Brief Communication
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Evaluation of Cisplatin as an Electrochemotherapy Agent for the Treatment of Incompletely Excised Mast Cell Tumors in Dogs

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Background: Electrochemotherapy (ECT) couples the administration of anticancer drugs with the delivery of electric pulses that increase the drug uptake through the cell membranes, resulting in an improved efficacy.

Hypothesis: To evaluate the tolerability and efficacy of cisplatin (CDDP) as an ECT agent to prevent recurrence of incompletely resected mast cell tumors (MCTs).

Animals: Thirty-seven dogs.

Methods: Prospective study recruiting dogs with incompletely excised MCTs as confirmed by surgeon and pathology reports. After debulking, the tumor bed and margins were infiltrated with CDDP, and then exposed to trains of biphasic electrical pulses under sedation. Five minutes after the injection of the chemotherapy agent, sequences of 8 biphasic pulses lasting 50 + 50 μs each, were delivered in bursts of 1,300 V/cm for sclerosed and of 800 V/cm for exposed lesions, with caliper or needle array electrodes, respectively. A second session was performed 1 or 2 weeks later based on clinical considerations.

Results: The treatment was well tolerated with minimal adverse effects. Twenty-nine dogs had no evidence of recurrence over the 6-year study period, 6 had tumor recurrence, 1 died of multiple cutaneous MCTs, and 1 died of unrelated causes. The estimated median time to recurrence was 1,200 days. Recurrence was not observed among the long-term (>1 year) treated dogs.

Conclusions and Clinical Importance: ECT with CDDP appears effective in the treatment of incompletely resected MCT in dogs and could be a useful addition to the current options based on its low cost, limited toxicity, and ease of administration.

Key words: Adjuvant; Biphasic pulses; Chemotherapy; Electroporation; Mastocytoma.

Mast cell tumors (MCTs) are among the most commonly diagnosed dermatological neoplasms in the dog, accounting for 7–21% of all skin tumors and for 11–27% of all cutaneous malignancies.[1] 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 other vasoactive substances.[1] MCTs have recurrence rates ranging from 22 to 54% after surgical excision.[1–3] Adjuvant treatments commonly include chemotherapy with prednisone, vincristine, vin blastine, lomustine, or multidrug protocols, and radiation therapy.[1–5] The rate and duration of response to adjuvant treatment is influenced by the presence of gross or residual disease. For dogs with gross disease, pooling together complete and partial responses, the overall response rates of chemotherapy range from 28 to 53%; however, these treatments carried short-lived responses and were frequently associated with variable degrees of hematologic, gastrointestinal, or hepatic toxicoses.[1,3–5] A higher degree of success has been reported for the treatment of incompletely excised MCTs with adjuvant chemotherapy, obtaining in 1 report a disease-free population of 57% at 1 and 2 years.[4] Radiation therapy resulted in high response rates and long-term remissions; however, the cost of the equipment confines this treatment mostly to referral hospitals.[1,5]

Abbreviations:

CDDP	cisplatin
ECT	electrochemotherapy
MCT	mast cell tumor

Electrochemotherapy (ECT) is an anticancer treatment that couples the local administration of antineoplastic drugs to the delivery of trains of electric pulses having appropriate waveform.[6–8]

The application of electric pulses that render the skin permeable leads to alterations in the cell membrane that increase cellular uptake of chemotherapy drugs, ultimately resulting in cancer apoptotic death.[8] In the past years, our group has tested the efficacy of this treatment in companion animals with spontaneous neoplasms, including melanoma, perianal tumors, transmissible venereal tumors, soft tissue sarcoma, and squamous cell carcinoma.[6–8] In a previous study, a high response rate was observed in dogs with incompletely excised MCTs treated with bleomycin-based ECT.[7] In this study, we evaluated the efficacy of cisplatin (CDDP) as an ECT agent in an adjuvant fashion after incomplete surgical resection of canine MCTs, because it has been already successfully used for ECT in combination with square electric pulses.[9]

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Material and Methods

Thirty-seven privately owned dogs presented to the Regina Elena Cancer Institute with histopathologically confirmed, debulked MCT were entered in the study between September 2002 and November 2008.

Of the 37 dogs, at the time of pre-ECT evaluation, 18 dogs presented with visible gross disease (4 of them were recurrences) and 19 dogs had only a surgical scar. The electroporation field for the dogs with the scar was calculated taking into account the presurgical images of the tumors, the surgical reports, and the interview of the owners. Nevertheless, wherever possible (this could not apply to interdigital lesions, for example), the scar and 1.5 cm of tissue in all directions around the scar were infiltrated.

Informed consent was obtained from the owners. In order to be enrolled in the study, dogs, staged according to the World Health Organization staging system, were considered eligible if they fulfilled the following criteria:

(1) An accessible location of the neoplasm.
(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) Overall performance status assessed according to the modified Karnowsky system of <3.

Dogs were staged through caliper measurement of the neoplasm or of its surgical field, fine-needle aspiration biopsy specimen of regional lymph nodes, complete blood cell count, chemistry profile, urinalysis, chest radiographs, and abdominal ultrasonography.

In order to confirm the diagnoses of MCT, histologic examination of the biopsy specimens was performed following standard protocols, with hematoxylin/eosin, hematoxylin/Van Gieson, and toluidine blue staining.

Dogs received 2 sessions of ECT 1 or 2 weeks apart (5 and 32, respectively) based on clinical considerations (ie, old dogs, dogs with mild renal insufficiency, etc.). During each treatment, the tumor bed and when possible 1.5 cm of normal tissue surrounding the surgical scar were injected with CDDP[a] at a concentration of 0.5 mg/mL. Five minutes after the injection, trains of biphasic pulses were administered with *Chemopulse* clinical electroporation equipment, kindly provided by the Centre of Biomedical Engineering of Sofia, Bulgaria.[6–8] The standard train was set to 8 biphasic pulses of $50 + 50\,\mu s$. 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.

Sequential bursts of 8 biphasic pulses were applied at a voltage of 1,300 V/cm for cutaneous lesions, or 800 V/cm for exposed lesions, with modified caliper electrodes or needle array electrodes. In addition, the tumor bed was pretreated with hyaluronidase[b] to allow a more uniform drug distribution.[6–8] Treatments were administered under general anesthesia induced with propofol[c] after premedication with medetomidine[d] and butorphanol[e] according to the manufacturers' instructions. During the ECT sessions, the dogs were monitored with an electrocardiogram and a pulse oximeter.

Response to treatment and local toxicosis were assessed by physical examination and hematologic analysis after the completion of the 2nd therapy and every 2 months thereafter. At the 2-month evaluations, dogs had complete blood cell count, chemistry profile, urinalysis, thoracic radiographs, and abdominal ultrasonography. Toxicosis was defined as adverse events that occurred in the cutaneous tissues within the treatment field. Response to treatment was assessed using the median time to terminal event and its 95% confidence interval. The terminal event was recurrence or death attributable to cancer or other noncancer causes. Time to recurrence was defined as time from the last ECT treatment and estimated according to the Kaplan-Meier method. Statistical analysis tested for relationship between tumor response and site, T stage, prior surgery, and duration of clinical signs before therapy. The statistical significance of the differences in survival distribution among the prognostic groups was evaluated by the log-rank test. *P*-values $< .05$ were regarded as significant in 2-tailed tests. SPSS software (version 10.00, SPSS, Chicago, IL) was used for statistical analysis.

Results

The breeds represented were 10 Boxers, 13 mixed breed, 2 Argentine Dogos, 2 Labrador Retrievers, 2 Irish Setters, 2 Pugs, 1 Pekingese, 1 Pitbull, 1 French Bulldog, 1 Dalmatian, 1 Giant Schnauzer, and 1 German Shepherd. There were 11 intact females, 11 intact males, 12 spayed female, and 3 castrated male dogs. Age ranged from 2 to 13 years, with a mean age at presentation of 7.6 years and a median age of 8 years. There were 7 grade I, 24 grade II, and 6 grade III MCTs. Fourteen tumors were located in the leg, 12 in the trunk, and 11 in the head/neck. Four dogs had multiple surgical treatments before referral. The treatment field (after debulking), ranged from 4 to 40 cm^2. CDDP dose ranged from 3 to 15 mg with a mean of 4.5 mg and a median dose of 3 mg. The mean dose of hyaluronidase was 175 IU.

Local Toxicoses

Twelve of 37 (32%) had local signs of mast cell degranulation within 10 minutes of ECT consisting of local edema coupled with mild erythema at the electroporation site that subsided within 30 minutes (Fig 1). These signs were compatible with degranulation of residual mast cells within the surgical field. One dog with aggressive, recurrent MCT had partial wound dehiscence and delayed healing that required a minor surgical debridement. None of the 37 dogs had mast-cell-induced gastrointestinal toxicosis or hypotension. Local drug-induced inflammation as per bleomycin's Raynaud phenomenon was not detected.[10]

Systemic Toxicoses

Cardiac arrhythmias were not detected. Dogs' oxygen saturation as measured by pulse oximetry remained stable during the procedure. Hematological and renal toxicoses were not detected.

Response to Treatment

Twenty-nine of 37 (78%) had no evidence of disease recurrence as defined as nodular regrowth within the ECT field during the study period. The median follow-up time was 365 days. The mean time to recurrence was 1,218 days, and the median time to recurrence was 1,200 days (range 190–2,200 days) (Fig 2). No recurrence has been observed in the 23 dogs with a follow-up period in excess of 1 year. Distant metastases were not observed among the dogs enrolled in the study.

The overall recurrence-free rate was 78% with a total of 29 dogs that are still disease free at different times (range 3 months to 6 years) from the end of therapy. One

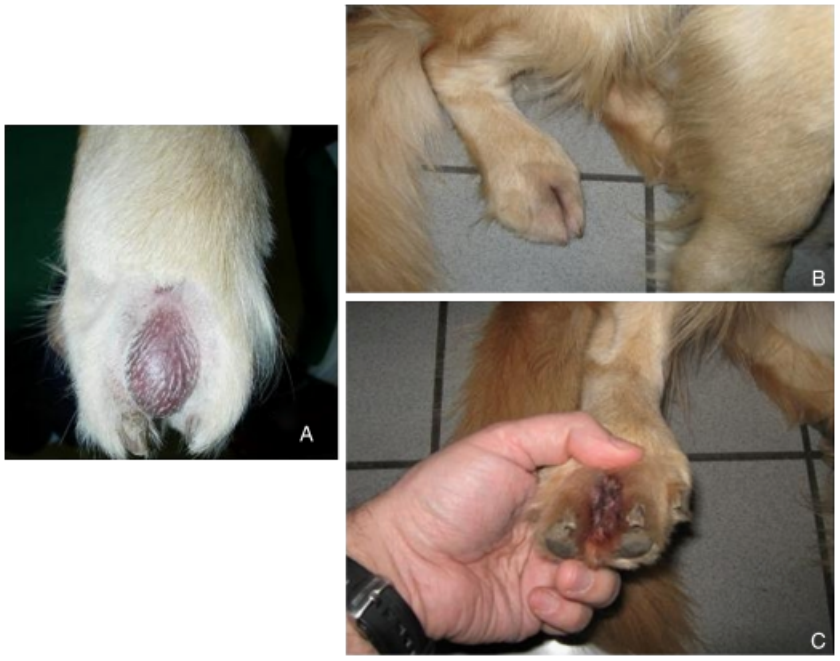

Fig 1. A 3-year-old Labrador with interdigital mast cell tumor at the time of surgery (**A**), at the time of first electrochemotherapy session (**B**), and after the session (**C**). Note the erythema and mild edema after treatment.

dog died of noncancer-related pathology (gastric dilatation volvulus) after 365 days from the successful completion of its therapy and was censored in the statistical analysis. One dog died of multiple cutaneous MCTs after 180 days from the last ECT treatment. Five dogs were euthanized at different times after completion of ECT because of tumor recurrence, and two of them had multiple surgeries before referral for ECT. Two dogs out of 8 were successfully retreated upon recurrence with a combination of surgery and ECT and are in remission after 9 and 15 months after the completion of the second cycle of ECT, respectively (Fig 3).

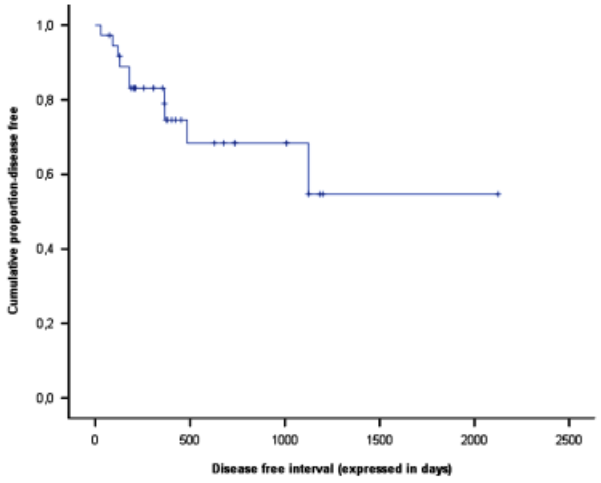

Fig 2. Kaplan-Meier curve for disease-free interval for 37 dogs with mast cell tumor, treated with 2 sessions of cisplatin-based adjuvant electrochemotherapy.

Interestingly, there was no statistically significant effect on local control by any of the factors evaluated (Table 1).

Discussion

ECT was capable of achieving local control in 78% (29/37) of the dogs with minimal adverse effects. Nine dogs with 12 MCTs, treated by other investigators, with ECT not associated with surgery, obtaining a response rate of 77% (7/9) that compared well with the control group receiving surgery as single treatment.[9] A limit of that study was the lack of grading of MCTs in the ECT group, because histologic analyses were not performed and the tumors were diagnosed only by cytology and thus limiting the evaluation of prognostic factors to tumor stage. The choice of combining pulse-mediated chemotherapy with surgical excision in our study lies in the fact that MCTs are biologically active neoplasms that can spontaneously release cytoplasmic vasoactive substances potentially leading to life-threatening conditions.[1] It is our opinion that until more data are gathered that would allow identification of canine MCTs at risk of histamine release upon electroporation, such neoplasms, when of large size, should not be treated with electric pulses as primary therapy in order to avoid local and systemic complications.

MCTs are aggressive neoplasms that exhibit a variable response to chemotherapy.[1,4] Wide and deep excision (lateral margins of 2–3 cm, deep excision up to 1 fascial plane deeper than the visible edge of the MCT), coupled with radiation therapy when margins are insufficient, is the treatment that carries the best outcome. Median remission durations after local radiation therapy, regardless

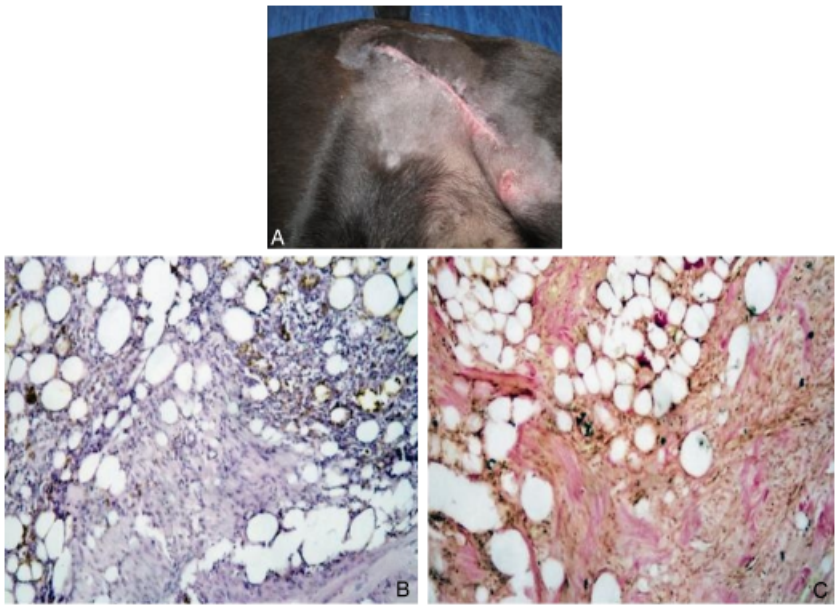

Fig 3. A 5-year-old mixed-breed dog with recurring mast cell tumor along the surgical incision (**A**). The histological appearance of the tumor shows a discrete amount of scar tissue surrounding the tumor cells (**B**, hematoxylin/eosin, original magnification 40×, **C**, Van Gieson, original magnification 40×).

of grade or completeness of resection, range from 2 to 125 months (median 60 months) and are strongly dependent on prognostic factors such as tumor grade, location, T stage, etc.[1,4] However, this combined approach might not be possible in several anatomic regions such as the lower extremities, oral cavity, eye canthus, ear canal, and anus because of limited availability of excisable tissue as was the case in many of the dogs enrolled in our cohort. In addition, acute and late toxicities to normal tissues within the radiation field may lead to severe morbidity or even to loss of function.[1,5]

The rate and duration of disease free intervals obtained in dogs in our study compares favorably to those described after surgery and radiation therapy. Of interest, CDDP, the chemotherapy agent used in combination with ECT, is unable to obtain local control of MCTs without the application of electric pulses. The enhanced effect of CDDP with pulse therapy probably happens because most MCTs are very sensitive to electropermeabilization because of their round shape and to their modest content of connective scaffolding that allows a smoother electrical transition.[6–8] Nevertheless, the coupling of trains of biphasic pulses with local administration of CDDP clearly enhanced the antitumor action of CDDP as suggested by the episodes of Darier's signs observed in our dogs. It is possible, in consideration of the large body of literature attesting to the efficacy of radiation therapy at controlling canine MCT, that ECT could enhance the radiation mimetic properties not only of bleomycin but of CDDP as well.

Incompletely excised MCTs pose a clinical dilemma regarding the necessity of adjunctive local therapies. As reported, local recurrence rates for grade II MCT were 17.3, 22.1, and 33.3% at 1, 2, and 5 years, respectively, raising the possibility that dogs with incomplete resection of grade II MCT may not always need adjunctive treatment for local tumor control.[2] Despite these results, the authors still suggested that adjunctive therapy could be considered for incompletely excised grade II MCT. While we did not have a control population treated with surgery alone, we feel it is appropriate to compare our results to those from papers that report results for any adjunctive therapy for incompletely excised MCTs and use such literature data as a historical control.[4,5] When considering the results of other therapies, the high efficacy shown by *Chemopulse* to deliver anticancer molecules within cancer targets led, in our cohort, to an apparent improvement in local control independently of the above mentioned prognostic factors. Another advantage of our approach, in selected dogs with recurrence, is the ability to perform electroporation a 2nd time, potentially resulting in additional local control without damage to the skin and the underlying tissues. We would like to stress that dogs enrolled for the study had tumors located in anatomical areas difficult to be treated with surgery. This could explain why we do not have a close relationship between tumor grade and recurrence (eg, we

Table 1. Characteristics of the 37 dogs treated with adjuvant ECT.

No. of Dogs for Each Grade[a]	Recurrences	Mean DFI (Days) ± Standard Error[b]
Grade 1 = 7	1	585 ± 86
Grade 2 = 24	6	1242 ± 228
Grade 3 = 6	0	612 ± 259

ECT, electrochemotherapy.
[a]According to Patnaik.
[b]Median value not yet achieved due to low number of events.

did not register recurrences in grade III tumors). Nevertheless, the majority of the tumors analyzed were grade II, thus unbalancing our cohort. Finally, we did not observe in our small cohort of grade III dogs any instances of metastasis. We do not have an explanation for the low rate of metastasis in this group apart from the small number of dogs with grade III tumors. This observation is also in accordance with similar results seen in other potentially highly metastatic tumors treated with ECT.[6–8] The molecular mechanisms responsible of this phenomenon have not been elucidated yet; however, we hypothesized that ECT-induced tumor apoptotic death could stimulate the immune system by uncovering tumor antigens.[6–8]

The preliminary results of this study suggest that ECT could be of benefit for MCT tumors located in difficult areas such as the perineum, the genitals, or the head, but these results need to be confirmed in larger trials that also evaluate different drugs and waveforms.

Footnotes

[a] Cisplatino Ebelwe, Ebelwe, Rome, Italy
[b] Lido-Hyal B, Laboratori Farmacutici Ogna & figli, Milan, Italy
[c] Rapinovet, Intervet Italia, Milan, Italy
[d] Domitor, Pfizer Italia, Milan, Italy
[e] Dolorex, Intervet Italia

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