

Hematoporphyrin-based photodynamic therapy for cutaneous squamous cell carcinoma in cats

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Conflict of Interest

There are no conflicts of interest.

Abstract

Photodynamic therapy (PDT) using a haematoporphyrin derivative (Photogem®, General Physics Institute and clustes Ltda) as photosensitizer and light emitting diodes (LEDs) as the light source was evaluated in 12 cats with cutaneous squamous cell carcinoma. Lesions were illuminated with LEDs, (300 J/cm for 30 min) 24 h after the administration of the photosensitizer. Clinical responses were classified as complete disappearance of the tumour with total re-epithelialization; partial response (a reduction greater than 50%); and no response (less than 50% reduction). Tumours localized to the pinna treated with one ($n = 3$) or two ($n = 4$) applications of PDT yielded no response. Highly invasive tumours of the nose and nasal planum also showed no response, after two treatments ($n = 2$). A combination of PDT and surgery was performed in three cases. Two cats showed partial response and one complete response with one application of therapy 30 days after nasal surgery. Small and noninfiltrative lesions ($n = 3$) of the nasal planum showed a PR with one application ($n = 2$) and a CR with two applications ($n = 1$). This study shows that PDT using Photogem® and LEDs can provide local control of low-grade feline squamous cell carcinoma. The addition of PDT to surgery in more invasive cases may help prevent recurrence.

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Introduction

Squamous cell carcinoma (SCC) is the most common malignant cutaneous tumour in cats^{1,2} with a predisposition

in white-haired breeds. Prolonged exposure to ultraviolet light contributes to neoplasia development.^{1,3} The treatment modalities should be determined according to tumour staging, general health state of the cat, owner co-operation, and availability of equipment and drugs. Early diagnosis has a profound effect on the prognosis.²⁻⁵

One treatment option for cutaneous SCC is photodynamic therapy (PDT).^{2,6} This was first used in animals with spontaneous tumours where haematoporphyrin derivative (HpD) was administered intravenously followed by laser irradiation.⁵ These studies included animals with various tumour types, the majority of which were considered responsive, indicating the clinical potential of this therapy in cancer treatment and identifying PDT as an important tool for veterinary oncologists and dermatologists.^{7,8}

Photodynamic therapy induces cytotoxicity of proliferating cells through the interaction of a photosensitizing agent (PS), light of appropriate wavelength and molecular oxygen.^{6,9,10} The PS accumulates mainly in neoplastic cells and is activated in the presence of light,⁴ to an excited state.¹⁰ One of the ways by which excited molecules return to the ground state is by energy transfer directly to cellular oxygen, forming highly reactive singlet oxygen that is responsible for cell death; this is known as the type II reaction.^{10,12} Both vascular and direct cell damage contribute to the destruction of the tumour.^{11,13,14} Alternatively, excited molecules can transfer energy to intermediate molecules that then react with oxygen to produce free radicals (type I reaction).⁹ In addition, the role of inflammatory processes and the immune system has been studied with regard to PDT.^{13,14}

A number of PS agents including 5-aminolevulinic acid, aluminium phthalocyanine tetrasulphonate, 5-ethylamino-9-diethylaminobenzo[a]phenothiaziniumchloride, pyropheophorbide- α -hexyl-ether, and meta-tetra (hydroxyphenyl) chlorin, have been used to treat animal cancer although HpDs, which are activated at a wavelength of 630 nm,^{6,14} have been most commonly used.^{6,9,15-17} The PS must be localized preferentially in cancerous cells to be effective. However, the mechanism by which this occurs is still not well understood, but it is believed to be associated with the tumour characteristics including increased numbers of low-density lipoprotein receptors, the presence of macrophages, low pH, large interstitial space, leaky vasculature, large amounts of newly synthesized collagen, and high lipid content.^{8,13}

The light sources are usually lasers with the disadvantage of being rather expensive.^{12,14,18,19} However, less expensive, more versatile, battery powered and hence portable light-emitting diodes (LEDs) are also available for clinical use.¹⁴ Prototypes for LED use in PDT are currently under development for use in veterinary cases and provide a safer and less expensive light source.^{7,9} When HpDs are

Table 1. Summarized data on 12 cats with squamous cell carcinoma treated with photodynamic therapy according to tumour site and TNM (tumour, lymph node metastasis, distant metastasis) system, follow-up, treatment response and outcome

Case (no.)	Cat signalment	Tumour site	Follow up	Treatment response	Outcome
1	3.8 kg 6-year-old female crossbred	Right pinna: T4N0M0 Left pinna: T4N0M0 Right lower eyelid: T2N0M0 Left lower eyelid: T2N0M0 Nose: T2N0M0	2 months	NR	Euthanasia
2	2.8 kg 10-year-old female crossbred	Nose: T2N0M0	1.5 months	NR	Dead after second therapy
3	6.6 kg 10-year-old male crossbred	Nose: T1N0M0	2 years	CR	
4	4.5 kg 7-year-old female crossbred	Nose: T2N0M0; Right pinna: T4N0M0	1 year	Nose: CR Pinna: NR	Pinna Amputation
5	5.0 kg 8-year-old male crossbred	Nose: T1N0M0	2 years	CR	
6	5.0 kg 10-year-old male crossbred	Nose: T2N0M0 Right pinna T4N0M0	4 months	Nose: PR Pinna: NR	Pinna amputation
7	4.5 kg 9-year-old male crossbred	Nose: T2N0M0 Left pinna: T1N0M0	2 months	Nose: PR Pinna: NR	
8	5.0 kg 10-year-old male crossbred	Nose: T2N0M0	2 months	Nose: CR	New tumour: medial canthus of right and left lower eyelid
9	4.5 kg 6-year-old female crossbred	Nose: T1N0M0 Left pinna: T4N0M0	1 year	Nose: CR Pinna: NR	Pinna amputation Dead as a result of metastasis
10	5.4 kg 10-year-old female crossbred	Right pinna T4N0M0 Left pinna T4N0M0 Left lower eyelid: T2N0M0	1 year	Pinna: NR Eyelid: PR	New tumor: spreading in direction to the lateral canthus of left lower eyelid
11	4.0 kg 5-year-old female crossbred	Nose: T1N0M0 Right pinna: T4N0M0	6 months	Nose: CR Pinna: NR	Dead as a result of fibrosarcoma in right hind limb
12	3.0 kg 7-year-old female crossbred	Right pinna apex T4N0M0 Left pinna apex T4N0M0	2 months	R Pinna: PR L Pinna: PR	Pinna amputation

NR, no response; PR, partial response; CR, complete response.

utilized, illumination is carried out 24 to 72 h after PS administration.¹²

The principal drawback of this therapy is the prolonged photosensitization when HpDs are used as PS agents.^{11,12,21} Although PS is rapidly removed from the serum, it may be retained in the skin for 4 to 8 weeks. Thus, the animal must be kept out of sunlight for a minimum of 2 weeks.⁶ Facial oedema, erythema and secondary necrosis may also occur at the site of application, but such adverse effects usually recede over time.² General anaesthesia is necessary during light administration, and the treated area requires protection to avoid self-trauma (e.g. Elizabethan-collar).⁷ Important causes of treatment failure are inadequate light dose and unsatisfactory PS action.¹⁰

Because there are few studies using haematoporphyrin as the photosensitizer and LEDs as the light source in cats with cutaneous SCC, the aim of this study was to evaluate the merit of this therapy in this species.

Materials and methods

This study was approved by the Ethics Committee of the School of Veterinary Medicine and Animal Science (UNESP), and all owners provided informed consent. Twelve cats with naturally occurring cutaneous SCC (Table 1) were clinically staged according to World Health Organization (WHO) classification guidelines using the TNM (tumour, lymph node metastasis, distant metastasis) system.²² After anamnesis and general physical examination of each cat, the lesions were submitted to cytological examination after fine needle

aspiration for diagnostic confirmation. Histological examination was performed only in cases in which cytological examination was not conclusive. Four cats had received previous therapy. Cat 3 had nasal surgery and chemotherapy with doxorubicin and bleomycin for a tumour localized to the nose and nasal planum. Cats 6, 7 and 8 had nasal surgery because of extensive and highly invasive tumours located on the nose and nasal planum 15 days before PDT. For the PDT, haematoporphyrin (Photogem®; Photogem LLC Company, Moscow, Russia) at a concentration of 1.5 mg/kg (dose recommended by the manufacturer²⁴) was administered intravenously to all cats. Once reconstituted with 0.9% sodium chloride the drug was protected from bright light.

After 24 h, the lesions were uniformly illuminated with custom-made LEDs (wavelength 630 nm) developed by the São Carlos Physics Institute (University of São Paulo Department of Physics and Science of the Materials, Trabalhador São-carlense Street, São Carlos, SP, Brazil) at a dose of 300 J/cm² for 30 min. The hair was clipped in areas with a high degree of hair covering. Aluminium foil was used to protect the eyes.

For illumination, cats were premedicated with acepromazine (Acepran 0.2%; Univet S.A., Sao Paulo, Brazil), 0.1 mg/kg, im, followed by ketamine (Vetaset; Fort Dodge, Campinas, Brazil), 10 mg/kg, and midazolam (Dormire; Cristália, Itapira, Brazil), 0.5 mg/kg, administered im. General anaesthesia was maintained with halothane (Halothano; Cristália, Itapira, Brazil), or isoflurane (Isoforine; Cristália, Itapira, Brazil) in case of extensive tumours. Ringer's solution was administered intravenously throughout the procedure. Meloxicam (Maxican; Ouro Fino, Ribeirão Preto, Brazil), 0.1 mg/kg, po, q24 h, was administered for 3 days and buprenorphine hydrochloride (Temgesic™; Schering Plough, Rio de Janeiro, Brazil), 0.01 mg/kg, im, was administered as required. An Elizabethan collar was used in all cases immediately after illumination and maintained as necessary.

Lesions were evaluated periodically from 3 days to 2 years with a mean of 8.6 months and a median of 5 months depending on the availability of the owners, who were instructed to keep the cats indoors and out of sunlight for 4 weeks. Thirty days after PDT all lesions were aspirated and re-submitted for cytological examination. If the presence of tumour was confirmed and if the owners agreed, the therapy was repeated after 1–2 months (mean of 1.5 months). The following classifications were used: complete disappearance of the tumour with total re-epithelialization (CR); partial response with tumour reduction greater than 50% (PR); and no response with less than 50% reduction (NR). Loss of appetite, behavioural changes and signs of photosensitization were classified as adverse effects.

Results

Table 1 shows the tumour site (pinna, eyelid, nose), follow-up (1.5 months to 2 years), treatment response (CR, NR, PR), and outcome after PDT of all cats (*n* = 12).

Cases 1, 5, 6, 7, 8 and 12 received one PDT administration, whereas cases 2, 3, 4, 9, 10 and 11 received two PDT administrations. Neither the appetite nor the behaviour worsened after therapy, and in the cases that had regression of lesions both improved. Immediately after illumination, all cats showed erythema and local oedema, and darkening of the cancerous tissue (vascular damage). After approximately 5 days, necrotic areas were observed in the irradiated area. Starting from 15 to 20 days after treatment the crusts came off spontaneously, and the healing process was more evident (Figure 1).

In cases with extensive nasal lesion (cases 1 and 2) signs of inspiratory dyspnoea resulting from temporary

obstruction of the nostrils by oedema were observed. Cat 2 died 24 h after the second PDT application. With the exception of severe oedema in the nasal region, necropsy did not detect any other alteration that could indicate the cause of death. Cat 7 showed signs of photosensitization (alopecia, reddening, pruritus) in the dorsal thoracic region, which was resolved after removal from sun exposure.

Discussion

Among the local effects caused by PDT, such as oedema, erythema and necrosis,^{2,6} the most significant observed acute side effect in the present study was oedema, which caused dyspnoea in cases of tumours in the nasal area. This was probably the cause of death in case 2, given that necropsy failed to reveal another lesion that could indicate the *causa mortis*. Even though it was a localized side effect it has to be considered a severe one. Signs of photosensitization were observed only in one cat (number 7), because the owner had not confined it adequately. Although in this case the problem was resolved after removal from sun exposure, it demonstrated that client education is an essential factor, because cutaneous photosensitivity is an issue.⁴ The photosensitizer 5-Aminolevulinic Acid (ALA) can be used topically, thereby preventing generalized photosensitivity;²⁵ it also accumulates in neoplastic tissue after oral application.²⁶

In the present study, the tumours localized to the pinna and treated with one (*n* = 3) or two (*n* = 4) applications of



Figure 1. Cat number 10: SCC medial canthus of left lower eyelid. (a) Before treatment, (b) necrotic areas within treated area 15 days after first PDT, (c) clinical improvement 1 month after first PDT, (d) partial response 1 month after second PDT with tumour spreading towards the lateral canthus of the left lower eyelid.

PDT had reductions of less than 50%, indicating absence or failure of response; although one case treated with one application of PDT had a reduction > 50%, indicating a partial response. Tumours on the pinna may show a lower degree of vascularization, resulting in a lower porphyrin accumulation. This is not true for tumours of the ear base. Highly invasive tumours of the nose and nasal planum also failed to respond to one ($n = 1$) and two applications ($n = 2$) of PDT. PDT permits repeated treatment without resistance to drugs and without cumulative toxicity and can be used in combination with other treatments.⁴ It was, therefore, combined with surgery in cats number 6 to 8 with the goal of reducing recurrence rates. In cases 6 and 7 a partial response was noted. In case 8 the tumour regressed after one PDT treatment although 2 months later another lesion was detected on the medial canthus of the left and right lower eyelid. This was probably associated with perpetuation of the causal factor. It is known that exposure to ultraviolet irradiation from sunlight contributes to development of neoplasia^{1–3} and must be avoided; however, the owner claimed to have no control over the animal's errant habits.

Cat 3 responded the most favourably to therapy, with no recurrence at approximately 3 years after two applications. Because the cat had already undergone surgery and chemotherapy, the probability of successful PDT was expected to be compromised. However, this case reinforces the possibility of better prognosis through a combination of therapies. Similarly, it was observed that small and noninfiltrative lesions ($n = 3$) located to the external nose had partial regression with one application and total regression with two, indicating that the extension of the lesion has a direct effect on the success of the treatment as has been shown previously by Magne *et al.*²⁷

The present study raises several questions. First, in extensive and deep lesions the wavelengths of the red LEDs may not reach the entire depth of the neoplasm, as most of the lesions had T2 or higher classification. Therefore, invasive SCC may not provide the ideal indication for PDT, and case selection should be based on tumour invasiveness for successful PDT. For SCCs that are too invasive, other options such as radiation therapy should be considered.⁵

Second, the action peak of the porphyrin derivative may have occurred prior to 24 h after its application. Pharmacokinetic studies in felines are required as for other PSs.¹⁶ Also, if a second PDT is administered, the optimal interval between the two consecutive treatments has yet to be established.

Third, the type of porphyrin used in this study may not be active in cats. Nevertheless, it is noteworthy that a previous study¹⁸ reported local control of 62% after a 1-year evaluation period employing PDT with Photofrin (Wyeth-Ayerst Lederle, Inc. for Axcan Pharma (Ireland) Ltda. Distributed by Axcan Pharma USA), with an argon laser, in cats with facial SCC. Furthermore, the authors did not observe biochemical, haematological or clinical signs of toxicity.

This study demonstrated that PDT with Photogem® and LEDs for cutaneous SCC in cats provided control only for low-stage tumours located on the external nose. When combined with surgery it appeared to decrease the

recurrence rate for invasive lesions. However, more information concerning the optimal interval between photosensitizer administration and illumination is necessary to improve PDT using this photosensitizer in cats.

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Résumé la thérapie photodynamique (PDT) utilisant un dérivé de l'hématoporphyrine (Photogem®) comme photosensibilisant et des LEDs comme source de lumière a été évaluée chez 12 chats présentant un carcinome épidermoïde. Les lésions ont été illuminées par les LEDs, (300 J/cm² pendant 30 min) 24 heures après l'administration du photosensibilisant. Les réponses cliniques ont été classées comme disparition complète de la tumeur avec re-epithelialization totale (CR); réponse partielle (une diminution de plus de 50% – PR); et absence de réponse (moins de 50% de réduction – NR). Les tumeurs localisées aux pavillons traitées avec une ($n = 3$) ou deux ($n = 4$) applications de PDT ont présenté une absence de réponse. Les tumeurs invasives de la truffe et du chanfrein ont également montré une absence de réponse après deux traitements ($n = 2$). Une combinaison de PDT et de chirurgie a été réalisée adns trois cas. Deux chats ont montré PR et un CR avec une application de traitement, 30 jours après la chirurgie. Les petites lésions non invasives ($n = 3$) de la truffe ont montré PR après une application ($n = 2$) et CR avec deux applications ($n = 1$). Cette étude montre que la PDT utilisant Photogem® et les LEDs peut provoquer un contrôle local des carcinomes épidermoïdes félines de bas grade. L'ajout de la PDT à la chirurgie dans les cas plus invasifs pourrait aider à contrôler les récidives.

Resumen Se evaluó la terapia fotodinámica utilizando un derivado de la hematoporfirina (Photogem®) como fotosensibilizador y diodos de emisión de luz (LEDs) como la fuente de luz en 12 gatos con carcinoma de células escamosas cutáneo. Las lesiones se iluminaron con LEDs, (300 J/cm² durante 30 minutos) 24 horas tras la administración del fotosensibilizador. Las respuestas clínicas se clasificaron como desaparición completa del tumor con reepitelización total (CR); respuesta parcial (a una reducción mayor del 50% – PR); y falta de respuesta (menos del 50% de reducción – NR). Los tumores localizados en la oreja tratados con una ($n = 3$) o dos ($n = 4$) aplicaciones de PDT no presentaron respuesta. Tumores altamente invasivos de la nariz y el plano nasal tampoco mostraron respuesta tras dos tratamientos ($n = 2$). La combinación de PDT y cirugía se realizó en tres de los casos. Dos gatos mostraron respuesta parcial, y uno respuesta total con una aplicación de terapia 30 días tras la cirugía nasal. Lesiones pequeñas y no infiltrativas ($n = 3$) del plano nasal presentaron respuesta parcial tras una aplicación ($n = 2$) y respuesta total con dos aplicaciones ($n = 1$). Este estudio muestra que PDT utilizando Photogem® y LEDs puede proporcionar un control local de carcinomas de células escamosas de bajo grado. La adición de PDT a la cirugía en casos más invasivos puede ayudar a prevenir recidivas.

Zusammenfassung Eine photodynamische Therapie (PDT), die ein Hämatoporphyrin Derivat (Photogem®) als Photosensibilisator und lichtemittierende Dioden (LEDs) als Lichtquelle benützt, wurde bei 12 Katzen mit kutanem Plattenepithelkarzinom evaluiert. Die Veränderungen wurden mit den LEDs (300 J/cm², 30 Minuten lang) 24 Stunden nach der Verabreichung des Photosensibilisators angestrahlt. Die klinischen Reaktionen wurden in ein totales Verschwinden des Tumors mit völliger Reepithelisierung (CR); in eine Teilreaktion (eine Reduzierung von mehr als 50% – PR); und keine Reaktion (weniger als 50% Reduzierung – NR) eingeteilt. Tumoren, die an den Ohrmuscheln lagen und mit einer ($n = 3$) oder mit zwei ($n = 4$) PDT Anwendungen behandelt wurden, erbrachten eine NR. Hochinvasive Tumoren der Nase und des Planum nasale zeigten nach zwei Behandlungen ($n = 2$) ebenfalls eine NR. Bei drei Fällen wurde eine Kombination aus PDT und Chirurgie durchgeführt. Zwei Katzen zeigten eine PR und eine CR bei einer einmaligen Behandlung 30 Tage nach der Operation an der Nase. Kleine und nicht-infiltrative Veränderungen ($n = 3$) des Planum nasale zeigten nach einer Behandlung ($n = 2$) eine PR und nach zwei Behandlungen ($n = 1$) eine CR. Diese Studie zeigt, dass PDT mittels Photogem und LEDs eine lokale Kontrolle eines niedriggradigen felinen Plattenepithelkarzinoms darstellen kann. Bei invasiveren Fällen könnte die zusätzliche Verwendung von PDT bei chirurgischer Intervention dabei helfen, ein Wiederauftreten zu verhindern.