Adjuvant Electrochemotherapy for the Treatment of Incompletely Resected Canine Mast Cell Tumors

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Abstract. *Background: Electrochemotherapy (ECT) is a novel anticancer therapy that combines the delivery of trains of appropriate waveforms with the local administration of chemotherapy agents. The purpose of this investigation was to assess the adjuvant potentials of ECT for the treatment of incompletely excised mast cell tumors (MCT). Materials and Methods: Twenty-eight privately-owned dogs with incompletely removed MCT were treated with intralesional bleomycin (1.5 IU/cm^2) followed by the application of trains of biphasic pulses (8 pulses, 1300 V/cm, 50 + 50 μs duration, 1 Hz frequency). Results: The overall response rate was 85% with a mean estimated time to recurrence of 52.76±6.5 months (range: 39.99 to 65.54 months, 95% CI). At the time of writing this report, the median survival time was not reached. Three dogs died of metastatic disease that they developed at the same time of local recurrence, one developed multiple cutaneous nodules at different locations and one with recurrence was re-treated and is currently disease-free after 22 months. No major local or systemic toxicities were noted for the duration of the study. Conclusion: ECT is a safe and effective therapy for incompletely excised MCTs in companion animals. Its ease of administration, lack of toxicities and low cost make it an attractive alternative to standard treatments and warrants further investigation.*

Mast cell tumors are among the most commonly diagnosed dermatological neoplasms in the dog, accounting for 7% to 21% of all skin tumors and 11% to 27% of all cutaneous malignancies (1-4). These tumors show a remarkable variability in terms of location, biological behavior and response to treatment. Furthermore, the morbidity associated with these neoplasms is due not only to local invasion or distant metastasis, but also to the release of cytoplasmic granules containing substances, such as histamine, heparin and several poorly-characterized vasoactive substances (4). Mast cell tumor degranulation is a phenomenon that may occur during surgical manipulation or even during palpation performed during physical examination leading to local swelling and edema (Darier's sign), hemorrhagic abnormalities, impaired wound healing or severe hypotension. Several prognostic factors have been identified over the years: histological grade, clinical stage, duration of disease, location, nucleolar organizer regions stained with silver (AgNORs), completeness of excision, proliferation cell nuclear antigen staining (PCNA), breed, DNA ploidy and growth rate (5-11). Mast cell tumors have been reported to have recurrence rates ranging from 22% to 54% after surgical excision (10, 11). Adjuvant treatments included chemotherapy with prednisone, vincristine, vinblastine, lomustine or multi-drug protocols and radiation therapy. The overall response of chemotherapy pooling together complete and partial responses ranged from 28% to 53%, however these treatments carried short lived responses and were frequently associated with severe hematological, gastrointestinal or hepatic toxicity (12-17). Radiation therapy resulted in high response rates and long term remissions; however this modality was associated with acute and late toxicities. Furthermore, the cost of the equipment confines this treatment mostly to research institutions (8, 18). A recent study pointed out the relationship between angiogenesis (scored as microvessel density) and mast cell malignancy, suggesting a potential target for new therapy strategies (19). Electrochemotherapy (ECT) is a novel anticancer treatment that couples the local administration of antiblastic drugs to the delivery of trains of electric pulses having an appropriate waveform (20, 21).

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The application of permeabilizing electric pulses leads to the opening of pores in the cell membrane, thus, resulting in increased uptake of chemotherapeutic drugs, ultimately resulting in cancer apoptotic death. Several articles have described the efficacy of this treatment in companion animals with spontaneous neoplasms (22-25). In this study the efficacy of ECT as adjuvant therapy combined with surgery was evaluated in a spontaneous canine mast cell tumor model.

Materials and Methods

Twenty-eight privately owned dogs presented to the Regina Elena Cancer Institute with histopathologically confirmed, incompletely excised mast cell tumor, were entered in the modified phase II study between October 2001 and November 2005.

Previous informed consent was obtained from the owners. In order to be enrolled in the study, according to the Italian law (116/92) and the guidelines defined by the ethical committee of the National Cancer Institute " Regina Elena" of Rome, Italy, patients, staged according to the World Health Organization (WHO) grading system, were considered eligible if they fulfilled the following criteria:

1) Accessibility of the neoplasm location.
2) Absence of distant metastases.
3) Compliance of the owner for follow-up rechecks.
4) Absence of other life-threatening diseases, such as cardiac disease, renal failure, *etc*.
5) Absence of bone involvement.
6) Overall performance status assessed according to the modified Karnowsky system, less than 3 (23).

Patients were staged through caliper measurement of the neoplasm or of its surgical field, histopathological revision of tumor biopsy, regional lymph nodes fine needle aspiration biopsy, complete blood cell count, chemistry profile, urinalysis, chest radiographs and abdominal ultra-sonography. In order to confirm the diagnoses, histological examination of the biopsies were performed following standard protocols, using Hematoxylin/Eosin, Hematoxylin/Van Gieson and toluidine blue staining.

Canine patients received two sessions of ECT one week apart. During each treatment the tumor bed and 1 cm of normal tissue surrounding the surgical scar were injected with bleomycin at the concentration of 1.5 IU/ml. Five minutes after the injection, trains of biphasic pulses were administered using a *Chemopulse* clinical electroporation equipment, kindly provided by the Centre of Biomedical Engineering of Sofia, BG (21). The standard train was set to 8 pulses of 50 + 50 μs. The pulse repetition frequency was 1 Hz while the frequency of burst repetition was 1 kHz, resulting in a total burst duration of 7.1 ms (23-25).

Five minutes after the injection of the antiblastic agent, sequential bursts of 8 biphasic pulses lasting 50+50 μs were applied at a voltage of 1300 V/cm using modified caliper electrodes. Adherence of the caliper electrodes to the lesions was maximized using an electrophoresis gel.

Treatments were administered after local anesthesia with lidocaine coupled with epinephrine and general anesthesia with pentothal, with prior pre-treatment with medetodimine or association of ketamine and diazepam, following the manufacturers' instructions. During the ECT sessions the patients were checked using cardiac monitor and pulse oxymeter.

Response to treatment and local toxicity were assessed prior the second therapy and every two months thereafter. At that time thoracic radiographs and abdominal ultrasonography were performed to check for spread. Toxicity was defined as disease processes that occurred secondary to changes of the cutaneous tissues within the treatment field. Response to treatment was estimated using the median time to terminal event and its 95% confidence interval. Survival analysis was estimated according to the Kaplan-Meier method (26). The terminal event was recurrence or death attributable to cancer or other non-cancer causes. Statistical analysis tested for relationship between tumor response and site, T stage, prior surgery and duration of symptoms prior to therapy. The statistical significance of the differences in survival distribution among the prognostic groups was evaluated by the log-rank test (27). *P*-value <0.05 was regarded as significant in two tailed tests. SPSS software (version 10.00 SPSS Chicago, USA) was used for statistical analysis.

Results

Local toxicities. Two dogs experienced local edema coupled with mild erythema at the electroporation site that subsided within 30 min. These symptoms were compatible with degranulation of residual mast cells within the surgical field. One dog with aggressive, recurrent mast cell tumor experienced partial wound dehiscence and delayed healing that required a minor surgical debridement. None of the twenty-eight dogs experienced mast cell induced gastrointestinal toxicity or hypotension.

Response to treatment. Individual data of the 28 dogs enrolled in the study are summarized in Table I. The median age at presentation was 8 years (range 2-14 years), there were 14 male intact, 2 male castrated, 6 female and 6 female spayed dogs. Electroporation field ranged from 5 to 400 cm^2 (average 28 cm^2), one patient had two grade III lesions that were considered together for homogeneity purposes. In our cohort boxer dogs are over-represented, probably due to this breed predisposition to develop MCT, as well as its popularity in Italy. Fifteen patients had previous surgery that failed to achieve local control. The overall response rate was 85% with a total of 23 patients that are still in remission at different times from the end of therapy. One dog died of non-cancer-related pathology (leptospirosis) after 14 months from the successful completion of its therapy and was censored in the statistical analysis. Three dogs had both local recurrence and distant spread and were euthanatized shortly thereafter. One dog experienced local recurrence of a mucosal grade II MCT localized on the lower lip and was retreated with a combination of surgery and ECT obtaining a remission that lasted 22+ months. At the time of writing this report the median survival was not reached by our cohort of veterinary patients. Figure 1 shows a Kaplan-Meier survival curve of the treatment receiving population. The estimated mean survival is 52.76±6.5 months (range: 39.99

Table I. *Individual data and response to ECT in 28 dogs with cutaneous mastocytoma.*

Age Breed	Sex	Site	Grade Outcome
3 Boxer	FS	Anus II	In remission
13 Mixed Breed	F	Leg II	In remission
11 Argentinean Dogo	M	Head II	In remission
2 Boxer	M	Trunk II	In remission
12 Dalmatian	M	Head II	Recurrence
13 Pug	M	Leg II	In remission
10 Boxer	M	Trunk III	In remission
6 Argentinean Dogo	MC	Leg III	In remission
13 Mixed Breed	M	Leg III	In remission
6 Boxer	M	Leg II	In remission
6 Schnautzer	F	Leg II	In remission
4 Mixed Breed	M	Leg II	In remission
8 Boxer	M	Leg II	In remission
10 Mixed	F	Digit II	In remission
6 Labrador	F	Leg I	In remission
8 Boxer	M	Leg II	In remission
10 Boxer	M	Anus II	In remission
3 Poodle	F	Trunk II	In remission
2 Boxer	F	Trunk II	In remission
7 Great Pyrenees	F	Digit I	In remission
10 Mixed Breed	FS	Leg I	In remission
6 Boxer	FS	Leg II	In remission
14 Yorkshire	M	Head II	Recurrence, retreated, in remission for 2 months
14 Syberian Husky	M	Leg I	Recurrence, progressive disease
7 Setter	M	Trunk III	In remission
10 Setter	M	Digit II	In remission, dead of leptospirosis
7 Setter	FS	Trunk III	Recurrence, dead of metastases
10 WHWT	FS	Trunk III	Recurrence, dead of metastases

F, female; FS, female spayed; M, male; MC, male castrated; WHWT: West highland white terrier.

to 65.54 months, 95% CI). Interestingly, local control was not influenced by the factors evaluated for significance.

Discussion

To the best of our knowledge, this is the first study of adjuvant ECT in the treatment of cutaneous MCT. ECT was capable of achieving local control in 85% of the patients without side-effects. The choice of combining pulse-mediated chemotherapy with surgical excision is based on the fact that the mast cell tumor is a biologically active neoplasm that can spontaneously release its cytoplasmic vasoactive substances potentially leading to life-threatening conditions (28). Until more data are gathered, MCT greater than 1 cm should not be directly attacked with electric pulses in order to avoid local and systemic complications. Mast cell tumors are aggressive neoplasms that show a limited and short living response to chemotherapy, with reported median survivals ranging from 53 to 154 days (12-

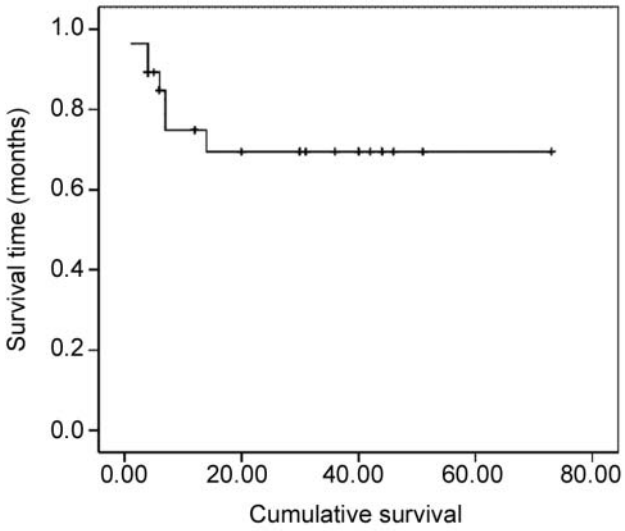

Figure 1. *Kaplan-Meier disease-free survival curve of 28 dogs with cutaneous mastocytoma treated with electrochemotherapy.*

17). Radiation therapy, coupled with wide and deep excision (lateral margins of 2 to 3 cm, deep excision up to one fascial plane deeper than the visible edge of the MCT), is the treatment that carries the best outcome. Median durations of remission after local radiation therapy, regardless of grade or completeness of resection, range from 2 to 125 months (median 60 months) and is strongly dependent on prognostic factors, such as tumor grade, location, T stage *etc.* (9, 18, 29, 30). However, this combined approach might not be pursuable in several anatomic districts, such as the lower extremities, oral cavity, eye canthus, ear canal and anus, due to limited availability of excisable tissue. Furthermore, the acute and late toxicities to the normal tissues within the radiation field may lead to severe morbidity or even to loss of function (4).

In our cohort of canine patients, ECT resulted in long term control without long term consequences. Rate and duration of obtained responses can be favorably compared to that of radiation therapy. It is interesting that bleomycin, the chemotherapeutic agent that is more commonly used for ECT, is not capable to achieve local control without the application of permeabilizing pulses (Spugnini, unpublished). This probably occurs because most MCT lack membrane carrier proteins that are necessary to transport the chemotherapeutic molecules to their site of action. Nevertheless, the coupling with trains of biphasic pulses clearly enhanced the anti-tumor action of bleomycin, as suggested by the two episodes of Darier's sign observed in our dogs.

The preliminary results of this ongoing study suggest that ECT may be useful for high grade MCT tumors located in sites such as the perineum or the head.

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