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ORIGINAL ARTICLE

Intraoperative versus postoperative electrochemotherapy in high grade soft tissue sarcomas: a preliminary study in a spontaneous feline model

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Abstract Feline soft tissue sarcomas are spontaneous, rapidly growing, and aggressive neoplasms that mimic their human counterpart. The purpose of this study was to evaluate the feasibility and efficacy of electrochemotherapy (ECT) in an adjuvant fashion for the treatment of feline sarcomas, and the possibility of repeated treatments in the case of recurrence. Cats with fibrosarcoma (FSA) were assigned to receive surgery or surgery plus ECT. Feline patients recruited in the ECT study were enrolled in a microscopic arm (39 patients) or a macroscopic arm (19 patients) on the basis of their tumor status (absence or presence of gross disease). Patients received local injection of bleomycin followed by bursts of eight biphasic pulses at a voltage of 1,300 V/cm for postoperative and of 800 V/cm for intraoperative treatments. The median time to recurrence was 4 months for cats treated with surgery alone, 19 months for the postoperative cohort, and 12 months for the intraoperative group. Moreover, ten patients with recurring neoplasms were retreated and experienced responses lasting 6 to 28+ months. Side effects were minimal. Of interest, the metastatic rate (1.7%) in our patients was negligible: only one cat had distant spread. The results suggest that ECT is a well tolerated and potentially useful addition to surgery in controlling high-grade sarcomas. On the basis of these results, additional evaluations are warranted in pets and in humans.

Keywords Adjuvant · Bleomycin · Cat · Electrochemotherapy · Sarcoma

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Introduction

Local management of soft tissue sarcoma (STS) in humans generally involves multimodality approaches whose cornerstones are surgery combined with radiation therapy [10]. The usual radiation protocols are based on preoperative, intraoperative, or postoperative external beam treatment or adjuvant brachytherapy [19]. The aim of these strategies is to maximize tumor control while minimizing side effects, especially in the case of limb sarcomas. For these reasons, low dose external beam fractionation is usually preferred; however in the case of large neoplasms that involve deep underlying structures, preoperative radiation therapy might be chosen [14]. Unfortunately, the rate of local wound complication associated with aggressive surgical management and radiation therapy is still elevated [2].

Recent publications advocated a trend toward increased disease-free interval and survival in patients receiving chemotherapy within a multimodality treatment as a result of improved systemic and intrarterial chemotherapy [4]. Our report describes a novel adjuvant strategy to STS based on the combination of chemotherapy with trains of biphasic electric pulses in a spontaneous feline model. Naturally occurring STS in cats is very similar to human invasive STS in histopathological characteristics, biological behavior and response to therapy [3, 5, 7, 12].

Electrochemotherapy (ECT) is a new approach to solid neoplasms that associates the administration of a chemotherapy agent with the application of square or biphasic electric pulses (EP) so as to increase the uptake of drug by the cancer cells [1].

On the basis of the promising results obtained in previous studies performed in companion animals with a broad spectrum of neoplasms [16–18], the authors decided to evaluate adjuvant ECT in a spontaneous feline model. Purpose of this study was to evaluate the efficacy and toxicity of adjuvant ECT in cats with incompletely excised or recurring sarcomas and to evaluate the possibility of a repeated protocol (surgery plus ECT) in the case of treatment failure.

Materials and methods

Patients selection

The Regina Elena Cancer Institute Ethical Committee approved this study that was performed according to the Italian Law (116/92). Seventy-two privately owned cats with histopathologically confirmed incompletely excised or recurring STS were entered in the modified phase II study. Evidence of recurrence was demonstrated upon the diagnostic histopathology analysis of bioptic specimens excised by the referring veterinarians.

Previous informed consent was obtained from the owners. In order to be enrolled in the study, patients had to fulfill 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.
5. Overall performance status assessed according to the modified Karnowsky system, had to be less than 3 [18].

Staging process included a thorough anamnesis, histopathologic assessment of surgical margins, physical examination, complete blood cell count (CBC), serum biochemistry profile, and thoracic radiographs (three projections). In order to confirm the diagnoses, histological examination of the biopsies was performed following standard protocols, using Hematoxylin/Eosin and Hematoxylin/Van Gieson stainings.

Patients with cancer located in the flank or abdominal wall had also an ultrasonographic evaluation. Patients with gross disease were randomized to receive surgery or surgery plus pulse-mediated chemotherapy; otherwise they were enrolled in the microscopic arm following tumor resection by their referring veterinarians, as per preoperative or intraoperative radiation protocols [19].

Random assignment of the eligible patients to either the control arm or the experimental one was carried out centrally by an independent randomization service at the Regina Elena Cancer Institute.

Of the 14 cats assigned to the surgery group 2 were stage T2N0M0, 9 were stage T3N0M0, and 3 were T4N0M0. Four of them had recurring tumors. Of the 19 cats enrolled in the intraoperative group, 4 were stage T2N0M0, 13 were stage T3N0M0, and 2 were T4N0M0. Only two cats had recurring tumors. The cats receiving postoperative ECT were staged as follows: 6 T2N0M0, 30 T3N0M0, and 3 T4N0M0.

Treatment

Surgical procedures were carried out by two of the authors (FB and CB), following the recommendations available in the literature [7].

Cats were entered in the ECT arms of the study on the basis of their tumor status. The macroscopic arm received the first ECT at the time of surgical reduction. Briefly, the tumor's bed and the margins for 1 cm in all directions were infiltrated with bleomycin[1] at a concentration of 1.5 IU/ml [18]. Before the local chemotherapy, multiple injections of hyaluronidase[2] (average dose: 300 IU) were performed to ensure a more uniform drug distribution through the ground substance as suggested by the literature [8] and confirmed by one of the authors observations (Spugnini EP, unpublished). Five minutes after the infiltration of the antiblastic agent, trains of eight biphasic electric pulses (EP) lasting 50 + 50 μs each, with 1 ms interpulse intervals, were delivered through needle array electrodes [18]. The EP (800 V/cm) were generated by a *Chemopulse* device kindly provided by the Center of Bioengineer-

[1] Bleomicina solfato, 15 UI vial; Aventis Pharma S.p.A., Milano, Italy.

[2] Jaluran, Pfizer Italia S.p.A., Latina/Roma, Italy.

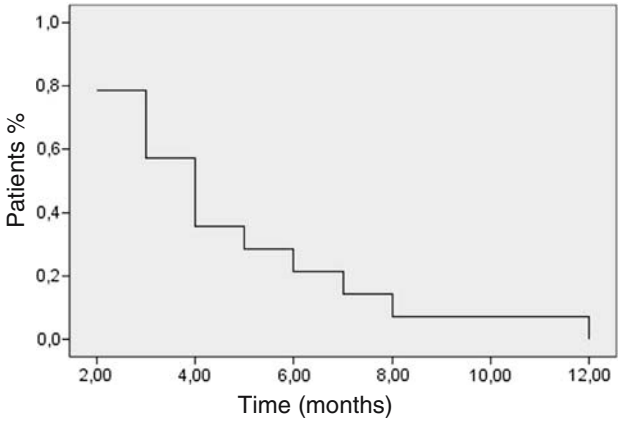

Fig. 1 Kaplan–Meier disease-free survival curve of 14 cats with soft tissue sarcoma treated with surgery

ing of Sofia [16]. Within the *Chemopulse*, a toroidal core transformer allows to generate rectangular pulses. The primary winding is split into two, allowing the generation of biphasic pulses by sequentially driving the two halves. Finally, the pulses are not singularly delivered, but are driven as a burst. For the intraoperative ECT, a six-needle array flexible electrode with a plastic handle was adopted. The characteristics of the electrode have been elsewhere described [17]; briefly, the needle length is 28 mm and the array diameter is 25 mm. The electrode comes with six movable disks (4 mm thickness) that allow modulation of needle insertion. The needles were inserted so as to overlap the electroporing fields within the tumor bed.

One week after the first session the patients received a second treatment using caliper electrodes; in this case, EP were delivered at a voltage of 1,300 V/cm on the basis of previous experience [16–18]. These electrodes come as a modified monolateral compass electrode made of steel, bachelite, plastic with perforated metal plates. Dimensions are (length × height × width): 22 × 10 × 1 mm. The validation of this electrode for electroporation has been extensively described [16–18].

Patients in the microscopic arm received two rounds of ECT 1 week apart at a voltage of 1,300 V/cm delivered through caliper electrodes, beginning 1 week after tumor excision. The surgical suture and 3 cm of apparently normal tissue (up to 1.5 cm depth) were injected with bleomycin. Patients in both arms received two additional sessions if the tumor/surgical escar extension was greater than 50 cm^2. All the intraoperatory treatments were administered while the patients were under general anesthesia with halothane, while the postoperative ECTs were given after sedation with medetodimine and ketamine following the manufacturers' instruction. During the ECT sessions the patients were checked using cardiac monitor and pulse oxymeter. Endpoint was the time of tumor recurrence; however at that time a second course of surgery and ECT was offered to the owner.

Follow-up evaluations

A CBC, BUN, creatinine, and urinalysis were performed prior and 1 week after the second dose of bleomycin. A monthly recheck was scheduled after the completion of the treatment. Thoracic radiographs were taken on a 3 months base until a 1 year follow-up was reached. Recurrence was proved by cytopathology or histopathology.

Endpoints

Primary endpoint was the presence or absence of wound complications secondary to ECT, defined as a second operation performed under anesthesia for debridement, placement of a drainage or secondary wound closure by means of rotationplasty or skin grafts or wound management without additional surgery. Wound management involved aspiration of seroma,

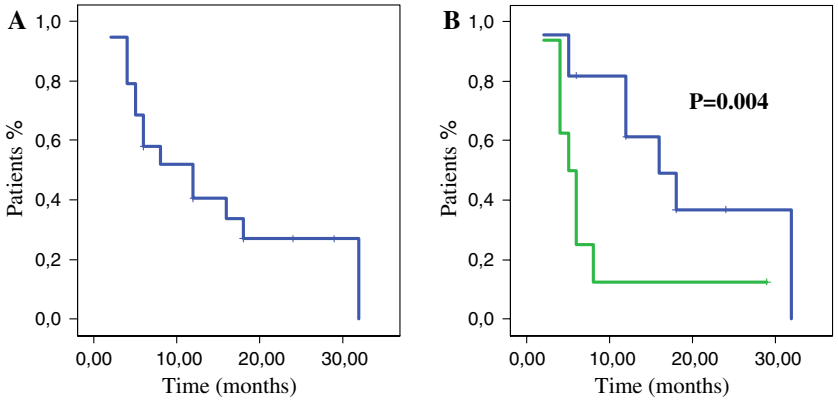

Fig. 2 **a** Kaplan–Meier disease-free survival curve of 19 cats with soft tissue sarcoma treated with intraoperative electrochemotherapy. **b** Kaplan–Meier showing the prognostic significance of tumor size on the disease-free survival curve of 19 cats with soft tissue sarcoma treated with intraoperative electrochemotherapy

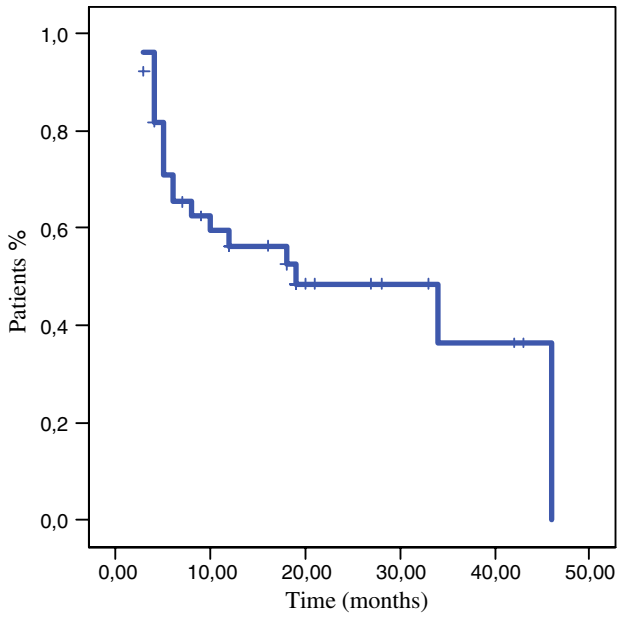

Fig. 3 Kaplan–Meier disease-free survival curve of 39 cats with soft tissue sarcoma treated with postoperative electrochemotherapy

long-term administration of antibiotics (in excess of 2 weeks) or persistent deep packing for a month or longer.

Secondary endpoints were evaluated in all eligible patients and included local control, overall survival and treatment failure due to metastatic spread. Skin toxicity was assessed using a modified scale for radiation-induced toxicity.

Statistical analysis

A univariate survival analysis for each prognostic variable on overall survival was estimated according to the Kaplan–Meier method [6]. The terminal event was death attributable to cancer or noncancer causes. The statistical significance of the differences in survival distribution among the prognostic groups was evaluated by the log-rank test [11].

All the prognostic variables were used for the survival analysis as dichotomized variables. In particular, tumor size was dichotomized using the median value as the cut-off.

P values < 0.05 was regarded as statistical significant in two-tailed tests. SPSS software (version 11.5, SPSS, Chicago) was used for the statistical analysis.

Results

Toxicities

Hematological, hepatic, or gatrointestinal toxicosis were not noted among the 58 patients of the ECT study.

Muscular All the patients experienced transient muscular contractions at the time of the treatment which were more pronounced in those cats whose tumor was located nearby nerve roots. These contractions did not result in pain or discomfort for the patients when they recovered from the sedation and did not require additional therapy.

Cardiorespiratory Neither cardiac arrythmia nor hypoxia were detected during the study.

Cutaneous Despite the use of electroconductive gel, five patients had small electrode-induced burns which exited in 1 cm long, 1 mm wide discolored scars. These scars tended to disappear within 2–3 weeks. One patient developed a circular (1.5 cm width) area of alopecia at the site of electroporation.

Soft tissue One patient experienced a focal necrosis at the treatment site which required surgical debride-

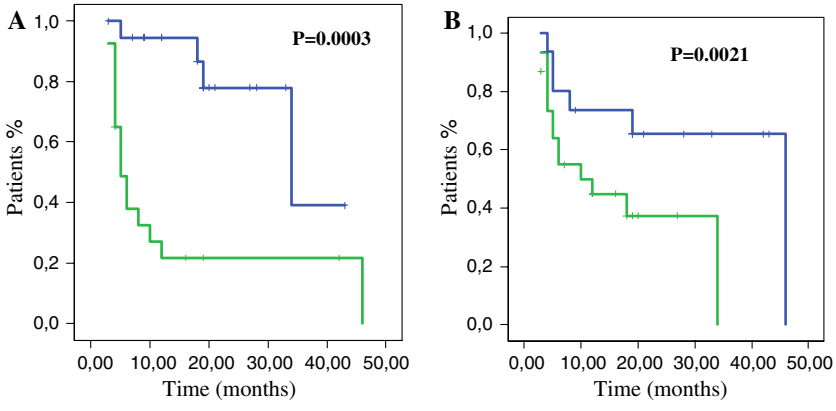

Fig. 4 **a** Kaplan–Meier showing the prognostic significance of previous treatment on the disease-free survival curve of 39 cats with soft tissue sarcoma treated with postoperative electrochemotherapy. **b** Kaplan–Meier showing the prognostic significance of tumor size on the disease-free survival curve of 39 cats with soft tissue sarcoma treated with postoperative electrochemotherapy

ment, two cats had a local inflammation involving the deep subcutaneous connective tissues which led to wound dehiscence, and a fourth cat had a subcutaneous inflammation which spontaneously healed after 6 weeks.

Renal Two patients died of renal insufficiency during the follow-up of the study: one patient developed a renal infection which exited in an irreversible kidney failure, the other patient was a 14-year-old DSH with signs of renal disease (elevated BUN and creatinine) which worsened and caused his death 1 year after the completion of his therapy. Apparently these events were correlated neither with the ECT nor with the tumor recurrence.

Efficacy

Surgical arm 14/14 had recurrence of the tumor at different times. Median time to recurrence was 4 months (Fig. 1); mean time to recurrence was 4.6 months (range 2–12 months). Due to the aggressive nature of the tumors, the owner of these cats declined further treatments at the time of local recurrence.

ECT (macroscopic arm) 12/19 (63%) had recurrence of the tumor at different times; one patient experienced also metastatic disease. Median time to recurrence was 12 months (Fig. 2a). Of all the prognostic factors, only tumor size carried prognostic significance ($P = 0.004$) as shown in Fig. 2b: cats with lesions smaller than 25 cm^2 had a longer control time than cats with tumor beds greater than 25 cm^2 (median 16 and 5 months, respectively). Previous treatment was not associated with outcome ($P = 0.58$) in this cohort; however the number of pretreated patients was low. Three patients with recurring disease were eligible to further ECT treatment. The cats were retreated with surgery and two additional rounds of ECT that ultimately resulted in further remissions of 6, 14 and 14+ months.

ECT (microscopic arm) 18/39 (46%) had tumor recurrence. Median time to recurrence was 19 months (Fig. 3). As shown in Fig. 4a, cats that had no previous treatment fared much better than pretreated patients (median 33 and 5 months, respectively). Finally, cats with tumor fields smaller than 10 cm^2 had a longer response (46 months) than those having larger areas (10 months) (Fig. 4b). Out of 18 cats that experienced recurrence, 7 were retreated: two patients developed another primary neoplasm (appendicular osteosarcoma and intestinal lymphoma) that caused their death after 10 and 22 months respectively. All the remaining five patients are still in remission at 2, 12, 24, 26, and 28 months after the completion of the second course of ECT. Finally, two cats developed renal failure and were censored in the survival analysis.

Discussion

The goal of combined approaches (surgery and radiation ± chemotherapy) in STS is to achieve local control with the lowest possible side effects. This study demonstrates the usefulness of adjuvant ECT in controlling high-grade sarcoma even in the case of heavily pretreated patients and compares favorably with other modalities adopted to eradicate this tumor in cats. In fact, our cohort of patients treated with surgery had a median time to recurrence of 4 months which is comparable with data from the literature claiming median times to recurrence ranging from 60 to 270 days [5]. Radiation therapy has been reported to extend the disease-free interval up to 398 days; however the cost of the equipment confines this modality to referral institutions [3]. The role of chemotherapy is still controversial since it prolonged the disease-free interval from 93 to 388 days in one paper [12] and carried no advantage compared to surgery in another [7]. Interestingly, ECT is associated with a low percentage of local complications (6.9%) that, however, are mostly self-limiting. A possible explanation of the efficacy of our treatment may lay in the peculiar ways through which bleomycin exerts its anticancer action. This drug can penetrate the cell membrane only through protein receptors due to its lipophobic nature, thus resulting in a slow and quantitatively limited uptake under normal conditions [13]. Following internalization by the cell, this drug induces single and double strand DNA breaks that can ultimately lead to cell death [13]. However, the addition of electric pulses can increase its cytotoxicity by several orders of magnitude, at least in cell lines [1]. Moreover, it should be highlighted the effectiveness of a short burst of biphasic pulses in comparison with monophasic EP, thanks to an extremely proficient drug delivery system. In fact, the particular arrangement of neoplastic cells, caused by the lack of size and shape homogeneity within cancers and by their frequently different orientations with respect to the field polarity, create a peculiarly difficult environment for tumor targeting [18]. As described, both these parameters usually allow an increased permeability transition when biphasic pulses are used [16, 18]. So far, only one study of ECT for feline sarcomas has been reported in the literature [9]. In that clinical

trial, cats with fibrosarcoma (FSA) treated with systemic bleomycin and square EP were involved. While tumor control seemed not to be achieved by the treatment, the authors described a prolonged survival in 12 cats receiving ECT versus 11 untreated controls. It needs to be stated that, unlike the tumors in that study, none of the neoplasms treated in our cohorts had previously received radiation therapy. This fact might partially account for the increased success rate we reported, since resistance to radiation therapy through increased DNA repair is one of the described mechanisms of resistance to bleomycin as well, at least in cell lines [16]. Moreover, in our study ECT had to deal with residual rather than bulky disease; as a consequence, there was a lower number of cancer cells to be eradicated. Therefore, in our paper the treatment efficacy was evaluated by measuring the time from therapy to tumor recurrence. Since all the ECT treatments were combined with surgery, in this paper was not possible to assess if ECT could induce stable disease. However, these data have been obtained in previous studies [16–18].

The authors believe that the major reason for the improved therapeutic efficacy of their therapy could be explained by the removal of significant amount of cancer-associated connective tissue. It has been suggested by studies on electromobility of plasmid DNA in tumor tissues that the tumor collagen content was the major obstacle to the mobility of the construct; this is likely to apply to drug molecules as well [8, 20]. Finally, a previous experience with hyaluronidase as an antidote to chemotherapy extravasation in companion animals [15], suggested its use to digest the connective tissue prior to the pulse delivery, further decreasing the amount of ground substance within the infiltrated tissues, thus allowing a more uniform and more efficacious drug delivery. Furthermore, the peculiar destruction of cancer cells by means of apoptotic death may lead to the recruitment of the immune system following the uncovering of deep tumor antigens, resulting in extended control [18]. As reported in humans, tumor size carried prognostic significance together with the exposure of the cancer to previous treatments. A surprising finding in our study was the extremely low incidence of pulmonary metastases experienced by our feline patients in contrast with the data from current literature. Among the possible explanations for this event, we considered a different behavior of this neoplasm in our population; however, a recently published work from another group of investigators reported an incidence of metastases similar to that of American groups [7]. We hypothesize that early ECT might decrease the number of cancer cells released during surgery, helping to sterilize the tumor bed thus preventing metastatic spread. There is some evidence that ECT performs a selection among the different tumor subpopulations [18], in fact in two of our patients retreated for local failure, the tumor recurred as a less aggressive histotype: a neurofibroma-like lesion rather than an high-grade sarcoma. The clinical outcome of both patients was very favorable since they are still in remission in excess of 2 years.

In conclusion, adjuvant ECT is a well tolerated and efficacious addition to oncological surgery and warrants further investigations on new drugs and improved delivery systems in companion animals and in humans as well.

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