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Electrochemotherapy Compared to Surgery for Treatment of Canine Mast Cell Tumours

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Abstract. *The aim of this study was to evaluate the effectiveness of local treatment electrochemotherapy (ECT) with cisplatin and to compare it with effectiveness of surgery for treatment of mast cell tumours (MCT) in dogs. Materials and Methods: In the present retrospective study, 25 dogs of different breeds with MCT were divided into two treatment groups: surgery (16 dogs with 16 tumours) and those whose owners refused surgery being included into the ECT group (9 dogs with 12 tumours). Response rate and duration of response to the treatment were evaluated and comparison between groups was made. Results: The clinical stages of the tumours were stage I in 4 (45%) and stage III in 5 (55%) dogs treated by ECT; 12 (75%) dogs treated by surgery were stage I and 4 (25%) dogs were in clinical stage III. The median size of the tumours was 5.2 cm^3 and 2.9 cm^3 of tumours treated by surgery and ECT, respectively. ECT resulted in as comparable antitumor effectiveness as surgical treatment. However, the estimated median duration of response in dogs treated with complete surgical excision was 31.5 months, while it was not reached for the ECT group at the time of writing. Clinical significance: ECT is an easy, effective and safe local treatment of MCT. It can be an alternative treatment to surgery, specifically for smaller nodules in which a complete response with long duration can be obtained after only one treatment session, or when the nodule is unresectable because of the location.*

Mast cell tumours (MCT) are malignant cutaneous tumours that account for 11-27% of all malignant cutaneous tumours in dogs. They have a wide range of appearance and behaviour, making these tumours challenging to diagnose and to treat. MCTs predominantly occur in middle-aged dogs of many breeds, but more frequently in Boxers, Staffordshire Bull Terriers, Labradors, Golden Retrievers, Weimeraner and Schnauzers, with no gender predisposition (1). Several factors have been evaluated as prognostic factors, including histological grade, which is the most accurate predictor of behaviour, as well as clinical stage, size, growth rate, breed, completeness of surgical excision, presence of systemic signs, argyrophilic nuclear organizer region count, DNA ploidy, matrix metalloproteinases, microvessel density and abnormal expression of the *p53* tumour suppressor gene. The morbidity in affected animals is not only due to the local invasion or distant metastases, but also to the systemic effects that occur after the release of cytoplasmic granules containing substances such as histamine, heparin and similar vasoactive substances (1). Handling MCT may cause degranulation and release of inflammatory mediators resulting in local reaction called Darier's syndrome ("wheal and flare" reaction) (2).

Surgical excision remains the treatment of choice. After surgery, the distant tumour recurrence rates are from 22-54% (3, 4). Complete surgical excision of grade II MCT was associated with effective local control in 89% and local tumour recurrence developed in 11% of dogs (3). Similar results were obtained in a retrospective study of 55 dogs with 60 MCT grade II, treated with surgery alone. In that study, 95% of the tumours did not recur during the follow-up period. Eighty four percent of dogs were free of MCT during the study period while 5% developed metastases (5). In a large retrospective study of 340 cutaneous MCT in 280 dogs with different histological grades, the authors reported recurrences in 23 dogs (6). Fulcher *et al*. (7) evaluated completeness of excision and clinical outcome in dogs with MCTs excised with a lateral margin of 2 cm and a deep margin of one fascial plane. In 91% of 23 dog complete excision with MCT, free margins was confirmed. In a recent study with a median follow-up time of 811.5 days, the recurrence rate in 28 dogs with MCT grade II

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with incomplete surgical excision without any adjunctive treatment except prednisone was evaluated. Local recurrence occurred in 23.3% of dogs during the study and 11 dogs developed MCT at other cutaneous locations (8). Postsurgical survival can be predicted on the basis of histological grade and completeness of surgical excision. Therefore, for incomplete surgical excision, an additional local therapy, such as irradiation, is needed. In addition, adjuvant systemic chemotherapy resulted in 28 to 53% objective responses, without long-term response and with pronounced side-effects (9-13). Currently, vinblastine/prednisolone is the most widely used protocol with less evidence of adverse effects. Nevertheless, gastrointestinal toxicity (nausea/vomiting) and myelosupression (more so than with vincristine) are the most frequent side-effects of vinblastine (14).

Electrochemotherapy (ECT) is a novel local tumour treatment that combines administration of chemotherapeutic drugs, cisplatin or bleomycin, with application of electric pulses to the tumour. It is based on the local application of short and intense electric pulses to the cells or tissues that transiently permeabilise the cell membrane and thus allow influx of foreign molecules from the extracellular space into the cytoplasm. To date, its main application has been the treatment of tumours where the electric pulses are associated with non-permeant or poorly permeant drugs having high intrinsic cytotoxicity. The most convenient drugs are bleomycin and cisplatin, which are non-permeant and poorly permeant drugs, respectively (15). Results of clinical trials demonstrated that ECT is an easy, highly effective and safe treatment approach for cutaneous and subcutaneous tumours of various malignancies in human patients resulting in up to 100% complete regression of tumours (16).

ECT is a local treatment and the presence of the injected drug in the target tissues determines ECT efficiency. When injecting, the sign of good tissue retention is tissue whitening, which can be verified visually. Some leakage of solution could occur through the holes produced by the needle in the tumour or in necrotizing tissues during further ECT sessions. Reaching 2 mg/cm^3 (cisplatin) or 3 mg/cm^3 (bleomycin) concentrations could be then purely theoretical in such cases and the goal is to try to retain as much solution as possible in the target tissues. In addition, electric pulses should be applied as quickly as possible in order to obtain the vasoconstricting effect which contributes to solution retention (17, 18). The vasoconstricting effect of electroporation could be beneficial in treatment of MCT, since it can prevent release of inflammatory mediators into the bloodstream due to possible degranulation of neoplastic cells during manipulation.

There are few studies that have already demonstrated the effectiveness, convenience and safety of ECT with either cisplatin or bleomycin for the treatment of spontaneous tumours in companion animals and horses (19-29). ECT using biphasic electric pulses and bleomycin injected in surrounding tissue was performed as an adjuvant treatment in incompletely excised MCT in dogs, resulting in an 85% overall response rate (22).

Based on these promising results, the aim of the present study was to evaluate the effectiveness of ECT with cisplatin as a single treatment and to compare it with the effectiveness of standard surgical treatment of MCT in dogs.

Materials and Methods

Animals. In this retrospective study, all the dogs that were referred to the University of Ljubljana hospital between January 2002 and August 2004 for management of MCT were included. The study cohort comprised twenty-five dogs, 20 males and 5 females, of different breeds. Sixteen dogs with 16 tumour nodules were operated on, using 2 cm surgical margins with a fascial plane, with complete surgical margins of between 2 to 5 mm, confirmed by histology. Antibacterial treatment was given to these patients prior to surgery and no other medications were administered. The remaining 9 dogs with 12 tumour nodules were treated with ECT after their owners refused surgical treatment. In all cases, the clinical stage was established according to tumour size, lymph node involvement and presence of distant metastases using modified WHO staging criteria (2).

Before the treatment, for each tumour fine-needle aspiration biopsy and cytological examination were performed. In all cases, most of the cells had clearly visible eosinophilic granules in the cytoplasm and a definitive diagnosis of MCT was confirmed with toluidine blue staining (30). Histological examination of tumours and surgical margins was performed in tumours that were excised surgically. Histological examination was not performed in ECT-treated tumours due to the owners' refusal of surgical treatment. Histological grade was established using Patnaik histological criteria (31).

Medical treatment. The patients included in the ECT-treated group had to possess one or more measurable cutaneous or subcutaneous nodules, cytologically confirmed as MCT and owners had to refuse standard treatment, *i.e.* surgical excision of the tumour. Eligibility criteria also included patients with normal blood tests and biochemistry, without visible visceral metastases assessed by thorax radiography and abdominal ultrasound examination and no evidence of chronic renal dysfunction or serious cardiovascular diseases. Dogs were premedicated with a combination of acepromazine (Promace, Fort Dodge Animal Health, Iowa, USA; 0.02 mg/kg) and methadone (Heptanon, Pliva Zagreb, Croatia; 2 mg/kg). General anaesthesia was induced using thiopental (Nesdonal, Merial, Lyon, France; 5 mg/kg) and maintained with isoflurane (Forane, Abbott Laboratories LTD, Queensborough, UK). During the anaesthesia, the animals received Hartmann's solution (B. Braun Melsungen AG, Melsungen, Germany) at a rate of 10 ml/kg/hour.

ECT consisted of intratumoural administration of cisplatin (*cis*-diamminedichloroplatinum II, Cisplatyl; Aventis, Paris, France) and exposure of tumours to electric pulses. In this study, ECT with cisplatin was used as a single treatment; therefore it was important to treat adequate margins, as is recommended for surgical excision, to obtain a complete response with long duration.

Cisplatin was dissolved in distilled water at a concentration of 2 mg/ml and was given intratumourally at the dose ~1 mg/cm^3 of tumour. Tissue blanching was used as an indicator of good tumour infiltration with cisplatin. If tissue blanching was not observed, the

Figure 1. *Electrical pulses were delivered through the electrodes that were placed on the tumour nodule during the procedure. If the tumour was bigger than the distance between the electrodes, several trains of electrical pulses were applied in order to cover whole tumour area and safety margins, using criteria for surgical excision.*

intratumoural injection was repeated, which happed very rarely (few cases). Special care should be given when preparing and applying the drug due to its mutagenic and carcinogenic potential. For drug dosage calculation, the volume of tumour nodules was calculated by the formula $V=ab^2\pi/6$, where a was the larger diameter of the tumour nodule and b the diameter of the tumour nodule perpendicular to a. The interval between cisplatin administration and the application of electric pulses was 1-2 min. Electrical pulses (8 pulses of 100 μs pulse duration, amplitude to electrode distance ratio 1300 V/cm and frequency 1 Hz) were generated by an electrical pulse generator (Jouan GHT 1287; Jouan, St Herblain, France) and delivered through two parallel stainless steel electrodes (IGEA S. r. l., Carpi, Italy; plate electrodes: thickness, 1 mm; width, 7 mm; length, 8 mm, with rounded tips and an inner distance between them of 7 mm) (Figure 1). Each run of electric pulses was delivered in two trains of four pulses, at 1 s intervals, in two perpendicular directions. Electrical pulses were first delivered at the tumour margin in order to reduce the blood flow to the tumour and then continued in concentric circles to the centre of the tumour nodule (17). Good contact between the electrodes and the skin was assured by depilation and application of a conductive gel to the treatment area. In the case of tumours bigger than the distance between the electrodes, several trains of electrical pulses were applied in order to cover the whole tumour area and safety margins, using criteria for surgical excision. If the tumour did not respond completely after the first session, additional sessions were performed at 2-4 weeks interval. Patients treated with ECT did not receive any other medication before, during or after treatment.

Evaluation of response and statistical analysis. After treatment with ECT, the patients were kept in the clinic for about 2 to 4 hours. After that, the patients were examined after 2 and 4 weeks and thereafter monthly in order to evaluate the treatment effectiveness and possible local and systemic side-effects. The maximal observation time was 43 months for the ECT-treated group and 29.5 months for the surgically-treated group. At each visit, tumours were measured with Vernier calliper and photographed. For evaluation of treatment response, the tumour size was calculated by the formula A=ab, in accordance with WHO Handbook for Reporting Results of Cancer Treatment (32). Response to the treatment was scored after 4 weeks and at the end of the observation period as a complete response (CR) when the tumour was not palpable and as a partial response (PR) when a decrease of more than 50% in the products of the largest perpendicular diameters of the measurable lesions was determined. Less than 50% reduction and up to 25% increase in the above measurements was defined as no change (NC). For all response definitions a minimum of 4-weeks' duration was required for qualifying each type of response. Progressive disease (PD) was defined by an increase of more than 25% . Surgically treated patients were also examined monthly and checked for tumour relapse. Duration of local tumour control (*i.e.* time to relapse) was calculated as the interval between the data of the treatment and the date of recurrence.

Statistical analysis was performed using SPSS 11.0 software (Statistical Packages for Social Sciences, Chicago, USA). Local tumour control was estimated as a function of time by the Kaplan-Meier product limit method, and the difference between the curves was analysed by means of log rank test. Local tumour recurrence

Table I. *Summary of dog characteristics*.

Pt. no.	Breed	Age (years)/ Gender	Duration of clin. signs (days)	Tumour location	Clinical stage	Cytology	Histological grade	Previous treatment	Treatment	No. of tumours
1	Labrador retriever	8.0/M	14	Neck	I	MCT	I	None	Surgery	1
2	Boxer	6.0/F	10	Thorax	I	MCT	I	None	Surgery	1
3	Rhode ridgeback	0.5/M	7	Head	III	MCT	II	None	Surgery	1
4	German shepherd pointer	8.0/M	20	Back	I	MCT	II	None	Surgery	1
5	Cross-breed	9.0/F	30	Hindleg	I	MCT	II	None	Surgery	1
6	Labrador retriever	6.0/M	30	Hindleg	I	MCT	II	None	Surgery	1
7	Boxer	3.0/F	14	Hindleg	I	MCT	II	None	Surgery	1
8	Boxer	5.0/M	20	Head	I	MCT	II	None	Surgery	1
9	Riesenschnauzer	3.0/M	14	Perianal	III	MCT	II	None	Surgery	1
10	Shar pei	5.0/F	45	Back	I	MCT	III	None	Surgery	1
11	Labrador retriever	3.0/M	20	Head	I	MCT	III	None	Surgery	1
12	Doberman pinscher	7.0/M	14	Abdomen	I	MCT	III	None	Surgery	1
13	Airdale terrier	2.5/M	30	Inguinal	I	MCT	III	None	Surgery	1
14	Golden retriever	4.0/M	20	Head	I	MCT	III	None	Surgery	1
15	Irish setter	12.0/M	30	Scrotum	III	MCT	III	None	Surgery	1
16	Briard	14.0/F	30	Foreleg	III	MCT	III	None	Surgery	1
17	Boxer	4.0/M	14	Hindleg	III	MCT	ND	None	ECT	2
18	Irish setter	11.0/M cas.	30	Hindleg	I	MCT	ND	None	ECT	1
19	Stafford terrier	4.0/M	40	Hindleg	I	MCT	ND	None	ECT	1
20	Cross-breed	9.0/M	14	Thorax	III	MCT	ND	None	ECT	2
21	Rottweiler	9.0/M	30	Foreleg	III	MCT	ND	None	ECT	1
22	Bernese mountain dog	9.0/M	20	Abdomen	III	MCT	ND	None	ECT	1
23	Yorkshire terrier	10.0/F	14	Abdomen	I	MCT	ND	None	ECT	1
24	Stafford terrier	8.0/F cas.	10	Foreleg/thorax	III	MCT	ND	None	ECT	2
25	Cross-breed	7.0/F	14	Foreleg	I	MCT	ND	None	ECT	1

ND, Not determined; MCT, mast cell tumour; ECT, electrochemotherapy.

represented the endpoint of interest and was thus scored as event. Data from patients that were in CR at the end of follow-up, dead due to other reasons or lost from follow-up, were used as censored data. Data are presented as median with range. The difference in median size of tumours was evaluated by Mann-Whitney Rank Sum test, as the data were not normally distributed. Statistical significance was tested at the 5% level.

Results

Treatment effectiveness. Twenty-five dogs of different breeds and both genders, with cytologically confirmed clinical diagnosis of MCT were treated either by surgery or ECT with cisplatin. The demographic data of the dogs (age, gender, breed), clinical stage and duration of clinical signs prior to treatment are presented in the Table I.

In the first group of patients, treated with complete surgical excision, the median tumour size prior to the treatment was 5.2 cm^3 (ranging from 0.52 to 43.98 cm^3), the mean duration of clinical signs was 20 days (ranging from 7 to 45 days) and the most prevalent location was the head (Table I). The median time of follow-up was 18.3 months, ranging from 0.7 to 30 months. Overall, the estimated median duration of local tumour control for dogs treated by surgery was 31.5 months. The median time to local recurrence was 22.5 months (range 15.0 to 22.5 months) in two dogs with MCT grade I, 8.5 months (range 0.7 to 29.5) in 7 dogs with MCT grade III and could not be calculated with the Kaplan-Meier method for 7 dogs with MCT grade II (range 3.0 to 28.5 months). Results obtained by standard surgical treatment, using 2 cm surgical margins with a fascial plane are presented in Table II and Figure 2. Surgical margins were determined by histology and clear margins between 2 to 5 mm were determined. In 8/16 dogs, the tumours recurred after 0.7 to 22.5 months. The highest recurrence rate was obtained in MCT grade III (5/7 dogs).

In Table III, the results of 9 patients with 12 nodules treated with ECT are presented. The median tumour size prior to the treatment was 2.9 cm^3 (ranging from 0.02 to 30.23 cm^3), the mean duration of clinical signs was 25 days (ranging from 10 to 40 days) and the most prevalent location was a hindleg (Table I). The median time of follow-up was 26.0 months, ranging from 2 to 43 months. The estimated median duration of local tumour control was not reached at the time of writing (follow-up range 2 to 43). Overall, 62.5% CR was obtained. In most cases, the CR was obtained after 4-5 weeks, when the scab, which formed at the tumour site fell off (Figure 3). In 2/9 dogs the tumours did not respond to the ECT treatment; these dogs possessed big tumours (>8

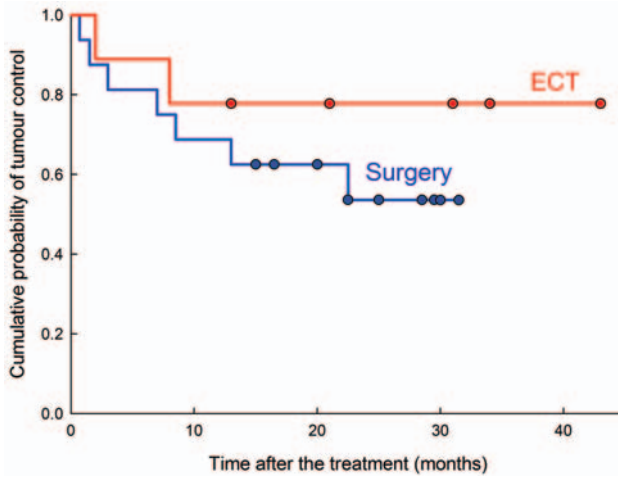

Figure 2. *Kaplan-Meier survival curves for tumour regression for patients treated with surgery and ECT with cisplatin. Circles represent censored data. At the end of the study, 7 dogs were still in CR of the ECT-treated group and 8 dogs treated by surgery.*

Table II. *Tumour volumes, histology and response to treatment in dogs treated with complete surgical excision.*

Patient no.	Histological grade	Tumour volume (cm^3)*	Follow-up time (months)	Response at the end of follow-up
1	I	0.7	22.5	PD
2	I	1.8	15.0	CR
3	II	44.0	3.0	PD
4	II	0.5	28.5	CR
5	II	9.4	16.5	CR
6	II	9.4	30.0	CR
7	II	4.2	25.0	CR
8	II	1.8	20.0	CR
9	II	6.3	13.0	PD
10	III	4.2	24.0	PD
11	III	6.3	27.5	CR
12	III	1.8	29.5	CR
13	III	4.2	7.0	PD
14	III	6.3	1.5	PD
15	III	18.9	0.7	PD
16	III	6.3	8.5	PD

PD, progressive disease; CR, complete response; *at the beginning of treatment.

cm^3) and were euthanized 2 and 8 months after the treatment on owner request without histopathological analyses.

In 7 patients with 9 tumours, 100% of CR was obtained. However, two dogs were euthanized due to other reasons, but at the time of euthanasia were without local tumour recurrence. In one (No. 22), the disease progressed after 24 months, with multiple pulmonary metastases of MCT and the other (No.18) was euthanized 36 months, post-treatment because of severe orthopaedic and neurological problems.

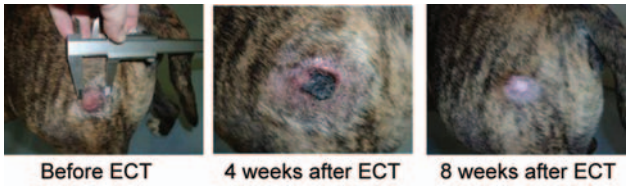

Figure 3. *Patient No. 18 with a tumour nodule in the hindleg before and 4 and 8 weeks after ECT treatment. After treatment, in some cases superficial scab is formed that fell off within 8 weeks. The tumour completely regressed and the dog has been free of disease for more than 3.5 years.*

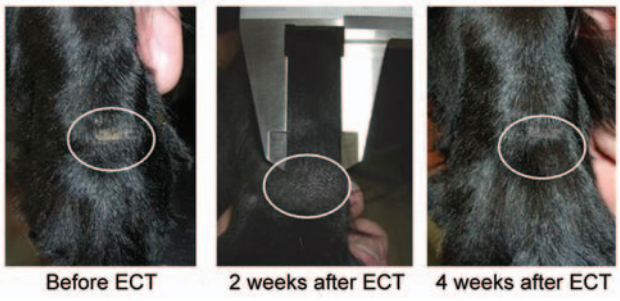

Figure 4. *The tumour nodule in some cases (patient No. 26) disappeared without evidence of local necrosis and without superficial scab formation. The position of the tumour is denoted by a circle.*

The comparison of the results between surgical excision and ECT showed that median size of tumours at the beginning of treatment did not differ between the treatment groups (Mann-Whitney Rank Sum test, p=0.44), the same was obtained for the median follow-up time (Mann-Whitney Rank Sum test, p=0.16). Furthermore, the local tumour control probability curves did not differ between the ECT-treated group compared to dogs that were treated surgically (Figure 2; Logrank test, p=0.14) and the same was time for cumulative probabilities of survival at 22.5 months (0.53±0.65 (surgery group) *vs*. 0.78±0.42 (ECT group); Figure 2). However, the duration of local tumour control was longer in the ECT-treated group compared to the surgery group. The Kaplan-Meier method calculated estimated median duration of response (time to recurrence) was 31.5 months for the surgery group, while for the ECT-treated group it was not yet reached at the time of writing.

Treatment side-effects. The patients tolerated ECT well and no major local or general side-effects were noted. Muscle contractions were observed during the application of electrical pulses, which dissipated immediately after the end of the last electric pulse. The treatment with cisplatin given

Table III. *Tumour volumes, details about treatment and response to treatment in dogs treated with ECT.*

Patient no.	Nodule	Number of sessions	Cisplatin dose (mg/nodule)	Number of trains of pulses/nodule	Tumour volume (cm^3)	Follow-up time (months)	Response at the end of follow up
17	1	1	1.0	8	1.0	21.0	CR
	2	1	1.0	8	1.0	21.0	CR
18	1	1	1.2	3	1.7	43.0	CR
19	1	2	6.0/3.0	17/21	5.3	43.0	CR
20	1	1	3.0	10	0.5	34.0	CR
	2	2	3.0/3.0	10/10	4.2	34.0	CR
21	1	2	8.0/21.0	23/24	25.4	2.0	NC
22	1	2	5.4	18	14.6	31.0	CR
23	1	1	0.2	1	0.02	43.0	CR
24	1	3	10.0/7.0/9.0	22/17/29	30.2	8.0	PD
	2	3	2.0/2.0/3.0	8/7/5	8.1	8.0	NC
25	1	1	0.5	4	0.8	6.0	CR

PD, progressive disease; NC, no change; CR, complete response; dose of cisplatin and the number of trains of electric pulses were adjusted according to the tumour volume.

intratumourally did not result in any local or systemic toxicity. In most cases, partial necrosis of the tumours was noticed within the first week, with formation of a superficial scab in the second to fourth week after treatment, which fell off within 5 to 8 weeks (Figure 3). In some cases, the tumour nodules regressed without any evidence of necrosis (Figure 4). In addition, treatment with ECT did not cause degranulation of tumour cells.

Discussion

The results of the study demonstrate that ECT with cisplatin as a single treatment is an effective treatment of MCT, with as comparable antitumour effectiveness as standard surgical treatment. Furthermore, the duration of local tumour control was longer for tumours that were treated with ECT. ECT was well tolerated by the patients and is a minimally invasive procedure.

ECT has already been demonstrated to be a suitable treatment for various tumour types in companion animals as well as in equides. The results of these different studies have demonstrated ~80% long lasting objective response of the ECT-treated tumours. Mainly, primary tumours of different histological origin, such as fibrosarcomas, perianal tumours, sarcoids, mammary carcinoma and melanoma were treated with ECT with either cisplatin or bleomycin (20-29). Recently, Spungnini and his co-workers published a clinical study using ECT as an adjuvant treatment in incompletely excised MCT in dogs. In that study, ECT using biphasic electric pulses and bleomycin injected in peritumoural tissue was successfully used as an adjuvant treatment. The overall response rate was 85%. The estimated mean time to recurrence time was 52.76±6.5 months, with an observation time up to 75 months (22). In the present study, ECT with cisplatin was tested as a single treatment option and compared its effectiveness with the effectiveness of standard surgical treatment. At 30 months post-treatment, treatment of MCT with ECT in this study resulted in 70% of CR, while surgery resulted in 50% of CR, which did not differ significantly. On the other hand, the mean duration of local tumour control was longer in the ECT-treated group. Whether this difference in the duration of local tumour control is reliable and accurate is impossible to demonstrate, as for the ECT-treated tumours histological grading, which is one of the prognostic factors, could not be performed. Another prognostic factor that influences the outcome of the treatment, tumour size, did not significantly differ between the two treatment groups.

According to the literature, surgery remains the first treatment of choice for MCT (3-8). The results obtained after complete surgical excision alone are not completely in agreement with the published data, most probably due to the low number of animals included; however, the difference could be also due to the inadequate size of clear margins. In the present study, recurrence occurred in one dog with MCT grade I (50%), in two with MCT grade II (28%) and most frequently in 5 out of 7 dogs with MCT grade III (71%). In general, grade I MCT has an excellent prognosis when treated by adequate surgical excision (7, 33). Complete surgical excision of grade II MCT resulted in 89% to 95% local control (3, 5). The recurrences in a large retrospective study in 280 dogs with 340 cutaneous MCTs with different histological grades were confirmed in 23 dogs, 19% with grade III, 6% with grade II and in just 1% with grade I. It should be noted that among these 23 dogs, only three had complete surgical excision, two had narrow margins, 14 had incomplete margins and in 4 the margins at the first excision were not evaluated (6). In a recent study in dogs with MCT

grade II, treated only with incomplete surgical excision, the recurrence rate was 23.3% . During the follow-up time, the median time to local recurrence was not reached and was estimated to be 1,713 days. Furthermore, the estimated proportions of local recurrence were 7.3% at the first year, 22.1% at the second and 33.3% at the 5th year after treatment (8).

Additional treatment of MCT including lymph nodes for local control of the disease and for prevention of metastatic disease is beneficial (34). Unfortunately, the owners of the patients refused any of the recommended postsurgical adjuvant treatments, such as systemic chemotherapy or radiotherapy.

The present study demonstrates that ECT with cisplatin can be an alternative treatment for MCT to surgery. In contrast to surgical excision, in dogs treated with ECT, no local recurrence was observed during the observation time, which was up to 43 months. ECT can be used as a single treatment or as an adjuvant or neoadjuvant treatment to surgery to improve local control and to obtain longer duration of response (22, 26, 27), especially in grade II and III MCT as it is minimally invasive and a one-time treatment. ECT or application of electrical pulses alone can be used as neoadjuvant pre-surgical treatment. Namely, it has been already demonstrated that the application of electrical pulses to the tumours induces a profound, but transient reduction of blood flow, which lasts up to 24 h. The reduction of blood flow is more pronounced after ECT (17). This reduction could be beneficially exploited in combined treatment with surgery that can help to prevent blood-borne shedding of the released vasoactive substances produced by mastocytes.

The mechanisms responsible for the antitumor effectiveness of ECT are diverse. The principal mechanism is an enhanced delivery of chemotherapeutic drugs to tumour cells due to the increased membrane permeability of cells in tumours by electroporation. Secondly, it has also been established that application of electrical pulses alone or ECT also results in tumour vascular disruption, resulting in cessation of blood flow in tumour and retention of chemotherapeutic drugs within. Application of electrical pulses alone to the tumour results in rapid reduction of tumour blood flow that is restored to control values within 24 h. This reduction is reflected in a small reduction of tumour growth without clinical benefit (35). Intratumoral cisplatin chemotherapy is effective and resulted in 20% long-term complete responses, but to a much lesser extent compared to ECT with cisplatin, which resulted in 80% long-term CRs of malignant melanoma in patients (36). On the other hand, intratumoural injection of bleomycin does not exhibit any antitumor effects, due to the highly hydrophilic nature of bleomycin, preventing it from entering the cells without electroporation. The third mechanism involved in antitumor effectiveness of ECT is the immune system. The studies performed in immunodeficient mice pointed out the necessity of immune response to obtain tumour cures. Namely, ECT resulted in up to 80% complete reduction of tumour growing in immunocompetent mice, while no tumour cures were obtained in immunodeficient mice.

In conclusion, the results of this study demonstrate that ECT with cisplatin as a single treatment is a highly effective local treatment of MCT with as comparable antitumor effectiveness as standard surgical treatment. Therefore, it can represent an alternative to surgical treatment, specifically in those cases when owners do not consent to surgery.

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