Photodynamic Therapy of Naturally Occurring Tumors in Animals Using a Novel Benzophenothiazine Photosensitizer

Angela E. Frimberger, Antony S. Moore, Louis Cincotta, Susan M. Cotter, and James W. Foley

The Harrington Oncology Program, Tufts University School of Veterinary Medicine, North Grafton, Massachusetts 01536 [A. E. F., A. S. M., S. M. C.]; The University of Massachusetts Cancer Center, Worcester, Massachusetts 01605 [A. E. F.]; and The Rowland Institute for Science, Cambridge, Massachusetts 02142 [L. C., J. W. F.]

ABSTRACT

5-Ethylamino-9-diethyaminobenz[α]phenothiazinium chloride (EtNBS) is a novel photodynamic therapy (PDT) photosensitizer with efficacy against experimental murine tumors. In this preliminary study, dogs and cats with naturally occurring tumors were treated with EtNBS-PDT to determine safety and efficacy. Fifteen treatments were performed on 13 animals (9 treatments in 8 cats and 6 treatments in 5 dogs), generally using 400 J of 652 nm light. Two feline sublingual squamous cell carcinomas (SCCs) responded briefly (minor response). Six feline facial SCCs were treated, resulting in two partial responses and four long-term complete responses (CR). Two canine intraoral SCCs were treated; one responded minimally for 2 weeks (minor response), and one achieved long-term CR. One canine cutaneous mast cell tumor achieved CR, and one canine ocular mast cell tumor responded briefly. One canine ocular melanoma did not respond to treatment. Systemic reactions included nausea associated with photosensitizer injection in two cats and two dogs, elevated body temperatures during treatment in two dogs, elevated body temperature 2 days after PDT in one cat, and inappetance for 2 weeks in one cat. A peripheral neuropathy of undetermined cause occurred in one cat 2 weeks after PDT and resolved without treatment. Local reaction was well tolerated in 13 of 15 treatments. All animals were exposed to normal daylight after less than 5 days (mean, 3.5 days) without residual photosensitization. EtNBS-PDT is safe for dogs and cats and has activity against selected naturally occurring tumors, with an overall objective response rate (partial response + CR) of 61.5%.

INTRODUCTION

PDT is a local antineoplastic therapy that is based on the interaction of a photosensitizing drug with light. The concept of photodynamic therapy is not new; however, its utility has been greatly expanded in recent years because of technical developments, chiefly in the area of light delivery systems such as lasers and fiberoptics (1–4). Compared with ionizing radiation therapy, PDT is generally safer for the surrounding normal tissues because photosensitizers ideally are preferentially accumulated in tumor cells. It also requires fewer treatments, often only one, but because of the sparing of the normal tissues, it can be safely repeated if needed. The photosensitizer is usually given systemically and should be inert unless activated by light of the appropriate wavelength. Upon activation, the photosensitizer can undergo type I reaction in which the excited photosensitizer reacts directly with substrates to produce free radicals. Alternatively, the excited photosensitizer can react with molecular oxygen to generate cytotoxic reactive oxygen species, primarily singlet oxygen (1^O_2), in the type II reaction (5). As with ionizing radiation, tumor bulk (6) and hypoxia (7) are important limiting factors for PDT. Because molecular oxygen is not only required for, but consumed by, the photodynamic reaction, tissue oxygenation is critical for a photodynamic effect (8). Tumor bulk limits efficacy because it contributes to tissue hypoxia, and because light penetration is only 5–10 mm, even at the best wavelengths. The wavelengths of light that penetrate tissue best are the red to infrared wavelengths, 600–900 nm (the “therapeutic window”); therefore, an ideal photosensitizer would be excited by a wavelength in this range.

The best characterized photosensitizers, Photofrin and hematoporphyrin derivative, are limited by a relatively inefficient absorption band in the therapeutic window, and more importantly, by prolonged cutaneous photosensitization (1–3). Furthermore, the mechanism of action of these photosensitizers appears to rely more on damage to the tumor vasculature, which would increase both hypoxia (and thus resistance to ionizing radiation and further PDT) and risk of tumor recurrence when the vasculature is repaired through angiogenesis, than on direct photocytotoxicity (2–3). To address these problems, a number of second generation photosensitizers have been developed, including benzoporphyrins, chlorins, purpurins, naphtha-

The abbreviations used are: PDT, photodynamic therapy; EtNBS, 5-ethylamino-9-diethyaminobenz[α]phenothiazinium chloride; CR, complete response; PR, partial response; MR, minor response; SCC, squamous cell carcinoma; MCT, mast cell tumor; ALT, alanine aminotransferase.

Received 5/5/98; revised 6/23/98; accepted 6/24/98.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Funding for this research was provided by The Rowland Foundation and The Rowland Institute for Science. Partial salary support for A. E. F. was provided by the University of Massachusetts Cancer Center, NIH Grants P01 HL56920-01 and DK 94-05, NASA Grant NRA-94-OLMSA-02, and the Our Danny Cancer Fund.

2 To whom requests for reprints should be addressed, at University of Massachusetts Cancer Center, Two Biotech, Suite 202, 373 Plantation Street, Worcester, MA 01605. Phone: (508) 856-1316; Fax: (508) 856-1310; E-mail: Angela.Frimberger@banyan.ummed.edu.

3 The abbreviations used are: PDT, photodynamic therapy; EtNBS, 5-ethylamino-9-diethyaminobenz[α]phenothiazinium chloride; CR, complete response; PR, partial response; MR, minor response; SCC, squamous cell carcinoma; MCT, mast cell tumor; ALT, alanine aminotransferase.
locyanines, and phthalocyanines (9). Despite the fact that drugs derived from dissimilar families of dyes would be expected to have different efficacy and toxicity profiles, and therefore to complement one another more effectively, the vast majority of photosensitizers that have been developed are porphyrin based (9).

EtNBS (Fig. 1) is a novel, nonporphyrin, benzophenoxazine-derived photosensitizer that is chemically unrelated to other photosensitizers. Photodynamically inactive benzophenoxazine dyes were originally investigated in the 1940s as chemotherapeutic agents, and although they were not found to possess long-term antitumor efficacy, they did exhibit the property of tumor cell localization (10–12). Because of this property, photoactive red-absorbing derivatives of the benzophenoxazines have been synthesized in recent years and studied for their potential as PDT photosensitizers (13–18). On the basis of empirical findings, EtNBS was selected for further development. Those preliminary studies demonstrated its safety and tissue distribution in mice, as well as its efficacy and optimal light dosimetry for the treatment of RIF and EMT-6 tumor cell lines, both in vitro and as solid tumors in mice (17, 18). No disadvantages relative to other photosensitizers were identified. The therapeutic margin is wide because of a tumor: normal cell distribution ratio of approximately 3.5:1, perhaps augmented by relatively increased deactivation of the drug in normal skin by NADH enzymes (17). The absorption spectrum peaks at 652 nm (extinction coefficient (ε), 68,600 /cm · M), the photocytotoxic reaction spares the vasculature, the lipophilicity of the drug is good enough to allow excellent tissue distribution but not strong enough to prevent easy aqueous solution, and the drug is taken up by tumor tissues rapidly (3 h after s.c. administration in mice), and cleared from all tissues of mice within 24 h (17, 18).

The encouraging results of those studies led to this preliminary, preclinical trial of EtNBS-PDT in dogs and cats with naturally occurring tumors. Dogs and cats develop spontaneous tumors that are similar to those in humans, with characteristics suitable for PDT. The use of pet animals with naturally occurring tumors as animal models for human disease has become increasingly accepted in recent years (19–22). Specifically, PDT trials of other photosensitizers have been carried out using spontaneous animal tumor models (23–30). The goals of this trial were to determine the tolerance of dogs and cats to EtNBS-PDT and to determine the efficacy of this treatment in naturally occurring tumors.

SUBJECTS AND METHODS

Animals. Thirteen pet animals with naturally occurring tumors that were presented to the Harrington Oncology Program at Tufts University School of Veterinary Medicine were treated. The tumors were required to be superficial for ease of treatment and re-evaluation and to have a low expected metastatic rate. Each tumor was diagnosed (and for the mast cell tumors, histological grades were assigned) by biopsy and histopathology, except the intraocular melanoma, which was diagnosed by an ophthalmologist based on its appearance. Each was assigned a clinical stage according to established WHO criteria (31) by physical examination, lymph node palpation and fine needle aspiration cytology where indicated, thoracic radiographs, and in the case of the mast cell tumors, abdominal radiography, bone marrow aspirate, and/or bone marrow aspiration cytology. Primary tumor measurements were taken directly using calipers except in cat 3, where the tumor thickness was determined by computed tomography scan, and in dog 5, where the tumor depth was determined by oculary ultrasonography. Animals with metastases were excluded from treatment. In addition, the animals were required to be otherwise healthy, determined by general clinical examination, complete blood cell count, serum chemistry profile, and other tests as indicated by the individual clinical situation. The animals were treated as described below and hospitalized under standard conditions in the clinical wards of the Foster Hospital for Small Animals at Tufts University School of Veterinary Medicine until discharged. Thereafter, they were housed and cared for by their owners.

Drug Source. EtNBS was synthesized, purified by column chromatography, and shown to be homogeneous by TLC and nuclear magnetic resonance spectroscopy at the Rowland Institute for Science.

Treatment. Each animal was examined physically and tested as described above. Animals were admitted either the night before or the morning of the procedure to the Foster Hospital. Animals were not fed the morning of the procedure, as routine preparation for general anesthesia. Prior to treatment, a blood sample was drawn for evaluation of methemoglobin and Heinz body levels, and an indwelling i.v. catheter was placed.

EtNBS was administered i.v. in a solution of 1.25 mg/ml in 5% dextrose in water as a constant rate infusion for a period of 25–30 min. The dose of EtNBS used was either 2.5 or 2.0 mg/kg of body weight, except for the first animal (cat 1), which received 5.0 mg/kg (see “Results”).

Three h after the infusion, animals were anesthetized under dim lighting and positioned for photoirradiation. Anesthesia for cats consisted of induction with a combination of ketamine and diazepam administered i.v. and maintenance with isoflurane administered via endotracheal tube, except for 1 cat (cat 6), which received preanesthesia butorphanol i.m., which was induced with i.v. propofol, and maintained with isoflurane. Another cat (cat 7) also received preanesthetic buprenorphine i.m. For dogs, anesthesia consisted of a preanesthetic combination of butorphanol, acepromazine, and glycopyrrolate administered i.m., induction with i.v. pentothal, and maintenance with halothane administered via endotracheal tube, except for dogs 2 and 5, which were maintained with isoflurane.

Photoirradiation was performed using a diode laser (Applied Optronics Corp., South Plainfield, NJ) producing a wavelength of 652 ± 3 nm wavelength unless otherwise noted (see “Results”) and a 400-μm fiberoptic fitted with a micro lens (PDT Systems, Santa Barbara, CA). Because of availability of
lasers, the first animal (cat 1) was treated with light of 670 ± 5 nm wavelength, and the remaining animals were treated with light of 652 nm wavelength. Because 670 nm is not optimal for activation of EtNBS, cat 1 was treated using the same fluence rate but double the photodestruction time (66 min) as subsequent animals. In general, a fluence rate of 200 mW/cm² was used for 33 min, for a total light dosage of 400 J/cm², except where noted (see above and “Results”). Wherever geometrically possible, the beam was directed perpendicular to the surface of the tumor. The beam was centered at the midpoint of the widest part of the tumor, with the radius of the beam extending at least 1 cm beyond the visible extent of tumor. Local temperature measurements were not taken during photodestruction because data obtained in murine systems indicated that thermal effects were not responsible for the antitumor effect of EtNBS-PDT (18), and we assumed the same would be true for these tumors.

From the start of EtNBS infusion, the animals were considered photosensitive and were protected from bright light until evidence was seen in the urine or stool that the photosensitizer had been excreted, or for 5 days, whichever occurred first. Thereafter, the animals were exposed to full normal light and released from the hospital. Rechecks were performed at least weekly for the first month after PDT, biweekly for the second month, every 2 months for the first 6 months, and every 6 months thereafter. Complete blood cell counts and serum chemistry panels were repeated 1 and 2 months after the treatment.

**Toxicity and Response.** At each re-examination, particular attention was paid to the owner’s description of the animal’s appetite, energy, and subjective well-being. A complete physical examination was performed, and tumors were re-evaluated at each visit (except for one intraocular tumor, which was re-evaluated by ultrasonography only at 8 weeks after PDT). Because many of the treated tumors were plaque-like, they were assessed in terms of area (the product of the largest diameter and the diameter perpendicular to it, in cm²) rather than volume. The criteria for tumor response were as follows: CR, absence of detectable tumor after healing of treatment field; PR, greater than 50% reduction in tumor; MR or brief response, photodynamic response that resulted in tumor reduction of <50%, or one that could not clearly be determined to be >50% (the initial tumor was too small to accurately measure or was postoperative tumor bed), or a response that did not last beyond healing of the treatment field; no response, no change in the tumor; or progressive disease. The criteria for local normal tissue toxicity were as follows: grade 0, normal or localized alopecia only; grade 1, erythema or edema; grade 2, skin or mucous membrane erosion or corneal edema; and grade 3, full-thickness skin or mucous membrane ulceration or corneal erosion or ulcer. Any changes in serum chemistry or hematological parameters compared with pretreatment levels and any other adverse changes in the animal’s condition were noted.

**RESULTS**

Nine treatments were performed in eight cats, and six treatments were performed in five dogs (Table 1). Six cats were treated for facial SCC (one treated twice), and two cats were treated for sublingual SCC. Two dogs were treated for intraoral SCC (one treated twice), one dog for grade II cutaneous MCT of the eyelid, one for grade II MCT of the bulbar conjunctiva, and one for pigmented intraocular limbal melanoma. The cats ranged in age from 6 to 16 years (median, 14 years), and the dogs ranged in age from 2 to 12 years (median, 9.5 years). Of the cats, six were castrated males and two were spayed females (including the one treated twice). Of the dogs, two were castrated males (including the one treated twice), and three were spayed females. In addition to the testing described above, a computed tomography scan of the nasal planum lesion in one cat (cat 3) showed that its thickness was <4 mm, and ocular ultrasonography of one dog’s (dog 5) tumor showed that its dimension was 8 mm deep × 7 mm wide. Furthermore, one dog was tested by abdominal radiography and bone marrow aspiration cytology and one by buphy coat smear examination to rule out spread of MCT, and 4 cats underwent echocardiograms as a precaution before anesthesia because of cardiac murmurs that were detected on physical examination.

**Local Toxicity.** Local toxicity was dependent on the dose of photosensitizer and light, the size of the patient, and the location of the treatment field. See Fig. 2 for examples of local tissue reactions. The initial dosage used, extrapolated from the murine preclinical studies, was 5 mg/kg of EtNBS and 800 J/cm² of 670-nm wavelength light with a fluence rate of 200 mW/cm² for 66 min in cat 1. As noted above, the duration of photodestruction was doubled for this cat compared with subsequent animals to provide a total light dosage of 800 J/cm², because the wavelength of the available laser, 670 nm, is not optimal for activation of EtNBS. This treatment produced severe burns of the normal tissues surrounding the tumor bed, resulting in painless exposure of maxillary alveolar bone and, 10 days after treatment, sloughing of the rostral third of the tongue. Following this experience, the dosage of EtNBS was reduced to 2.5 mg/kg, and the dosage of light was reduced to 400 J/cm² with a fluence rate of 200 mW/cm² for 33 min, using a wavelength of 652 nm. After six treatments had been performed at that dosage, and good efficacy and tolerable toxicity were demonstrated, the dose of EtNBS was further reduced to 2.0 mg/kg for seven of the remaining eight treatments, although no further untoward effects had been seen, in an attempt to minimize drug exposure while maintaining efficacy.

At 2.0 and 2.5 mg/kg of EtNBS and 400 J/cm² of light, the effects to the surrounding normal tissues were found to be acceptable in all cases except 1 (dog 1). On skin, the local effects generally consisted of edema and erythema (grade 1) for the first week, then localized alopecia with regrowth of hair over 1–6 months. When oral mucous membranes were treated, the surrounding normal tissues in the field became ulcerated (grade 3), with rapid complete healing in less than 4 weeks, even for the largest ulceration. Halitosis was severe in some cases, but only lasted approximately 1 week, until the necrotic tissue sloughed. In one dog (dog 1) and one cat (cat 1), gingiva was present in the treatment field, and in both cases the gingiva became necrotic and sloughed, exposing the alveolar bone. Surprisingly, this did not appear to cause any pain to the animals.

The eyelids were treated in one dog (dog 2) and one cat (cat 6), using a photo-opaque corneal shield. Neither experienced any visual problems after the treatment. The dog was treated for cutaneous MCT and experienced a deep ulcer of the lid (grade
Table 1  Summary of patient characteristics and responses to EtNBS-PDT

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Breed</th>
<th>Sex*</th>
<th>Tumor type</th>
<th>Previous tx</th>
<th>Size at tx (cm)</th>
<th>Body wt (kg)</th>
<th>EtNBS (mg/kg)</th>
<th>Infusion problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat 1</td>
<td>14</td>
<td>DSH</td>
<td>CM</td>
<td>Sublingual SCC</td>
<td>debulk</td>
<td>1 × 1 bed</td>
<td>5.0</td>
<td>5.0</td>
<td>Slight nausea related to infusion rate</td>
</tr>
<tr>
<td>Cat 2</td>
<td>16</td>
<td>DSH</td>
<td>CM</td>
<td>Sublingual SCC</td>
<td>debulk</td>
<td>1.5 × 1.5 bed</td>
<td>3.4</td>
<td>2.5</td>
<td>None</td>
</tr>
<tr>
<td>Cat 3</td>
<td>14</td>
<td>DSH</td>
<td>SF</td>
<td>Nasal planum SCC</td>
<td>no</td>
<td>1.5 × 1.5</td>
<td>3.4</td>
<td>2.5</td>
<td>None</td>
</tr>
<tr>
<td>Cat 4</td>
<td>14</td>
<td>DSH</td>
<td>SF</td>
<td>Nasal planum SCC</td>
<td>no</td>
<td>1.5 × 1.5</td>
<td>3.4</td>
<td>2.5</td>
<td>None</td>
</tr>
<tr>
<td>Cat 5</td>
<td>6</td>
<td>DSH</td>
<td>CM</td>
<td>Nasal planum SCC</td>
<td>no</td>
<td>1.5 × 1.5</td>
<td>3.4</td>
<td>2.5</td>
<td>None</td>
</tr>
<tr>
<td>Cat 6</td>
<td>16</td>
<td>DLH</td>
<td>CM</td>
<td>Nasal planum SCC</td>
<td>no</td>
<td>1.5 × 1.5</td>
<td>3.4</td>
<td>2.5</td>
<td>None</td>
</tr>
<tr>
<td>Cat 7</td>
<td>10</td>
<td>DSH</td>
<td>CM</td>
<td>Nasal planum SCC</td>
<td>no</td>
<td>1.5 × 1.5</td>
<td>3.4</td>
<td>2.5</td>
<td>None</td>
</tr>
<tr>
<td>Dog 1</td>
<td>10</td>
<td>G ret</td>
<td>CM</td>
<td>Maxillary gingival SCC</td>
<td>debulk</td>
<td>1.5 × 1.5</td>
<td>3.4</td>
<td>2.5</td>
<td>None</td>
</tr>
<tr>
<td>Dog 2</td>
<td>9</td>
<td>Y Lab</td>
<td>SF</td>
<td>GrII MCT subconj OD</td>
<td>debulk</td>
<td>0.8 × 0.7</td>
<td>27.6</td>
<td>2.0</td>
<td>None</td>
</tr>
<tr>
<td>Dog 3</td>
<td>2</td>
<td>G shep</td>
<td>SF</td>
<td>Scleral melanoma OS</td>
<td>no</td>
<td>0.8 × 0.7</td>
<td>27.6</td>
<td>2.0</td>
<td>None</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient</th>
<th>Fluence (mW/cm²)</th>
<th>P1 (min)</th>
<th>ED (J/cm²)</th>
<th>PI problems</th>
<th>Days dark</th>
<th>Toxicity grade</th>
<th>Response</th>
<th>Reason off study</th>
<th>DFI (days)</th>
<th>DFI (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat 1</td>
<td>200</td>
<td>66</td>
<td>800</td>
<td>Marked edema of field</td>
<td>2</td>
<td>3</td>
<td>MR</td>
<td>PD*</td>
<td>48</td>
<td>1.6</td>
</tr>
<tr>
<td>Cat 2</td>
<td>200</td>
<td>33</td>
<td>400</td>
<td>Mild edema of field</td>
<td>3</td>
<td>1</td>
<td>MR</td>
<td>PD</td>
<td>22</td>
<td>0.7</td>
</tr>
<tr>
<td>Cat 3</td>
<td>200</td>
<td>33</td>
<td>400</td>
<td>None</td>
<td>3</td>
<td>1</td>
<td>CR</td>
<td>Marginal CIS</td>
<td>283</td>
<td>9.4</td>
</tr>
<tr>
<td>Cat 4</td>
<td>200</td>
<td>33</td>
<td>400</td>
<td>None</td>
<td>3</td>
<td>1</td>
<td>CR</td>
<td>DOC in CR</td>
<td>276</td>
<td>9.2</td>
</tr>
<tr>
<td>Cat 5</td>
<td>200</td>
<td>33</td>
<td>400</td>
<td>None</td>
<td>3</td>
<td>1</td>
<td>PR</td>
<td>PD</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Cat 6</td>
<td>200</td>
<td>33</td>
<td>400</td>
<td>Apparent discomfort</td>
<td>3</td>
<td>3</td>
<td>PR</td>
<td>PR</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Cat 7</td>
<td>200</td>
<td>33</td>
<td>400</td>
<td>None</td>
<td>3</td>
<td>1</td>
<td>CR</td>
<td>516</td>
<td>17.2</td>
<td></td>
</tr>
<tr>
<td>Cat 8</td>
<td>200</td>
<td>33</td>
<td>400</td>
<td>None</td>
<td>2</td>
<td>1</td>
<td>CR</td>
<td>327</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>Dog 1</td>
<td>200</td>
<td>25</td>
<td>303</td>
<td>Hyperthermia</td>
<td>2</td>
<td>3</td>
<td>MR</td>
<td>PD</td>
<td>14</td>
<td>0.5</td>
</tr>
<tr>
<td>Dog 2</td>
<td>200</td>
<td>33</td>
<td>400</td>
<td>Marked edema of field</td>
<td>5</td>
<td>3</td>
<td>CR</td>
<td>Chemo for other MCT</td>
<td>126</td>
<td>4.2</td>
</tr>
<tr>
<td>Dog 3</td>
<td>200</td>
<td>33</td>
<td>400</td>
<td>Apparent discomfort</td>
<td>5</td>
<td>3</td>
<td>CR</td>
<td>PD</td>
<td>28</td>
<td>0.9</td>
</tr>
<tr>
<td>Dog 4</td>
<td>50</td>
<td>33</td>
<td>100</td>
<td>None</td>
<td>3</td>
<td>1</td>
<td>MR</td>
<td>PD</td>
<td>40</td>
<td>1.3</td>
</tr>
<tr>
<td>Dog 5</td>
<td>100</td>
<td>33</td>
<td>200</td>
<td>Hyperthermia 106°F</td>
<td>3</td>
<td>1</td>
<td>NR</td>
<td>NR</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

| Mean    | 3.5             | 3.0       | 170        | 5.7         | 48         | 1.6       |

* DSH, domestic short hair; DLH, domestic long hair; Y Lab, yellow Labrador retriever; G ret, Golden retriever; S poo, standard poodle; G shep, German shepherd.

** CM, castrated male; SF, spayed female.

† tx, therapy; cryo, cryosurgery; RT, ionizing radiotherapy; wt, weight.

‡ GrII, grade II MCT; subconj OD, subconjunctival right eye; OS, left eye.

§ PI, photoirradiation time in minutes.

‖ Energy density.

* DFI, disease-free interval.

* PD, progressive disease (see text for definitions of toxicity and response criteria); DOC, died of other causes; Chemo, chemotherapy; NR, no response.

3) 1 week after PDT. The ulcer healed completely by the fourth week, leaving a very mild cosmetic and functional defect with a slight external roll of the lid caused by scarring. This dog developed mild inflammation of the conjunctiva 2 months after PDT, which resolved with topical steroid/antibiotic ointment. Tear production in the affected eye was normal, no corneal ulceration was detected by fluorescein staining, and the episode was judged to be due to reduced blinking ability caused by the slight deformity of the lid rather than due to direct toxicity of the PDT. The cat that was treated at the eyelid did develop a corneal ulcer after treatment (grade 3). This cat had received a total of 80 Gy ionizing radiation to the area over the 2 years before PDT in an effort to control its SCC and furthermore had an anatomical defect of the lower lid caused by the tumor; therefore, the cat already had a history of keratitis and corneal ulceration before PDT.

Two dogs (dogs 4 and 5) were treated directly to the globe, and the light dosage was reduced for both. In the first, the fluence rate used was 50 mW/cm², and in the second, a fluence rate of 100 mW/cm² was used. Both dogs were photoirradiated.

Downloaded from clincancerres.aacrjournals.org on April 20, 2017. © 1998 American Association for Cancer Research.
for the standard 33 min, so the total energy density was 100 and 200 J/cm², 25% and 50% of that used for the majority of the animals in the study, respectively. Local conjunctival reaction was mild in both, consisting of chemosis and hyperemia (grade 1) that subsided within 2 weeks. No intraocular changes could be detected in either dog after PDT.

Five animals were judged to be in pain during anesthetic recovery and were treated with analgesia consisting of butorphanol (2), butorphanol (4), carprofen (1), and acepromazine (1), either alone or in combination. These five animals included dog 2 and cat 6, which were treated at the eyelid, one dog (dog 4) of two that were treated directly to the globe, one cat (cat 8) of five that were treated at the nasal planum, and dog 3 after the first of two treatments in the buccal cavity. Dog 1 was agitated and had an elevated body temperature but was not judged to be in pain and was treated with tranquilizer (acepromazine) only. No animal required analgesia beyond 12 h after PDT.

Systemic Toxicity and Photosensitization. Systemic toxicity was minimal. Cats 1 and 3 experienced nausea and vomited once, during the drug infusion of 5 and 2.5 mg/kg EtNBS, respectively. The nausea appeared to subside when the rate of infusion was slowed. Dog 2 vomited once immediately after finishing the infusion at 2 mg/kg. Dog 5 developed an elevated body temperature (105.0°F) during the drug infusion at 2 mg/kg and vomited twice just after finishing. This dog’s body temperature rose again during photoradiation (106.0°F), but this resolved on recovery from anesthesia and did not recur. For dog 2, photoradiation was stopped because of elevated body temperature under general anesthesia after only 76% of the planned dose of light (approximately 303 J/cm²) had been administered.

Several delayed systemic effects were seen, and again all were judged to be tolerable. Cat 5 had an elevated body temperature on the second day after PDT, and this resolved within 24 h, either spontaneously or in response to i.v. cephalothin. Cat 7 was inappetant for 2 weeks after treatment and regained its appetite spontaneously. Cat 8 developed a symmetrical peripheral neuropathy beginning 2 weeks after PDT. Signs consisted of hind leg weakness and inability to jump; on neurological examination, the withdrawal reflex was intact but the patellar reflexes were markedly decreased, and the cat was unable to extend its stifles, while electromyography did not detect any abnormalities. The dysfunction was bilaterally symmetric and very isolated, suggestive of bilateral femoral nerve neuropathy. The cause could not be determined, and the cat recovered partially without treatment, relapsed once, then recovered fully over the next 2 months. The cat had also been exposed to an herbicide (glyphosphate, not known to cause neurotoxicity) shortly before both occurrences.

Pre- and posttreatment Heinz body levels were available for 12 treatments, and there was no increase in Heinz bodies in any of these cases. In six cases, the Heinz body count was 0 both before and after treatment, in two cases Heinz bodies were “rare” both before and after treatment, in one case they were “occasional” both before and after treatment, in one case they were “rare” before and “very rare” after treatment, in one case they were “trace” before and “rare” after treatment, and in one case they were “few” before and 0 after treatment. Pre- and posttreatment methemoglobin levels were available for nine treatments, and posttreatment-only levels were available for an additional two treatments. For the latter two, the levels were 1.3 and 0%; for the nine cases with both pre- and posttreatment levels, the percentage of methemoglobin went from −0.5 to 1.4, from 0.2 to 0.7, from 0.5 to −0.2, from 0.7 to 0.5, from 0.8 to 1.9, from 2.4 to 5.3, from 15 to −12.6, from −0.6 to 0.9, from 0 to 1.4, and from 29.8 to 2.4. The median increase in methemoglobin level was 1.1%, and if the two cases with large decreases are excluded as possible technical failures, then the median increase was 1.4%.

Serum chemistry and complete blood cell counts were repeated 4 and 8 weeks after PDT, and only minor fluctuations were found; no changes were associated with clinical abnormalities. Dog 2 had mildly increased alkaline phosphatase of 350 units/l (normal, 20–200 units/l) and ALT of 88 units/l (normal, 10–70 units/l) at 4 weeks after PDT, but at 8 weeks, they had returned to normal. Cat 3 experienced a transient mild increase in creatinine levels (normal, 0.6–1.6 mg/dl) from 1.5 mg/dl before PDT to 2.5 mg/dl 8 weeks after the first treatment, which returned to 1.7 mg/dl by 6 months after treatment. After a second treatment, the same cat’s creatinine level rose from 1.5 mg/dl before to 1.7 mg/dl 8 weeks after PDT. Four cats (cats 3, 6, 7, and 8) had mildly increased globulin levels after PDT (range, 4.2–5.3 g/dl; normal, 1.5–4.0 g/dl). Such increased globulin levels are commonly associated with chronic inflammation in cats, and in two of these cats, the globulin was elevated prior to PDT (4.2 and 4.9 g/dl). In cat 4, hepatocellular enzymes that were increased before PDT (ALT: 522 units/l; normal, 10–130 units/l; AST: 131 units/l; normal, 8–35 units/l) were decreased by 4 weeks after treatment (ALT: 218 units/l; AST: 63 units/l). The serum biochemical changes in this cat are presumed to have been associated with hyperthyroidism that was brought under control shortly after PDT.

Dog 3 developed multiple mild hematological abnormalities beginning 4 weeks after the first PDT; therefore, we continued to monitor this dog’s hematology. The changes consisted of mild anemia, with the RBCs ranging from 4.55 to 5.41 × 10⁹/μl (normal, 5.5–8.5 × 10⁹/μl), with slight anisocytosis and slight poikilocytosis occasionally noted on red cell morphology, and mild thrombocytopenia ranging from 114 to 185 × 10⁹/μl (normal, 200–500 × 10⁹/μl). Four months after the hematological abnormalities began appearing, a splenic mass was palpated. The dog underwent splenectomy, and the mass was examined histologically and found to be a benign lymphofollicular hyperplastic nodule.

In general, excretion of EtNBS was visible as green-colored urine or feces. For dog 3 at the second treatment and cat 4, no colored excretions were recorded; dog 4 did have colored urine on days 2, 3, and 4 after the first treatment. These two animals were held in subdued light for 5 days and then exposed to normal light with no evidence of photosensitization. Of the remaining animals, nine had colored urine during the first 3 days after PDT (day 1 only, one dog; days 1 and 2, one cat; day 2 only, four cats; days 2 and 3, 2 dogs and 1 cat). Dog 2 had both colored urine on days 3 and 4 and a colored bowel movement on day 3, and cat 3 had a colored bowel movement on day 3 after the first treatment and colored urine on day 3 after the second treatment. After these signs of EtNBS clearance were noted, the
Fig. 2 Selected examples of antitumor responses and local toxicities following EtNBS-PDT. a–g: cat 3, progression through PDT. a, before treatment. Note large stage 1 SCC of nasal planum. b, 48 h after PDT. Note sharply demarcated necrosis of tumor and edema and erythema of surrounding normal tissues. c, 7 days after PDT. The tumor forms a dry scab. Edema is resolving, and alopecia is beginning. d, 14 days after PDT. Alopecia of the treatment field is marked. The necrotic tumor scab is sharply defined. e, 4 weeks after PDT. The scab has lifted, and the bed has healed. Nasal planum defect exists where normal tissue had been effaced by tumor. f, 7 days after second treatment (18 months after initial treatment), showing location of residual carcinoma in situ. Again necrosis is sharply demarcated. Note that area of necrosis is much smaller than after the first treatment, because the extent of tumor was much less. g, 1 year after second treatment; CR. h and i: dog 3, healing of PDT reaction. h, 3 days after PDT. Note necrosis and sloughing of entire treatment field. i, 14 days after PDT. Ulcer has completely epithelialized, and only erythema remains. j–l: cat 4, progression through PDT. j, before PDT. Note small stage 1 SCC of naris with spot of CIS above. k, 24 h after PDT. Note sharply demarcated photodynamic reaction of tumor and carcinoma in situ and edema and erythema of surrounding normal tissues. l, 8 weeks after PDT. CR with good cosmetic outcome.
Fig. 2 continued. m–o: cat 7, progression through PDT. m, before PDT. Note moderately sized stage 1 SCC of nasal planum. n, 7 days after PDT. Note sharply demarcated necrosis of tumor and edema and erythema of surrounding normal tissues. o, 8 weeks after PDT. CR with fair cosmetic result. p–s: dog 2, progression through PDT for eyelid MCT. p, 24 h after PDT. Note marked edema of entire treatment field, with some erythema. q, 7 days after PDT. Large thick scab in area of tumor (larger than apparent tumor but not the entire treatment field) sloughs. r, 14 days after PDT. Ulcer is contracting and healing rapidly. s, 4 months after PDT. CR. Eyelid is fully healed with slight external rolling defect. t–u: cat 8, progression through PDT. t, before PDT. Note moderate stage 1 SCC of nasal planum. u, 6 weeks after PDT. CR with good cosmetic result. v, dog 1, 7 days after PDT. Note necrosis and sloughing of entire treatment field (compare with h) and full thickness necrosis of gingiva above the canine tooth, constituting unacceptable grade 3 local toxicity. w and x, dog 4, healing of PDT reaction after treatment for bulbar conjunctival MCT. w, 48 h after PDT. Note chemosis and hyperemia of bulbar conjunctiva. x, 14 days after PDT. Conjunctival reaction almost fully resolved.
animals were released into full normal light (mean days in subdued light, 3.5) with no evidence of photosensitization in any animal.

**Efficacy.** Every tumor treated except 1 showed at least some short-term response. However, as expected, efficacy was dependent on the tumor type and size treated. Of all 13 tumors treated, there were 6 CR (46.2%) and 2 additional PR (15.4%), for an overall response rate of 61.5%. In general, intraoral tumors became necrotic and sloughed along with the surrounding normal tissues, and the antitumor effect consisted of an absence of tumor regrowth as the normal tissues healed. In contrast, cutaneous tumors exhibited sharply demarcated necrosis characterized by the formation of a leathery black eschar, while the surrounding normal tissues merely became edematous and erythematous for the first week and alopoeic thereafter. The eschar was shed after 3–6 weeks, and the resulting wounds healed by granulation. Also see Fig. 2 for examples of tumor responses.

Of the two dogs with intraoral SCC, dog 1 had a stage 2 tumor and received only 76% of the prescribed dose of light. This dog initially had complete disappearance of the tumor bed with ulceration of the entire treatment field, followed by recurrence after only 2 weeks, coincident with healing of the normal tissues (MR). The second dog (dog 3) was treated with 2.0 mg/kg EtNBS and standard photoradiation for a stage 1 tumor and experienced an initial small recurrence after 4 weeks. It was then treated a second time with 2.5 mg/kg EtNBS and a fluence rate of 100 mW/cm² for 66 min and achieved CR that remained until he died of other causes 142 days after starting PDT.

The dog with cutaneous MCT (dog 2) remains in CR at the treated site for 24 months to date but was started on chemotherapy for two cutaneous MCTs at other sites 4 months after PDT and, therefore, is excluded from efficacy analysis after that time. The dog with periorcular MCT (dog 4) was treated with 50 mW/cm² for 33 min because of concern about local toxicity to the eye and had tumor recurrence after 7 weeks (considered MR rather than PR because the initial tumor was too small to accurately calculate the reduction in tumor size). The dog with ocular melanoma (dog 5) was treated with 100 mW/cm² for 33 min again because of concern about local toxicity. The visible part of the tumor became paler, and the corneal reaction decreased slightly but tumor diameter, measured by ocular ultrasonography, was 8 mm deep × 8 mm wide 2 months after PDT compared with 8 mm deep × 7 mm wide before PDT.

Two cats with debulked sublingual SCCs responded poorly to treatment (both MR). The first (cat 1) received a high dose of EtNBS (5 mg/kg) and light (800 J/cm²), although the wavelength used was 670 nm, and did not have recurrence in the site of the original tumor but did develop a nodal metastasis after 2 months. At postmortem examination, microscopic nests of tumor cells were seen at the primary tumor site. The second (cat 2) was treated with the lower dose (2.5 mg/kg EtNBS and 400 J/cm²) and developed primary tumor recurrence after 22 days.

Of six cats with facial SCCs, four achieved CRs. One of the two that did not (cat 6) had previously received two courses of ionizing radiation therapy over 2 years, and the tumor was stage 3 (invasive) at the time of PDT. This cat achieved PR for 3 months in response to PDT. The other (cat 5) was infected with feline immunodeficiency virus and had a very large tumor (stage 3) at the time of PDT. This cat achieved PR for 4 months. Of the six that achieved CRs, all had stage 1 tumors at the time of PDT. Cat 4 died of other causes while still in CR 9 months after treatment, cat 8 remains in CR for 11 months to date, cat 7 remains in CR for 17 months to date, and cat 3 had marginal recurrence of carcinoma in situ after 9 months, received a second treatment, and now remains in CR 39 months after starting PDT.

**DISCUSSION**

These findings demonstrate that PDT using the novel photosensitizer EtNBS is safe and well tolerated by dogs and cats. This is a preliminary study, however, in that no pharmacokinetic studies were performed, the timing of the photoradiation has not been optimized, nor is the optimal fluence rate known for dogs and cats. The only lasting untoward effect of EtNBS-PDT that was discovered in this study is gingival necrosis, which occurred following 2 of the 15 treatments performed (cat 1 and dog 1), the only cases where gingiva was present in the treatment field. Cat 1 was treated at the initial high dose of both EtNBS, which was extrapolated from the murine studies, and light of 670 nm wavelength at twice the duration and total dose as subsequent animals, because of laser availability. However, dog 1 was treated at the reduced EtNBS dosage that resulted in acceptable local tissue effects in all other 13 treatments, and in fact this dog received only 76% of the prescribed dose of light because of hyperthermia under anesthesia. The gingival necrosis led to exposure of the alveolar bone, but this was fortunately not painful to the animals, presumably because the necrosis also affected sensory nerves in the periosseum. The occurrence of this sequela in a case where the reduced dosage of EtNBS and a low dose of light were used, as well as one where the initial high dosage was used, suggests a tissue-specific sensitivity of the gingiva. This could be due to locally increased levels of photosensitizer accumulation or a local metabolic difference leading to increased sensitivity. Alternatively, thin tissues overlying bone may be exposed to an increased effective dose of PDT because of light reflected from the bone back into the tissue. The response of the other cases that were treated for intraoral tumors (discussed in more detail below) indicate that oral mucous membranes are more sensitive to the effects of this treatment than skin is; therefore, perhaps gingival necrosis is the result of a combination of increased tissue sensitivity and increased light reflected from bone. Regardless of the cause of the necrosis, obviously every effort must be made to avoid its occurrence. Without further trials, it is impossible to determine whether the effect is specific to gingiva; therefore, we recommend avoiding the use of EtNBS-PDT on gingiva and treating any thin tissue overlying bone with caution.

All other local effects of EtNBS-PDT seen in this study were acceptable. Whenever oral mucous membranes were treated, the entire field became necrotic and ulcerated; however, it did not appear to have a significant clinical impact on the animals. None of the animals treated in the oral cavity (except for cats 1 and 2, which were already dysphagic prior to treatment because of their tumors) had any dysphagia or evidence of oral pain after the treatment. In our subjective opinions, the clinical impact to these animals was much less than that of oral
mucositis caused by ionizing radiation therapy, which is often quite painful and commonly does cause dysphagia. Therefore, although local toxicity that fell under the predetermined criteria for grade 3 (ulceration) occurred after intraoral treatments, it was considered acceptable because it was not painful, did not cause dysphagia, and healed rapidly and completely.

Treatment of skin tumors resulted in acceptable local effects as well. Only two treatments to skin resulted in grade 3 toxicity (ulceration), and both of these involved the eyelid (dog 2 and cat 6). Cat 6 had undergone four cryosurgeries and two courses of ionizing radiation therapy in the area over the preceding 2 years to control its tumor. It is likely that the ionizing radiation left the skin with increased fragility and impaired healing ability. It is also possible, however, that EtNBS-PDT caused a "radiation recall" effect similar to what has been described for doxorubicin and other chemotherapy drugs (32). Therefore, although it would be appealing to use this treatment for tumors that have failed ionizing radiotherapy, until more experience is gained with EtNBS-PDT in this setting, we recommend that such tumors be treated with caution.

Dog 2 was treated for a MCT of the eyelid. Although no study has been published specifically addressing this question, many veterinary oncologists and radiation oncologists feel that MCTs exhibit more severe radiation reactions than other types of skin tumors do, presumably because of damage to surrounding tissue when mast cells die and degranulate. If this is true, then such a reaction would be expected to be even more severe after PDT, because cell death occurs at a faster rate. Alternatively, it is possible that the eyelids also have an increased sensitivity to the local toxic effects of EtNBS-PDT. This dog’s skin ulcer did not appear painful and healed completely and rapidly by second intention and is therefore considered acceptable, although it met the criteria for grade 3 toxicity. However, until further experience is gained, clinicians should be aware that the risk of local toxicity may be increased when using EtNBS-PDT for cutaneous MCTs and for eyelids. On the other hand, it should be pointed out that in many cases (as in this one), the other options for treatment of an eyelid tumor also bear a significant risk of sequelae. Extensive surgical resection risks a cosmetically and functionally imperfect result, and for MCT, surgery alone carries a significant risk of tumor recurrence. Radiotherapy risks painful and potentially blinding acute and chronic ocular toxicity. After PDT, this dog had a slightly imperfect eyelid because of scarring (see Fig. 2). The defect led to mild conjunctivitis because of decreased blinking ability, which was easily treated with topical medication, and no other ocular toxicity. This is a much better outcome than would have been expected with radiation therapy.

The remaining animals treated for skin tumors had only grade 1 toxicity consisting of edema and erythema for the first 1–2 weeks after PDT, then local alopecia in the treatment field. The alopecia resolved over 1–6 months after PDT. The alopecia was comparable to that seen with ionizing radiation of the same areas. These local toxic effects of EtNBS-PDT are considered acceptable as side effects of cancer treatment.

The two dogs that were treated directly to the globe (dogs 4 and 5) both had mild reactions to PDT. The dose of light was reduced prophylactically in both cases because of concern about local toxicity. For dog 4, the light dose was reduced 75% by using a fluence rate of 50 mW/cm² instead of 200 mW/cm². This dog’s local reaction consisted of mild chemosis and hyperemia (grade 1) and resolved within 2 weeks; therefore, the next dog was treated at 50% of the normal dose by using a fluence rate of 100 mW/cm². Although both dogs had reactions that met the criteria for grade 1, dog 5’s reaction was milder than that of dog 4. Two possible explanations for this are suggested. One is that dog 4 had a MCT, and as discussed above, mast cell degranulation may have exacerbated the reaction. Another possible explanation is that dog 4 was lightly pigmented (a yellow Labrador retriever) and dog 5 was darkly pigmented (a German shepherd dog), and pigmentation decreases light penetration into the tissues and, therefore, the effective photodynamic dose. The difference may also simply reflect individual variation in sensitivity to EtNBS-PDT.

Systemic toxicity of EtNBS-PDT was also mild in this study. Acute systemic effects consisted of nausea associated with EtNBS infusion in 4 of 15 treatments and elevated body temperatures in 2 of 15 treatments. In the two cats (cats 1 and 3) that experienced nausea during the infusion, it appeared to subside when the rate of infusion was slowed and disappeared when the infusion was finished. In the two dogs that vomited (dogs 2 and 5), nausea was not apparent until after the infusion was finished and subsided within 5–10 minutes. One (dog 5) of the two dogs that developed elevated body temperatures did so during EtNBS infusion, returned to normal after the infusion, and then the temperature rose again during photoirradiation. The other dog (dog 1) did not have an elevated body temperature until it was undergoing photoirradiation. This required stopping anesthesia early; therefore, this dog received only 76% of the prescribed light dose. In both cases, the temperature returned to normal upon anesthesia recovery and remained so for the duration of hospitalization. Both dogs were excluded from receiving a second treatment. In these cases, the complication resolved itself quickly and without lasting effects; however, body temperature should be monitored during EtNBS-PDT.

The later effects that were seen were also mild and without lasting impact. One cat (cat 5) had an elevated body temperature for 1 day, 48 h after PDT. This cat had a stage 3 facial SCC, and thus, a very large volume of tumor was treated. It was felt that the most likely explanation for this cat’s elevated body temperature was massive tumor cell necrosis. However, bacterial infection had not been ruled out as a possible explanation; therefore, i.v. antibiotics were administered. The elevated body temperature could also have been a reaction to EtNBS-PDT directly, as was seen in the two dogs that developed elevated body temperatures during the treatment. The cause could not be definitively determined, but the temperature returned to normal in 1 day and remained so. Cat 7 had a poor appetite after the treatment and returned to normal within 2 weeks.

Cat 8 developed acute symmetrical peripheral neuropathy of the femoral nerves 2 weeks after PDT. The cat improved partially, had one relapse, and then recovered fully over 2 months. Because the signs developed very suddenly, a relatively long time after PDT, we believe it is unlikely that it was caused by the treatment; however, no other definitive diagnosis could be made. Therefore, we feel it must be reported as a possible toxicity of PDT. The cat was also exposed to an herbicide, glyphosate, shortly before both the initial onset of signs and...
the relapse. Glyphosate is generally considered a relatively safe herbicide, and no references to peripheral neurotoxicity associated with exposure to it could be found. Therefore, despite the temporal association, it is also unlikely that the neuropathy was caused by the herbicide because it would have been an idiosyncratic reaction. Cranial nerve palsy 1 week after PDT with another photosensitizer has been reported (26), and the authors of that report were unable to determine the cause of the neuropathy. The cat in that report died of other causes 6 weeks later, and the neuropathy had not resolved by that time.

All other changes that occurred in the condition of the animals after PDT consisted of mild alterations in blood chemistry levels. None of these alterations lasted, and none of them were associated with clinical complaints. No hematomatologic changes were detected in any of the animals, except for multiple mild changes in dog 3, which was found to have a benign splenic mass. No changes in either Heinz body or methemoglobin levels were detected in this trial.

This study demonstrates that EtNBS is functionally cleared, with no residual photosensitization, from dogs and cats within 2–5 days. After 13 of the 15 treatments, evidence of photosensitizer was seen in the urine or bowel movements. The route of excretion did vary, even within the same animal in one case (cat 3). After evidence of EtNBS excretion was seen, the animals were no longer protected from light, and none of them showed any evidence of photosensitization. After 2 of the 15 treatments, no colored excretions were recorded. It is possible that they occurred but were not recorded; however, it is also possible that a small percentage of animals metabolize EtNBS to colorless products. These two animals were protected from light for 5 days, and when released into light, they showed no evidence of photosensitization. Overall, the animals were protected from bright light for a mean of 3.5 days. Preclinical murine studies showed that normal tissues were protected from PDT effects even more than would be expected from the ratio of EtNBS in normal versus tumor tissue. This was hypothesized to be because of inactivation of EtNBS, either by photoreduction or enzymatic reduction that occurs at greater levels in normal skin than in tumor tissues (18). Because of those findings, we believe it is likely that even this short period of protection is unnecessary, but because these were pet animals, we did not expose them to any excess risk by testing this hypothesis.

The results of this study also demonstrate clearly that EtNBS-PDT possesses antitumor activity against certain naturally occurring tumors. As expected, efficacy varied with both tumor type and size. Both cats (cats 1 and 2) that were treated for sublingual SCC responded poorly to the treatment. Feline sublingual SCC is extremely refractory to treatment, with a median survival of approximately 2 months, regardless of the form of therapy attempted (33). Also, it is generally agreed that the difficulty of attaining accurate dosimetry in PDT is a major cause of treatment failure, and the geometry of the tumor bed for the feline sublingual tumors was particularly difficult. Therefore these results, although disappointing, were not surprising.

Dog 5 was treated for a pigmented ocular melanoma, and this was the only tumor that exhibited no response at all to PDT, although the associated corneal reaction decreased somewhat and the visible part of the tumor became slightly paler. Again, these results were not surprising, because melanin would be expected to absorb a proportion of the light needed to achieve a photodynamic effect, and furthermore, this dog was treated with a reduced dose of light because of concern about toxicity to the eye. Successful photodynamic therapy of ocular melanomas has been reported in a transplanted tumor model in albino rabbits (34), but the largest of those tumors was 4.6 mm, and dog 5’s tumor was 8 mm deep. Also, this dog’s normal tissues were heavily pigmented and may have absorbed some of the light. Because of the light absorption qualities of melanin, pigmented melanomas would be expected to respond better to PDT using longer wavelengths (35).

Of two dogs treated for intraoral SCC, one (dog 1) had a rapidly growing stage 2 tumor that had been excised once 3 weeks before presentation and already required debulking again for PDT. This dog’s tumor recurred as quickly as the surrounding normal tissues healed. The other (dog 3) had a plaque-like stage 1 tumor that was discovered on routine dentistry, and this tumor achieved CR after two treatments. The dosage of EtNBS was increased slightly for the second treatment from 2.0 to 2.5 mg/kg, but a major change was made in the photoradiation protocol, although the total dose of light used was the same, by using a fluence rate of 100 mW/cm² for 66 min instead of 200 mW/cm² for 33 min. Theoretically, a lower fluence rate would be expected to be more effective because it would allow more time for oxygen delivery to the tumor cells to fuel the photodynamic production of oxygen radicals (35).

The tumor type that was treated with the most success (and in the highest numbers) in this study was feline facial SCC. Other treatment modalities are also effective against this tumor. Surgical excision can be effective but must be very aggressive to avoid local recurrence. Feline facial SCC is sensitive to ionizing radiation; 25 cats with precancerous plaques or early lesions <2 mm deep treated with 90Sr brachytherapy had a average tumor-free period of 34 months (36), whereas 90 cats with more advanced lesions of the nasal planum treated with orthovoltage external beam teletherapy enjoyed tumor control for a median of 14 months (37). Superficial SCC in 13 of 19 treated cats responded to local hyperthermia for 2–6 months (38), and 11 of 15 treated by cryotherapy had tumor control for a median of 6 months (39). Chemotherapy is not generally considered a modality of choice for this tumor, but intralaseional cisplatin (systemic cisplatin is fatal toxic to cats) was effective in resolving 64% of 118 feline SCCs after six treatments (40); systemically administered carboplatin may also be effective but has not been evaluated. PDT using other photosensitizers has also been used successfully in two other studies; in one (26), 7 of 11 treated feline facial SCCs resolved completely, and in another (27), 12 of 17 cats had responses of 3–18 months.

Of the six cats with facial SCCs treated in this study, four achieved CRs. At the time of PDT, the four tumors that eventually achieved CR were stage 1, and the two that achieved only PR (cats 5 and 6) were stage 3. This agrees with previous studies that have found correlation between tumor stage and therapeutic responsiveness of feline facial SCC (30, 41). Furthermore, both cats 5 and 6 had other complicating factors. Cat 5 was concurrently infected with feline immunodeficiency virus. Previous studies with EtNBS-PDT in mice suggested that immune response plays a role in the antitumor activity of this treatment (18). Therefore, it is possible that effects of the feline immuno-
deficiency virus infection suppressed this cat’s ability to mount an antitumor response. Cat 6 had been treated previously with four cryosurgeries and two courses of ionizing radiation therapy and the tumor had recurred again, indicating that this was a highly refractory tumor. The other four cats all achieved lasting CRs and remained in remission either until the cat died of other causes or until the time of writing.

Like many other forms of cancer therapy, PDT is probably best used in combination with other modalities (1), such as intraoperative PDT or PDT in combination with chemotherapy drugs or ionizing radiation therapy. Other approaches being explored to optimize the efficacy of PDT include the use of oxymimetic agents to help overcome hypoxic tumor cell resistance, optimizing light delivery by varying fluence rate and light fractionation, interstitial photoradiation to help manage the problem of tumor bulk, and improved dosimetry systems (1–4). Also, combination PDT using different photosensitizers together may present another avenue for optimizing efficacy (42).

The results of this preliminary study indicate that EtNBS-PDT has a role in the treatment of selected tumors of cats and dogs. The results show that the treatment is safe and well tolerated by these animals. Most had no untoward effects at all, and the majority of those that did occur were mild and self-limiting. The only unacceptable toxicity that occurred was gingival necrosis, and treatment of the gingiva using this modality should be avoided. Furthermore, the results show unequivocal evidence of the antitumor activity of this treatment in feline cutaneous SCC, canine intraoral SCC, and canine cutaneous MCT. Other tumor types not studied here may be equally sensitive, and preclinical studies with other tumor types in mice are ongoing. These positive results in spontaneous animal tumors strongly support the development of EtNBS-PDT for use in the treatment of human cancer.

ACKNOWLEDGMENTS

We thank Drs. Philip DuBois and Franklin Loew for helping to initiate this study; Dr. Peter Quesenberry for support during the course of the research; Drs. P. Anthony Moore, Kenneth Rassnick, and Cheryl London for referring cases for study and providing clinical support; Amy Bengtson for technical assistance; the anesthesia staff at Tufts University School of Veterinary Medicine for their expert anesthetic care of the animals; and Robert Brown and Brian Delgijuice for photographic assistance.

REFERENCES

Photodynamic therapy of naturally occurring tumors in animals using a novel benzophenothiazine photosensitizer.

A E Frimberger, A S Moore, L Cincotta, et al.


Updated version  Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/4/9/2207

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.