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ELECTROCHEMOTHERAPY IS HIGHLY EFFECTIVE FOR THE TREATMENT OF CANINE PERIANAL HEPATOID ADENOMA AND EPITHELIOMA

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Perianal tumors are common in older male dogs. The usefulness of electrochemotherapy in veterinary oncology has already been demonstrated by clinical studies on different malignancies in companion animals. In a prospective non-randomized study, we evaluated the effectiveness of electrochemotherapy in 5 male dogs with 26 perianal adenocarcinomas and 16 male dogs with 40 benign tumors. After premedication and under general anesthesia, the dogs were treated with intratumoral administration of a chemotherapeutic drug (cisplatin or bleomycin) and exposure of tumors to electric pulses, delivered by two different electroporation protocols (Protocol 1 or Protocol 2). At the end of the observation time (median 14 months), an objective response (OR) was obtained in 62/66 tumors (94%) with 87.9% complete responses (CR). No statistically significant difference in OR rate was observed based on histological type ($p = 0.110$), previous castration ($p = 0.088$), chemotherapeutic drug used ($p = 0.657$), and electroporation protocol ($p = 0.337$). Tumor size at the beginning of the treatment was the only parameter that influenced the treatment outcome ($p = 0.04$). No major local or general side-effects were noted. We can conclude that electrochemotherapy is an easy, highly effective, safe and cost-effective local approach for the treatment of primary perianal tumors of dogs, especially hepatoid adenoma and epithelioma.

Key words: bleomycin, cisplatin, dog, electrochemotherapy, perianal tumor

INTRODUCTION

Perianal tumors arise from the perianal glands (circumanal or hepatoid glands) and are very common in older, intact male dogs, but rare in female dogs (Holt, 1985; Berrocal *et al*., 1989; Shelley, 2002; Pisani *et al*., 2006). Perianal tumors may occur as solitary or multiple lesions (Shelley, 2002). Perianal benign

tumors (adenoma and epithelioma) constitute one of the most common canine skin tumors and predominantly occur in male dogs due to the androgenic dependency of the perianal glands and their tumors (Berrocal *et al*., 1989). Perianal adenocarcinomas occur less frequently representing only 3-7% of all perianal neoplasms (Berrocal *et al*., 1989; Shelley, 2002; Pisani *et al*., 2006). Perianal adenocarcinoma occurs in castrated or intact males, suggesting no hormonal dependency (Withrow, 1996). Recent studies, however, demonstrated an increased androgen receptor expression in perianal adenocarcinomas, indicating the need for further studies to evaluate the hormonal control of this neoplasm (Shelley, 2002; Pisani *et al*., 2006). Perianal adenocarcinomas look similar to benign tumors but tend to grow faster, are firmer, more frequently ulcerated, usually adhere to the anal and rectal tissues, and frequently recur following treatment (Withrow, 1996). According to the World Health Organization (WHO) International Histological Classification of Tumors of Domestic Animals perianal tumors can be classified in three groups: adenomas, carcinomas, and tumor-like hyperplasias – epitheliomas. Epitheliomas, which are low-grade malignant tumors, are clinically still considered as benign entity (Weiss and Frese, 1974; Goldschmidt *et al*., 1998; Pisani *et al*., 2006).

The treatment for benign perianal tumors in the male dog is tumor removal by surgery or cryosurgery in combination with castration (Liska, 1980; Holt, 1985; Withrow, 1996). Some authors describe also castration alone as an effective treatment of perianal benign tumors (Wilson and Hayes, 1979; Thomas and Fox, 1998). In addition, the growth of benign perianal tumors can be slowed down following estrogen therapy. However, only the temporary effect for the neoplasm regression and the potential risk of severe myelosuppression following estrogen therapy, limits its use (Wilson and Hayes, 1979; Thomas and Fox, 1998).

Perianal adenocarcinomas do not regress following castration and are not responsive to estrogen therapy. Dogs with perianal adenocarcinomas without lymph node involvement and distant metastases are usually treated by a wide surgical excision in combination with cryosurgery or radiation (Vail *et al*., 1990; Withrow, 1996; Thomas and Fox, 1998). For a limited number of cases, following surgical excision, lymphadenectomy, intraoperative radiation to the lymph node bed, and external beam radiation to the lymph node may be useful in slowing down disease progression, although the cost and availability of radiation make this approach a rare alternative for most clinicians (La Rue *et al*., 1995; Withrow, 1996).

Electrochemotherapy is a new tumor treatment modality facilitating intracellular delivery of non-permeant drugs. It is based on the local application of short and intense electric pulses to the cells or tissues that transiently permeabilize cell membranes (Gehl, 2003). To date, its main application has been in the treatment of tumors with non-permeant or poorly permeant drugs having high intrinsic cytotoxicity. The most convenient drugs are bleomycin and cisplatin (Sersa, 2006; Sersa *et al*., 2008). Results of clinical trials in humans have demonstrated that electrochemotherapy is an easy, highly effective and safe treatment approach for cutaneous and subcutaneous tumors of different types resulting in up to 74 % complete regression (Marty *et al*., 2006, Sersa *et al*., 2008).

There are few studies that have already demonstrated the effectiveness, convenience and safety of electrochemotherapy with either cisplatin or bleomycin for the treatment of spontaneous tumors in companion animals (Cemazar et al., 2008). In a previous study on a small number of tumors (n = 26) without confirmatory histology, electrochemotherapy resulted in 65% complete responses (Tozon *et al*., 2005). Because of these promising results, the aim of the present study was to comprehensively evaluate the effectiveness of electrochemotherapy for the treatment of primary perianal tumors in dogs. For this purpose, we determined the effectiveness of electrochemotherapy according to tumor histology (benign tumors versus adenocarcinoma), previous castration, chemotherapeutic drug (bleomycin versus cisplatin), electroporation protocol, and tumor size at the beginning of treatment.

MATERIALS AND METHODS

Selection of dogs

Between March 2000 and September 2006, 21 male dogs for a total of 66 perianal tumors were included in the study (Table 1). This study was a prospective non-randomized study conducted in accordance with protocols for electrochemotherapy that were based on previous experience in human and animal clinical studies (Tozon *et al*., 2005; Sersa, 2006). National Ethics Committee approval and written informed consent from each owner were obtained before the beginning of treatment. The dogs had to possess measurable cutaneous or subcutaneous perianal tumor nodules and owners had to refuse standard treatment (i.e., surgical excision of the tumor), although some dogs had been castrated before the start of electrochemotherapy. In spite of owner's refusal of surgical treatment, they agreed to incisional biopsy just before electrochemotherapy. Eligibility criteria included dogs with normal hemogram and biochemistry results. Dogs with radiographic or ultrasonographically visible visceral metastases, without fine needle aspiration biopsy confirmation, allergic reactions to previous treatments with cisplatin or bleomycin, chronic renal dysfunction or serious cardiovascular diseases, and expected survival of <3 months, were not included in the study.

Histological evaluation

Samples were taken for histopathology by incisional biopsy prior to treatment. Tissue samples were fixed in 4% buffered formalin and embedded in paraffin. Histological classification of tumors was made on 4 μm hematoxylin and eosin (HE) stained paraffin tissue sections according to the World Health Organization (WHO) International Histological Classification of Tumors of Domestic Animals. For the purpose of this study, tumors were classified using the WHO classification published in 1974 that classifies hepatoid (perianal) glands tumor in three groups: adenomas, adenocarcinomas, and tumor-like hyperplasias (Weiss and Frese, 1974). Five carcinomas diagnosed in our series of tumors were morphologically classified as tumors of low-grade malignancy, thus corresponding to the hepatoid (perianal gland or circumanal) gland epitheliomas,

as they are defined in the second edition of WHO classification (Goldschmidt *et al*., 1998). Diagnosis of high-grade carcinoma was based on features such as distinctive cellular atypia, nuclear pleomorphism, higher number of nucleoli and frequent mitoses. To avoid misinterpretation of the malignancy grade due to limited amount of tissue available and clinical classification, it was decided to define a group of adenocarcinomas and a single group of benign perianal tumors (adenomas and epitheliomas) (Figure 1).

Table 1. Patient's and tumor characteristics

Patient No.	Breed	Age/sex	No. of tumors	Tumor type (BT[a] or CA[b])	Castration	Observation time (months)
1	Middle Schnauzer	9/Male	2	BT	no	30
2	Karst Shepard	11/Male	8	CA	no	24
3	Cross-breed	12/Male	2	BT	no	17
4	Cross-breed	13/Male	7	BT	no /yes	20
5	Middle Schnauzer	11/Male	3	BT	no	4
6	Cross-breed	14/Male	2	BT	yes	31
7	Fox Terrier	14/Male	3	BT	no	12
8	Cocker Spaniel	13/Male	3	BT	no	5
9	Pekinese	14/Male	1	BT	no	11
10	Samoyed	11/Male	3	BT	yes	29
11	English Bulldog	10/Male	6	BT	yes	32
12	Labrador Retriever	8/Male	1	BT	no	30
13	Middle Schnauzer	12/Male	9	CA	no	21
14	Karst Shepherd	12/Male	2	BT	no	2
15	German Shepherd	8/Male	2	BT	no	36
16	Cross-breed	12/Male	1	BT	yes	7
17	Cross-breed	12/Male	1	BT	yes	7
18	Small Schnauzer	13/Male	1	CA	yes	7
19	Samoyed	10/Male	3	CA	yes	6
20	Beagle	10/Male	1	BT	yes	5
21	Samoyed	10/Male	5	CA	yes	16

[a] Perianal benign tumor
[b] Perianal adenocarcinoma

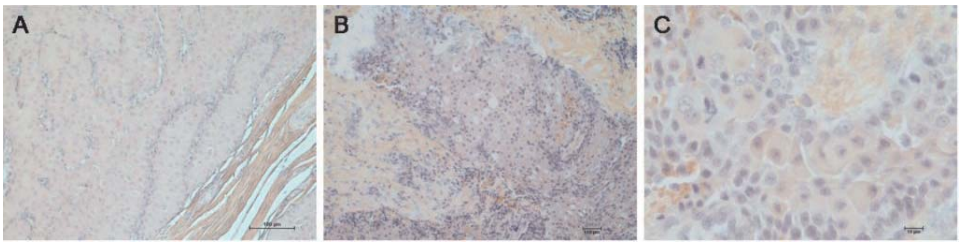

Figure 1. Perianal gland, dog. (A) Adenoma with well-differentiated glandular tissue arranged in cords and limited from surrounding tissue with a fibrous capsule. Reserve cells are scarce and located at the periphery of the cords. HE stain, magnification x 100. (B) Epithelioma with local invasion of tumor cells, but no confirmed invasion of lymphatic or blood vessels. Pleomorphism of hepatoid and reserve cells and clustering of reserve cells are evident. HE stain, magnification x 40. (C) Carcinoma. Grade was not clearly determined in this sample. Cellular and nuclear pleomorphism of hepatoid and reserve cells is evident, as well as several mitoses, however lymphatic or blood invasion was not confirmed. HE stain, magnification x 400

Treatment protocol

Dogs were premedicated with a combination of acepromazine (Promace, Fort Dodge Animal Health; 0.02 mg/kg) and methadone (Haptanon, Pliva; 2 mg/kg). Thirty minutes later, general anesthesia was induced using thiopental (Nesdonal, Merial; 5 mg/kg) and maintained with isoflurane (Forane, Abbott Laboratories). During anesthesia, the animals were receiving Hartmann's solution (B. Braun Melsungen AG) at a rate of 10 mL/kg/h.

The volume of the tumor nodules was calculated by the formula $V = ab^2\pi/6$ (where "a" is the larger diameter of the tumor nodule and "b" the diameter of the tumor nodule perpendicular to "a"), and used for drug dosage calculation when cytotoxic drugs were injected intratumorally.

Electrochemotherapy consisted of intratumoral administration of cisplatin cis-diamminedichloroplatinum II (Cisplatyl, Aventis) followed by exposure of tumors to electric pulses. Cisplatin was used as the first choice drug because of its broader use as a single chemotherapeutic drug or in combined chemotherapy schedules for treatment of different malignancies in veterinary oncology. Cisplatin was dissolved in distilled water at a concentration 2 mg/mL. Cisplatin was given intratumorally at an approximative dose ~2 mg/cm^3 of tumor. The other chemotherapeutic drug used in the study was bleomycin (Blenoxane, Bristol-Myers), which was dissolved in physiological saline at a concentration 3 mg/mL and was given intratumorally at a dose of ~3 mg/cm^3 of tumor. Bleomycin was also used as a second choice drug, when the treatment with cisplatin was not successful. The interval between cisplatin or bleomycin administration and the application of electric pulses was 1-2 min.

Two different electroporation protocols were used in the study based on differences in the type of electrodes and parameters of electric pulses used. Protocol 1 comprised eight electric pulses of 100 μs duration, 1300 V/cm amplitude to electrode distance ratio, and 1 Hz frequency. Electric pulses were

generated by an electric pulse generator Jouan GHT 1287 and delivered through two parallel stainless steel plate electrodes (thickness, 1 mm; width, 7 mm; length, 8 mm, with rounded tips and an inner distance between them of 7 mm; IGEA S. r. l.). Each run of electric pulses was delivered in two trains of four pulses with a 1 s interval in two perpendicular directions. Good contact between the electrodes and the skin was assured by depilation and application of a conductive gel to the treated area. Protocol 2 consisted of eight electric pulses of 100 μs duration, 1000 V/cm amplitude to electrode distance ratio, and 5000 Hz frequency. The electric pulses were delivered through needle electrodes (four needles in a row, two rows, 4 mm apart; IGEA S. r. l.). The use of the second electroporation protocol was enabled by the newly designed electric pulse generator Cliniporator (IGEA S. r. l.) that was developed in the frame of a European Union Commission funded project for clinical use.

Treatment evaluation

After treatment, dogs were kept at the clinic for about 2-4 h. The dogs were then examined twice with a two-week interval, and monthly thereafter in order to evaluate the treatment effectiveness and possible local and systemic side effects. In addition, clinical examination and ultrasound evaluation were performed to evaluate the possible metastatic spread of the disease. At each visit, tumors were measured with Vernier caliper and photographed. For evaluation of treatment response, the tumor size was calculated by the formula $A = ab$, in accordance with the WHO Handbook for Reporting Results of Cancer Treatment (1997). Response to the treatment was scored after four weeks and at the end of the observation period, as complete response (CR) when the tumor was not palpable or as partial response (PR) when a decrease >50% of the largest perpendicular diameters of measurable lesions was determined. A reduction <50% and an increase <25% of the above measurements was defined as no change (NC). Progressive disease (PD) was defined by an increase >25%. In cases where it was not possible to obtain measurements because tumors were ulcerated or covered with crusts, they were rated as non-evaluable. The number of objective responses (OR) was determined by combining the number of CR and PR. Observation time was calculated as the interval between the date of the first treatment and the date of the last examination of the patient. All data and parameters of the treatment procedure were stored in an electronic database storing the electronic Case Record Forms (CRF) (Pavlovic and Miklavcic, 2007). The electronic CRF included measurements and photographs of the treated tumors before and after the treatment and reports on side effects that were evaluated by NCI-CTC toxicity scale (NCI-CTC toxicity grade, 2008).

Statistical analysis

Statistical analysis was performed using the Statistical Packages for Social Sciences (SPSS) 11.0 software. The differences in the distribution of OR of the tumors in the analyzed groups were tested by contingency tables and Mann-Whitney test. $P \leq 0.05$ was considered statistically significant.

RESULTS

Selection of dogs

In the present study, 21 male dogs for a total of 66 tumors were treated with electrochemotherapy (median 2 tumors per dog, range 1-9). The median age was 11.5 years (range 8-14) and the most frequent breeds were cross-breed, Middle Schnauzer, Samoyed and Karst Shepherd. Treated tumors were classified by histological types into perianal adenocarcinomas (26 nodules; 39%) and benign tumors (40 nodules; 61%; adenomas: 35 nodules, epitheliomas 5 nodules). At the end of the observation time, in none of the dogs with perianal adenocarcinomas, metastatic spread of the disease was observed.

In most cases, one electrochemotherapy session was sufficient to obtain good response and more than one session was performed only in 13 tumors (two sessions in 6 tumors and three sessions in 7 tumors, Table 2). Subsequent treatments were repeated 4 weeks after the first treatment with electrochemotherapy if CR was not achieved after the first session. The decision to repeat the treatment with electrochemotherapy was made after the first evaluation of response after 4 weeks, which was a minimum duration for qualification to a certain response. Summary of treatment parameters and tumor response are presented in Table 2.

Effect of treatment at 4 weeks and at the end of observation time regardless of the tumor type

Overall, results for dogs that completed the response evaluation demonstrated good treatment effectiveness. Four weeks after electrochemotherapy, an objective response (OR) was achieved in 51/55 tumors available for evaluation (92.7%) with a 81.8% (45/55) CR, a 10.9% (6/55) PR and a 7.3% (4/55) NC. At the end of the observation period, the OR was obtained in 62/66 tumors evaluated and improved to 93.9% with a few PR (4/66, 6.1%) and CR being the prevalent response (58/66, 87.9%) compared to the response rate seen 4 weeks after electrochemotherapy. Negative responses were rare with few NC (4/66, 6.1%) and none of the treated tumors progressed (PD) (Figure 2, Table 3).

Effect of treatment according to the tumor type, size and electroporation protocol

In the study, 26 adenocarcinomas and 40 benign tumors of different sizes were treated using two different electroporation protocols, and there was no significant difference ($p = 0.110$) at the end of the observation period in OR rate between adenocarcinomas (25/26, 96%) and benign tumors (37/40, 83%, Table 3) regardless of the electroporation protocol and chemotherapeutic drug used. In order to evaluate whether the size of the treated tumors regardless of the histological type, affected treatment outcome, electrochemotherapy treated tumors were alloted into two categories according to their size at the beginning of the treatment (60 tumors <3 cm^2, six tumors ≥ 3 cm^2). A statistically significant difference between the treatment responses to electrochemotherapy according to tumor size was found ($p = 0.04$). At the end of the observation period, tumors

Table 2. Summary of treatment parameters and tumor response

Patient No.	Tumour type (AD[a] or CA[b])	Tumor	No. of ECT[c] sessions	CDDP[d] (mg/tumor)	BLEO[e] (IU/tumor)	Protocol of electric pulses (1[f] or 2[g])	Tumor size before ECT[c] (cm^2)	Tumor size after 4 weeks (cm^2)	Response after 4 weeks (CR[h], PR[i], NC[j] or NA[k])	Response at the end of observation (CR[h], PR[i], NC[j] or NA[k])
1	BT	a1	1	0.6	no	1	0.36	0	CR	CR
		b1	1	0.4	no	1	0.25	0	CR	CR
2	CA	a1	2	1	no	1	1.56	1.1	PR	CR
		a2	1	0.8	no	2	0.72	0	CR	CR
		a3	1	0.8	no	2	0.56	0	CR	CR
		a4	1	no	300	2	0.16	NA	NA	CR
		b4	1	no	900	2	1.95	NA	NA	CR
		a5	2	1	no	2	1.95	NA	NA	CR
		b5	2	0.5	no	2	0.48	NA	NA	CR
		c5	2	0.5	900 - sess. 2	2	0.36	NA	NA	CR
3	BT	a1	1	0.6	no	1	1.54	0	CR	CR
		a2	1	no	1500	1	1.30	0	CR	CR
4	BT	a1	3	2.5	1000 - sess.3	1	1.54	1	PR	CR
		a2	1	0.8	no	2	0.88	NA	NA	NC
		b2	1	0.4	no	2	0.56	NA	NA	PR
		c2	1	0.6	no	2	0.42	NA	NA	CR
		a3	1	no	3000	2	1	0	CR	CR
		b3	1	no	900	2	0.36	0	CR	CR
		c3	1	no	300	2	0.16	0	CR	CR
5	BT	a1	3	0.5	600 – sess. 3	2	0.88	0	CR	PR
		b1	3	0.4	300 – sess. 3	2	0.72	0	CR	PR
		c1	3	0.2	no	2	0.16	0	CR	CR
6	BT	a1	2	1.4	no	1	3.06	1.30	PR	CR
		b1	2	0.4	no	1	1	0.72	NC	CR

Cont. Table 2.

Patient No.	Tumour type (AD[a] or CA[b])	Tumor	No. of ECT[c] sessions	CDDP[d] (mg/tumor)	BLEO[e] (IU/tumor)	Protocol of electric pulses (1[f] or 2[g])	Tumor size before ECT[c] (cm^2)	Tumor size after 4 weeks (cm^2)	Response after 4 weeks (CR[h], PR[i], NC[j] or NA[k])	Response at the end of observation (CR[h], PR[i], NC[j] or NA[k])
7	BT	a1	1	1	no	1	1.44	0	CR	CR
		b1	1	0.3	no	1	0.54	0	CR	CR
		c1	1	0.4	no	1	0.84	0	CR	CR
8	BT	a1	3	2	no	1	0.90	0	CR	CR
		b1	3	6	600 – sess. 3	1	9	8.12	NC	NC
		c1	3	2	no	1	1.10	0	CR	CR
9	BT	a1	1	no	900	1	0.30	0	CR	CR
10	BT	a1	1	no	300	2	0.25	0	CR	CR
		b1	1	no	300	2	0.49	0	CR	CR
		c1	1	no	1800	2	1.80	0.90	PR	PR
11	BT	a1	1	1	no	2	0.36	0	CR	CR
		b1	1	1	no	2	0.49	0	CR	CR
		c1	1	1	no	2	1.10	0	CR	CR
		d1	1	1	no	2	1	0	CR	CR
		e1	1	1	no	2	0.36	0	CR	CR
		f1	1	3	no	2	2.40	0	CR	CR
12	BT	a1	1	no	3000	2	1	0	CR	CR
13	CA	a1	1	no	900	2	1.32	1.08	NC	NC
		b1	1	no	900	2	1.3	0	CR	CR
		c1	1	no	1500	2	1.44	0.56	PR	CR
		d1	1	no	600	2	0.35	0	CR	CR
		a2	1	1	no	2	1.08	0	PR	CR
		b2	1	0,6	no	2	0.56	0	CR	CR
		c2	1	0,4	no	2	0.16	0	CR	CR
		a3	1	1	no	2	0.56	NA	NA	CR
		b3	1	0,4	no	2	0.04	NA	NA	CR

Cont. Table 2.

Patient No.	Tumour type (AD[a] or CA[b])	Tumor	No. of ECT[c] sessions	CDDP[d] (mg/tumor)	BLEO[e] (IU/tumor)	Protocol of electric pulses (1[f] or 2[g])	Tumor size before ECT[c] (cm^2)	Tumor size after 4 weeks (cm^2)	Response after 4 weeks (CR[h], PR[i], NC[j] or NA[k])	Response at the end of observation (CR[h], PR[i], NC[j] or NA[k])
14	BT	a1	1	4	no	1	5	0	CR	CR
		b1	1	3	no	1	2.7	0	CR	CR
15	BT	a1	1	1	no	1	0.77	NA	NA	CR
		a2	1	no	3000	1	3	2,52	NC	NC
16	BT	a1	1	1.2	no	2	1.44	0	CR	CR
17	BT	a1	1	no	3000	2	3.06	0	CR	CR
18	CA	a1	1	no	1800	2	2.52	0	CR	CR
19	CA	a1	1	no	1500	2	1.21	0	CR	CR
		b1	1	no	300	2	0.56	0	CR	CR
		c1	1	no	1500	2	1.21	0	CR	CR
20	BT	a1	1	2.5	no	2	7.5	0	CR	CR
21	CA	a1	1	no	150	2	0.04	0	CR	CR
		b1	1	no	300	2	0.09	0	CR	CR
		c1	1	no	150	2	0.09	0	CR	CR
		d1	1	no	300	2	0.25	0	CR	CR
		e1	1	no	300	2	0.25	0	CR	CR

[a] Perianal benign tumor; [b] Perianal adenocarcinoma; [c] Electrochemotherapy; [d] Cisplatin; [e] Bleomycin;
[f] Plate electrodes, amplitude 1300 V/cm, frequency 1 Hz; [g] Needle electrodes, amplitude 1000 V/cm, frequency 5000 Hz
[h] Complete response; [i] Partial response; [j] No change; [k] Not evaluable

<3 cm^2 responded better to the treatment with a 96.7% (58/60) OR rate and a high CR rate (54/60, 90%), compared to the tumors ≥3 cm^2 that responded with a 66.7% (4/6) OR rate (4/6 tumors responded with CR, Table 3).

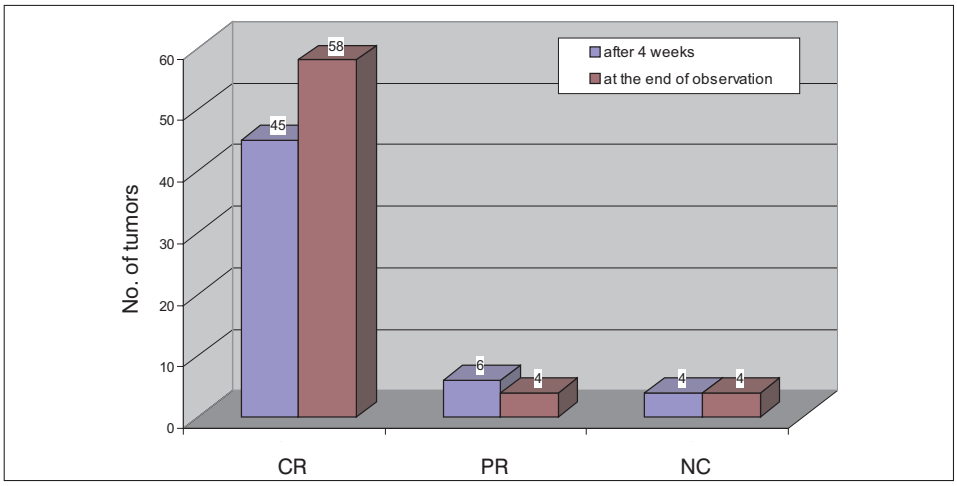

Figure 2. Therapeutic efficacy of electrochemotherapy on perianal tumors after four weeks and at the end of observation time. CR = complete response, PR = partial response, NC = no change

Table 3. Summary of tumor response according to the electric pulses protocol and tumor type and size

	CR	PR	NC
Protocol 1			
large adenoma	2		2
large carcinoma			
small adenoma	14		
small carcinoma	1		
Protocol 2			
large adenoma	2		
large carcinoma			
small adenoma	15	4	1
small carcinoma	24		1
Response at 4 weeks*	45	6	4
Response at the end of obs. period	58	4	4

*response of 11 tumors to treatment was not evaluable

For the electroporation protocols, we treated 19 tumors with Protocol 1 and 47 tumors with Protocol 2 (Table 3). Statistical analysis of the treatment response according to the electroporation protocol did not show a difference in responsiveness of tumors to electrochemotherapy ($p = 0.337$). At the end of the observation period, the OR rate was 89.5% for Protocol 1 and 95.7% for Protocol 2.

Effect of treatment based on previous castration

We treated 23 tumors in nine dogs castrated before starting electrochemotherapy and 36 tumors in 11 dogs previously not castrated. One patient (no. 4) started electrochemotherapy without previous castration, but was castrated later during the treatment (before treatment of tumors a3, b3, c3). Among castrated 10 patients 7 dogs had benign tumors and 3 dogs had adenocarcinomas. Three patients (6, 10 and 16) with benign tumors were castrated one to 12 months before electrochemotherapy and 4 dogs simultaneously with electrochemotherapy. All 3 dogs with adenocarcinoma were castrated simultaneously with electrochemotherapy due to benign prostatic hyperplasia. At the end of the observation period, tumors in previously castrated dogs responded with a 96.7% (29/30) OR rate, a 90.0% (27/30) CR rate, a 6.7% (2/30) PR rate and 3.3% (1/30) NC rate. Tumors from non-castrated dogs responded with a 91.7% (33/36) OR rate, an 86.1% (31/36) CR rate, a 5.6% (2/36) PR rate, and a 8.3% (3/36) NC rate. No significant difference in OR rate between castrated and non-castrated dogs was observed ($P = 0.088$).

Effect of treatment based on the chemotherapeutic drug used

Electrochemotherapy was performed using two chemotherapeutic drugs, cisplatin (35 tumors) or bleomycin (26 tumors). Five tumors were treated with cisplatin first and continued with bleomycin in later sessions. When the tumors were treated with a single chemotherapeutic drug, results of OR rate at the end of the observation period showed no statistically significant difference ($p=0.657$) between the OR rate after electrochemotherapy with bleomycin (92.3%) or cisplatin (97.1%). Among the five tumors that were treated with both cisplatin and bleomycin, two responded with CR (40%), two with PR (40%), and one tumor remained unchanged (NC, 20%).

Side effects

Dogs tollerated the treatment well and no major general side effects were noted. Muscle contractions were observed after the application of electric pulses in both electroporation protocols. The contractions were instantaneous, disappearing immediately at the end of each electric pulse. Therefore, in the Protocol 2 due to the high repetition frequency of applied electric pulses (5 kHz), only one muscle contraction was observed, compared to eight contractions with Protocol 1. The treatment with cisplatin or bleomycin given intratumorally did not result in any local or systemic toxicity. In some cases, we noticed partial necrosis of the tumors after a week with formation of a superficial scab, which fell off within

4 weeks, while in some cases the scab was not formed (Figure 3). After treatment, none of the patients suffered from local or systemic infections.

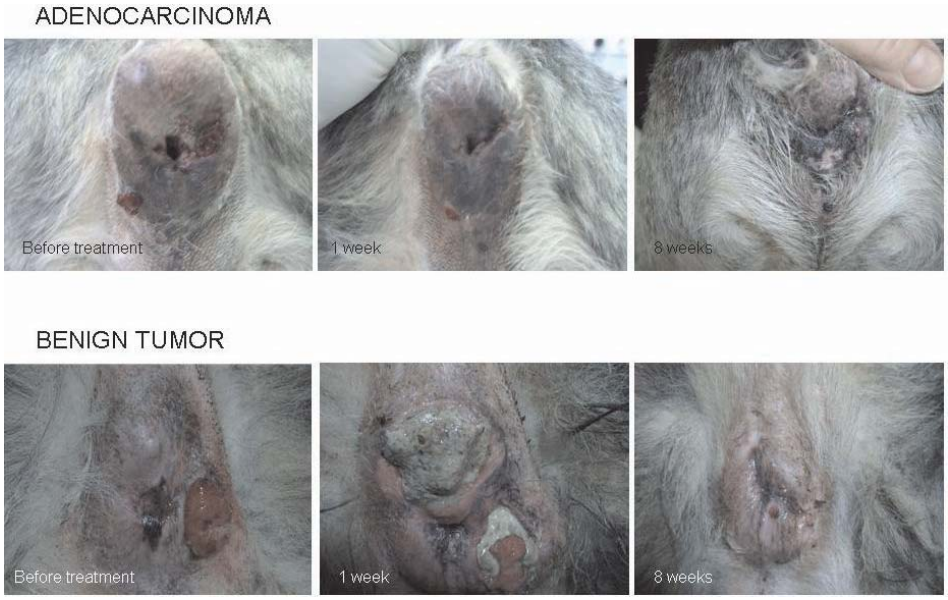

Figure 3. Antitumor effectiveness of electrochemotherapy with bleomycin in patient no. 13 with 9 adenocarcinoma nodules of which 3 were exulcerated (upper panel) and patient no. 14 with large perianal adenoma (lower panel) before electrochemotherapy, one week post-treatment (note necrosis of the tumor nodules), and the complete regression of the tumor nodule after eight weeks

DISCUSSION

Our study demonstrates that electrochemotherapy of primary perianal tumors in dogs, especially hepatoid adenoma and epithelioma is a highly effective treatment with an overall OR rate of 93.9%. Tumor size at the beginning of the treatment was the only parameter that influenced treatment outcome; treatment effectiveness of electrochemotherapy was less pronounced in tumors ≥ 3 cm^2, although still resulting in a 66.7% OR. Furthermore, this highly efficient treatment resulted in long term CR that lasted up to 36 months.

In our previous study, we demonstrated that electrochemotherapy was effective with a 92% overall treatment response at the end of the observation time (median observation time 12 months) (Tozon *et al.*, 2005). The main difference between the two studies is that in the present study, we evaluated more comprehensively the effectiveness of electrochemotherapy for the treatment of perianal tumors in dogs. No difference in the success rate between adenocarcinomas and benign tumors in the present study confirms observations

from previous clinical studies on electrochemotherapy, although in the present study most of the treated tumors were benign. These studies demonstrated that when the whole tumor mass is electroporated and sufficient drug is used, electrochemotherapy is effective regardless of the tumor type (Marty *et al*., 2006; Sersa, 2006). In the present study, tumors <3 cm^2 resulted in significantly better treatment responses compared to bigger ones. In larger tumors, several applications of electric pulses were needed to cover the whole tumor area and in two patients (no. 5 and no. 8), more than one electrochemotherapy session was needed for further reduction of the tumor. In bigger tumors, the response of the tumors to electrochemotherapy was good, but with less CR, and in some cases, with more complications (larger necrotic area, but without systemic signs of inflammation). Our results are in agreement with the results of another study in which the prognosis of smaller perianal carcinomas (<5 cm in diameter) surgically treated was better than in bigger tumors, having a survival rate in excess of 70% after two years (Vail *et al*., 1990; Withrow, 1996; Thomas and Fox, 1998).

Previous clinical studies on different malignancies in companion animals demonstrated the usefulness of electrochemotherapy with either bleomycin or cisplatin. In the first clinical trial conducted in 1997, electrochemotherapy increased the lifespan of cats with spontaneous large soft-tissue sarcomas (Mir *et al*., 1997). In another study, cutaneous and subcutaneous tumors of various histological types in cats and dogs responded with an 84% OR to electrochemotherapy. Significant prolongation of the duration of response in electrochemotherapy treated tumors was observed compared to tumors treated with cisplatin only (Tozon *et al*., 2001). In another study with companion animals affected by spontaneous large neoplasms, electrochemotherapy resulted in a high response rate (overall response >80%), superior to the group of patients treated only with intratumoral injections of bleomycin (Spugnini and Porrello, 2003). Similar effectiveness was confirmed also in several other recently published clinical studies, where canine oral melanomas, canine mast cell tumors, feline hemangiopericytoma, and feline soft tissue sarcomas were treated with electrochemotherapy (Baldi and Spugnini, 2006; Spugnini *et al*., 2006a, 2006b, 2007). Electrochemotherapy is also very successful in the treatment of horse sarcoids. The clinical trials conducted in horses confirmed that electrochemotherapy with cisplatin is a highly effective treatment against sarcoids with long-lived anti-tumor effects and good treatment tolerance (Rols *et al*., 2002; Tamzali *et al*., 2001, 2003).

Veterinary medicine is still searching for alternative treatments of inoperable tumors in the perianal region and metastatic adenocarcinoma in that area. Excision with surgery, cryosurgery or CO_2 laser in combination with castration is still the preferred treatment for perianal tumors (Liska, 1980; Shelley, 2002). Radiation therapy, which can prevent spread to regional lymph nodes in perianal carcinoma, has local disadvantages, since it can be performed only in specialized centers and is also very costly (Liska, 1980; La Rue *et al*., 1985; Withrow, 1996). In a retrospective study, 41 dogs with perianal carcinoma were treated with surgical excision, debulking and cryosurgery, or debulking and radiation. Treatment type did not influence either disease-free interval or survival. Two-year disease free

intervals approached 75% and 60% in stage T1N0M0 (tumor <2 cm in diameter) and T2N0M0 (tumor 2-5 cm in diameter), implying that surgical removal of the tumor mass with or without cryotherapy is a good option for controlling these tumors. In that study, dogs treated with surgical debulking and external beam radiation were all of advanced stage (T4N0M0) and their number was too low to evaluate the response; however the authors concluded that perianal carcinomas are not highly radiation responsive (Vail *et al*., 1990). Results of our study demonstrate that antitumor effectiveness of electrochemotherapy is comparable to the effectiveness of surgical treatment. Namely, 96.8% of OR with 93.6 % of CR lasting up to 29 months were achieved for perianal carcinomas, regardless of the size of the tumor at the beginning of the treatment. Furthermore, tumors ≥ 3 cm^2 (T2N0M0 and advanced) responded well to electrochemotherapy with a 66.7% OR rate.

Other treatment modalities for perianal adenocarcinomas include estrogen therapy and systemic chemotherapy with actinomycin D. These treatment modalities are not frequently used due to side effects and limited effectiveness (Wilson and Hayes, 1979; Liska, 1980; Hammer *et al*., 1994; Thomas and Fox, 1998). Hyperthermia was tested in one study as an alternative treatment for perianal tumors. In that study treatment of six perianal adenomas with hyperthermia resulted in six complete responses. The observation time in the study was short (2-6 months) and it is therefore difficult to assess the potential success and applicability of this treatment for perianal adenoma (Grier *et al*., 1980). Electrochemotherapy proved effective for local tumor control in both perianal adenocarcinomas and benign tumors resulting in long lasting complete responses (median 14 months) and can as such represent a good alternative for standard treatments of tumors in the perianal region. However, it should be noted that treatment of adenocarcinomas with surgery alone can be effective, but patients often die from metastatic disease to the local lymph nodes.

Regarding the treatment procedure, electrochemotherapy is easy and quick to perform, and is inexpensive. The requirements are: a suitable room for patient preparation and treatment, and an electric pulse generator with different sets of electrodes. After treatment, patients do not require special care or post-treatment medication. In human patients treated with electrochemotherapy, pain is a limiting factor (Sersa *et al*., 2008). In animals, pain associated with injection of the chemotherapeutic drug and application of electric pulses is avoided with short-term anesthesia, which lasts no more than 10-15 min depending on the size and numbers of the nodules to be treated. Namely, each application of electric pulses lasts a maximum of 8 s. Another advantage of electrochemotherapy is that lower doses of chemotherapeutic drugs are needed for pronounced antitumor effectiveness, which does not result in systemic side effects. All possible known systemic side effects were not observed after electrochemotherapy. These side effects were avoided due to selective tumor drug delivery (Sersa *et al*., 2008). In the present and our previous studies, tumor necrosis is a consequence of successful treatment; however it represents a side effect of electrochemotherapy. The size of tumor necrosis depends on the size of the treated tumor. It is important to note that animals did not show any signs of pain due to the presence of

necrosis. However, the animal owners had to be willing to maintain the wound toilet, although special wound dressing was not required. For owners, unpleasant wound care was needed only for a short period of time, since approximately one week after therapy, the superficial scab developed and fell off within 5 weeks in the case of CR of the tumor nodule.

CONCLUSIONS

Clinical studies in humans have already demonstrated that electrochemotherapy is an easy, highly effective, safe and cost-effective approach for the treatment of cutaneous and subcutaneous tumors of different malignancies. The results of the present study on electrochemotherapy of perianal tumors in dogs, with a 93.9% OR rate (87.9% CR), long lasting responses and insignificant side effects, are in-line with clinical studies in humans. Specifically, electrochemotherapy was highly effective regardless of tumor histological type, drug used, previous castration of the patient, and electroporation protocol. Therefore, these results indicate that electrochemotherapy is a good local alternative to current treatment modalities for non-metastatic canine perianal tumors, especially hepatoid adenomas and epitheliomas.

CONFLICT OF INTEREST STATEMENT:
None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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ELEKTROHEMOTERAPIJA JE VEOMA EFIKASNA U TRETMANU PERINEALNOG HEPATOIDNOG ADENOMA I EPITELIOMA PASA

TOZON NATAŠA, KODRE VERONIKA, JUNTES POLONA, SERSA G i CEMAZAR MAJA

SADRŽAJ

Perianalni tunori su relativno česti kod starijih pasa muškog pola i primenjljivost elektrohemoterapije u veterinarskoj onkologije je već opisana u nekoliko studija. U ovoj prospektivnoj studiji mi smo procenjivali uspeh elektrohemoterapije kod 5 pasa sa 26 perianalnih karcinoma i 16 pasa sa 40 benignih tumora. Posle premedikacije i uvođenja u opštu anesteziju, psi su tretirani aplikacijom hemoterapeutika (cisplatin ili bleomicin) u tkivo tumora a zatim su tumori izlagani električnim impulsima na osnovu dva različita protokola. Na kraju perioda opservacije (u proseku 14 meseci), povoljan ishod je zapažen u 94 % slučajeva, a potpun uspeh je postignut u 87,9% slučajeva. Nisu uočene statistički značajne razlike u odnosu na histološki nalaz, prethodnu kastraciju i elektroterapijski protokol. Jedini parametar koji je bio u vezi sa ishodom je bila veličina tumora ($p<0,04$). Takođe, nisu zapaženi sporedni lokalni ili opšti efekti. Zaključeno je da je elektrohemoterapija jednostavan, efikasan i ekonomski opravdan način tretmana primarnih perinealnih tumora pasa, posebno hepatoidnih adenoma i epitelioma.