trials may be unnecessary for evaluating novel PDT protocols. (*Am J Vet Res* 2007;68:399–404)

Photodynamic therapy is a method of treatment for neoplasia that relies on light activation of a photosensitizing drug that preferentially accumulates within a tumor.[1] Cell death occurs when the photosensitizer, visible light of the appropriate wavelength, and molecular oxygen are present simultaneously. Therefore, PDT is a highly selective form of cancer treatment, compared with systemic chemotherapy or external beam radiotherapy.[1] In veterinary medicine, PDT has been used to treat a variety of neoplasms, most commonly squamous cell carcinoma.[2-13] Although clinical PDT is still considered an investigational cancer treatment in veterinary medicine, it is efficacious for most of the tumor types treated.[14,15]

For various reasons, including lack of a cost-effective, FDA-approved photosensitizer for animals and the expense and safety issues that attend use of laser systems, PDT is not widely used in veterinary

ABBREVIATIONS	
PDT	Photodynamic therapy
ALA	Aminolevulinic acid
$ZnPcS_4$	Zinc phthalocyanine tetrasulfonate

medicine. Many photosensitizers used in veterinary PDT have limitations. For example, porfimer sodium is not available in small vials appropriate for veterinary patients, making the cost prohibitive for most pet owners. Moreover, the prolonged cutaneous photosensitization associated with administration of porfimer sodium[16] creates practical difficulties in managing a photosensitive dog or cat for several weeks. The prophotosensitizer 5-ALA has been given to both dogs and cats and, upon metabolism, is potentially useful for selectively photosensitizing carcinomas. However, oral administration of ALA to dogs can cause acute vomiting and transient increases in serum liver-derived enzyme activity, whereas IV administration of ALA to healthy cats causes thrombocytopenia, anorexia, and hepatotoxicosis.[8,17] Aluminum phthalocyanine tetrasulfonate is an effective photosensitizer for PDT in cats,[12,18] but idiosyncratic hepatotoxicosis occurs.[19] Pheophorbide-a-hexylether is an apparently safe and efficacious photosensitizer for dogs and cats but is not commercially available.[7,9-11,20]

In considering photosensitizers for clinical use, several characteristics are important. The candidate

photosensitizer must efficiently elicit reactive oxygen species with wavelengths of light that are readily propagated through tissue (> 630 nm) and not cause systemic toxicosis or prolonged cutaneous photosensitization.[21] Cost is also an important consideration for veterinary applications. Zinc phthalocyanine tetrasulfonate, a candidate photosensitizer for veterinary PDT applications, has many desirable characteristics.[22-26] The compound is water-soluble and is activated by 675-nm wavelength light. The effectiveness of $ZnPcS_4$ in the clinical setting is predicted by in vitro and in vivo results, but toxicity data are lacking.[22,25,27-31] The purpose of the study reported here was to evaluate the acute toxic effects of parenterally administered $ZnPcS_4$ in mice as a starting point for a phase I clinical trial of $ZnPcS_4$-based PDT in pet dogs with naturally occurring tumors.

Materials and Methods

Photosensitizer preparation—Zinc phthalocyanine tetrasulfonate powder[a] was dissolved in saline (0.9% NaCl) solution to yield solutions with concentrations of 1, 10, and 25 mg/mL. The $ZnPcS_4$ solutions were sterilized by filtration[b] and placed in sterile vials. Vials were stored at –20°C until ready for use.

Mouse acute toxicosis study—After a 7-day period of acclimatization to the housing facilities, adult male Swiss-Webster mice weighing 25 to 30 g were given graded doses of $ZnPcS_4$ solution by IP injection. Control mice were given IP injections of sterile saline solution. Mice were injected in the morning and were observed hourly throughout the day. Mice that became or remained immobile when given a gentle stimulus or had labored breathing were euthanatized and necropsied. Twenty-four hours after $ZnPcS_4$ injection, mice were euthanatized via CO_2 asphyxiation and exsanguination. Blood was collected into heparinized syringes, and plasma was separated by centrifugation. Samples of heart, lungs, liver, kidneys, spleen, pancreas, and duodenum from each mouse were fixed in neutral-buffered 10% formalin and processed routinely for H&E staining and histologic evaluation. Plasma from each mouse was frozen for biochemical analysis. The Purdue Animal Care and Use Committee approved all animal studies.

Phase I clinical trial design—To assess the safety of $ZnPcS_4$ as a photosensitizer for PDT in dogs, an accelerated titration phase I study design was used.[32] In this study design, small cohorts (n = 1) are used initially for each drug dose. If no signs of toxicosis are observed, the dose is increased by 100% for the next cohort. This rapid dose escalation continues until 1 dog has signs of toxicosis, at which point the cohort is expanded to 3 dogs. If no further signs of toxicosis are observed, the dose escalation becomes 40% between cohorts and 3 dogs are entered into each subsequent cohort. If 1 of the 3 dogs has signs of toxicosis, the cohort is expanded to 6 dogs. If no other dogs within the cohort develop signs of toxicosis, the dose escalation continues. The study is terminated when 2 dogs within a cohort have dose-limiting signs of toxicosis. This study design typically identifies the maximum tolerated dose within the first 24 subjects.

Tumor-bearing dogs were eligible for entry into the phase I clinical trial if they had previously untreated localized disease with no evidence of metastasis (ie, $T_{is-3}N_0M_0$), had a histopathologic diagnosis, and were otherwise healthy and if the owners provided written informed consent. The owners were cautioned that dogs could be photosensitive for an unknown period of time after $ZnPcS_4$ injection and were advised to keep dogs out of direct sunlight for at least 7 days after treatment. The study was conducted with the approval of the Purdue Animal Care and Use Committee, and dogs were treated at the Purdue University Veterinary Teaching Hospital. Clinical staging of disease included physical examination; CBC; serum biochemical analyses; urinalysis; analysis of a lymph node aspirate; thoracic and abdominal radiography; abdominal ultrasonography; and computed tomography, when indicated by tumor type.

PDT protocol—Dogs that met the eligibility criteria for entry were hospitalized and given $ZnPcS_4$ (concentration, 1 mg/mL) as a slow IV bolus. After injection, they were kept in a low-light environment and monitored for adverse reactions attributable to the photosensitizer. Twenty-four hours after injection, dogs were anesthetized and tumors were irradiated with 675 ± 0.2-nm–wavelength light (100 mW/cm², 100 J/cm²) delivered from a diode-pumped, solid state[c]–pumped tunable dye laser[d] launched into a 400-μm-diameter quartz optical fiber terminating in a microlens or cylindrical diffuser.[e] Laser spectral emission was confirmed with a stacked photodiode spectrometer,[f] and laser power output was determined with a thermopile detector and digital power analyzer.[g]

The endpoints of the study were hematologic, biochemical, and clinical evidence of toxicosis (primary endpoint) and tumor response (secondary endpoint). Blood samples were collected from each dog immediately before and 24 and 48 hours after $ZnPcS_4$ administration and every 7 days thereafter for the following 3 weeks. Routine hematologic and serum biochemical analyses were performed at each time point. Dogs also underwent physical examination at each time point, and their owners were questioned about cutaneous photosensitivity and other potential signs of toxicosis. Any relevant hematologic or serum biochemical abnormality or clinical sign of toxicosis, such as vomiting, was sufficient to stop dose escalation and expand the cohort.

As a secondary endpoint for the study, tumor response was evaluated 4 weeks after PDT. Complete response was defined as resolution of all evidence of tumor, partial response was defined as $\geq$ 50% decrease in tumor volume, stable disease was defined as $<$ 50% change in tumor volume, and progressive disease was defined as $\geq$ 50% increase in tumor volume. Tumor response was assessed by use of 3-dimensional caliper measurements. Advanced imaging (ie, computed tomography) was used for tumors in locations not amenable to manual measurements (eg, intranasal tumors).

Results

Mouse acute toxicosis study—Three experiments were conducted to determine the minimum acute toxic

dose for mice. In the preliminary range–finding study, doses of ZnPcS$_4$ ≥ 250 mg/kg resulted in severe toxicosis and death within hours after administration. The ZnPcS$_4$ stained the skin and other soft tissues blue. In the second experiment, groups of 4 mice each received

doses of 0, 50, 100, 150, or 200 mg of ZnPcS$_4$/kg. All mice that received 0, 50, 100, or 150 mg of ZnPcS$_4$/kg survived for the 24-hour study period, whereas 1 mouse that received 200 mg of ZnPcS$_4$/kg died within the first 24 hours. The mice that received 0 to 100 mg of ZnPcS$_4$/kg had no clinical signs of toxicosis. The 4 mice that received 150 mg of ZnPcS$_4$/kg and the 3 surviving mice that received 200 mg/kg had clinical signs of toxicosis ranging from mild to severely decreased responsiveness at 24 hours. In the third and final acute toxicosis experiment, 2 groups of 3 mice received 150 or 200 mg of ZnPcS$_4$/kg. Two of the mice that received 150 mg/kg and all 3 mice that received 200 mg/kg died within the first 24 hours.

Blue discoloration of the serum precluded biochemical analyses. The tissue discoloration in specimens dissipated during histologic processing and was not evident in microscopic sections. Renal tubular lesions were histologically evident at doses of ZnPcS$_4$ ≥ 100 mg/kg (**Figure 1**). Patchy epithelial necrosis affected proximal tubules, especially in the outer portion of the medulla, and extended into the renal cortex. In more severely affected areas, necrosis extend-
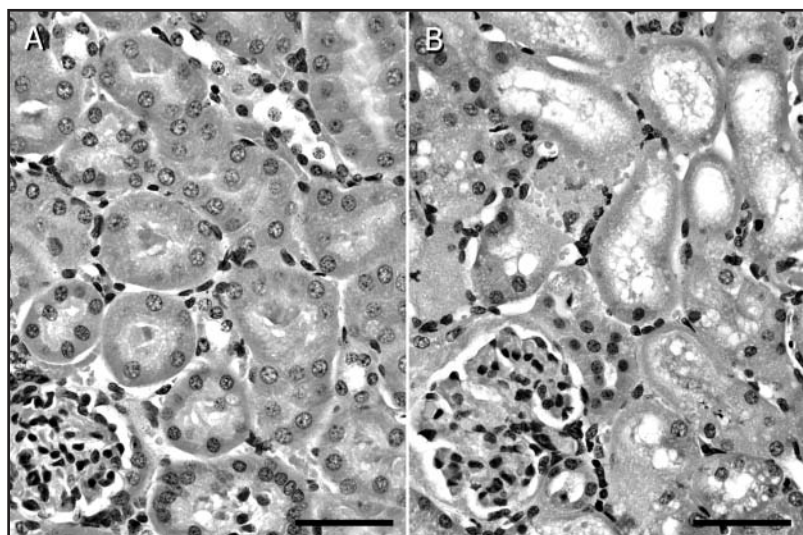

Figure 1—Photomicrograph of sections of kidneys from Swiss-Webster mice 24 hours after IP administration of saline (0.9% NaCl) solution or 100 mg of ZnPcS$_4$/kg. A—Histologically normal renal tissue from control mouse injected with saline solution. B—Renal tissue from mouse that received ZnPcS$_4$. Notice the coagulative necrotic changes involving the proximal renal tubular epithelial cells. H&E stain; bar = 50 µm.

Table 1—Summary of data from 12 tumor-bearing dogs in a phase I clinical trial of ZnPcS$_4$-based PDT.

Case No.	Breed	Age (y)	Sex	Tumor type	Tumor location	ZnPcS$_4$ dose (mg/kg)	Light treatment surface area (cm^2)	Tumor response
1	Golden Retriever	10	SF	Melanoma	Soft palate	0.25	2.0	CR
2	Golden Retriever	11	CM	Malignant fibrous histiocytoma	Subcutis, elbow	0.5	176.7	PR
3	Mix	12	SF	Squamous cell carcinoma	Pharynx, tonsil	1.0	12.6	PR
4	Labrador Retriever	13	CM	Spindle cell sarcoma	Subcutis, carpus	2.0	22.9	PR
5	Cocker Spaniel	12	CM	Mast cell tumor	Gingiva	4.0	18.8	PR
6	Mix	7	SF	Viral papilloma	Mouth, vulva	4.0	4.5, 3.0	PD
7	Mix	14	SF	Undifferentiated sarcoma	Intranasal	4.0	9.4	CR
8	Golden Retriever	12	M	Squamous cell carcinoma	Intranasal	4.0	9.4	CR
9	Golden Retriever	12	CM	Squamous cell carcinoma	Nasal plane	4.0	9.6	PR
10	Labrador Retriever	10	CM	Squamous cell carcinoma	Nasal plane	4.0	15.9	CR
11	Golden Retriever	14	CM	Squamous cell carcinoma	Nasal plane	4.0	12.6	PD
12	Boxer	4	CM	Squamous cell carcinoma	Cutaneous, multiple	4.0	11.5, 4.5, 12.6, and 0.9 × 4	CR, all lesions

SF = Spayed female. CM = Castrated male. M = Sexually intact male. CR = Complete response. PR = Partial response. PD = Progressive disease.
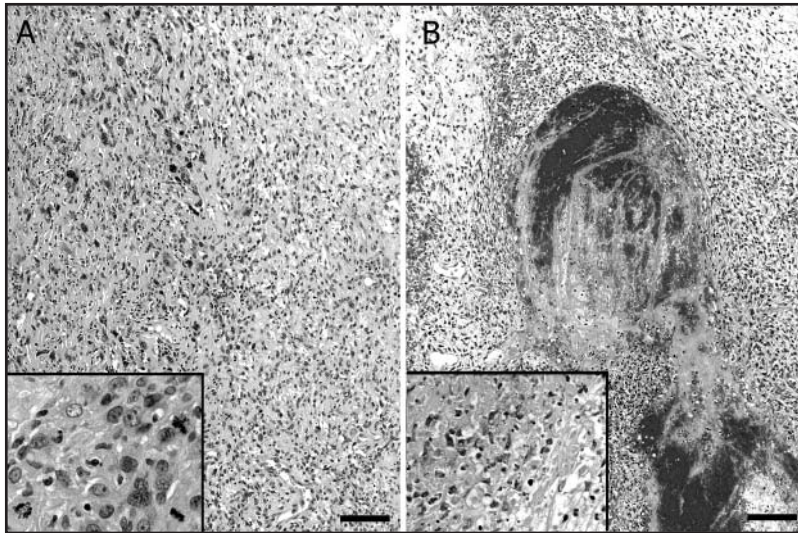
Figure 2—Photomicrographs of sections of subcutaneous malignant fibrous histiocytoma from a dog. A—Section of viable neoplastic tissue before PDT. H&E stain; bar = 200 μm. Inset: Detail of pleomorphic and mitotically active tumor cells. B—Section of neoplastic tissue 4 days after ZnPcS₄-based PDT. Notice the thrombosis and necrotic changes in neoplastic tissue. H&E stain; bar = 200 μm. Inset: Detail of necrotic tumor.

ed to surrounding renal parenchyma, including adjacent glomeruli. Aside from apoptosis in splenic follicles from mice with severe tubular nephrosis, histologic lesions were not detected in other tissues.

Phase I clinical trial in tumor-bearing dogs—The phase I clinical trial in tumor-bearing dogs was initiated at a dose of 0.25 mg of $ZnPcS_4$/kg, which represented 0.25% of the observed minimum toxic dose in mice. The calculated dose was administered IV as a slow bolus. Twelve dogs with various tumor types were enrolled in the study (**Table 1**). No adverse effects were observed during administration of the $ZnPcS_4$, and no owners reported photosensitivity or vomiting.

Serum biochemical values were within laboratory reference ranges for all dogs at all time points. Three dogs had hematologic changes after $ZnPcS_4$ administration. One dog that received 4 mg of $ZnPcS_4$/kg developed fever (rectal temperature, 39.8°C [103.6°F]) and neutrophilia (24,680 neutrophils/μL; reference range, 3,000 to 12,000 neutrophils/μL) 24 hours after drug administration but before PDT. This required no treatment and resolved 24 hours later; however, the cohort was expanded to 3 dogs. The second dog in the cohort developed mild neutropenia (2,160 cells/μL) 24 hours after administration of the photosensitizer. The third dog in the group had no signs of toxicosis, but to be conservative, this cohort was expanded to 8 dogs and the dose escalation was stopped at 4 mg of $ZnPcS_4$/kg. The urine of all dogs that received 2 or 4 mg of $ZnPcS_4$/kg had blue-green discoloration for several hours after drug administration. Swelling in and around the treatment area at 1 and 24 hours after treatment was minimal. Tissue within the treatment field darkened during the first 24 hours after PDT with eschar formation in the irradiated area.

The overall observed response rate (complete and partial remission) was 83% and included both mesenchymal and epithelial tumors. One dog that had malignant fibrous histiocytoma was treated with 0.5 mg of $ZnPcS_4$/kg, and because the tumor was 15 cm in its longest axis, it received a combination of interstitial and surface irradiation. Massive tumor necrosis occurred during the first week after PDT, requiring surgical debridement. Microscopic evaluation of this tissue confirmed extensive necrotic changes with hemorrhage and thrombosis (**Figure 2**).

Discussion

The results of the mouse acute toxicosis study indicated that $ZnPcS_4$ damages the kidney with a minimum acute toxic dose of approximately 100 mg/kg. Nephrotoxicosis likely results from renal excretion of this water-soluble drug, but we are unaware of studies of the biodistribution or pharmacokinetics of $ZnPcS_4$ in mice, dogs, or other species. The observation of blue-green urine discoloration in dogs given 2 and 4 mg of $ZnPcS_4$/kg suggests that renal filtration or excretion of the drug occurs, and the generalized blue tissue discoloration in $ZnPcS_4$-treated mice suggests that the drug is widely distributed throughout the body.

The traditional starting dose used in human phase I clinical trials is one tenth of the LD_{10} (ie, the dose that is lethal to 10% of animals) in the most sensitive animal species identified in toxicologic studies.[32] However, because the present study population was composed of client-owned dogs, safety was the chief concern. In the absence of LD_{10} data in mice, the phase I clinical trial in dogs was started at 0.25% of the observed minimum toxic dose in mice, a dose judged to be conservative. All dogs in the study tolerated the $ZnPcS_4$ administration. It is unclear whether the mild neutrophilia and fever observed in the dog that received 4 mg of $ZnPcS_4$/kg were related to potential contamination of the vial, subclinical preexisting illness, paraneoplastic syndrome, or toxicity of the drug. Similarly, the cause of the neutropenia observed in the dog with oral papillomatosis that received 4 mg of $ZnPcS_4$/kg is unknown. In all dogs, the hematologic changes were transient and unassociated with any clinical signs. The minimal degree of posttreatment swelling observed in these dogs was different than findings reported with pheophorbide-a-hexylether–based PDT in cats with facial squamous cell carcinoma,[9] in which edema extending beyond the treatment site resulted in nasal obstruction, stertor, and dyspnea for up to 5 days after treatment. Changes in those cats may have reflected photosensitizer or species differences in post-PDT responses.

Although $ZnPcS_4$ doses ≤ 4 mg/kg appeared to be tolerated well, dose escalation was discontinued before the maximum tolerated dose was reached because tumor responses were observed at each photosensitizer dose tested, including the lowest dose. Furthermore, there was a concern that high tissue concentrations of photosensitizing drugs may interfere with the efficacy of PDT.[33,34] This phenomenon is thought to occur because at high tissue concentrations, the photosensitizer quickly absorbs the incident light, thereby decreas-

ing the depth of penetration into the target tissue. Although determination of the maximum tolerated dose may be a requisite for approval by regulatory agencies, photosensitizer dose and antitumor effect may not have a positive linear relationship; therefore, clinical trials designed to determine the maximum effective biological dose may be more clinically relevant.

Treatment efficacy is not the primary endpoint of a phase I clinical trial. However, $ZnPcS_4$-based PDT resulted in partial or complete remission of both epithelial and mesenchymal tumors in 10 of 12 dogs at 4 weeks after a single treatment. Biopsy of the treated areas in dogs with complete remission would have been useful to determine whether $ZnPcS_4$-based PDT had successfully ablated all neoplastic cells. Because this was a short-term safety study in dogs, follow-up intervals beyond the 4 weeks varied substantially. However, long-term follow-up was available for 2 dogs with complete responses; 1 dog with oral melanoma and 1 dog with cutaneous squamous cell carcinoma were free of disease 1 year after PDT without any subsequent treatment. None of the dogs was retreated with $ZnPcS_4$-based PDT.

Results of the present study suggest that $ZnPcS_4$-based PDT may be effective against many tumors in dogs and may provide long-term local disease control. Given the absence of published data in veterinary medicine about $ZnPcS_4$-based PDT, further investigation in dogs is warranted to determine its clinical usefulness. Carefully designed phase II clinical trials will be necessary to determine which tumor types are best treated with $ZnPcS_4$-based PDT and may identify prognostic factors that can help predict outcome. Knowledge of the pharmacokinetics and biodistribution of $ZnPcS_4$ in dogs will be useful in optimizing PDT protocols. Importantly, idiosyncratic reactions, such as those reported with aluminum phthalocyanine tetrasulfonate,[19] may not be detected until more animals are treated.

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a. Frontier Scientific, Logan, Utah.
b. 0.22-μm pore, Whatman Inc, Florham Park, NJ.
c. Verdi 10-W laser, Coherent Laser Group, Santa Clara, Calif.
d. 599 tunable dye laser, Coherent Laser Group, Santa Clara, Calif.
e. Pioneer Optics, Windsor Lock, Conn.
f. WaveMate, Coherent Laser Group, Santa Clara, Calif.
g. FieldMaster GS, Coherent Laser Group, Santa Clara, Calif.

References

1. Brown SB, Brown EA, Walker I. The present and future role of photodynamic therapy in cancer treatment. *Lancet Oncol* 2004;5:497–508.
2. Cheli R, Addis F, Mortellaro CM, et al. Photodynamic therapy of spontaneous animal tumors using the active component of hematoporphyrin derivative (DHE) as photosensitizing drug: clinical results. *Cancer Lett* 1987;38:101–105.
3. Frimberger AE, Moore AS, Cincotta L, et al. Photodynamic therapy of naturally occurring tumors in animals using a novel benzophenothiazine photosensitizer. *Clin Cancer Res* 1998;4:2207–2218.
4. Gloi AM, Beck E. Evaluation of porfimer sodium in dogs and cats with spontaneous tumors. *Vet Ther* 2004;5:26–33.
5. Jacobs TM, Rosen GM. Photodynamic therapy as a treatment for esophageal squamous cell carcinoma in a dog. *J Am Anim Hosp Assoc* 2000;36:257–261.
6. Lucroy MD, Bowles MH, Higbee RG, et al. Photodynamic therapy for prostatic carcinoma in a dog. *J Vet Intern Med* 2003;17:235–237.
7. Lucroy MD, Long KR, Blaik MA, et al. Photodynamic therapy for the treatment of intranasal tumors in 3 dogs and 1 cat. *J Vet Intern Med* 2003;17:727–729.
8. Lucroy MD, Ridgway TD, Peavy GM, et al. Preclinical evaluation of 5-aminolevulinic acid-based photodynamic therapy for canine transitional cell carcinoma. *Vet Comp Oncol* 2003;1:76–85.
9. Magne ML, Rodriguez CO, Autry SA, et al. Photodynamic therapy of facial squamous cell carcinoma in cats using a new photosensitizer. *Lasers Surg Med* 1997;20:202–209.
10. McCaw DL, Payne JT, Pope ER, et al. Treatment of canine hemangiopericytomas with photodynamic therapy. *Lasers Surg Med* 2001;29:23–26.
11. McCaw DL, Pope ER, Payne JT, et al. Treatment of canine oral squamous cell carcinomas with photodynamic therapy. *Br J Cancer* 2000;82:1297–1299.
12. Peaston AE, Leach MW, Higgins RJ. Photodynamic therapy for nasal and aural squamous cell carcinoma in cats. *J Am Vet Med Assoc* 1993;202:1261–1265.
13. Roberts WG, Klein MK, Loomis M, et al. Photodynamic therapy of spontaneous cancers in felines, canines, and snakes with chloro-aluminum sulfonated phthalocyanine. *J Natl Cancer Inst* 1991;83:18–23.
14. Lucroy MD, Edwards BF, Madewell BR. Veterinary photodynamic therapy. *J Am Vet Med Assoc* 2000;216:1745–1751.
15. Lucroy MD, Magne ML, Peavy GM, et al. Photodynamic therapy in veterinary medicine: current status and implications for applications in human disease. *J Clin Laser Med Surg* 1996;14:305–310.
16. Wolfsen HC, Ng CS. Cutaneous consequences of photodynamic therapy. *Cutis* 2002;69:140–142.
17. Lucroy MD, Edwards BF, Peavy GM, et al. Preclinical study in cats of the pro-photosensitizer 5-aminolevulinic acid. *Am J Vet Res* 1999;60:1364–1370.
18. Hahn KA, Panjehpour M, Legendre AM. Photodynamic therapy response in cats with cutaneous squamous cell carcinoma as a function of fluence. *Vet Dermatol* 1998;9:3–7.
19. Leach MW, Peaston AE. Adverse drug reaction attributable to aluminum phthalocyanine tetrasulphonate administration in domestic cats. *Vet Pathol* 1994;31:283–287.
20. Reeds KB, Ridgway TD, Higbee RG, et al. Non-coherent light for photodynamic therapy of superficial tumours in animals. *Vet Comp Oncol* 2004;2:157–163.
21. Lucroy MD. Photodynamic therapy for companion animals with cancer. *Vet Clin North Am Small Anim Pract* 2002;32:693–702, viii.
22. Fingar VH, Wieman TJ, Karavolos PS, et al. The effects of photodynamic therapy using differently substituted zinc phthalocyanines on vessel constriction, vessel leakage and tumor response. *Photochem Photobiol* 1993;58:251–258.
23. Howe L, Zhang JZ. The effect of biological substrates on the ultrafast excited-state dynamics of zinc phthalocyanine tetrasulfonate in solution. *Photochem Photobiol* 1998;67:90–96.
24. Margaron P, Gregoire MJ, Scasnar V, et al. Structure-photodynamic activity relationships of a series of 4-substituted zinc phthalocyanines. *Photochem Photobiol* 1996;63:217–223.
25. Reddi E, Lo Castro G, Biolo R, et al. Pharmacokinetic studies with zinc(II)-phthalocyanine in tumour-bearing mice. *Br J Cancer* 1987;56:597–600.
26. Spikes JD, Bommer JC. Zinc tetrasulphophthalocyanine as a photodynamic sensitizer for biomolecules. *Int J Radiat Biol Relat Stud Phys Chem Med* 1986;50:41–45.
27. Fabris C, Valduga G, Miotto G, et al. Photosensitization with zinc (II) phthalocyanine as a switch in the decision between apoptosis and necrosis. *Cancer Res* 2001;61:7495–7500.
28. Haywood-Small SL, Vernon DI, Griffiths J, et al. Phthalocyanine-mediated photodynamic therapy induces cell death and a G0/G1 cell cycle arrest in cervical cancer cells. *Biochem Biophys Res Commun* 2006;339:569–576.
29. Shopova M, Mantareva V, Krastev K, et al. Comparative pharmacokinetic and photodynamic studies with zinc(II) phthalocyanine in hamsters bearing an induced or transplanted rhabdomyosarcoma. *J Photochem Photobiol B* 1992;16:83–89.
30. van Leengoed HL, van der Veen N, Versteeg AA, et al. In vivo photodynamic effects of phthalocyanines in a skin-fold observation chamber model: role of central metal ion and degree of sulfonation. *Photochem Photobiol* 1993;58:575–580.

31. Villanueva A, Dominguez V, Polo S, et al. Photokilling mechanisms induced by zinc(II)-phthalocyanine on cultured tumor cells. *Oncol Res* 1999;11:447–453.
32. Simon R, Freidlin B, Rubinstein L, et al. Accelerated titration designs for phase I clinical trials in oncology. *J Natl Cancer Inst* 1997;89:1138–1147.
33. Bown SG, Tralau CJ, Smith PD, et al. Photodynamic therapy with porphyrin and phthalocyanine sensitisation: quantitative studies in normal rat liver. *Br J Cancer* 1986;54:43–52.
34. Wilson BC, Patterson MS, Burns DM. Effect of photosensitizer concentration in tissue on the penetration depth of photoactivating light. *Lasers Med Sci* 1986;1:235–244.

Correction: Epidemiologic cutoff values for antimicrobial agents against *Aeromonas salmonicida* isolates determined by frequency distributions of minimal inhibitory concentration and diameter of zone of inhibition data

In the report, "Epidemiologic cutoff values for antimicrobial agents against *Aeromonas salmonicida* isolates determined by frequency distributions of minimal inhibitory concentration and diameter of zone of inhibition data" (AJVR 2006;67:1837–1843), Figure 2D should appear as follows:

MIC (μg/mL)	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	> 61
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Diameter of zone of inhibition (mm)

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