Electrochemotherapy in Veterinary Oncology

State-of-the-Art and Perspectives

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KEYWORDS

• Biphasic pulses • Bleomycin • Carcinoma • Cisplatin • Electroporation
• Mast cell tumor • Pets • Sarcoma

KEY POINTS

- The cell membrane is the major obstacle to be overcome by chemotherapy agents in order to reach their biological targets. This is especially true for lipophobic agents like bleomycin.
- Electroporation is a technique that greatly increases the uptake of such drugs by tumors. The combination of permeabilizing pulses and chemotherapy is called electrochemotherapy (ECT).
- ECT has been successfully used in combination with bleomycin and cisplatin to treat solid tumors such as carcinoma, sarcoma, and hematologic malignancies such as mast cell tumor.
- Novel applications include the treatment of visceral tumors under ultrasonographic guidance and the delivery of molecular compounds such as oligonucleotides, plasmids, and small proteins.

INTRODUCTION

Achieving local tumor control in veterinary patients with cancer affected by solid neoplasms represents one of the major challenges for veterinary oncologists, frequently due to late referrals or rapid tumor growth.[1–4] This clinical presentation often prevents the achievement of local control with surgery alone, needing a multimodality approach involving adjuvant therapies, such as chemotherapy, radiotherapy, or electrochemotherapy (ECT).[1–5]

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Chemotherapy can be adopted as a strategy to reduce the tumor volume, thus permitting a wide excision, or can be used, in selected histotypes, as a tool to decrease the probability of local recurrence after surgery.[3] Finally, its major application is to reduce the chances of distant dissemination.[3] Radiation therapy is the cornerstone of combined approach to solid tumors, usually as an adjuvant therapy or as palliation for inoperable neoplasms.[1–3] ECT has been extensively investigated over the past 15 years as an additional treatment modality for local control of solid neoplasms, evidencing high rates of responses with limited side effects.[5–8] It involves the combination of chemotherapy agents (mostly lipophobic molecules) with the application of permeabilizing electric pulses that promote the uptake of these drugs by cancer cells.[5–8] This therapy is rapidly becoming popular among the veterinary community because of its favorable characteristics: ease of administration, effectiveness, low morbidity, and relative inexpensiveness.[5–8]

TECHNICAL ASPECTS OF ELECTROCHEMOTHERAPY

Electroporation is the creation of aqueous pathways (electropores) in the cell membrane following the exposition to short intensive electric fields having appropriate waveforms. This temporary permeabilization permits free transit of molecules, ions, and water between the 2 sides of the cell membrane (**Fig. 1**).[9,10] After the exposure to the permeabilizing pulses, the cell has 2 possible fates: (1) reversal of the process and return to the previous steady state, and (2) impossibility to reverse the ion fluxes and activation of the caspase apoptotic or necrotic pathways with progression to

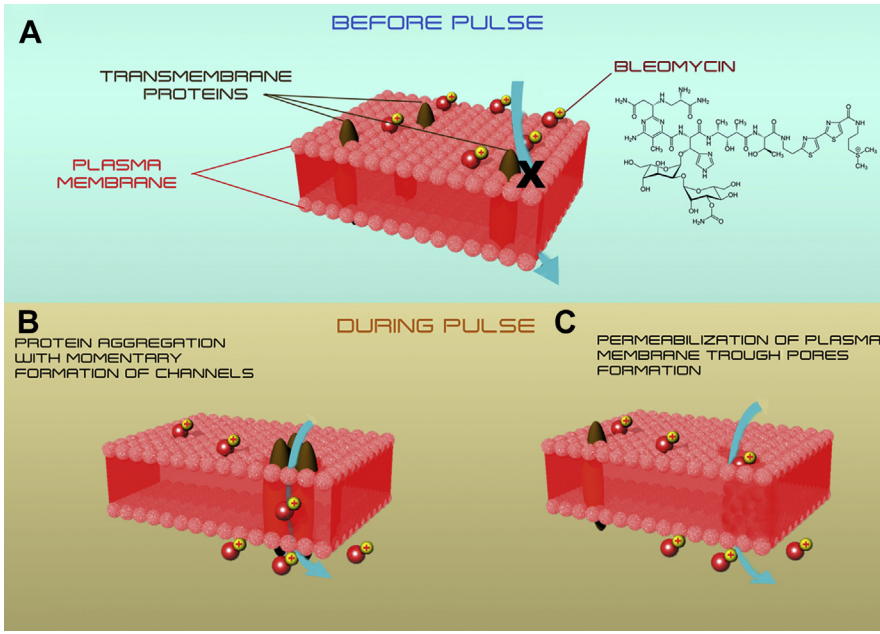

Fig. 1. Mechanisms of drug perfusion following electroporation. (*A*) Cytoplasmic membrane before the application of permeabilizing pulses: the lipophobic drug cannot enter the cell without the intervention of a transmembrane carrier. (*B*) The permeabilizing pulses induce aggregation of the transmembrane proteins and/or (*C*) formation of pores in the lipid bilayer of the membrane.

cellular death. The crossroad between the 2 destinies is the ability to pump the calcium ions outside the membrane and sequester them within the endoplasmic or the sarcoplasmic reticulum.[9,11] The first phenomenon is defined as reversible electroporation; the latter is named irreversible electroporation.[11] Electroporation can be structured, for convenience purposes, in 5 phases: induction, expansion, stabilization, resealing, and memory effect.[12] Another effect of cell exposure to permeabilizing electric fields is the transient clustering of transmembrane proteins, with the formation of pseudotunnels that contribute to the cross-flow of material.[13] Electroporation has been defined as a threshold phenomenon occurring when the permeabilizing pulses exceed the value of the transmembrane potential ($\Delta\phi m$) accordingly to the formula: $\Delta\phi m = 1.5 \times E_{ext} \times r \times \cos \Theta$ (Θ = polar angle at the membrane with respect to the E_{ext}, the external electrical field).[14,15] This physical model is too crude for ECT of neoplasms. In fact, even if shown to be qualitatively acceptable, it does not consider the cellular heterogeneity within of neoplastic tissue, the presence of neoplastic foci, or the variable content of connective tissue, as well as the different orientation of the cancer cells in terms of field polarity, parameters that would prevent an unopposed permeabilization.[15] For these factors, ECT trains are split into 2 perpendicular trains of 4 pulses or administered as trains of biphasic pulses.[16–18] Length, number, and shape of the pulses are key factors for a successful electroporation. Further experiments elucidated the phenomenon of electroporation, showing 2 phases of relevant clinical application: (1) an early phase of pore induction, and (2) a later phase of pore enlargement.[19,20] These early pores are called "transient electropores," which, after the disappearance of the electric field, shrink in size and stabilize to form the so-called long-lasting electropores.[20] In terms of uptake, large molecules (greater than several kilodaltons) can cross the cell membrane mostly through the transient electropores, and in lesser quantity, through the protein pseudotunnels.[21] Conversely, only smaller molecules can transit through the "long-lasting electropores" that are characterized by a much longer half-life.

CHEMOTHERAPY AGENTS USED IN VETERINARY ELECTROCHEMOTHERAPY

Bleomycin

The cornerstone of ECT is the combination of electrical pulses with the administration of bleomycin.[5–8] This drug is lipophobic and uses protein receptors to penetrate the cell membrane; therefore, the uptake is slow and quantitatively limited under normal conditions.[5–8] The loss of this membrane receptor by tumor cells is the main mechanism of chemoresistance against bleomycin. A way to bypass this obstacle is the permeabilization of the cell membrane by using electric pulses that can promote bleomycin captation by a factor of 700-fold.[5–8] **Fig. 1** shows the 2 proposed mechanisms of membrane permeabilization following application of electric pulses.

The mechanism of action of bleomycin is the fragmentation of DNA, under normal conditions, which results in G2-M phase arrest of the neoplastic cell cycle. When used in combination with ECT, the number of DNA lesions is dramatically increased, leading to apoptosis.[5–8]

Cisplatin

Cisplatin and its analogues interact with cancer cells through binding with DNA bases,[5–8] leading to cross-linking of DNA that causes cell death. Electroporation enhances the transmembrane passage of this drug by a factor of 4 to 8, thus increasing the number of cross-links.[5–8] This property of ECT has allowed its topical

use in cats, which are notoriously intolerant to this agent. Presently, cisplatin is the second most extensively adopted drug for ECT.

Doxorubicin

Doxorubicin belongs to the anthracycline class. Mechanisms of action include various interactions with tumor DNA, such as base intercalation, DNA strand breakage, and topoisomerase II inhibition. Other mechanisms consist of DNA polymerase activity inhibition, altered regulation of gene expression, and release of free radicals.[22] Preclinical studies inferred that ECT could improve doxorubicin efficacy in in vitro and in vivo models.[22] This agent has been also adopted for ECT palliation in pets affected by mammary cancer with acceptable results (Enrico Pierluigi Spugnini, personal observation).

Mitoxantrone

Mitoxantrone is a synthetic anthracycline analogue with much less cardiotoxicity than the other members of the family, and its mechanisms are similar to doxorubicin. It diffuses through the cell membrane according to the composition of the lipid bilayer. ECT has been shown to increase its efficacy in human and veterinary patients.[23]

CLINICAL ELECTROCHEMOTHERAPY PROTOCOLS IN VETERINARY ONCOLOGY

There are 3 major considerations that differentiate veterinary ECT from human ECT:

1. In humans, it is mostly limited to the palliation of cancer cutaneous metastases or for the treatment of primary skin tumors. In veterinary oncology, ECT is adopted as first-line treatment of solid tumors and of the treatment of selected visceral neoplasms.
2. Although in humans, ECT is frequently administered under local anesthesia, in veterinary applications, ECT is performed with the patients under heavy sedation or general anesthesia.
3. Veterinary ECT can be palliative, adjuvant, or neoadjuvant and can be administered simultaneously with surgery (intraoperative ECT).

The protocols involve patient sedation using a combination of different drugs, including butorphanol, medetomidine, acepromazine, methadone, and ketamine, as per standardized induction protocols.[24] After the induction, propofol or barbiturates are intravenously administered for greater the depth of anesthesia. In cases of intraoperative ECT, the patient is generally maintained with gases such as isoflurane or sevoflurane.[24] With the patient properly sedated, the chemotherapy agents are administered systemically and/or locally. As a general rule, bleomycin is given as an intravenous bolus at the concentration of 20 to 30 mg/m^2 (or mitoxantrone at the dose of 5 mg/m^2).[5–8,24] Locally, cisplatin can be injected within tumor or in tumor beds at the concentration of 0.5 to 1 mg/mm^3 or, alternatively, bleomycin at the concentration of 1.5 mg/mm^3. Administration of the 2 agents has been successfully combined to increase therapeutic efficacy. Subsequently, following a given time interval (usually 5 minutes), sequences of 8 permeabilizing pulses are administered. **Fig. 2** summarizes an ECT session in a veterinary patient. The 2 most popular waveforms are square and biphasic.[5–8,24] The permeabilizing protocols can vary in terms of electrical shapes, voltage, amperage, frequency, and interpulse duration.[5–8,24] The authors' previous setting adopted a train of 8 biphasic pulses lasting 50 + 50 microseconds each, with 1-ms interpulse intervals, delivered in trains of 1300 V/cm (800 V/cm for intraoperatory ECT), 1-Hz frequency, using caliper or needle array electrodes. Currently, the

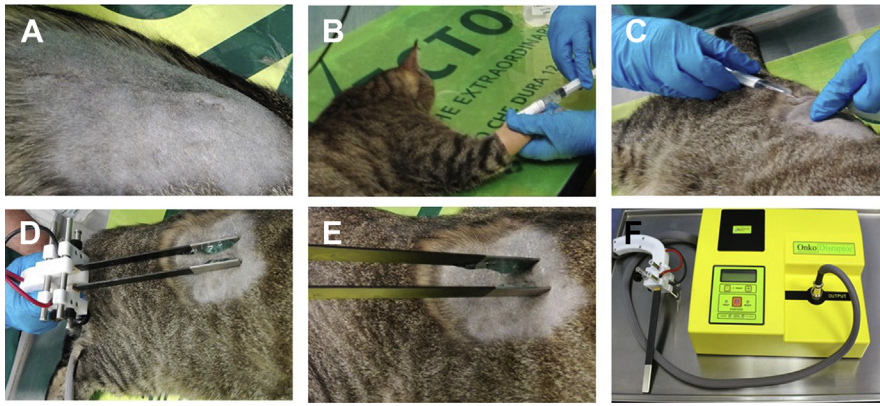

Fig. 2. Different phases of an ECT session. (*A*) The patient is shaved to expose the underlying scar of an incompletely excised fibrosarcoma. (*B*) After sedation, the patient is treated with a bolus of bleomycin. (*C*) The surgical scar is injected with cisplatin. (*D*) Electrical pulses are administered with the larger surface of a plate electrode. (*E*) Electrical pulses are administered with the smaller surface of a plate electrode. (*F*) An overview of an ECT instrument.

authors reduced the interpulse to a 300-μs interpulse (total treatment time per train 3.2 ms) and adopted an amperage dampener that significantly reduced the morbidity.[8] Contact of the external electrodes to the lesions and electrical conduction is improved using electroconductive gel. The treatment is repeated at 2-week intervals. In cases of postoperative ECT, the number of sessions is limited to 2 treatments, whereas in the case of direct ablation of gross disease, ECT is administered until a complete response is reached or tumor progression occurs.

Protocol for Ultrasound-Guided Electrochemotherapy for Intracavitary Tumors

After the induction of patient sedation, bleomycin is administered intravenously, and then paired needles with electrical shielding are inserted within the tumor using ultrasonographic guidance. Imaging assistance allows for avoidance of necrotic and hemorrhagic foci, and Doppler technique consents to avoid major vessels. At this time, 5 series of biphasic electric pulses are delivered to the tumor with a starting voltage of 800 V and modified according to the tissue conductivity.[25] This protocol combines the Joule effect (direct thermal ablation) with the effects of ECT. Treatment is repeated until tumor coverage is achieved. The sessions are repeated every 2 weeks until a tumor response is observed (**Fig. 3**).

CLINICAL RESULTS OBTAINED BY ELECTROCHEMOTHERAPY IN VETERINARY ONCOLOGY: SOLID TUMORS

Mesenchymal Tumors

Feline soft tissue sarcoma

Feline patients have a special record in veterinary ECT, being the very first pets to be enrolled in a clinical trial. In the late 1990s, a group of cats with recurring soft tissue sarcomas following adjuvant cobalt radiation therapy and surgical ablation was enrolled in a compassionate ECT study and compared with untreated controls.[26] This trial reported a single responder with partial remission, likely as a consequence of acquired chemoresistance by relapsing tumors. The need for additional preliminary investigations resulted in 2 studies enrolling pets with various neoplasms (including several

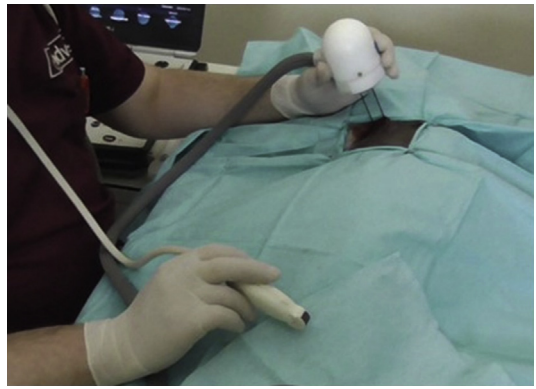

Fig. 3. An ultrasonography-guided ECT in a canine patient; the impulses are delivered through needle array electrode.

soft tissue sarcomas), directly targeted using ECT. Different from other histotypes, mesenchymal tumors evidenced a lower response rate and rare complete remissions.[27,28] Preliminary observation in a cat with an incompletely excised hemangiopericytoma receiving 2 postoperative sessions of ECT resulted in long-term control.[29] The necessity for a different approach to further ameliorate the effectiveness of ECT in sarcomas led to the adoption of adjuvant ECT (both intraoperatively and postoperatively) following surgical tumor excision. A cohort of feline patients was enrolled to receive intraoperative or postoperative ECT using local injection with bleomycin. These cats were matched against a cohort treated with surgery alone.[30] A total of 72 cats were assigned to the 3 experimental groups. Results evidenced that ECT led to extended local tumor control and survival, independently by its modality of administration. Numerous prognostic factors, including previous treatments and tumor size, were identified in this study, and side effects were limited to local inflammation and occasional wound dehiscence.[30] Notably, a cat with a previously radiated sarcoma experienced a radiation recall following ECT.[31] Over the years, perfecting of ECT protocols has allowed the clinical recovery of chemotherapy drugs with narrow therapeutic indices. An early case involved the treatment of a cat with bilateral facial rhabdomyosarcoma using cisplatin-based adjuvant ECT.[32] The swift transmembrane translation of the drug during ECT deters the toxicities (fatal pulmonary edema) reported in felines receiving cisplatin. Last, a broader confirmatory study further investigated the use of cisplatin with adjuvant ECT for feline sarcoma. A total of 64 cats with sarcomas were recruited and treated with ECT, and their responses were compared with a control group of 14 patients receiving surgery alone.[33] ECT resulted in increased local control with a mean time to recurrence of 666 days versus 180 of controls.

Canine soft tissue sarcoma

In dogs, the use of adjuvant ECT for the treatment of incompletely excised soft tissue sarcomas was described in a cohort of 22 dogs treated with intralesional bleomycin at the tumor site.[34] The protocol was well tolerated and resulted in a median time to recurrence of 730 days. Negligible side effects were noted. A case report described the effectiveness of neoadjuvant ECT in a dog with an inoperable high-grade soft tissue sarcoma by shrinking the tumor mass to an operable size. Neoadjuvant ECT allowed the removal of the residual tumor and the sterilization of the surgical field resulting in long-term tumor control.[35] A very recent investigation from the

authors' group used the combination of systemic bleomycin with local administration of cisplatin in a cohort of 30 dogs for the treatment of incompletely excised soft tissue sarcomas. At the time of this writing, 26 dogs had no evidence of recurrence; 3 dogs had recurrence, and 1 dog had both local recurrence and pulmonary metastases. Median estimated time to recurrence was 857 days. ECT using a combination of bleomycin and cisplatin appears to be well tolerated and highly effective in the treatment of incompletely resected soft tissue sarcomas in dogs and is the current standard protocol in the authors' group.[36]

Unusual mesenchymal neoplasms: canine fibromatosis

Canine fibromatosis is a rare neoplasm of the soft tissues. It is characterized as rapidly growing and invasive and is prone to scar tissue–like recurrence. Because of growth patterns and tumor locations, these lesions pose a clinical challenge. One report describes the positive outcome of a Great Dane diagnosed with recurring fibromatosis after incomplete surgical excision. The dog achieved complete remission after 4 courses of ECT and died 3 years later of unrelated disease.[37]

Epithelial Tumors

Canine perianal and anal sac tumors

Canine hepatoid glands tumors, especially adenomas, are exquisitely responsive to ECT. The treatment can be used to treat large perianal neoplasms that do not regress following castration. In selected cases, the authors performed ECT with spinal anesthesia with good patient tolerability. The first report using cisplatin-based ECT for the treatment of large perianal tumors in dogs reported an overall response rate of 82% with 41% achieving complete response.[38] A subsequent report described the use of bleomycin-based ECT for these neoplasms with a total of 91% responders and 83% attaining complete response.[39] Considering the involvement of deep underlying tissues by these tumors (especially those involving the anal sacs) and the frequent ulceration and bleeding that could affect proper drug distribution, an adjuvant approach has been recently proposed for these aggressive neoplasms combined with surgical excision.[40] For invasive anal sac carcinoma, especially those extending to the abdominal cavity, an ultrasound approach is deemed more appropriate. These strategies are the proof of concept that ECT can be successfully adopted in sensitive tissues, such as the anus, allowing tumor control and preservation of function with conservative surgery.

Feline head and neck carcinoma

White cats are extremely prone to sun-induced malignancies, in particular, squamous cell carcinoma (SCC), affecting the nasal planum, the eyelid, and the head.[41] Patients are often diagnosed with these tumors in an advanced condition that precludes radical excision. Therefore, these tumors are generally treated with radiation therapy.[42] ECT has been successfully proposed as an alternate therapy for these tumors. The proof of concept has been a preliminary investigation treating a small cohort of 9 cats with intralesional bleomycin and ECT. Seven of the cats (77.7%) had a complete response of various durations.[43] Subsequent work adopted the systemic administration of bleomycin within an ECT protocol, again reporting a good control rate with preservation of the anatomic structures.[44] A larger cohort of feline patients with advanced SCC of the head and neck was treated with systemic bleomycin potentiated by permeabilizing electric pulses (ECT) and matched against a control group receiving bleomycin alone. The ECT group had a significantly better outcome than the control. Median times to progression were 30.5 and 3.9 months, respectively.[45] Generally speaking, side effects are confined to local

inflammation; however, cats with advanced nasal planum SCC (ie, T4 stage) can develop significant scar tissue that can potentially result in anosmia (E.P. Spugnini, personal observation). This study verified the ability of ECT to attack extensive carcinomatous lesions, even in challenging areas, such as the eyelids and the periocular area. **Fig. 4** shows the outcome of a cat with ocular SCC successfully treated with ECT.

Miscellaneous skin tumors

Cutaneous tumors are generally treated with surgery. ECT is currently limited to the treatment of inoperable tumors, tumors with regional metastatic cascade, or tumors affecting areas where a conservative strategy could not be pursued.

Scientific literature reports the ECT treatment of an apocrine gland carcinoma with cervical lymph node metastases, a ganglioneuroblastoma of the footpad, and a trichoblastoma of the digit. The dog with the apocrine gland carcinoma was treated with systemic mitoxantrone-based ECT that resulted in tumor control in excess of 6 months.[23] The cat with the ganglioneuroblastoma was treated with 3 sessions of ECT and achieved a complete remission in excess of 450 days.[46] The digital trichoblastoma was successfully treated with 3 sessions of ECT using systemic bleomycin. The tumor volume decreased throughout the ECT sessions, with a complete response reached after 81 days. The patient achieved a disease-free interval of 700 days.[47]

CLINICAL RESULTS OBTAINED BY ELECTROCHEMOTHERAPY IN VETERINARY ONCOLOGY: ROUND CELL TUMORS

Canine Melanoma

Melanoma was among the first round cell tumors to be treated with ECT. A small study investigated intralesional bleomycin–based ECT in a group of 10 dogs, which resulted in a response rate of 80%, and 40% of responders was controlled in excess of 1 year.[48] A factor that promoted a longer response was the tumor location in soft areas of the oral cavity, where the lack of bone allowed a more homogeneous drug distribution. Remarkably, complete responders showed persistent vitiligo-like discolorations at the tumor sites, possibly secondary to ECT-induced triggering of an immune response. More recently, a case report described the successful palliation of an anal melanoma in a dog, whereby ECT allowed preservation of the anal functionality for 3 months.[49]

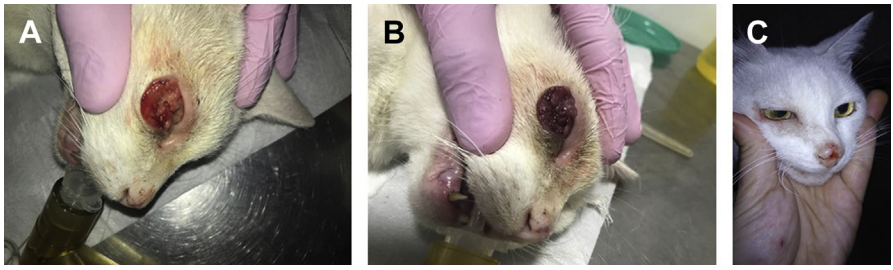

Fig. 4. Outcome of a sun-induced palpebral SCC in a cat treated with ECT. (*A*) Patient at presentation. (*B*) Patient after 1 session of ECT: the mass is reduced and appears less vascularized. (*C*) Patient after 2 sessions of ECT, showing complete resolution of the palpebral neoplasm. (*Courtesy of* D. Santos dos Anjos, DVM, MSc, PhD student (Unesp-Jaboticabal), Brazil, Mexico.)

Canine Mast Cell Tumors

Mast cell tumors (MCTs) are among the most common neoplasms in veterinary medicine and may be difficult to control because of growth rate, location, and high content of histamine and other vasoactive substances.[50] ECT can be used to directly attack these tumors or as an adjuvant following surgical excision. The first article described the successful use of locally administered bleomycin-based ECT in a cohort of 28 dogs with incompletely excised mast cell neoplasms.[51] Another study compared the results of directly attacking MCTs with ECT to surgery, inferring that ECT could be a potential alternative to surgery.[52] A third prospective study treated 37 dogs with incompletely excised grade II and grade III MCTs using cisplatin-based ECT and showed a 78% response rate with minimal side effects.[53] However, another report describes the use of ECT in dogs with MCTs as either first-line therapy or an adjuvant to surgery. In this retrospective study, 51 dogs with MCTs were classified in the following treatment groups (ECT-only, 15 cases; intraoperative ECT, 11 cases; ECT adjuvant to surgery, 14 cases; surgery followed by ECT, 11 cases).[54] In this study, the intraoperative group of dogs showed the best disease-free interval. Pooled, these observations showed that ECT is a very effective and pliable technique that can be used alone or in combination with other therapies.

Canine and Feline Lymphoma

ECT literature for lymphoma in pets is limited to a single article assessing the potential of bleomycin-based ECT in the treatment of localized canine and feline lymphoma.[55] A total of 6 patients were recruited in this study, having clinical responses that ranged from 1 week up to 3 years. ECT was well tolerated, and side effects were limited despite the treatment of sensitive tissues, such as the nasal cavity and the retrobulbar space. Feline patients with nasal or retrobulbar lymphoma were the best responders in terms of degree and duration of response. The authors' group is mostly adopting this approach for the palliation of chemoresistant oral lymphoma.

Sticker Sarcoma

Sticker sarcoma is a transmissible venereal tumor (TVT) that is sexually transmitted within the canine population. This neoplasm is successfully treated with vincristine. Rarely, chemoresistance has been reported, and doxorubicin is suggested as second-line treatment. Alternatively, radiation therapy has been successfully used for local control. A clinical report describes the possible use of ECT as a rescue, reporting 3 chemoresistant TVT cases that successfully responded to intralesional bleomycin and electroporation.[56]

IMAGING-BASED ELECTROCHEMOTHERAPY

The frontier of ECT is now its application in the treatment of visceral and intracavitary neoplasms. The first application of ultrasound-assisted ECT has been the treatment of a clear cell thymoma in a cat.[25] The patient was treated under general anesthesia with systemic bleomycin, and then permeabilizing electric pulses were administered through a specifically designed ecoreflective electrode. The tumor had a long-term partial response that resulted in a 14+ months' survival. Another novel field of ECT application is the treatment of nasal cavity tumors. A recent article describes the use of a single-needle electrode to treat deeply sited neoplasms.[57] A total of 11 dogs with miscellaneous nasal tumors were treated using ECT, with 91% of patients responding. Overall survival rates of 60% at 1 year and 30% at 2 years were reported. Endoscopic ECT has been successfully attempted on 2 canine patients with rectal

neoplasms (adenocarcinoma and lymphoma), achieving good tumor control. These therapeutic avenues are currently being investigated, but the preliminary reports show promise as novel technologies are developed and field tested.[58]

SUMMARY

ECT combines the use of some standard chemotherapy agents with electroporation to promote their efficacy. It allows the use of lipophobic drugs with narrow therapeutic indices to obtain high response rates. while sparing the patient from toxicosis. In veterinary oncology, this approach is becoming a first-line therapy for various cancer histotypes, because of its high efficacy and low toxicity.[59] An additional advantage of this approach is the possibility of repeated courses of ECT in case of recurrence. Care must be exerted in selecting adequate patients for this therapy in order to avoid undesirable complications. In particular, patients with bulky neoplasms might be prone to toxicosis secondary to the massive destruction of neoplastic tissues (ie, tumor lysis syndrome, thromboembolism, disseminated intravascular coagulation) or to delayed wound healing, cheloids, and local necrosis.

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