

ELECTROCHEMOTHERAPY: A REVIEW OF ITS USE AND LIMITATIONS IN SMALL ANIMAL ONCOLOGY

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Abstract

Electrochemotherapy (ECT) is a recent anticancer treatment used for solid tumours in which square wave electric pulses are combined with a chemotherapeutic drug administered either intravenously or intratumorally. The drugs most frequently used in veterinary medicine are bleomycin and cisplatin. Due to the advanced cancer stage in which companion animals are usually diagnosed, treatment with surgery alone is either inefficient or not accepted by the owner, either due to loss of function or cosmetic effect. This is where multimodal therapies come in, by combining surgery with chemotherapy, radiation therapy and other therapies. Unfortunately, these are currently not available in our country or are cost-prohibitive. The only major disadvantage of ECT is the need for general anesthesia, especially when the treatment has to be repeated. This paper reviews 13 articles on ECT in small animal medicine so far in order to establish the method's current indications, limitations and success rates for different types of cancer. Electrochemotherapy has numerous advantages: it is a simple method, with almost insignificant side effects (muscle contractions during the application of electric pulses and in some cases local edema or necrosis after therapy), it can be applied as single therapy or adjuvant to surgery and can be used for inoperable tumours, it can be repeated several times without being less effective and, last but not least, it is an affordable method. In conclusion, ECT is a welcome addition in the fight against cancer in animals as the incidence of diagnosed malignancy in veterinary medicine is ever rising.

Key words: electrochemotherapy, small animal, veterinary oncology, bleomycin, cisplatin.

INTRODUCTION

Electrochemotherapy (Figure 1) combines chemotherapy and electroporation in order to increase the number of chemotherapeutic drug molecules that penetrate the cell and thus greatly improve the efficiency of the treatment. The term electroporation was coined in the 1980s, but the phenomena was observed as early as 1754 (Rubinsky, 2007). The first experiments on cell membranes using electroporation began in the 1970s (Lee et al., 2011) and as the first pulse electroporators became available, more and more researchers began to study the method worldwide.

The different amplitude over voltage ratios of the electric field applied to a cell can permeabilize the cell reversibly (the cell membrane returns to its initial state) or irreversibly (irreversible electroporation, which leads to cell death) (Rubinsky, 2010).

Reversible electroporation is used in medicine to facilitate the access of molecules that cannot naturally enter the cell or do so in very small

numbers, such as: drugs, genes and chemotherapeutic agents. Irreversible electroporation is being investigated as a means for non-thermal tumour ablation (Jourabchi et al., 2010). The first clinical trial on electrochemotherapy applied to spontaneous tumours in veterinary

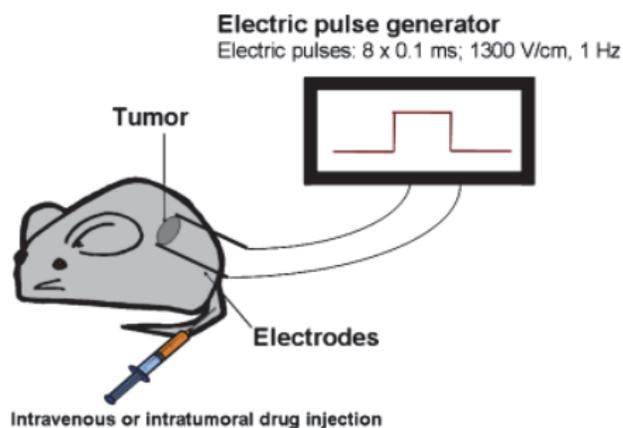


Figure 1. Protocol of electrochemotherapy of tumors presented schematically. The drug is injected either IV or intratumorally at doses that do not exert an antitumor effect. After an interval that allows sufficient drug accumulation in the tumors, electric pulses are applied to the tumor by plate, contact, or needle electrodes (Cemazar, 2008)

medicine took place in 1997 (Mir et al., 1997) and since then more than 50 articles have been published. Veterinary experience confirms that the increased uptake of chemotherapeutic drugs leads to neoplastic cell death, which prolongs the patients' lifespan and quality of life through local control of the tumour (Spugnini et al., 2007). The drugs whose cytotoxicity and efficacy increase through electroporation are those that are either nonpermeant (bleomycin) or low-permeant (cisplatin) (Lee et al., 2011).

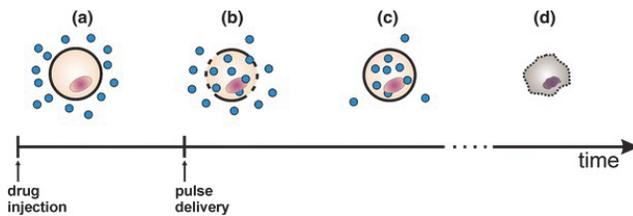


Figure 2. Basic concept of electrochemotherapy: a) after injection drug surrounds the cells; b) formation of pores after pulse delivery, drug enters the cells; c) membrane resealing, drug entrapped inside the cells; d) drug kills the cells (Miklavcic et al., 2012)

Electrochemotherapy not only has a cytotoxic effect on the tumour (Figure 2), but it also induces a 'vascular lock' effect, where blood stops flowing through the tissue which has been electroporated, for less than two minutes in normal tissues but longer in neoplastic tissue (Lee, Gehl and Kee, 2011; Sersa et al., 2006). In immunocompetent animals, there is a strong immune response determined by the massive antigen release from the dying tumour cells (Jaroszeski et al., 2000) which has been shown to be essential in obtaining a cure and not just local control of the tumour (Mir et al., 1991).

The aim of this study was to look at the different electrochemotherapy response rates obtained when applied to different tumour histotypes, taking into consideration the tumour grade and size, location, type of procedure, number of applications, previous treatments. We wanted to compare different clinical experiences and highlight authors' observations in order to create an introduction to ECT for a clinician who would like to start using the method for his patients.

MATERIALS AND METHODS

We searched scientific databases for relevant articles identified by the keywords 'veterinary',

'electrochemotherapy' and 'electroporation' and selected 13 articles which discuss the application of electrochemotherapy in veterinary patients for different types of superficial solid tumours. We excluded case reports from our review.

The different authors used electroporation in combination with either Cisplatin or Bleomycin in one or more applications using different pulse generators to apply 8 biphasic pulses lasting 50 + 50 μ s each or 8 monophasic electric pulses of 100 μ s each at a voltage of 1300 V/cm and a pulse frequency of 1 Hz. They recruited patients with normal blood biochemistry values and complete blood counts and applied ECT under general anesthesia. Tozon et al. (2016) defined operating procedures for safely applying ECT in treating cutaneous or subcutaneous tumours in dogs and cats using either cisplatin or bleomycin.

Most researchers describe the response to treatment they observe according to the WHO Handbook for Reporting Results of Cancer Treatment, the revised RECIST guidelines (ver. 1.1) or the criteria for solid tumours in dogs devised by the Veterinary Comparative Oncology Group. These guidelines similarly classify response into complete response, partial response, no change or stable disease and progressive disease. The WHO classifies measurable disease response into:

- Complete response (CR): The disappearance of all known disease, determined by two observations not less than 4 weeks apart.
- Partial response (PR): 50% or more decrease in total tumour size of the lesions which have been measured to determine the effect of the therapy by two observations not less than 4 weeks apart. In addition there can be no appearance of new lesions or progression of any lesion.
- No change (NC): a 50% or more decrease in total tumour size cannot be established nor has a 25% increase in the size of one or more measurable lesions been demonstrated.
- Progressive disease (PD): a 25% or more increase in the size of one or more measurable lesions, or the appearance of new lesions.

RESULTS AND DISCUSSIONS

Canine studies

Tozon et al. (2005) applied ECT using cisplatin as the drug of choice to 12 male dogs with a

total of 26 perianal adenomas and adenocarcinomas (in the absence of histological confirmation) whose owners did not want to pursue standard treatment. Bleomycin was used when tumour nodules did not respond or regrew after 4 weeks or when the tumours were ulcerative and had a tendency to bleed after ECT with cisplatin. The authors noted no major local or general side-effects or toxicity and the treatments had no effect on complete blood counts or biochemical analysis. They divided the tumours according to volume (less or more than 1 cm³) and noticed that ECT was more effective on smaller lesions, most of which obtained CR in one session. In comparison, large tumours needed a high number of applications in one session in order to cover the entire volume and most of them required additional sessions. The authors' conclusion was that ECT is safe and effective in treating perianal tumours in dogs.

Tozon et al. delivered another study on perianal tumours in 2010, this time describing their results with ECT on 21 male dogs: 5 with 26 perianal adenocarcinomas and 16 with 40 benign tumours using either plate electrodes or needle electrodes depending on tumour size. Their study confirmed their previous finding, that treatment response is significantly influenced by tumour size. An interesting observation was that the objective response did not greatly differ between malignant and benign tumours, between castrated or non-castrated dogs or between chemotherapeutic drugs used.

Spugnini et al. (2007) used adjuvant electrochemotherapy on incompletely excised canine soft tissue sarcomas. Of the 22 dogs treated, 11 were still in remission at the time of writing, 2 had died of unrelated causes and one due to hemangiosarcoma in a different location and 8 experienced local recurrence (the owner declined further treatment for one, 4 were euthanised due to distant metastases, one suffered a limb amputation and 2 were retreated successfully with surgery and ECT). The authors treated the tumours in two sessions one week apart with local injection of Bleomycin of both the tumour bed and adjacent margins (1 cm of normal tissue). The authors observed a significant difference in the duration of remission between tumour types, most evident

in the case of hemangiosarcoma, where all 3 tumours treated recurred and/or metastasised. Despite not being statistically significant, the authors also noticed that tumours located on the trunk were more likely to recur and/or metastasise as compared to those on the limbs. Kodre et al. (2009) compared surgery (25 dogs) to ECT (9 dogs) for the treatment of canine mast cell tumours. In 7 patients with 9 tumours, a 100% CR was obtained, whereas the other 2 had very large tumours (>8 cm) which did not respond to therapy and were euthanised at the owners' request (75% CR for the 9/12 tumors treated with ECT). What the authors observed was that the duration of local tumour control was longer in the ECT group versus the surgery group (median duration of response 31.5 months, while for ECT it was not yet reached at the time of writing). The advantage over surgery, considering the similar results, is that ECT is well tolerated by the dog and minimally invasive. The authors used tissue blanching as a good indicator of tumour infiltration with cisplatin and a concentric approach when applying the electric pulses. This ensured a reduced blood flow to the tumour (due to the vascular lock effect) which maintained the drug inside the tumour and prevented any bioactive molecules from being released from the cytoplasmic granules from reaching the systemic circulation.

Spugnini et al. (2006) also looked at ECT as an adjuvant treatment for incompletely resected mast cell tumors. Their study found that local control was not influenced by site, T stage, prior surgery and duration of symptoms prior to therapy. They also observed that the rate and duration of the response to adjuvant ECT is similar to that of radiation therapy, but it is more accessible, has no major side effects and might be better suited to tumors situated in highly functional areas (eg. limbs, head).

Spugnini et al. (2008) applied ECT to refractory canine transmissible venereal tumours, previously treated with vincristine with or without doxorubicin. Even though the authors only treated three such cases, all three responded to two ECT sessions with bleomycin, one week apart. The complete response lasted 28-48 months, stopped genital bleeding and improved urinary function, thus improving the animals' quality of life.

Feline ECT studies

Spugnini et al. (2007) compared intraoperative ECT, postoperative ECT and surgery (control) applied to different feline soft tissue sarcomas. The cats were distributed to one of three groups randomly by an independent service. The tumours treated were grade T2-T4, and none of the cats had lymph node involvement or distant metastasis. The intraoperative group also received multiple injections of hyaluronidase before ECT in order to improve distribution of bleomycin in the tumour bed and margins. Intraoperative ECT was administered after tumour removal and before suturing, while postoperative ECT was administered one week after surgery. Both were repeated a week later. The control group treated with surgery alone experienced local relapse in 2-12 months. The intraoperative group had a 63% recurrence at different times. Three of the recurrences were retreated with surgery and ECT and gained 6-14 months without disease. The postoperative group had a 46% tumour recurrence and in this group a lack of previous treatment was associated with a better response (median time to relapse 33 versus 5 months). In both types of ECT, tumour size negatively influenced the duration of remission. Seven relapsing tumours were treated again: two cats developed another histological type of tumour, while the rest were still tumour-free at the time of writing. The authors observed that their success rate could be partly attributed to the fact that ECT was applied on a small number of residual tumor cells remaining after surgery (confirming that tumor volume has a significant prognostic value) and to the fact that none of the cats had undergone radiation therapy. In the authors' opinion, the surgical removal of tumors prior to ECT significantly improves treatment outcome due to removal of tumour connective tissue, which impedes bleomycin distribution and pulse delivery. This is also the rationale behind using hyaluronidase prior to ECT. The authors also found that the patients they treated had fewer pulmonary metastases, which could be explained through the involvement of the immune system or the early intervention with adjuvant ECT which prevents metastasis. The authors observed that there is a possibility that ECT might determine a later recurrence of a less aggressive type of tumor (two of the

retreated recurrences had a subsequent milder histotype).

A similar study on adjuvant ECT using cisplatin after surgical removal of fibrosarcoma in cats was accomplished by Spugnini's team (Spugnini et al., 2011) on 64 cats. The authors prefer using adjuvant ECT to overcome the poor distribution of chemotherapeutic drugs inside tumour tissues as well as to be able to use highly toxic substances whose are rarely used in practice or cannot be used in some species (intravenous cisplatin, for instance, determines fatal pulmonary edema in cats, but used locally before ECT none of the 64 cats had any respiratory side effects).

Tozon et al. (2014) applied ECT using intravenous bleomycin to 16 squamous cell carcinomas in 11 cats. The authors used a high repetition frequency (5 kHz) which produced a single muscle contraction for each 8 electric pulses. The authors observed no toxicity due to the administration of intravenous bleomycin or any changes in hematological and biochemical parameters. They concluded that it is a safe and effective method for feline SCC, with a much better response of tumours in initial stages, independent of location, than for more invasive lesions (stages T3-4). The advantage of ECT over surgery is evident in the case of non-invasive nasal SCCs, where the radical surgery required would be an invasive and mutilating procedure that owners do not usually approve of, while ECT preserves the anatomy, function and cosmetic aspect.

Spugnini et al. (2015) compared intravenous bleomycin to intravenously bleomycin and electroporation in 21 cats with periocular carcinoma (9 cats treated with bleomycin and 12 with ECT) and 26 cats with advanced SCC of the head (12 with bleomycin only and 14 ECT). The authors note that none of the cats were previously treated. ECT use in the head and especially periocular region is limited both in human and veterinary medicine due to possible ocular side effects. The cats with periocular tumours treated with ECT showed no sign of damage to the retina, uvea or cornea, but three of the cats later presented epiphora, which made authors suspect lacrimal duct sclerosis. In cats treated with ECT for SCC of the head, 4/14 had small electrode-induced burns that healed in 2-3 weeks and 2/14 had local

inflammation of deep connective tissue which induced compulsive scratching (managed with a daily dose of meloxicam for 7 days). In comparison, there was no local effect due to iv bleomycin in either control group. Authors also recorded a mild neutropenia on day 7 in 2 cats in the ECT group. In the ECT cohort, 3 displayed progressive disease and were euthanised, while 4 died of metastasis to the lung after 4-7 months. In comparison, all cats treated with bleomycin only died as a result of disease progression in 0-16 months.

Canine and feline studies

Six cases (4 cats, 2 dogs) of localised lymphoma in various locations were treated with ECT (Spugnini et al., 2007). At one week, all animals had CR, that lasted 6 months to 2 years for the cats and 20 days for one dog (until it died in a car accident) or over 2 years (until it died due to heart disease).

Maglietti et al. (2016) used both intravenous and intratumoral bleomycin with electro- poration in 9 cases which they did not expect to respond favourably to ECT with one or the other (large tumours, schwannomas and tumours which had responded poorly to previous ECT sessions). Three patients that had previously obtained a PR and one SD responded to ECT with intravenously and it bleomycin with a CR. The authors hypothesise that previous failure is due to the uneven distribution of bleomycin intratumorally, either due to tumor vasculature or the inhomogenous application of multiple injections.

Lowe (2016) reviewed 176 cases treated with ECT in his practice during 2004-2014 for a variety of tumours which would have been very difficult to treat with surgery or surgery would have determined a cosmetic or functional compromise. ECT was applied as sole treatment, intraoperative ECT or as adjuvant ECT 2-4 weeks after surgery. ECT was not offered to patients with tumours amenable to surgery. The author observed that several patients avoided limb amputation due to treatment with ECT, when tumours were not very large or infiltrative. Of the 176 patients treated, 7 did not respond (<50% reduction in tumour or continued growth), while all others responded either with a CR (complete disappearance of tumour), or a SR (all measurable tumour was

removed surgically either during intra- operative ECT or prior to adjuvant ECT). The author applied a higher frequency (5 kHz) to larger lesions which could not be covered with electric pulses at 1 Hz within 20 minutes and observed a higher local toxicity in this group. The author observed recurrences in 56 cases out of 169, no success for osteosarcomas, chondrosarcomas and giant cell tumours (5 cases in all). The author concluded that ECT 'allows effective treatment for tumours where adequate surgery would lead to unacceptable functional or cosmetic effect'.

In Table 1 we summarise the different response rates obtained by using ECT for canine and feline solid superficial tumours, either as single treatment or adjuvant to surgery, either intra- operatively or postoperatively at a later date. Some of the authors used control groups treated with bleomycin or surgery alone in accordance with current veterinary standards, and compared the results to ECT.

CONCLUSIONS

The authors of the studies included in this review confirm the scientific literature in saying that electrochemotherapy is a safe and effective treatment for tumors of different histotypes

Electrochemotherapy, as a form of local chemotherapy, has the advantage of a lack of systemic toxicity, as demonstrated by studies in veterinary and human medicine.

ECT in veterinary has mostly been applied to cases where standard treatment had failed or the recommended treatment would determine an unacceptable lack of function or have a mutilating effect. There are authors that support the use of ECT in treating tumors with a known good response, such as perianal tumors. Owners made an informed decision to accept ECT after been informed of all possible courses of action.

Tumour size is an important prognostic factor for the success rate of ECT and most authors agree that for large tumours, adjuvant ECT, either immediately after surgical ablation or after surgical scar healing, is the best approach.

Tabel 1. Tumour response rates in different studies

Nr.	Species and number of animals	Nr. lesions	Type of tumour and location	Drug and route	Tumour response rates	Reference
1	DOG 12	26	adenoma, adenocarcinoma, perianal	C/B it	82% OR (CR 41%, PR 41%), 16% NC, 2% PD at 4 weeks after 1 st therapy. At the end of the observation period for each tumour, ranging from 1 to 34 months, 92% OR (CR=65%, PR=27%), 8% NC and no PD were obtained.	Tozon et al., 2005
2	DOG 21	66	26 M, 40 Be; perianal	C/B it	92.7% OR (CR 81.8%, PR 10.9%), 7.3% NC at 4 weeks after 1 st therapy. At the end of the observation period, 93.9% OR (CR 87.8%, PR 6.1%), NC 6.1%	Tozon et al., 2010
3	DOG 22	22	soft-tissue sarcoma, various	B adj.	95% OR response rate, with a mean time to recurrence of 730 days.	Spugnini et al., 2007
4	DOG 9	12	mast cell tumours, various	C it	75% CR, 17% NC, 8% PD, compared to surgery: 50% CR, 50% PD	Kodre et al., 2009
5	DOG 28	28	mast cell tumours, various	B adj.	85% in remission (CR)	Spugnini et al., 2006
6	DOG 3	3	TVT, genital	B it	100% CR	Spugnini et al., 2008
7	CAT 58	58	soft-tissue sarcoma, various	B it/adj.	Intraoperative: 63% recurrence, postoperative: 46% recurrence, compared to 100% for surgery	Spugnini et al., 2007
8	CAT 64	64	fibrosarcoma, various	C it	Postoperative ECT: 29.7% recurrence compared to 92% in control (surgery only), at a mean time of 666 days versus 180 for surgery alone	Spugnini et al., 2011
9	CAT 11	16	sq. cell carcinoma, nasal / pinnae	B iv	CR 87.5% (14/16) of nodules, lasting 2 months to >3 years; 2/16 (12.5%) had PD	Tozon et al., 2014
10	CAT 26	26	carcinoma, periocular or head	B iv	CR 81%, PR 7.5%, 11.5% PD, at two weeks after treatment, compared to control (bleomycin only) CR 19%, 14% PR, 29% SD, 38% PD	Spugnini et al., 2015
11	DOG 2, CAT 4	6	localized lymphoma various	B it	Local CR 100% at one week, lasting 20 (HBC) - 760 days	Spugnini et al., 2007
12	DOG 7, CAT 2	9	various, various	B iv+it	CR 55%, PR 33%, 11% PD	Maglietti et al., 2016
13	DOG 140, CAT 36	176	various, various	B iv	33% recurrence (56/169), 3.9% NR	Lowe, 2016

Abbreviations: ECT electrochemotherapy, D dog, C cat, M malignant, Be benign, B bleomycin, C cisplatin, adj. adjuvant, it intratumoural, iv intravenous, HBC hit by a car, OR objective response, CR complete response, PR partial response, NR no response, PD progressive disease, OR objective response = CR+PR

While ideally surgeons should perform tumour ablation according to the standards set for each histotype and presentation, in practice this is difficult due to the functional and cosmetic effect of ensuring histologically confirmed clear borders and depth. Some authors thus recommend intraoperative ECT when they cannot ensure obtaining tumour-free margins and at least one other session 1-4 weeks later. ECT can also be used in tumours of unknown histotype when the owner declines further investigations, with or without surgery.

ECT can be used with bleomycin intratumorally or intravenously and cisplatin intratumorally. Most authors prefer the intratumoural route due to the absence of general side-effects, as it requires a much lower dose to be effective. In practice, for non-responding tumours, authors replaced cisplatin with bleomycin or used bleomycin both intratumorally and intratumorally, and improved the clinical outcome.

An advantage of ECT in veterinary medicine is also related to cost. The late diagnosis of cancer in veterinary medicine means that the

veterinarian has to treat high-grade tumours, which require a multimodal approach that most owners cannot afford or cannot access (unavailability, distance, time etc.). ECT is cost-effective as it uses much lower doses of cytotoxic drugs and does not require expensive equipment or special facilities and staff (as is the case for radiotherapy, for instance). On the other hand, staff does have to be trained in the use of chemotherapeutic drugs and the safety measures this entails.

ECT is a quick method which can be reapplied multiple times if necessary, with low toxicity and a minimal effect on surrounding healthy tissue. It has been shown to improve and not decrease the animals' quality of life, as compared to aggressive surgery, chemotherapy or radiotherapy in treating primary tumors or superficial metastases. The only major disadvantage is the need for general anesthesia. Several ongoing studies are currently trying to optimise electrodes that can be applied to deep tumours either surgically or endoscopically (Impelizzeri et al., 2016)

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ABBREVIATION USED

ECT = electrochemotherapy

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