TREATMENT CONSIDERATIONS FOR PHOTODYNAMIC THERAPY IN THE CAT

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1. INTRODUCTION

The process of photodynamic therapy (PDT) and its application for the treatment of cancer has been reported for veterinary patients[1,2,3,4,5,6]. Multiple factors function together in the patient to influence the overall effectiveness of a PDT treatment, including tumor size, location and biology, efficiency of the photosensitizer to produce a cytotoxic reaction, systemic and tumor pharmacokinetics of the photosensitizer, tissue oxygenation, and light dosimetry. All of these factors need to be considered in evaluating a patient for possible PDT treatment, reviewing the design and results of any PDT clinical trial, and in proposing alterations to the approach of the clinical application of PDT where improvement of results is desired.

The use of PDT has been reported several times for the treatment of facial, solar-induced squamous cell carcinoma (SCC) in cats. Several of these studies have employed the use of chloro-aluminum sulfonated phthalocyanine (AlPcS) as a photosensitizer, with varying degrees of clinical response, which the authors in each case felt were encouraging for the employment of PDT for this condition in cats.[4,5,6] From our clinical experiences in employing AlPcS for this condition in cats and in comparing our experiences to those of others, we are attempting to better understand this disease and the factors which could influence the effectiveness of PDT for its treatment.

2. TUMOR SIZE, LOCATION AND BIOLOGY

Squamous cell carcinoma is a malignant neoplasm which arises from squamous epithelial cells. Histologically, these tumors are composed of epidermal cells arranged in irregular masses and cords which extend into dermal and subcutaneous tissue. Squamous cell carcinoma of the skin in cats occurs most commonly in poorly furred, unpigmented areas of the head, typically the pinnas of the ears, skin of the face, and the nasal planum. These tumors may be proliferative (papillary or keratinized) growths or, more commonly, ulcerative (invasive, erosive,) lesions [7] and may be clinically staged according to the World Health Organization T N M classification of

Stage	Definition	Stage	Definition
T_{is}	Preinvasive carcinoma (Carcinoma in situ)	T_3	Tumor > 5 cm maximal diameter, or with invasion of the subcutis, irrespective of size
T_0	No evidence of tumor	T_4	Tumor invading other structures, such as fascia, muscle, bone or cartilage
T_1	Tumor < 2 cm maximal diameter, superficial or exophytic	N_0	No lymph node involvement
T_2	Tumor 2 to 5 cm maximal diameter, or with minimal invasion irrespective of size.	M_0	No distant metastasis

TABLE 1. Clinical staging of feline tumors of epidermal or dermal origin.

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canine and feline tumors of epidermal or dermal origin (Table 1) [8].

In the one published study where tumor staging is reported, 14 nasal planum and 3 aural pinna SCC tumors were treated. All of the aural tumors had complete response regardless of stage. Of 8 nasal tumors staged T_{is} or T_0, 6 had a complete response to one treatment, and 2 had a partial response. Of 6 nasal tumors staged T_3 or T_4, only 1 had a complete response to one treatment while the other 5 responded partially [6]. These results are consistent with our clinical observations using a similar protocol[5] and clearly indicate that the size and location of this tumor in the cat will influence the likelihood of achieving a complete response to treatment as it has been employed.

Because the normal nasal planum of the cat generally does not reach 2 cm in any dimension, the decision as to the clinical stage of SCC tumors in this location must be based upon a judgment of the degree of invasiveness of the individual tumor. Close examination of these tumors, particularly those with an ulcerative surface 3 mm in diameter or greater, will usually reveal an area of slightly raised, sometimes slightly erythematous, otherwise normal looking nasal epithelial tissue at least 2 to 3 mm wide surrounding the tumor. This margin represents tumor presence under the normal epithelial surface and reveals that the tumor is at least that much wider in diameter than the ulcerative surface alone would indicate. Thus a 3 mm ulcerative lesion with a 2 mm raised margin would more likely suggest a tumor that is at least 7 mm in diameter.

In an attempt to better understand the extent of invasion of ulcerative SCC of the nasal planum of cats, we selected for study 3 cats with lesions where the ulcerative surface and surrounding raised margin measured a diameter of at least 5 mm. Each cat was treated by doing a complete resection of the nasal planum as described by Withrow et al.[9]. The resected nasal planums were then transected for gross examination and measurement. In each case, the grossly visible depth of the tumor was found to be at least equal to the full width of the ulcerative surface of the tumor, and the tumor was found to be wider than the ulcerative surface.

Ulcerative SCC tumors of the nasal planum of cats tend to be much more invasive than one might suspect without careful examination. The tumor should not be visualized as a half spherical mass with its ulcerative surface being the greatest diameter of the lesion. More typically, the ulcerative surface of the tumor is like the area of attachment of the stalk to the head of a mushroom with the tumor proliferating and spreading through the nasal planum in a configuration similar to the head of the mushroom.

Accurate judgment of the full size and extent of involvement of a SCC lesion of the nasal planum of a cat is important not only for prognostic considerations, but for designing an appropriate treatment plan for the patient, using an appropriate protocol for light dosimetry if PDT is to be employed, and for assessing the true efficacy of any photosensitizer for this condition.

3. PHOTOSENSITIZER EFFICIENCY AND PHARMACOKINETICS

Many chemical compounds have been and continue to be investigated for potential use as photosensitizers for PDT. The ability of a compound to produce photoinduced type I and/or type II cytotoxic reactions is an initial consideration in the selection of a photosensitizing agent. The efficiency of a compound's ability to generate cytotoxic products and the ability to achieve a concentration of the compound in target tissue which is substantially higher than in non-target

(normal) tissue are additional considerations in selecting a photosensitizer. While it would be ideal to have a high concentration of photosensitizer in the target tissue and none in the normal tissue, presence in both tissues is acceptable when a ratio of concentrations between the tissues exists where the level in normal tissue can be photobleached before producing cytotoxicity but is high enough in target tissue to elicit tissue necrosis[10,11,12].

The phthalocyanines are one family of compounds which have been proposed as potential clinical photosensitizers and have been reviewed several times[13,14,15,16]. Because of their photocytotoxic properties and several advantages over porfimer sodium, including lack of prolonged skin photosensivity following administration and excitation at a wavelength (675 nm) which has better tissue penetration, phthalocyanines have been considered promising photosensitizers. The addition of metal elements to and the degree of sulfonation of phthalocyanine will influence the tumor and cellular uptake as well as photoactivity of the derived compound. The process of sulfonating chloro-aluminum phthalcyanine results in replacement of the chlorine molecule with a hydroxy group in addition to the sulfonation; therefore, the resultant product more appropriately should be referred to as sulfonated aluminum phthalcyanine ($AlPcS_X$).

AlPcS obtained from Ciba-Geigy (Basel, Switzerland)[4,5] and from Porphyrin Products (Logan, Utah)[6] have been used and reported for PDT of feline SCC. The pharmacokinetics of these compounds in normal and some neoplastic canine and feline tissues have been studied[17,18]. While initially used and reported in cats at what was thought to be a dosage of 1 mg/Kg of body weight, the Ciba Geigy AlPcS (AlPcS-CG) was later found to be half as concentrated as thought, so it was actually administered at 0.5 mg/Kg of body weight. The Porphyrin Products AlPcS (AlPcS-PP), when given at 1 mg/Kg, did not produce clinical response which was judged as adequate by the investigators, so its dosage was increased to 2 mg/Kg of body weight. One reported and 2 subsequent cats treated with the AlPcS-PP developed fatal liver failure attributed to an idiosyncratic drug reaction[6,18]; however, none of the AlPcS-CG treated cats have experienced this problem.

A clonogenicity assay of each of the AlPcS products was performed in our laboratory and confirmed that the AlPcS-CG exhibited twice the cytotoxicity of the AlPcS-PP. Both products were then submitted to a laboratory for HPLC analysis. Analysis of samples revealed that the AlPcS-CG as prepared and used was in fact approximately 0.5 mg/ml while the AlPcS-PP was approximately 1.0 mg/ml. The AlPcS-CG was found to be composed of a mixture of mostly tri- and tetrasulfonated AlPc, along with mono-, di-, and an unidentified, highly sulfonated AlPc compound. The AlPcS-PP was determined to be composed of only tri- and tetrasulfonated AlPcS compounds.

While highly photocytotoxic and able to cross cell walls easily, non-sulfonated AlPc is hydrophobic and tends to aggregate in aqueous solution resulting in loss of photochemical activity. The addition of sulfonal groups to the AlPc compound increases the hydrophilic nature of the product, improving its solubility; however, its photocytoxicity and ability to cross cell walls are decreased as the degree of sulfonation increases[16]. Therefore, we conclude that the presence of the mono- and disulfonated AlPc compounds in the AlPcS-CG probably allowed that product to be used with equivalent clinical results at one fourth the dosage needed for the AlPcS-PP and that the four-fold difference in dosage may account for the differences in incidence of liver toxicity observed in the groups of cats treated.

In an attempt to determine the localization of the AlPcS-CG product in tumors of veterinary patients at the time of laser irradiation according to our protocol (48 hours following drug administration), we have collected tumor and normal tissue samples just prior to irradiation. Tissue samples were placed in embedding medium for frozen tissue specimens (OCT Compound, Tissue-Tek-Miles, Elkhart, IN) and frozen at -70°C. These specimens were then cut into 6 μm sections, mounted on glass slides, and stored at -20°C until examined by both phase contrast and fluorescence imaging. Additional tissue samples were fixed in 10% buffered formalin, infiltrated with and then embedded in paraffin, cut into 6 μm sections, cleared of paraffin, stained with hematoxylin and eosin, and examined under light microscopy.

An inverted microscope (Axiovert 10, Carl Zeiss, Oberkochen, Germany) was used with 10X and 40X objectives in order to visualize phase contrast and fluorescence images. A 100 W mercury lamp filtered through an interference filter (365 nm bandcenter, 30 nm FWHM, Carl Zeiss, Oberkochen, Germany) provided the excitation source. Excitation light was reflected onto the sample using dichroic filters (FT395, Carl Zeiss, Oberkochen, Germany) and the emission was isolated with a 615 nm long-pass filter (Carl Zeiss, Oberkochen, Germany). All images were recorded using a cooled, slow-scan CCD camera (576 X 384 pixel) system (Princeton Instruments, Trenton, NJ) interfaced to a computer. The camera is capable of 16 bits per pixel dynamic range. Instrument control and image processing were performed with IP lab software (Signal Analytics Corporation, Vienna, VA). A UniBlitz shutter and driver (model T132, Vincent Associates, Rochester, NY) were used to synchronize the CCD camera with the mercury lamp in order to minimize sample photobleaching.

In order to estimate light distribution, background images were acquired from blank slides with identical parameters. All fluorescence images were normalized in order to correct for non-uniform illumination by the algorithm: normalized fluorescent image = (Fluorescence - Background) / Background. Both fluorescence and background images were corrected for dark noise contributed during the exposure time.

Our examination of phase contrast and fluorescence imaged frozen tissue sections and light microscopic examination of hematoylin and eosin stained formalin fixed tissue sections has revealed AlPcS fluorescence in the highly vascular stroma and surface of SCC lesions of the nasal planum. The poorly vascular epidermal cell masses within these same SCC lesions show limited evidence of AlPcS fluorescence, except around clearly discernible vessels. These observations reveal that at 48 hours post administration, AlPcS is principally located in the perivascular structures of the nasal planum SCC tumors.

In contrast to the observation of AlPcS localization in the feline nasal planum SCC, similar studies performed on a feline fibrosarcoma and a canine hemangiopericytoma have revealed AlPcS fluorescence throughout each tumor without specific perivascular localization. Thus, it appears that tumor pharmacokinetics of a photosensitizer may vary between tumor types. This suggests that caution should be exercised in extrapolating information about photosensitizer pharmacokinetics from one tumor type to another.

When normal nasal planum tissue samples from the AlPcS-CG treated cats were examined, it was observed that AlPcS fluorescence was relatively absent from the subcutaneous areas but was strongly present in the perivascular tissues on either side of the cartilaginous structures of the nasal planum as well as associated with the vasculature and hair follicles of the dermis. Additionally, the presence of some smaller amounts of AlPcS fluorescence was detected within the cartilage structures of the planum.

While AlPcS fluorescence was observed strongly in the SCC lesions of the nasal planums examined, with a marked differentiation in fluorescence intensity observed between neoplastic and normal nasal planum tissues, the retention of AlPcS in some normal structures of the nasal planum would suggest that cytotoxic necrosis of normal tissue could be expected in those areas if exposed to sufficient light irradiation.

4. LIGHT DOSIMETRY

In administering PDT, it is not only important to be able to deliver therapeutic levels of photosensitizer to target tissue while minimizing normal tissue concentrations, but it is also necessary to deliver enough light energy of the appropriate wavelength to the entire target tissue in order to induce the desired photochemical reactions. Singlet oxygen generated in Type II photochemical reactions is considered to be the most important cytotoxic products of PDT. The ability of singlet oxygen to diffuse from its site of generation is typically less than 100 nm, due in large part to its life expectancy of only microseconds[19]. This means that the cytotoxic mechanism occurs at the site of induction and thus requires that enough light energy be delivered to all of the target tissue for necrosis of that tissue to be induced.

Light dosimetry has been studied and the current understanding well reviewed[20,21,22]. The distance light travels through tissue is both wavelength and tissue dependent, being influenced both by the concentration of compounds within the tissue that will scatter or absorb the specific wavelength and by the geometry of the irradiance. For a given tissue, scattering and absorption coefficients can be determined with hemoglobin and melanin being the principal absorbing chromophores within tissues of the body. The presence of photoactive compounds will also increase the absorption of specific wavelengths and may further decrease the depth of penetration of those wavelengths in treated tissue.

The depth of penetration (∂) of a given wavelength is the distance within the tissue traveled by the light where the fluence rate drops by 63%. This may be considered in increments where the depth of each increment represents an additional 63% decay in fluence rate. Thus, within two increments, the fluence rate drops by 87% and has decayed by 95% in three penetration depths.

The penetration depth does not necessarily correspond to the effective treatment depth but may be used to predict what an anticipated effective treatment depth may be. For example, if the irradiant fluence applied to the surface is 100 J/cm^2 and the photosensitizer at its tissue concentration requires 50 J/cm^2 to produce enough product to be cytotoxic, then the effective treatment depth will be less than ∂ where the internal radiant exposure would be 37 J/cm^2. On the other hand, if the surface irradiance were 150 J/cm^2 and the photosensitizer required 20 J/cm^2, effective therapeutic depth would be expected at two increments of penetration depth.

In addition to the optical properties of the tissue, the geometry of the irradiance will influence the fluence-depth distribution within the tissue. As the irradiant spot size increases, a higher percentage of photons are still to be located within the field of the primary beam after each scattering event. The result is that for the same incident irradiance, up to a spot size of approximately 4∂, the depth of fluence increases as does the effective treatment path.

To date, the optical properties of the nasal planum and SCC tumors of cats have not been investigated and have been assumed to be similar to other non-melanin containing soft tissues and tumors. What influence, if any, the cartilage planes of the planum may have on photon

migration similarly has not been determined. However, application of what information is known about the photosensitizer which is to be employed, irradiance in other soft tissues, and an accurate assessment of the size and distribution of a patient's tumor may be used to propose a dosimetry plan which would likely produce an effective treatment pattern that would encompass all of the target tissue.

For our AlPcS patient treatments, we have used an excitation wavelength of 675 nm and have chosen to use an incident irradiance of 100 J/cm^2, delivered at 50-150 mW/cm^2. As incident power densities of 150-200 mW/cm^2 are associated with temperature increases of 5-7^{o}C[23], we prefer to stay below 150 mW/cm^2 in order to prevent thermal injury. If we assume ∂ = 3-5 mm and a minimum required optical dose of 30-40 J/cm^2 for AlPcS, then 100 J/cm^2 would be expected to produce an effective depth of treatment which would approximate ∂. Assuming that an irradiance spot size of 4∂ (1.2-2.0 cm) diameter were to be used, then any target tissue at or beyond 5 mm from the irradiant surface could be expected to survive treatment. In consideration of our previously described observations regarding the size, location and biology of SCC lesions of the nasal planum of cats, it would seem, therefore, that T_3 or T_4 lesions which are in excess of 3-5 mm in diameter may not exhibit a complete response to a single surface irradiation. Additionally, if lesions of 3-5 mm or less are irradiated with a spot size that approximates or is only slightly larger than the diameter of the lesion, failure to obtain a complete response with a single irradiation may also be expected.

5. PATIENT CONSIDERATIONS

The type and duration of post treatment care will be dependent upon the size and location of the lesion(s) to be treated. Some degree of post treatment depression and anorexia can be expected as tissue swelling and local tumor necrosis are initiated. This should be considered in patient selection, both for appropriateness of this treatment mode for the individual patient and in being prepared for patient management following treatment.

An assessment of the patient's overall general health through at least a thorough physical examination, hematological and clinical pathologic testing, radiographic studies, and confirmatory pre-treatment histopathology are an important part of the patient's evaluation for the suitability of this therapy mode. If evidence is found of disseminated neoplasia, underlying hepatic or renal dysfunction, which might interfere with the normal metabolism and elimination of photosensitizer, or other concurrent disease, which might be exacerbated by a prolonged recovery time or by either physiologic or psychological stress, then the patient should be considered an unsuitable candidate for PDT treatment.

Treatment of periocular lesions should be given special evaluation and consideration. Small lesions of the eyelids may resolve nicely with PDT without complication, but where tissue slough may involve full thickness lid and/or loss of the nictitating membrane, corneal integrity must be protected during and following tissue slough. Similarly, if the conjunctiva or cornea have neoplastic involvement, surgical enucleation may be a preferred approach to management.

It should be remembered that if a PDT treatment is successful, any tissue within the treatment area will be lost if its predominant structure is neoplastic. This is an important point to consider in both pretreatment client education and in the planning of a treatment protocol for the individual patient. PDT using multiple irradiations at various angles to the tumor can produce a very nice photochemical nasal planectomy when the planum is substantially infiltrated with

tumor tissue. This tissue, however, may take 8-10 weeks to slough with continual nasal congestion and repeated debridement needed during this period of time.

In our treatment of almost 90 cats with SCC using AlPcS, we have experienced 3 post treatment fatalities, each occurring approximately 3 days following laser irradiation and after a period of continued post treatment lethargy and depression. All three cases were older cats (>15 years of age) which had received multiple facial treatments, including full nasal planum, at least one temporal area and at least one pinna. One of the three cats developed a severe hypoproteinemia following treatment while the other two displayed no clinical pathologic abnormalities. Because of these experiences, we have adopted a policy of staging treatments at separate sessions when a substantial number of sites must be treated, particularly in older animals.

In our treatment group, we have also experienced three cases where tissue slough has been considered excessive. Two of these cases involved the turbinates of the patient when internasal fiber optic diffusing tips were used for laser irradiation and one involved mucosa over the hard palate following direct laser irradiation of an oral lesion. The reason for these excessive tissue sloughs has not been determined but is suspected to be related to special pharmacokinetic considerations of these tissues and/or light dosimetry.

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