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 investigational 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 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 be effective in the treatment of the disease for which it is intended, safe, easy to administer and applicable, andof a dosing profile that can be achieved in a reasonable time period.

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

Antonella Borgatti-Jeffreys, DVM, MS; Stephen B. Hooser, DVM, PhD; Margaret A. Miller, DVM, PhD; Michael D. Lucroy, DVM, MS

**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 ≥ 100 mg of ZnPcS\(_4\)/kg resulted in renal tubular necrosis 24 hours after IP injection. In tumor-bearing dogs, ZnPcS\(_4\) doses ≤ 4 mg/kg induced no signs of toxicity 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)

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>PDT</th>
<th>Photodynamic therapy</th>
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</thead>
<tbody>
<tr>
<td>ALA</td>
<td>Aminolevulinic acid</td>
</tr>
<tr>
<td>ZnPcS(_4)</td>
<td>Zinc phthalocyanine tetrasulfonate</td>
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</tbody>
</table>
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. Cost is also an important consideration for veterinary applications. Zinc phthalocyanine tetrasulfonate, a candidate photosensitizer for veterinary PDT applications, has many desirable characteristics. The compound is water-soluble and is activated by 675-nm wavelength light. The effectiveness of ZnPcS₄ in the clinical setting is predicted in vitro and in vivo results, but toxicity data are lacking. The purpose of the study reported here was to evaluate the acute toxic effects of parenterally administered ZnPcS₄ in mice as a starting point for a phase I clinical trial of ZnPcS₄-based PDT in pet dogs with naturally occurring tumors.

Materials and Methods

Photosensitizer preparation—Zinc phthalocyanine tetrasulfonate powder was dissolved in saline (0.9% NaCl) solution to yield solutions with concentrations of 1, 10, and 25 mg/mL. The ZnPcS₄ solutions were sterilized by filtration 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 ZnPcS₄ 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 ZnPcS₄ injection, mice were euthanatized via CO₂ 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 ZnPcS₄ as a photosensitizer for PDT in dogs, an accelerated titration phase I study design was used. 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, TₐN₀M₀), 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 ZnPcS₄ 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 ZnPcS₄ (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 tumor were irradiated with 675 ± 0.2-nm–wavelength light (100 mW/cm², 100 J/cm²) delivered from a diode–pumped, solid state–pumped tunable dye laser launched into a 400-µm-diameter quartz optical fiber terminating in a microlens or cylindrical diffuser. Laser spectral emission was confirmed with a stacked photodiode spectrometer, and laser power output was determined with a thermopile detector and digital power analyzer.

The endpoints of the study were hematochemical, 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 ZnPcS₄ administration and every 7 days thereafter for the following 1 week. 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 ≥ 50% decrease in tumor volume, stable disease was defined as < 50% change in tumor volume, and progressive disease was defined as ≥ 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 ZnPcS₄ ≥ 250 mg/kg resulted in severe toxicosis and death within hours after administration. The ZnPcS₄ 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 ZnPcS₄/kg. All mice that received 0, 50, 100, or 150 mg of ZnPcS₄/kg survived for the 24-hour study period, whereas 1 mouse that received 200 mg of ZnPcS₄/kg died within the first 24 hours. The mice that received 0 to 100 mg of ZnPcS₄/kg had no clinical signs of toxicosis. The 4 mice that received 150 mg of ZnPcS₄/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 ZnPcS₄/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 ZnPcS₄ ≥ 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 ZnPcS₄/kg. A—Histologically normal renal tissue from control mouse injected with saline solution. B—Renal tissue from mouse that received ZnPcS₄. Notice the coagulative necrotic changes involving the proximal renal tubular epithelial cells. H&E stain; bar = 50 µm.](image)

**Table 1**—Summary of data from 12 tumor-bearing dogs in a phase I clinical trial of ZnPcS₄-based PDT.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Breed</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Tumor type</th>
<th>Tumor location</th>
<th>ZnPcS₄ dose (mg/kg)</th>
<th>Light treatment surface area (cm²)</th>
<th>Tumor response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Golden Retriever</td>
<td>10</td>
<td>SF</td>
<td>Melanoma</td>
<td>Soft palate</td>
<td>0.25</td>
<td>2.0</td>
<td>CR</td>
</tr>
<tr>
<td>2</td>
<td>Golden Retriever</td>
<td>11</td>
<td>CM</td>
<td>Malignant fibrous histiocyoma</td>
<td>Subcutis, elbow</td>
<td>0.5</td>
<td>176.7</td>
<td>PR</td>
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<tr>
<td>3</td>
<td>Mix</td>
<td>12</td>
<td>SF</td>
<td>Squamous cell carcinoma</td>
<td>Pharynx, tonsil</td>
<td>1.0</td>
<td>12.6</td>
<td>PR</td>
</tr>
<tr>
<td>4</td>
<td>Labrador Retriever</td>
<td>13</td>
<td>CM</td>
<td>Spindle cell sarcoma</td>
<td>Subcutis, carpus</td>
<td>2.0</td>
<td>22.9</td>
<td>PR</td>
</tr>
<tr>
<td>5</td>
<td>Cocker Spaniel</td>
<td>12</td>
<td>CM</td>
<td>Mast cell tumor</td>
<td>Gingiva</td>
<td>4.0</td>
<td>18.8</td>
<td>PR</td>
</tr>
<tr>
<td>6</td>
<td>Mix</td>
<td>7</td>
<td>SF</td>
<td>Viral papilloma</td>
<td>Mouth, vulva</td>
<td>4.0</td>
<td>4.5, 3.0</td>
<td>PD</td>
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<tr>
<td>7</td>
<td>Mix</td>
<td>14</td>
<td>SF</td>
<td>Undifferentiated sarcoma</td>
<td>Intranasal</td>
<td>4.0</td>
<td>9.4</td>
<td>CR</td>
</tr>
<tr>
<td>8</td>
<td>Golden Retriever</td>
<td>12</td>
<td>M</td>
<td>Squamous cell carcinoma</td>
<td>Intranasal</td>
<td>4.0</td>
<td>9.4</td>
<td>CR</td>
</tr>
<tr>
<td>9</td>
<td>Golden Retriever</td>
<td>12</td>
<td>CM</td>
<td>Squamous cell carcinoma</td>
<td>Nasal plane</td>
<td>4.0</td>
<td>9.6</td>
<td>PR</td>
</tr>
<tr>
<td>10</td>
<td>Labrador Retriever</td>
<td>10</td>
<td>CM</td>
<td>Squamous cell carcinoma</td>
<td>Nasal plane</td>
<td>4.0</td>
<td>15.9</td>
<td>CR</td>
</tr>
<tr>
<td>11</td>
<td>Golden Retriever</td>
<td>14</td>
<td>CM</td>
<td>Squamous cell carcinoma</td>
<td>Nasal plane</td>
<td>4.0</td>
<td>12.6</td>
<td>PD</td>
</tr>
<tr>
<td>12</td>
<td>Boxer</td>
<td>4</td>
<td>CM</td>
<td>Squamous cell carcinoma</td>
<td>Cutaneous, multiple</td>
<td>4.0</td>
<td>11.5, 4.5, 12.6, and 0.9 × 4</td>
<td>CR, all lesions</td>
</tr>
</tbody>
</table>

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 1 clinical trial in tumor-bearing dogs**—The phase 1 clinical trial in tumor-bearing dogs was initiated at a dose of 0.25 mg of ZnPcS₄/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 ZnPcS₄, 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 hemolytic changes after ZnPcS₄ administration. One dog that received 4 mg of ZnPcS₄/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 ZnPcS₄/kg. The urine of all dogs that received 2 or 4 mg of ZnPcS₄/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 ZnPcS₄/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 ZnPcS₄ 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 ZnPcS₄ in mice, dogs, or other species. The observation of blue-green urine discoloration in dogs given 2 and 4 mg of ZnPcS₄/kg suggests that renal filtration or excretion of the drug occurs, and the generalized blue tissue discoloration in ZnPcS₄-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 LD₅₀ (ie, the dose that is lethal to 10% of animals) in the most sensitive animal species identified in toxicologic studies. However, because the present study population was composed of client-owned dogs, safety was the chief concern. In the absence of LD₅₀ data in mice, the phase 1 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 ZnPcS₄ administration. It is unclear whether the mild neutrophilia and fever observed in the dog that received 4 mg of ZnPcS₄/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 ZnPcS₄/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, 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 ZnPcS₄ 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. This phenomenon is thought to occur because at high tissue concentrations, the photosensitizer quickly absorbs the incident light, thereby decreases...
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, ZnPcS₅-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 ZnPcS₅-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 ZnPcS₅-based PDT.

Results of the present study suggest that ZnPcS₅-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 ZnPcS₅-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 ZnPcS₅-based PDT and may identify prognostic factors that can help predict outcome. Knowledge of the pharmacokinetics and biodistribution of ZnPcS₅ 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.

References

4. 22-µm pore, Whatman Inc, Florham Park, NJ.
5. 699 tunable dye laser, Coherent Laser Group, Santa Clara, Calif.
7. WaveMate, Coherent Laser Group, Santa Clara, Calif.
10. 0.22-µm pore, Whatman Inc, Florham Park, NJ.
14. WaveMate, Coherent Laser Group, Santa Clara, Calif.
15. FieldMaster GS, Coherent Laser Group, Santa Clara, Calif.

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:

![Diagram](image-url)