in vivo *21*: 819-822 (2007)

Adjuvant Electrochemotherapy for the Treatment of Incompletely Excised Spontaneous Canine Sarcomas

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Abstract. *Electrochemotherapy (ECT) is a new therapeutical technique that combines the administration of trains of biphasic pulses with the local application of poorly permeant anticancer molecules, thus obtaining increased chemotherapy uptake. The purpose of this study was to prospectively assess the adjuvant potentialities of ECT for the treatment of different incompletely excised canine sarcomas. Twenty-two privately owned dogs with incomplete surgical excision of high grade sarcomas were treated with bleomycin injected within the tumor bed (1.5 IU/mg) followed by the sequential application of trains of biphasic pulses (8 pulses, 1300 V/cm, 50+50 μs duration, 1 Hz frequency). The overall response rate was 95% (21 out of 22 patients) with a mean time to recurrence of 730 days. At the time of writing 11 dogs were still in remission, three dogs had died of unrelated causes, one had local recurrence and the owner declined further treatment, one had limb amputation following recurrence, four had both local recurrence and distant metastases that led to euthanasia, and two were retreated following tumor recurrence and are disease free at 850 and 1947 days. The only observed toxicity was wound dehiscence in three patients. Electrochemotherapy is well tolerated and has effectiveness against incompletely excised sarcomas in companion animals. Further investigations are warranted to improve the currently available protocols.*

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Key Words: Biphasic pulses, bleomycin, dog, electrochemotherapy, sarcoma.

Soft tissue sarcomas (STS) are a heterogeneous group of neoplasms that are grouped together because of their clinical and histopathological similarities (1-9). STS account for 15% of all canine cutaneous and subcutaneous cancers, with an annual incidence of 35 cases per 100,000 patients at risk (1). These tumors usually appear as pseudoencapsulated soft or firm cancers that generally have poorly defined margins with infiltration of the surrounding tissues (6). As a consequence, conservative surgery usually yields infiltrated margins, thus resulting in tumor recurrence (2, 6, 7). Local recurrence is 10.5 times more likely in dogs with incomplete resection, compared with dogs with complete resection (2). Moreover, STS tend to metastasize through the hematogenous route in up to 20% of cases (1). Prognostic factors identified for this pathology include grade, non curative surgeries, incomplete surgical margins, AgNOR and Ki-67 scores (2, 7). Lesions greater than 5 cm in diameter tend to have a poor response to chemotherapy and radiation therapy (1). Nevertheless, in order to improve the rate of local control, especially in patients having incomplete resection margins, several adjuvant treatments have been proposed: radiation therapy (3), sometimes associated with hyperthermia (8), intracavitary cisplatin (9) and adjuvant chemotherapy (5). In general, it is well accepted that dogs with high grade STS are prone to early recurrence in case of incomplete excision.

Electrochemotherapy (ECT) is a novel anticancer therapy that has been recently developed and adopted to treat cutaneous neoplasms as a single modality of treatment (10-13), or as an adjuvant (14-16) in humans and in pets. ECT couples the administration of antiblastic drugs with the delivery of trains of electric pulses having an appropriate waveform (10, 11). The application of permeabilizing electric pulses leads to perturbation of the

cell membrane that ultimately opens pores in it, resulting in increased cross-flow of material through the cytoplasmic membrane (10-16). Among the other particles moving through the pores, an increased number of poorly permeant chemotherapy molecules enter the cell, ultimately resulting in tumor apoptotic death. Numerous articles report high efficacy of this technique for the treatment of neoplasms of pets (12-16). In this study, the efficacy of adjuvant ECT for the local control of incompletely excised STS was assessed in a spontaneous canine model of sarcoma.

Materials and Methods

Twenty-two privately owned dogs presented to the Regina Elena Cancer Institute with histopathologically confirmed, incompletely excised sarcomas were entered in a modified phase II study between August 1999 and October 2006.

Previous informed consent was obtained from the owners. In order to be enrolled in the study, according to 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: i) accessibility of the neoplasm location; ii) absence of distant metastases; iii) compliance of the owner for follow-up checks; iv) absence of other life-threatening diseases such as cardiac disease and renal failure; v) absence of bone involvement; vi) overall performance status assessed according to the modified Karnowsky system of less than 3 (12).

Patients were staged through caliper measurement of the neoplasm or of its surgical field, histopathological revision of tumor biopsy, regional lymph node fine-needle aspiration biopsy, complete blood cell count, chemistry profile, urinalysis, chest radiographs and abdominal ultrasonography. In order to confirm the diagnoses, histological examinations of the biopsies were performed following standard protocols, using hematoxylin/eosin and hematoxylin/Van Gieson.

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 a 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, Bulgaria (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 (10, 12-16). Hence, 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.

Prior to treatment, local anesthesia with lidocaine coupled with epinephrine and general anesthesia with pentothal after pretreatment with medetodimine or a combination of ketamine and diazepam, following the manufacturers' instructions, were administered. During the ECT sessions, the patients were monitored using a cardiac monitor and pulse oxymeter.

Response to treatment and local toxicity were assessed prior to the second therapy and every two months thereafter. After the second treatment, 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 (17). The terminal event was recurrence or death attributable to cancer or other non-cancer causes. Statistical analysis tested for any relationship between tumor response and site, tumor histotype, 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 (18). A p-value <0.05 was regarded as significant in two-tailed tests. SPSS software (version 10.00 SPSS; Chicago, IL, USA) was used for statistical analysis.

Results

Local toxicities. Three patients with large soft tissue neoplasms experienced wound dehiscence and delayed healing that required further surgical debridement in one patient. There were no systemic toxicities among the 22 enrolled dogs.

Response to treatment. Individual data of the 28 dogs enrolled in the study are summarized in Table I. The median age at presentation was 8.7 years (range 3-13 years), there were 10 male intact, 3 male castrated, 3 female and 6 female spayed dogs. The electroporation field ranged from 6 to 650 cm^2 (average 40 cm^2). The STS were mostly located in the leg (15/22), or in the trunk (5/22) with only one lesion sited at the neck; this is in accordance with the reported incidence of these neoplasms (1). The overall response in our patients was 95%, however there was a significant difference in the duration of remission among the different tumour histotypes. In particular, tumor control was short lived in the two patients affected by hemangiosarcoma (HSA). At the time of writing, 11 patients are still in remission (50%), while two have died of unrelated causes (renal failure and gastric dilatation-volvulus) and were censored in the statistical analysis. One patient died of splenic hemangiosarcoma while still in remission for a cutaneous HSA, two had tumor local recurrence and were retreated with a combination of surgery and ECT experiencing remissions lasting 850+ and 1947+ days, finally 6 dogs had local recurrence and/or distant metastases (see Table I). The mean time to recurrence was 977±187 days (range 609 to 1345 days), while the median time to recurrence was 730 days as shown in Figure 1. Local control was not influenced by the factors evaluated for significance, however one of the factors, the leg location compared to other anatomical sites, was just below significance ($p=0.08$).

Table I. *Individual data and response to ECT in 22 dogs with soft tissue sarcoma.*

Age (years)	Breed	Gender	Type	Site	Response	Outcome
10	Mixed Breed	FS	LipoSA	Thorax	CR 60+	In remission
4	Briard	F	MFH	Flank	CR 120	Recurrence, dead from metastases
10	Mixed Breed	M	FSA	Paw	CR 425+	In remission
9	Jack Russell	M	FSA	Leg	PD	Dead from metastases
10	Boxer	M	HPC	Neck	CR 150	Recurrence
12	Setter	FS	LeiomyoSA	Leg	CR 1461+	In remission
4	Husky	F	HPC	Leg	CR 730	Recurrence, retreated in remission for 1947+
11	Argentinean Dogo	MC	LipoSA	Leg	CR 200	Dead from GDV
8	German Shepherd	FS	FSA	Leg	CR 1765+	In remission
6	Great Pyrenees	M	HPC	Leg	CR 1735+	In remission
5	German Shepherd	M	HSA	Pelvis	CR 30	Recurrence, dead from metastases
10	Newfoundland	M	HSA	Thorax	CR 60	Dead, splenic HSA
12	Mixed Breed	FS	HPC	Leg	CR 365	Dead, renal failure
10	Newfoundland	M	HPC	Leg	CR 1826+	In remission
3	Doberman	F	FSA	Leg	CR 120	Recurrence, amputation
10	German Shepherd	FS	HPC	Leg	CR 1020+	In remission
13	Boxer	M	HPC	Leg	CR 365	Retreated, in remission for 850+
5	Briard	M	NFSA	Pelvis	CR 485+	In remission
7	Great Pyrenees	FS	NFSA	Pelvis	CR 300+	In remission
11	Labrador	MC	HPC	Leg	CR245+	In remission
11	Malinois	MC	FSA	Leg	CR 790+	In remission
10	Boxer	M	HSA	Pelvis	CR 150	Recurrence, dead from disease

F, female; FS female spayed; M, male; MC, male castrated; FSA, fibrosarcoma; GDV, gastric dilatation-volvulus; HPC, hemangiopericytoma; HSA, hemangiosarcoma; LeiomyoSa, leiomyosarcoma; LipoSA, liposarcoma; MFH, malignant fibrous histiocytoma; NFSA, neurofibrosarcoma; CR, complete remission; PD, progressive disease.

Discussion

To the best of our knowledge, this is the first study of adjuvant ECT in dogs with STS. ECT coupled with surgery was capable of achieving complete remission in more than 90% of our patients and obtained local control in excess of 300 days in 12 out of 22 (55%). The choice of combining ECT with surgery was based on our previous experience with pets having large neoplasms (12-16). Soft tissue sarcomas are surrounded by a pseudocapsule that is composed of an internal rim of compressed normal tissue, an outer rim of edema and a more external zone made by newly formed vessels (19, 20). Tumor invasion occurs along the normal tissue planes and tumor digitations often extend through the pseudocapsule. Because of such features, STS can appear to be easily extirpated by conservative surgery, but usually has a high recurrence rate. Sarcomas of the soft tissues carry an unfavorable prognosis and require high doses of radiation, or radical surgery (*i.e.* limb amputation) in order to increase the rate of local control (2-4). When total doses of 45 and 50 Gy were adopted to treat large STS, 1-year control rates of 48% and 62% were reported (21). More recently, median time to recurrence of 1082 days and 798 days have been described using a total dose ranging from 42 to 57 Gy respectively (3, 4). Despite the high reported metastasis rate, the role of adjuvant chemotherapy is still unclear (5), while slow release intracavitary cisplatin enhanced local control in another study (9). Overall, our results of 730 days median time to recurrence compares

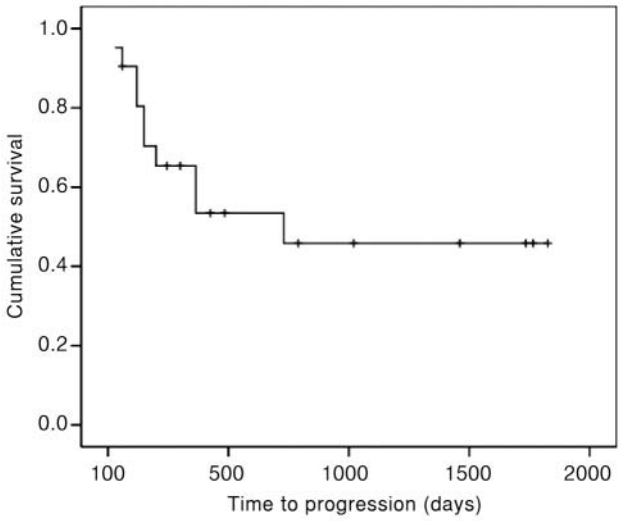

Figure 1. *Kaplan-Meier disease-free survival curve of 22 dogs with soft tissue sarcomas treated with electrochemotherapy.*

favorably with the results of other studies adopting different strategies for the local control of incompletely excised sarcoma in canine patients. These results are promising in consideration of the margins of improvement that are conceivable for ECT in the coming years, both in terms of equipment as well as of novel protocols. The ease of administration of ECT, its efficacy, lack of systemic toxicity and its low cost compared to other therapies make this novel technique very appealing to the veterinary oncologist. Further studies in this field might be valuable in speeding its transition to the treatment of humans as well.

Acknowledgements

This work was supported by "Grant 2005" of the Italian Ministry of Health to E.P.S and G.C., and by a FUTURA-onlus Grant and a Second University of Naples Grant to A.B. We thank the International Society for the Study of Comparative Oncology (ISSCO), Silver Spring, MD 1215, U.S.A. for its continuous support.

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Received March 12, 2007
Revised May 11, 2007
Accepted May 22, 2007