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Evaluation of long-term outcome and prognostic factors of feline squamous cell carcinomas treated with photodynamic therapy using liposomal phosphorylated meta-tetra(hydroxylphenyl)chlorine

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Abstract

Objectives To evaluate efficacy and long-term outcome and prognostic factors of feline squamous cell carcinomas (SCC) treated with photodynamic therapy (PDT)

Methods Cats with histologically verified SCC of the head and neck received an intravenous injection of liposomal phosphorylated meta-tetra(hydroxylphenyl)chlorine (mTHPC) and 4 hours later 652nm light was delivered by a diode laser. One group received ≤10J/cm^2, the other 20J/cm^2. Tumor response and duration were analyzed with stage, tumor diameter, location and treatment intensity as prognostic factors.

Results In total, 63 lesions in 38 cats underwent treatment with ≤10J/cm (n=22) and 20J/cm2 (n=41). Overall response rate was 84% (complete remissions 61%, partial remissions 22%) with a mean progression free interval of 35 months (median not reached) and a median overall survival time of 40 months (95%CI:33,

47). In regard to tumor stage, invasiveness yielded a highly significant worse outcome ($p<0.017$). All patients with invasive tumors showed progression at less than 6 months. Larger lesions were associated with inferior control and treatment intensity and tumor location did not influence response and duration.

Conclusions and relevance PDT using systemic photosensitizer leads to excellent long-term tumor control in the majority of cats. However, invasive and large tumors had a clear inferior outcome, even if treated with the higher dose intensity. This suggests that advanced lesions are not indications for photodynamic therapy.

Introduction

Squamous cell carcinomas (SCC) are frequently occurring skin tumors in cats. As in humans, there is a predisposition for non-pigmented skin areas and an etiologic correlation between tumor development and exposure to ultraviolet light is known. Squamous cell carcinomas are locally invasive but rarely metastasise[1]. The spectrum of lesions ranges clinically and histologically from actinic keratosis (pre-cancerous, mild dysplasia due to chronic sun exposure) to carcinoma in situ (cancer cells within their site of origin) to invasive SCC (penetration of basement membrane) (Figure 1). The most common locations for cats are the sparsely haired areas of the nasal planum, eyelids, and pinnae[2] - locations where surgical interventions lead to non-optimal cosmetics and function. Radiation therapy has been reported to achieve appealing response rates and control durations but is associated with multiple treatment sessions for the patient and should be reserved for larger, more invasive stages of the tumor[3-6]. Electrochemotherapy,

cryotherapy and strontium-90 plesiotherapy also achieve satisfying lesion control but are not widely available[7-9]. Photodynamic therapy (PDT) is a non-invasive treatment modality, which involves the use of a photosensitizer, light and endogenous molecular oxygen to kill cancer cells. Previous studies have shown the efficacy of photodynamic therapy to treat feline squamous cell carcinoma as well as other superficial tumors, yielding a superb cosmetic outcome and satisfying tumor control for superficial tumors[9-11]. PDT using Foslip (Biolitec AG Jena) or phosphorylated meta-tetra(hydroxylphenyl)chlorine (mTHPC), a second-generation photosensitizer is one of the most common systemic sensitizer for cats studied to date and a good tolerability has been described[10, 11].

The purpose of this retrospective study was to evaluate the efficacy and long-term outcome of systemic PDT using liposomal mTHPC on treatment naive and recurrent cutaneous carcinomas in a large cohort of cats and to evaluate tumor and treatment variables associated with local control and survival. It was hypothesized that tumor size/invasiveness would be one of the strongest prognostic factors regarding disease free interval (DFI) and overall survival.

Materials and methods

Animals Cats with histologically verified carcinoma in situ or infiltrative squamous cell carcinoma were entered in the study. Previous surgical interventions or photodynamic treatments were allowed, as were cats with multiple lesions. Tumors were staged according to a modification of the World Health Organization system and categorized into four groups. The first group (T1a) was non-invasive lesions measuring < 1.5cm in diameter. The second group

(T1b) included cats with invasive tumors < 1.5cm in diameter. The third group (T2a) were non-invasive neoplasms > 1.5cm in diameter and the fourth group comprised invasive tumors > 1.5cm in diameter[12]. Staging included tumor cytology or histology, complete blood count, serum biochemistry, regional lymph node aspiration and thoracic radiographs. All patients were treated at the Division of Radiation Oncology at the Vetsuisse Faculty at the University of Zurich, Switzerland, between April 2011 and July 2016.

Photodynamic therapy Liposomal m-THPC (Foslip, Biolitec AG Jena) was administered intravenously over five minutes at a dosage of 0.15mg/kg body weight. Six hours after injection, a 652nm diode laser was used as a light source. The light was delivered by an optical quartz fiber to noncontact surface illumination of the visible tumor with an additional security margin of 5mm. The laser was adjusted to 0.5W/cm^2 non-thermal power density. One group of patients received ≤10J/cm^2 over a treatment time of 100 seconds, the other group 20J/cm^2 within 200 seconds. Cats were otherwise protected from light as much as possible. After discharge, owners were instructed to keep the cat indoors away from strong light sources and direct sun light exposure for approximately two weeks.

Anesthesia and pain management Cats were anaesthetized with propofol (Propofol 1% Fresenius, Fresenius Kabi AG, Stans) administered in combination with midazolam (Dormicum, Roche Pharma AG, Reinach) or ketamine (Narketan 10, Vetoquinol AG, Bern) to effect and were maintained on isoflurane/oxygen (IsoFlo Abbott, Wiesbaden). For pain management, Fentanyl 7µg/kg (Fentanyl Sintetica, Mendrisio) was injected at induction, 10µg/kg buprenorphine

(Temgesic, ESSEX Chemie AG) was administered intravenously q8 hours overnight and at home meloxicam 0.1mg/kg SID po (Metacam for cats; Boehringer Ingelheim) for ten to fourteen days was prescribed.

Tumor response and control Complete response (CR) was defined as complete resolution of all lesions. Cats were considered to have had a partial response (PR) when there was at least a 50% decrease in the size of the measureable tumor and as no response when tumor size reduction was <50% or if tumor progression was present. Tumor size was measured with calipers.
Patients were examined 4 to 8 weeks after PDT and follow-up data was collected thereafter approximately every 3 months by direct examination, telephone communication, per mail and photos either with the owner or the referring veterinarian. A minimum follow up period of 6 months was requested.

Statistical analysis All data reported is given by mean (±standard deviation) unless otherwise specified. Progression free interval (PFI) was calculated from the date of the first PDT treatment until documentation of recurrence. Cases that were lost to follow-up or that remained free of recurrence were censored at the date of the last follow-up evaluation or death of the cat. Median time to recurrence was reported with the 95% confidence interval of 95%. A Kaplan-Meier survival analysis was performed using SPSS 24 (SPSS Inc., Chicago, IL, USA) to calculate PFI and overall survival time (OST). Tumor response and duration were analyzed with stage, tumor diameter, tumor location and treatment intensity with the log rank statistic after ensuring even distribution of cases per group. Results were considered significant at values of $P<0.05$.

Results

Animals The study population consisted of 38 cats with the majority being Domestic Short Hair cats (n=36), one Siamese and one Maine Coon. Sixteen of the cats were female spayed, and 22 were male neutered. The median age at time of treatment was 12.9 (± 3.8) years and the median weight was 4.8 (± 1.2) kg. Nineteen of the cats had one lesion, 13 had two and 6 cats had three lesions, resulting in a total of 63 lesions. Histologically, the majority (n=50) was classified as infiltrative squamous cell carcinomas and 13 as carcinoma in situ (of which 7 had Bowen's disease). All tumors were located on the head and neck, with 20 located on the eyelid, 19 on the nasal planum, nine in the temporal or ear base region and fifteen in the facial skin or on the neck. All tumors were diagnosed histologically. Most tumors were non-invasive with 41 categorized as T1a (<1.5 cm in diameter) and three as T2a (<1.5cm in diameter). Ten cats had T1b tumors (invasive, <1.5cm) and 9 had T2b tumors (invasive, >1.5cm). Lymph node aspiration was performed in eleven cats and thoracic radiographs were available for all cats. All staged free of metastatic disease (N0M0).

PDT Intravenous liposomal m-THPC was well tolerated by all patients and no systemic adverse events were noted. Mild acute local adverse effects including erythema and edema (grade 0-1) were seen in 42% of cats shortly after PDT and resolved within 2-7 days post therapy. In most cats, tumor necrosis and scab formation at the PDT site were observed within two weeks of treatment (Figure 2). Maximum response to therapy was documented after 8 weeks.

Post-treatment nasal obstruction and dyspnea occurred in one cat with a large T2b nasal planum lesion. This patient was euthanized after 1 month when there was no improvement on supportive therapy and no tumor response noted.

Tumor Response and Control In total, 63 lesions in 38 cats underwent treatment with ≤10J/cm (n=22) and 20J/cm2 (n=41). Overall response rate was 84% (CR 61%, PR 22%) with a mean PFI of 35mo (median not reached) (Figure 3) and a median OST of 40mo (95% CI:33, 47). Tumor stage, maximum tumor diameter tumor location and treatment intensity were evaluated as possible prognostic factors. In regard to tumor stage, invasiveness (T1b and T2b tumors) yielded a significantly worse outcome ($p<0.017$). All patients with invasive tumors showed progression at less than 6 months. Also, larger lesions were associated with inferior control – increasing tumor diameter resulted in decreased PFI. Treatment intensity (≤10J/cm^2 or 20J/cm^2) and tumor location did not influence response and duration ($p>0.05$).

Discussion

Results of the present study support previously reported data on the role of PDT with a systemically applied photosensitizer in the treatment of feline SCC[10-12]. We have shown that PDT using liposomal m-TPHC results in good control of cutaneous squamous cell carcinomas in cats obtaining an overall response rate of 84% with a mean progression free interval of 35 months.

From all prognostic factors analyzed, tumor invasiveness and maximum tumor diameter were the only ones shown to be of significance for treatment response. Larger and more advanced lesions (T1b and T2b) responded less often and

complete and relapsed sooner. Clearly one key element in eradicating tumor cells by PDT is adequate light delivery to all areas of the tumor. Penetration depth of the photo activating light is not sufficient in such large, invasive tumors. In the present study, local control rates for more advanced, invasive tumors were clinically unsatisfactory and PDT should not be recommended as treatment choice.

Among other factors, the success of PDT is further dependent among other factors on the total light dose delivered into the target tissue, and treatment intensity was evaluated for its influence on outcome[13]. No difference in outcome was observed whether tumors were treated with ≤10J/cm^2 or 20J/cm^2, indicating a saturation of photosensitizer-light combination already at the lower intensity. Also tumor location (pinnae/ear base, nasal planum, eyelid/peri-orbital, or other cutaneous sites) did not affect response to PDT ($p>0.05$).

Magne et al.[12] revealed that PDT based on a chlorophyll A derivative photosensitizer HPPH-23 (pyropheophorbide-alpha-hexyl-eter) was effective in early tumor stage (small, non-invasive) whereas the local control rate of large, invasive tumors was unacceptable. In our cohort all cats failed locally the latest within 6 months of PDT if an invasive lesion had affected them. But cats with non-invasive tumors remained free of disease for at least five years once they had achieved complete remission. In the same study of Magne et al. the morbidity associated with the application of HPPH-23 was reported to be high with all cats treated for T1b and T2b lesions showing post-treatment nasal obstruction and dyspnea. Further, anorexia affected approximately half of the treated cats and although easily controlled with antibiotics secondary bacterial infection was another common complication reported. In the present study, PDT with

intravenous liposomal m-THPC was well tolerated by the majority of treated patients. Local reactions following treatment with intravenous m-THPC have to be expected and mainly consist of scab formation and hypersensitivity to light for two weeks. In the population treated herein, these reactions were minimal and are easily managed symptomatically and with owner communication. Only one cat with a large T2b tumor lesion developed stertor and dyspnea due to nasal obstruction and had to be euthanized because of that complication. No other unacceptable side effects were observed in the current study or in the one by Buchholz et al. using a similar treatment protocol[11].

This study performed by Buchholz et al.[11] described an overall response rate of even 100% after systemic liposomal m-THPC. Four out of twenty (20%) treated cats had a recurrence within a median of 5.6 months but no impact of tumor stage was detected. However, number of treated cats was smaller than in our study, which might have limited the power of some analyses.

Although initial response rates were encouraging in the present study, systemic PDT did not lead to durable remission or cure in any of the invasive and large tumors. We propose to treat these advanced tumor stages with external beam radiotherapy which was shown to be a valuable treatment option resulting in long tumor control. The most recent study from our group evaluated an accelerated radiation protocol of 10x4.8 Gy delivered twice daily at least 6hr apart on 5 consecutive days for tumor and treatment associated variables[6]. Of the forty-four treated cats all showed complete response to radiotherapy with a median DFI of 916 days and a one-year DFI of 71%. Again, of all tested variables, also with external-beam radiation therapy, tumor size showed a tendency to influence DFI,

with larger tumors having a greater risk of recurrence than smaller ones. Acute and late toxicities were mild to moderate.

Limitations of the current study are certainly the retrospective nature, the total number of patients treated and the number of cats per stage. Follow-up information was inconsistently gained via direct examination, telephone or mail communication with the owner or the referring veterinarian, what might have resulted in incomplete data. Further, there was a pre-selection of cases as some owners had refused multiple treatment sessions under anesthesia as required for radiotherapy or had refused irradiation because of financial or logistical reasons.

PDT should be considered for treatment of SCC when there are multiple lesions, in areas where surgeries would result in morbidity (for example the eyelid) or diminished cosmetic function and when lesions are known to be non-invasive. The appealing advantages of PDT as opposed to other treatment modalities are a low toxicity profile, no cumulative toxicity after multiple sessions, that it is performed in a single treatment session and that the cosmetic outcome is very rewarding. The main limitation is the inability to treat large, invasive tumors effectively.

Conclusions PDT using liposomal m-THPC leads to excellent long-term tumor control in the majority of cats. However, invasive and large tumors had a clear inferior outcome, even if treated with the higher dose intensity. This suggests that advanced lesions are not indications for photodynamic therapy.

Figures:

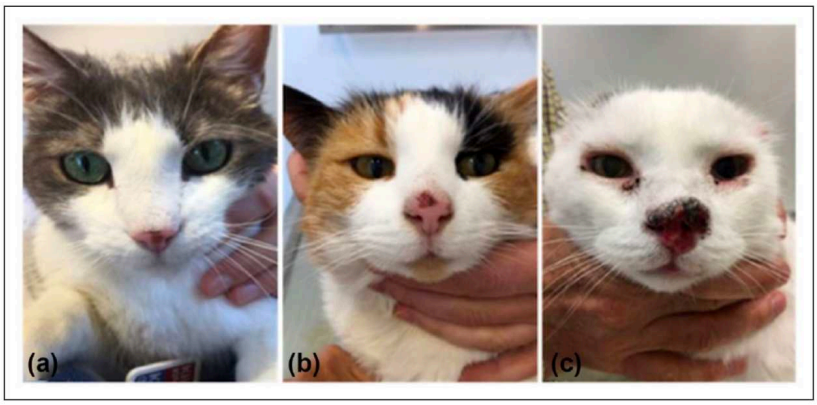

Figure 1 (a) Photograph demonstrating a pre-cancerous, mild dysplasia due to chronic solar exposure (actinic keratosis) involving the nasal planum. (b) Cat with carcinoma in situ and (c) cat with a T2b lesion affecting the nasal planum (>1.5 cm in diameter) and penetrating the deeper skin

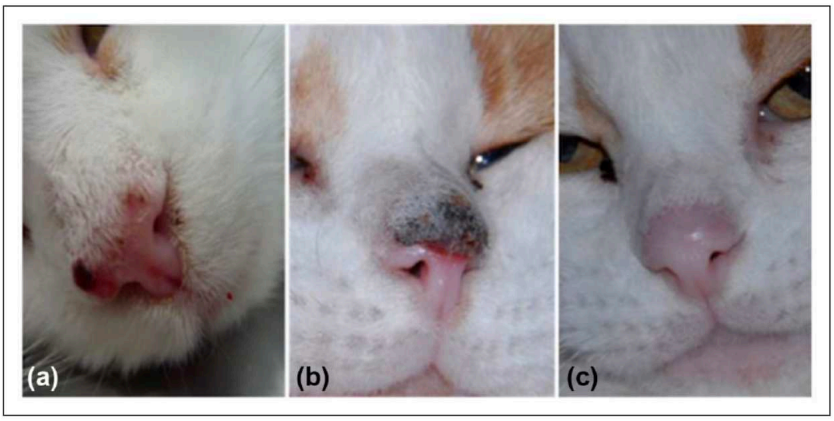

Figure 2 Photograph demonstrating a small carcinoma (T1a) in situ affecting the left and right nasal planum. Same cat (a) before, and (b) 2 weeks and (c) 6 weeks after photodynamic therapy

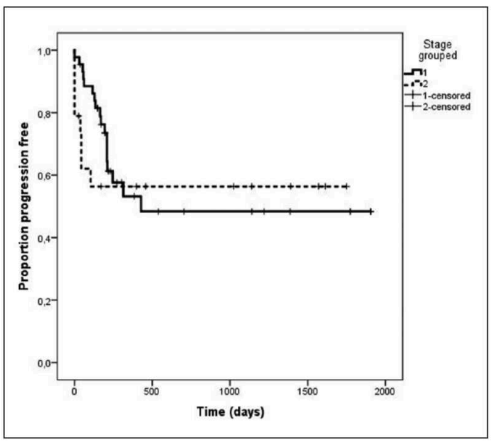

Figure 3 Progression-free survival of all cats

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