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Electroporation Enhances Bleomycin Efficacy in Cats with Periocular Carcinoma and Advanced Squamous Cell Carcinoma of the Head

E.P. Spugnini, M. Pizzuto, M. Filipponi, L. Romani, B. Vincenzi, F. Menicagli, A. Lanza, R. De Girolamo, R. Lomonaco, M. Fanciulli, G. Spriano, and A. Baldi

Background: Advanced carcinoma of the head represents a substantial health problem in cats for local control and overall survival.

Objectives: Evaluate the capability of electrochemotherapy (ECT) to improve bleomycin efficacy in cats with periocular carcinoma and advanced carcinoma of the head.

Animals: Twenty-one cats with periocular carcinoma (17 squamous cell carcinoma [SCC] and 4 anaplastic carcinoma) and 26 cats with advanced SCC of the head.

Methods: Nonrandomized prospective controlled study. Periocular carcinoma cohorts: 12 cats were treated with bleomycin (15 mg/m^2 IV) coupled with ECT under anesthesia; 9 cats were treated with bleomycin alone. Advanced head SCC cohorts: 14 cats were treated with bleomycin (15 mg/m^2 IV) coupled with ECT administered under sedation; 12 control cats were treated with bleomycin alone. ECT treatments (2–8) were performed every other week until complete remission (CR) or tumor progression occurred.

Results: Toxicities were minimal and mostly treated symptomatically. Overall response rate in the ECT treated animals was 89% (21 Complete Response [CR] and 2 Partial Response [PR]) whereas controls had response rate of 33% (4 CR and 3 PR). Median time to progression in ECT group was 30.5 months, whereas in controls it was 3.9 months ($P < .0001$). Median time to progression for ECT cohorts was 24.2 months for periocular cohort and 20.6 in advanced head SCC cohort, respectively.

Conclusions: Electrochemotherapy is well tolerated for advanced SCC of the head in cats; its use may be considered among loco-regional strategies for cancer therapy in sensitive body regions such as periocular region.

Key words: Biphasic Pulses; Carcinoma; Electroporation; Feline.

Companion animals are largely protected from the carcinogenic effects of ultraviolet light by their haircoat and dermal pigmentation. However, animals with pale skin and hair color are at increased risk of solar injury.[1] The 2 most prevalent neoplastic lesions induced by ultraviolet irradiation are cutaneous squamous cell carcinoma (SCC) in dogs and cats, and cutaneous hemangiosarcoma.[1–7]

Cutaneous carcinomas originate from epidermal stem cells that have the potential for self-renewal and multi-lineage differentiation; these cells are located in the hair follicle bulge and the basal layer of the interfollicular epidermis.[8] Depending on the study, cutaneous carcinomas account, for approximately 15–50% of all cutaneous tumors in cats, making it among the 4 most common skin tumors in cats.[2,3] These tumors frequently have an insidious progression characterized by nonhealing scabbing lesions on the eyelids, nasal planum or ears of light-colored cats that tend to progress over time to ulcers. These tumors are difficult to treat in a traditional clinical setting because of their advanced stage at time of presentation. As a result, these tumors that could be handled in their initial stages by surgery, cryotherapy or radiation therapy, require aggressive surgery combined with radiation therapy.[9–13] In patients with advanced disease, aggressive surgery may compromise the cosmetic appearance of the cat.[11] On the other hand, radiation therapy, when delivered to periorbital areas, such as the oral cavity or nose, sometimes has been associated with adverse ocular effects such as conjunctivitis, ulcerative keratitis and keratoconjunctivitis sicca.[14] Electrochemotherapy has been gaining popularity among veterinary oncologists over the past several years and currently is adopted in human oncology as well.[15] It aims at improving the efficacy of chemotherapy agents such as bleomycin or cisplatin by increasing their uptake into tumor cells by the administration of

Abbreviations:

CR	complete remission
ECT	electrochemotherapy
PD	progressive disease
PR	partial remission
SCC	squamous cell carcinoma
SD	stable disease

From the SAFU, Regina Elena Cancer Institute, Rome, Italy (Spugnini, Fanciulli); Centro Veterinario Gianicolense, Rome, Italy (Pizzuto, Menicagli, Lanza, De Girolamo); Centro Veterinario Casal Monastero, Rome, Italy (Filipponi, Romani); Medical Oncology, University Campus Bio-Medico, Rome, Italy (Vincenzi); Ambulatorio Veterinario Dr. Lomonaco, Rome, Italy (Lomonaco); Department of Head and Neck Oncology, Regina Elena Cancer Institute, Rome, Italy (Spriano); and Department of Environmental Biological and Pharmaceutical Sciences and Technologies, Second University of Naples, Naples, Italy (Baldi).

Corresponding author: E.P. Spugnini, SAFU, Regina Elena Cancer Institute, Rome, Italy; e-mail: info@enricospugnini.net.

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permeabilizing electric pulses.[16] This technique has been used successfully in cats to treat nasal planum carcinoma and as an adjuvant for the treatment of incompletely excised soft tissue sarcoma, obtaining a high percentage of responses as well as long term control.[17–20] Its use in the head region has been limited to date both in veterinary and human oncology because of concern for possible adverse effects for the eye and risk of massive tumor lysis.[21] Indeed, tear production could act as a facilitator for the delivery of electric pulses and in some cases result in the re-routing of current flow along the lines of least electrical resistance with potential secondary damage to the lens, the retina or both.[22] Patients also are risk for tumor lysis and fatal complications associated with rapidly shrinking tumor masses after ECT treatments.[21] Finally, larger tumors may have areas of necrosis or extensive fibrosis that could impede smooth electrical transition during treatment. The goals of this study were to compare ECT associated with bleomycin versus bleomycin alone to evaluate for increased drug efficacy and lack of adverse effects (both local and systemic) after the administration of permeabilizing electric pulses in 2 cohorts of cats with periocular carcinomas and advanced SCC of the head.

Materials and Methods

Animals

Twenty-one feral colony cats or cats owned in rural areas with periocular carcinoma (17 squamous cell carcinoma [SCC] and 4 anaplastic carcinoma) were included in the periocular cohort and 26 were enrolled in the advanced head SCC cohort. The tumors in these cohorts were presumed to be UV-induced. Registered volunteer associations provided follow-up and transportation for the cats.

Study Design

The study was a controlled prospective nonrandomized trial matching a currently adopted systemic bleomycin-based protocol versus the same protocol with standard electroporation in cats with periocular carcinoma and advanced SCC of the head.[16]

Inclusion Criteria

Cats were enrolled in the study if they fulfilled the following characteristics: a histopathologically confirmed diagnosis of $\geq T_2$ periocular carcinoma and advanced SCC of the head (Table 1); absence of distant metastases (lymph node metastases were not considered as exclusion factor); compliance of the owner for follow-up re-evaluation; absence of life-threatening diseases and overall performance status assessed according to the modified Karnowsky system of <3.[21] Cats with SCC localized to the oral cavity were not included in this study.

Patient Staging

All patients were staged by complete physical examination. A fine needle aspiration of mandibular lymph nodes, if palpable, was performed. Of the 47 cats enrolled in the study, 28 had both submandibular lymph nodes aspirated; 11 had the right lymph node and 8 had the left lymph node aspirated. Complete blood cell count, biochemistry profile, urinalysis, thoracic radiographs (3 projections) and abdominal ultrasonography also were performed.

Table 1. World Health Organization TNM classification system for feline tumors of epidermal origin.

Stage	Feature
T	
T_0	No evidence of tumor
T_{is}	Tumor *in situ*
T_1	Tumor <2 cm diameter
T_2	Tumor 2–5 cm diameter or minimally invasive
T_3	Tumor >5 cm diameter or with invasion of subcutis
T_4	Tumor invading other structures such as fascia, muscle or bone
N	
N_0	Absence of lymph node metastasis
N_1	Presence of lymph node metastasis
M	
M_0	Absence of distant metastasis
M_1	Presence of distant metastasis

To confirm the diagnosis, histological examination of the biopsy samples was performed following standard protocols, using ematoxylin andeosin and ematoxylin and Van Gieson stains. All patients were treated with bleomycin[a] systemically, ECT was additionally offered to the owners to be performed weekly under general anesthesia until CR or tumor progression was observed.[16] In the periocular cohort, 12 owners elected for their cats to be treated with ECT whereas 9 others declined to pursue further treatment beyond standard iv. chemotherapy because of emotional or financial concerns. The tumor types were distributed as follows: 10 SCC patients and 2 anaplastic carcinoma patients were assigned to bleomycin and ECT treatment and 7 SCC patients and 2 anaplastic carcinoma patients were assigned to bleomycin treatment alone. Similarly, in the advanced head SCC cohort, 14 owners chose bleomycin and ECT, whereas 12 chose bleomycin alone. Both treatments were performed on alternate weeks and continued until CR or tumor progression was observed. However, upon reaching a maximum accumulation dose of 200 mg/m^2 the drug was discontinued. The total dose was exceeded in a single cat upon request of the owner. Complete blood cell count, biochemistry profile and urinalysis were performed during staging and before each therapy. Thoracic radiographs were taken at 1's, 3's, 5's, 7's, 9's, 12's and 18 months after the first IV therapy.

Treatment Procedures

Bleomycin was diluted in 5 mL of saline solution and administered IV at a dosage of 15 mg/m^2.[16] In the ECT group, 5 minutes after the administration of the bleomycin, sequences of 8 biphasic electric pulses lasting 50 + 50 ms each, with a frequency of 1 Hz, and with 1-ms interpulse intervals (total treatment time: 7.1 ms/cm^2 of treated area), were generated by a Chemipulse III portable electroporator (EU patent application number 2221086) and delivered by means of modified caliper electrodes with 1 cm distance between the plates until complete coverage of the lesion and 1 centimeter of margin (Figs 1–3). The pulse repetition frequency was 1 Hz and the frequency of burst repetition was 1 kHz. All of the ECT treatments were administered under general anesthesia using propofol[b] at a dosage of 2 mg/kg after premedication with medetomidine[c] at a dosage of 40 μg/kg and butorphanol[d] at a dosage of 0.1 mg/kg. During the ECT treatments the cats were monitored using an electrocardiogram and a pulse oximeter.

Fig 1. A 12-year-old female spayed cat with palpebral carcinoma. Patient at presentation (**A**), during an electrochemotherapy treatment (**B**) and at the completion of treatment consisting with 6 treatments (**C** and **D**).

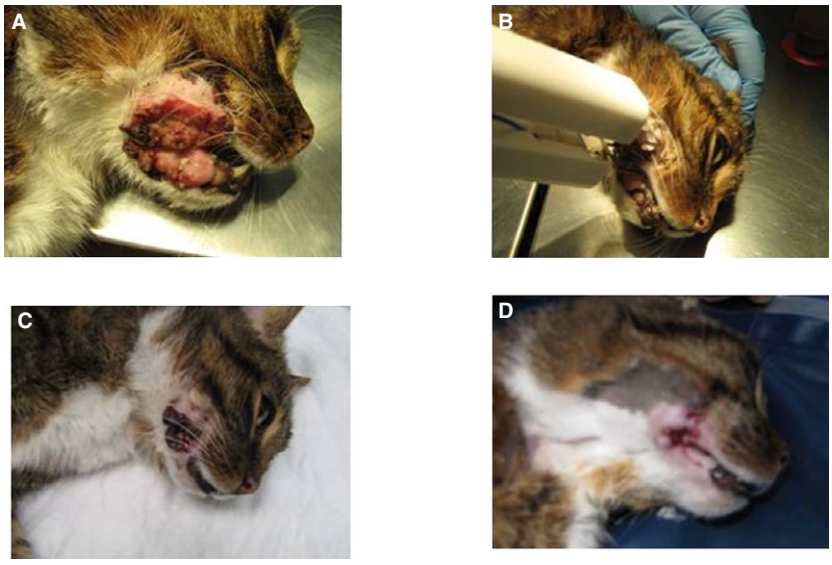

Fig 2. (**A**) Advanced lip and buccal squamous cell carcinoma in a 12-year-old cat at presentation. (**B**) The cat was treated with systemic bleomycin at a dosage of 15 mg/m^2 followed by permeabilizing pulses. (**C**) The cat at the time of the third treatment. (**D**) After 5 treatments, the tumor went on to resolve completely. Adverse effects were limited to scratching.

Tumor Response Evaluation

Tumor measurements were recorded before initiation of treatment and before each treatment, and assessed as previously described.[21] Although the VCOG RECIST system is the current method of tumor measurement in oncology,[23] in this study tumor volume was calculated as (height $\times$ width $\times$ depth) $\times$ (π/6) for consistency with the current human and veterinary ECT literature.[15,16] During the course of treatment, tumor measurements were made using calipers until remission or until relapse occurred and ultrasound examination was performed on large tumors to estimate their depth and margins. A complete remission (CR) was defined as total reduction in measured tumor volume and a partial remission (PR) as $\geq$50% reduction in tumor volume. Stable disease (SD) was defined as <50% reduction in tumor volume or <25% increase in tumor volume, and progressive disease (PD) was defined as $\geq$25% increase in tumor volume. A minimum duration of 2 weeks was required for a response to qualify as positive.

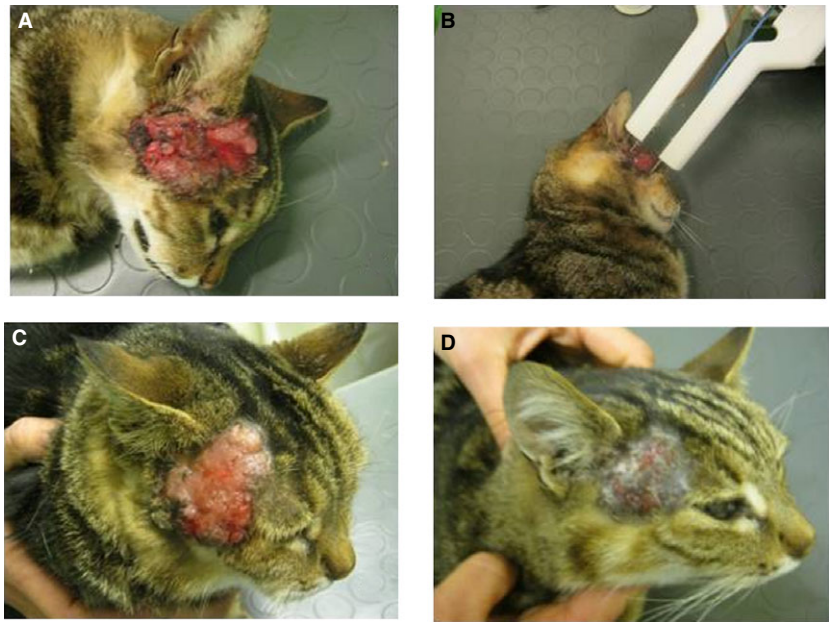

Fig 3. (**A**) Advanced head squamous cell carcinoma in a 13-year-old cat at presentation. (**B**) The cat was treated with systemic bleomycin at a dosage of 15 mg/m^2 followed by permeabilizing pulses. (**C**) The cat at the time of the third treatment. (**D**) After 5 treatments the tumor went on to resolve completely. Adverse effects were limited to scratching.

Treatment Toxicity

Toxicity was defined as pathological events that occurred systemically in the cutaneous tissues within the treatment field or in the eye. Ocular examinations were done by 1 of the authors (MP) at time of inclusion in the study and before every ECT treatment, and included Schirmer tear test, biomicroscopy, fluorescein stain uptake, applanation tonometry and indirect ophthalmoscopy.

Possible adverse ocular effects were categorized as follows: adnexal disease (periocular alopecia, blepharitis or both), conjunctival disease (conjunctivitis), tear production <15 mm/min or other lacrimal disorders, corneal disease (nonulcerative and ulcerative keratitis), lenticular disease (cataract development), uveal disease (iritis), and retinal disease (retinal atrophy). Acute adverse ocular effects were defined as clinical signs detected during or within the first 3 months after ECT treatment. Conversely, late adverse ocular effects were defined as clinical signs detected ≥3 months after the last ECT treatment. Meanwhile, in the cohort of bulky carcinomas, local adverse effects to be evaluated included necrosis, fistula formation, scarring and cheloids, and distant adverse effects included tumor lysis syndrome and thromboembolism.[16–21] Although late toxicities have been described for radiation therapy, this issue has not been clarified to date for ECT both in human and veterinary oncology, especially because limited information is available regarding possible damage inflicted by electric pulses to different ocular regions.[22,24] One concern when working with ECT is the involuntary induction of cardiac arrhythmia and this concern warrants cardiac monitoring during ECT treatment.[25] As a standard procedure, we monitor respiratory function at the same time for possible effects of ECT on anesthesia-induced respiratory depression.

Statistical Analysis

Response to treatment was assessed using the median time to terminal event and its 95% confidence interval. The terminal event was tumor progression, recurrence or death attributable to cancer or other noncancer causes. Time to recurrence was defined as the time from the observation of tumor disappearance and was estimated according to the Kaplan–Meier method. Statistical analysis for the relationship between tumor site (eyelids versus canthus or other cranial locations and T stage was performed using univariate analysis.[26] The statistical significance of the differences in time to recurrence among the prognostic groups was evaluated by the log-rank test.[27] P values <.05 were regarded as significant in 2-tailed tests. Commercial software[e] was used for statistical analysis.

Results

A total of 47 domestic shorthair cats entered the study. In the periocular cohort, 21 cats were entered into the study, 12 received bleomycin with electroporation (7 T_2 stage and 5 T_3 stage) and 9 received bleomycin alone (5 T_2 stage and 4 T_3 stage) (see Table 1 for TNM classification). There were 7 female spayed and 5 male castrated in the ECT group and ages ranged from 9 to 13 years (median, 11.6), whereas in control group (bleomycin alone) there were 5 female spayed and 4 male castrated cats, and ages ranged from 7 to 13 years (median, 11.1). The advanced head SCC cohort had 26 cats. Fourteen cats received bleomycin coupled with electroporation (2 T_2 stage and 12 T_3 stage) and 12 cats received bleomycin alone (5 T_2 stage and 7 T_3 stage). There were 9 female spayed and 5 male castrated cats in the ECT group and ages ranged from 9 to 14 years (median, 11), whereas in control group (bleomycin alone) there were 8 female spayed and 4 male castrated cats, and ages ranged from 7 to 13 years (median, 11.2).

No cat had prior treatment or concurrent medications. The neoplastic lesions, accordingly to the owners, had been present from 9 to 15 months before referral.

Toxicities

Local Toxicities. Cats in the periocular ECT cohort did not show any evidence of corneal, uveal, or retinal damage. Tear production did not change over time, but in 3 cats treatment resulted in epiphora, suspicious for lacrimal duct sclerosis (Figs 1, 4, 5). The cats in the periocular bleomycin cohort did not experience local adverse effects. In the advanced head SCC ECT cohort, despite the use of electroconductive gel, 4 cats had small electrode-induced burns that resulted in 1 cm long, 1 mm wide discolored areas. These lesions tended to disappear within 2–3 weeks as previously reported.[16,18,19] Cutaneous lesions were not reported in the controls. Two cats in the advanced head SCC ECT group had local inflammation involving the deep subcutaneous connective tissues, which led to compulsive scratching. In both cats the inflammation was successfully managed by administration of the non-steroidal anti-inflammatory drug meloxicam at a daily dosage of 0.05 mg/kg for a period of 1 week. All of the cats that underwent ECT experienced transient muscular contractions at the time of treatment, that were more pronounced in those cats with tumors located near nerve roots at the base of the skull. These contractions did not result in apparent pain or discomfort for the cats when they recovered from sedation and did not require symptomatic treatment.

Systemic Toxicities. As previously reported, neither cardiac arrhythmias nor respiratory treatment induced adverse effects were reported.[16–20] In the ECT cohorts, patient oxygen saturation remained stable throughout the procedure. Hematological toxicity was not observed in the 26 cats of the ECT study, except for mild neutropenia on day 7 in 2 cats (grade I accordingly to VCOG-CTAE guidelines). Gastrointestinal and renal toxicities were not detected. Toxicity was not observed in the bleomycin group.

Response to Treatment

The ECT groups had 2–9 treatments (median, 4), whereas the bleomycin group had 2–16 doses of bleomycin (median, 5). In the ECT groups 21/26 cats had CR (81%), 2 had a PR (7.5%) and 3 had PD (11.5%). In the bleomycin groups 4/21 patients achieved CR (19%), 3/21 had PR (14%), 6/21 had SD (29%) and 8/21 had PD (38%). Of the 26 cats enrolled in the ECT studies, 4 died of metastatic carcinoma to the lung after 4–7 months, approximately 1 month after lung metastases were detected, and 2 had tumor recurrence that was successfully retreated with additional ECT (2 and 3 treatments, respectively). The median time to progression for the pooled ECT groups was 30.5 months whereas in the bleomycin groups median time to progression was 3.9 months ($P < .0001$). The median time to progression for the 2 ECT cohorts was 24.2 months for the periocular cohort and 20.6 months for the advanced head SCC cohort respectively. Figures 1–5 show 5 representative cases of response in cats with advanced disease. In the bleomycin group, all of the cats died or were euthanized as a result of tumor progression at different times (range 0–16 months) with a mean time for the periocular cohort of 4.7 months and a mean time for the advanced carcinoma cohort of 3.2 months. In the ECT cohort f4 cats were euthanized because of metastatic disease and 3 because of progres-

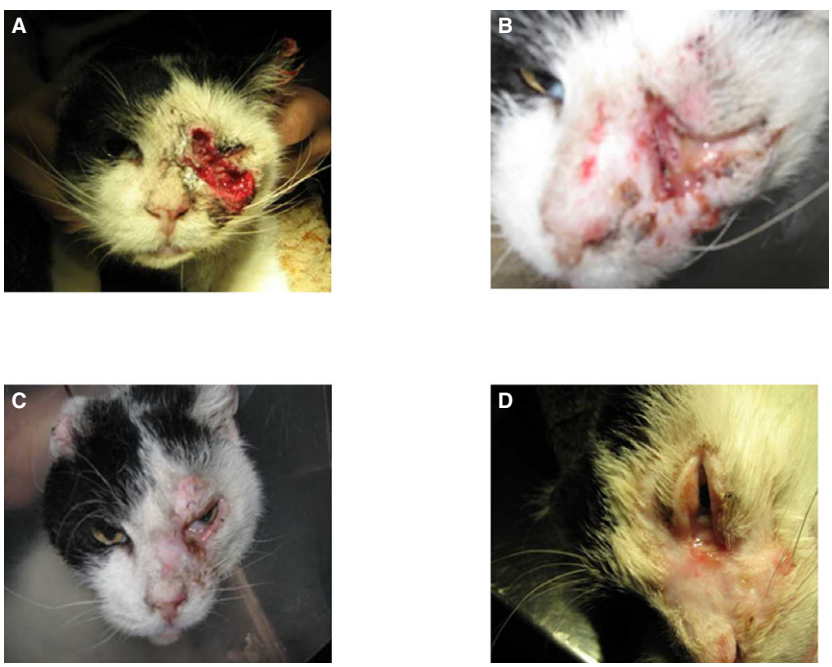

Fig 4. An 11-year-old male castrated cat with advanced periocular carcinoma. Patient at presentation (**A**), after 2 treatments of electrochemotherapy (ECT) (**B**), at the time of the 6th and last ECT (**C**) and at 1 month follow-up (**D**). The patient died of pulmonary metastases 5 months after completion of ECT.

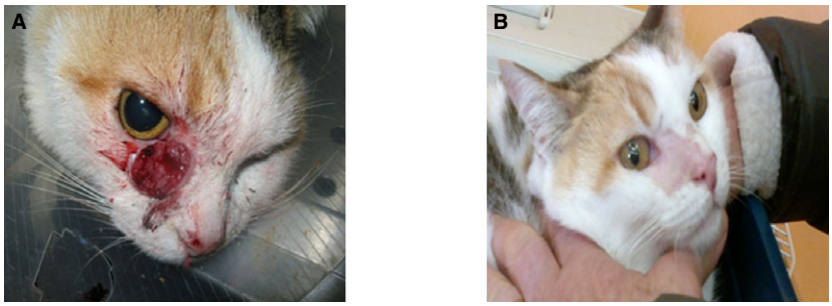

Fig 5. A 10-year-old female spayed cat with carcinoma at the canthus of the eye. Patient at presentation (**A**), and at 1 month follow-up after only 2 treatments of electrochemotherapy (**B**).

sive disease. Statistical analysis failed to identify prognostic factors in the groups and there was no significant difference between the responses of the 2 ECT cohorts. Figure 6 shows the Kaplan–Meier curve of the 2 cohorts of cats (ECT treated versus bleomycin alone).

Discussion

To the best of our knowledge, ours is the first study of the application of biphasic pulse ECT in the treatment of cats with periocular carcinoma and advanced SCC of the head. The only other reports in the veterinary literature describe ECT for the treatment of nasal planum and superficial carcinoma with intralesional or systemic bleomycin.[16,20] ECT was capable of achieving an overall response rate in 89% of the cats with acceptable adverse effects. Many reports have indicated that aggressive surgical excision or radiation therapy are the modalities that offer the highest chance of obtaining local control, but, tumor control and survival are highly dependent on reaching adequate surgical margins or on the degree of response to radiotherapy.[11,13] Surgery often is limited by anatomical and functional considerations because treatment of periocular carcinoma can have major cosmetic and functional effects on the patient. Cats undergoing surgical or radiotherapy management may suffer adverse events ranging from trichiasis and secondary corneal disease to partial or total disfigurement and ocular toxicoses that may result in vision loss.[11,13,14] In terms of efficacy, recent reports on the adoption of radiation therapy for facial and periocular carcinoma in cats reported median disease-free time of 414 days and mean disease-free time of 271 days, respectively.[13,28] Identified prognostic factors for radiation therapy in these studies were low Ki 67 and tumor stage, respectively.[13,28] In our study, ECT greatly improved the efficacy of bleomycin against advanced carcinoma. In fact, in our study, bleomycin alone resulted in inadequate tumor control, whereas ECT successfully induced tumor response in 21/26 cats with carcinoma. In our study, different cohorts of cats affected by solid tumors were treated with bleomycin with or without ECT allowing to clearly demonstrion of the improved efficacy of chemotherapy after the application of permeabilizing electric pulses.

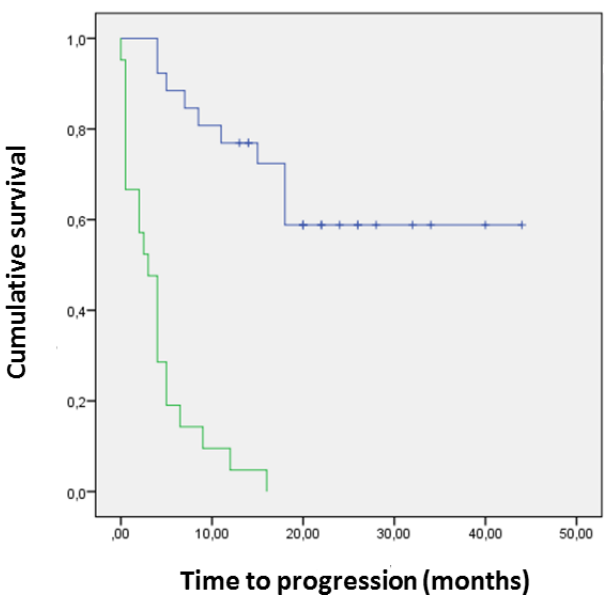

Fig 6. Kaplan–Meier curve for time to progression for 26 cats treated with electrochemotherapy (blue line) and for 21 cats treated with bleomycin alone (green line) for advanced head and periocular carcinoma. The censored cats are those still alive at the time of the analysis.

Bleomycin's mechanism of tumor destruction derives from its ability to induce single- and double- strand DNA cleavage, thus mimicking radiation-induced DNA cleavage.[14] Unfortunately, the efficacy of bleomycin is limited by its complex diffusion through the cell membrane's lipid bilayer because it can only enter the cell through protein membrane carriers, thus resulting in slow and limited quantitative uptake under normal conditions.[16,29] The lack of expression of these protein membrane carriers by tumor cells undergoing chemotherapy is the primary mechanism of cancer escape, making the degree and duration of tumor response unpredictable. On the other hand, *in vitro* and *in vivo* preclinical studies have shown that bleomycin's uptake can be increased 700-fold after electroporation, resulting in massive apoptotic death of cancer cells.[16,29] This effect ultimately results in increased local control after the application of permeabilizing electric pulses, altough its efficacy to counter distant metastases still is limited. Preclinical and clinical

studies had shown a lack of anti- tumor efficacy of electric pulses in the absence of systemic or intra-lesional chemotherapy. Therefore, we did not include a control group treated with electroporation alone.[30] We speculate that this might be a consequence of ECT-induced apoptotic death of tumor cells that might uncover hidden antigens and thus boost the immune response.

The study has some limitations. The relatively low number of cats and lack of randomization were influenced by the willingness of some owners to pursue a more aggressive therapy based on emotional or financial reasons. However, the degree of local control is promising, considering the relatively low cost of treatment and few adverse effects observed in our study. These results make this treatment a potentially affordable alternative to more standardized therapeutic options. Another point to be considered is that 4 ECT-treated animals died of metastatic disease, indicating that additional chemotherapy agents might be beneficial in the treatment of this neoplasm. Additional studies with larger number of patients are warranted to fully elucidate the potential role of ECT for SCC in cats, especially considering the extended duration of local control and the preservation of the normal facial architecture in the ECT groups.

Footnotes

[a] Bleomicina Solfato, Sanofi-aventis, Milan, Italy
[b] Rapinovet, Intervet Italia, Milan, Italy
[c] Domitor, Pfizer Italia, Milan, Italy
[d] Dolorex, Intervet Italia, Milan, Italy
[e] SPSS Chicago, version 10.00. SPSS Inc., Chicago, IL

Acknowledgments

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Conflict of Interest Declaration: EPS and AB are among the applicants of the patent for the Chemipulse III portable electroporator (EU patent application number 2221086).

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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