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Electrochemotherapy for Mammary Tumour in Dogs

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ABSTRACT

Background: Mammary neoplasms are the most common tumours in the bitch. Even though surgery remained the treatment of choice for mammary gland tumours except those with inflammatory carcinomas or distant metastasis; it did not seem to reduce the chances of recurrence in case of malignancy in dogs. Other alternatives to surgery are chemotherapy, immunotherapy, radiation therapy, hormonal therapy *etc*. The major disadvantage of chemotherapeutic agents was the lack of selectivity for tumour cells. To overcome this problem, electrochemotherapy could be used as a single or as an adjuvant therapy to surgery which could be used for treatment of inoperable tumours. Cisplatin and bleomycin are the two chemotherapeutic agents used in veterinary oncology for electrochemotherapy. This study was carried out on clinical cases of mammary tumours to study and compare the efficacies of electrochemotherapy using Cisplatin and bleomycin

Methods: The research was carried out on clinical cases of canine mammary tumour presented to the Small Animal Surgical Out-Patient Unit of Madras Veterinary College Teaching Hospital for a period of one year from September 2018 to May 2021. Routine Clinical, Haematological, Biochemical and Radiographic evaluations were performed. Twelve dogs with mammary tumours, with a single solitary lump were selected for the study irrespective of the age, breed and location. Group I Cases were treated by electrotherapy with cisplastin and Group II were treated by electrotherapy with bleomycin intralesionally following which surgical excision was performed.

Result: Most cases in the cisplatin group produced partial response (3/6) according to the response evaluation criteria, whereas most cases in the bleomycin group produced complete response (100% regression). Bleomycin was comparatively more efficient (1.7 times) than cisplatin in electrochemotherapy for mammary tumours. The time under anaesthesia was low which makes this procedure a suitable alternative to geriatric dogs that cannot be anaesthetised for a longer duration for surgery.

Key words: Canine, Cisplastin-bleomycin, Electrochemotherapy, Mammary tumors.

INTRODUCTION

Mammary neoplasms are the most common tumours in the bitch. Even though surgery remained the treatment of choice for mammary gland tumours except those with inflammatory carcinomas or distant metastasis; it did not seem to reduce the chances of recurrence in case of malignancy in dogs. The major disadvantage of chemotherapeutic agents was the lack of selectivity for tumour cells. Therefore, high doses of chemotherapeutic agents were needed to achieve the required anti-tumour effect which led to severe local and systemic toxicity. To overcome this electrochemotherapy as a novel treatment modality for tumours facilitated intracellular delivery of non-permeant drugs. Cisplatin and bleomycin are the two chemotherapeutic agents used in veterinary oncology for electrochemotherapy. Electrochemotherapy could be used as a single therapy or as an adjuvant therapy to surgery which could be used for treatment of inoperable tumours as a one-time treatment or could be repeated several times with equal or improved effectiveness in case of failure or partial tumour response. This study was carried out on clinical cases of mammary tumours to study and compare the efficacies of electrochemotherapy using Cisplatin and bleomycin.

MATERIALS AND METHODS

The research was carried out on clinical cases of canine mammary tumour presented to the Small Animal Surgical Out-Patient Unit of Madras Veterinary College Teaching Hospital for a period of three years from September 2019 to May 2021. Routine clinical, haematological, biochemical and radiographic evaluations were performed. Twelve dogs with mammary tumours, with a single solitary lump were selected for the study irrespective of the age, breed and location of the tumour.

Group I cases were treated by electrotherapy with cisplastin and Group II with bleomycin. Location and size of tumor,evaluation of lymphnodes and distant metastasis, WHO TNM classification of mammary tumors were performed to be followed by electrochemotherapy for the mammary tumors. The dose of the chemotherapeutic drug

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was calculated based on the volume of the tumour. Two perpendicular diameters of the tumour were measured using a digital vernier caliper and the volume of the tumour was calculated using the formula,

$$V = \frac{ab^2 \pi}{6\ cm^3}$$

Where,
"a"= Argest diameter.
"b"= Diameter of the nodule perpendicular to "a".

Following a safe anesthetic protocol, Cisplatin (Khandelwal Laboratories Ltd.) was administered intra-tumourally @ 1 mg/ cm^3 of tumour for tumours < 1 cm^3 and 0.5 to 1 mg/cm^3 for tumours > 1 cm^3 whereas, bleomycin (Cipla ltd.) was given @ 1500 IU/cm^3 of tumour for tumours < 1 cm^3 and 1500 to 3000 IU /cm^3 for tumours > 1 cm^3.

Application of electric pulses

The application of electric pulses was done immediately following intra-tumoural administration within 1 to 2 minutes. Electrical pulses were generated by ELECTRO vet S13 (Leroy biotech, France) (Fig 1) and delivered through two parallel stainless steel plate electrodes (Fig 2) at pulse duration 100msec, amplitude to electrode distance ratio 1,300 V/cm, frequency 1 Hz (Fig 3). Additional sessions were performed at 2-4 weeks interval, if the tumour was observed unresponsive post first session of treatment.

Post-chemotherapy follow-up

Tumour size evaluation

Evaluation of tumour volume

The size of the tumour was also measured periodically at the end of 30 days following electrochemotherapy response was calculated based on Response Evaluation Criteria in Solid Tumours (RECIST) and classified under complete response (CR), where there was disappearance of target lesion; partial response (PR), where there was at least a 30% decrease in sum of diameters of target lesions; progressive disease (PD), where there was at least a 20% increase in the sum of diameters of target lesions and stable disease (SD), neither progressive disease, nor partial response. The tumour size was measured on 3rd, 7th, 14th, 21st and 30th postoperative days. Two perpendicular diameters (a, b) were measured (Fig 4). The response was calculated according to the response evaluation criteria in solid tumours.

$$\text{Response} = \frac{(a_0+b_0)-(a_{30}+b_{30})}{(a_0+b_0)} \times 100$$

Where
a_0= Largest diameter of the tumour on day 0.
b_0= Diameter perpendicular to a_0.
a_{30}= Largest diameter on day 30.
b_{30}= Diameter perpendicular to a_{30}.

At the end of four weeks, fine needle aspiration cytology was performed at different sites of the tumour mass to find any neoplastic cells and to detect any cellular criteria of malignancy. Post-chemotherapy complications, if any, were recorded and treated accordingly. The adverse effects were categorized as groups based on Veterinary Co-operative Oncology Group-Common Terminology Criteria for adverse events (VCOG-CTCAE) (2016) and were graded from grades 1 to 5. The tumours that have partially responded to the electrochemotherapy were surgically removed by simple or regional mastectomy. Separate surgical instruments were used to prevent seeding of tumour cells in the healthy tissues. The surgically excised tumour masses were subjected to histopathological evaluation.

RESULTS AND DISCUSSION

A retrospective study was conducted on the clinical cases of tumours in dogs brought to the Madras Veterinary College Teaching Hospital between September 2019 to May 2021. These included 629 cases of dogs with cutaneous or subcutaneous tumours. Mammary tumour constituted to 27.66% of all canine tumours (174 cases) as also reported earlier (Johnson 1993; Dorn *et al.* 1968 and Moulton 1990). The mean size of the tumours in Group I was found to be 3.70±0.94 cm and Group II 2.52±0.45 cm. All the tumours in

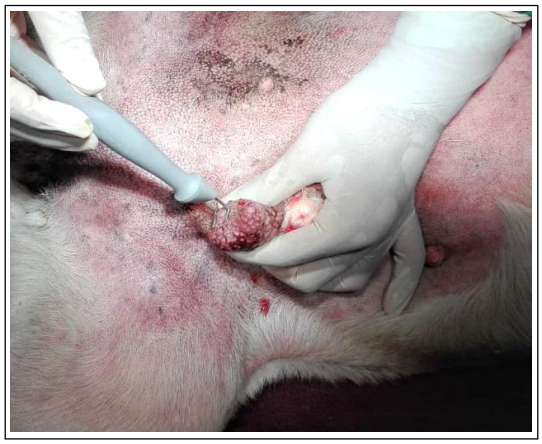

Fig 1: Application of electrodes over the tumour mass.

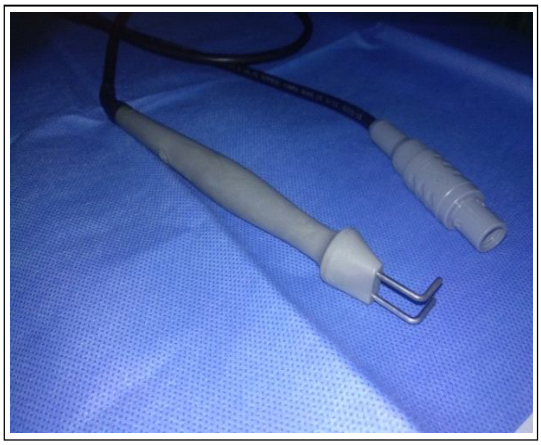

Fig 2: Parallel stainless steel plate.

the present study were non-ulcerated, round, freely movable and non-invasive solitary tumours. Absence of palpable enlargement of the lymph nodes was observed in the cases under study. There was no change in consistency of the lymph nodes (Table 1). According to Misdorp (2002), metastasis to the regional lymph node was the earliest step in metastasis. Absence of distant metastasis was noticed on a lateral and ventro-dorsal thoracic radiography in all the 12 cases. Among the 12 cases, 2 cases in Group I were categorized under stage III because the tumour sizes were more than 5 centimetres. Three of the cases in Group I and 2 cases in Group II were categorized under stage II, since the tumour size was between 3 and 5 centimetres (Table 2). This clinical staging helped in providing a realistic prognosis to the owner. Bleomycin and cisplatin were chosen for the study since only these two drugs were identified as potential drugs for electrochemotherapy by Mir (2006) and Lowe *et al.* (2017). The cytotoxicity of cisplatin as observed by Patrick *et al.* (1984) was due to direct binding to the DNA and inhibition of its synthesis and was proven to combat a variety of malignancies like sarcomas, cancers of soft tissues, bone, muscle and blood vessels observed by Desoize and Madoulet (2002). The cytotoxicity of bleomycin was mainly due to direct DNA damage. The presence of injected drug in the target tissue determined the efficacy of electrochemotherapy. The electric pulses were applied using stainless steel plate electrodes in two perpendicular directions covering the entire surface of the tumour which was said to generate a transmembrane potential difference according to Neumann *et al.* (1982). This transmembrane potential difference resulted in cell electroporation by generation of hydrophilic long-lived pores, according to Weaver and Chizmadzhev (1996) and Teissie *et al.* (2005). Bharathidasan *et al*. (2019) however opined that the uptake of drugs proceeded through an endo-cytic-like mechanism instead of electroporation.

The advantage noticed in the procedure was its short duration of time, simplicity and low dose of cytotoxic drugs with insignificant side effects and hospitalization was not required in any of the cases. There was an apparent decrease in the mean value of tumour volume in both the groups on the 30th day when compared to day 0 (Table 3). This could possibly be attributed to the fact that electric pulsation increased the toxicity of bleomycin by several thousand folds and the fact that several hundred molecules

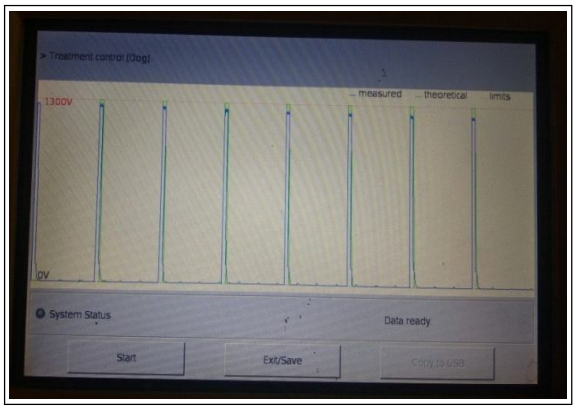

Fig 3: Pulses parameters and waves depicting transmission of pulses.

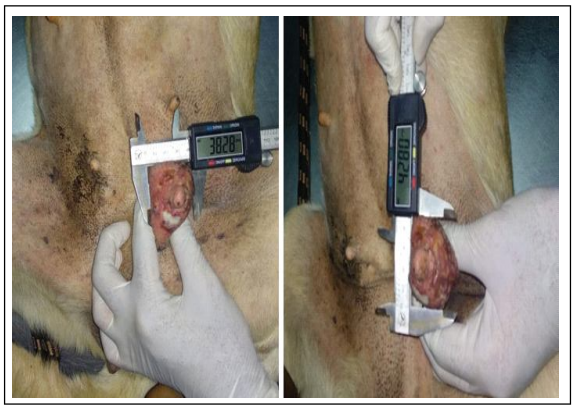

Fig 4: Diameters of the tumour using vernier callipers electrodes.

Table 1: FNAC, RLN cytology and nipple aspiration cytology.

Group	Case no.	FNAC- Tumour mass	FNAC- regional lymph node	Nipple aspiration cytology
Group I	1	Mammary adenocarcinoma	Negative	Positive
	2	Mammary adenocarcinoma	Negative	Positive
	3	Mammary adenocarcinoma	Negative	Positive
	4	Mammary adenocarcinoma	Negative	Negative
	5	Mammary adenocarcinoma	Negative	Negative
	6	Mammary adenocarcinoma	Negative	Negative
Group II	1	Mammary adenocarcinoma	Negative	Negative
	2	Mammary adenocarcinoma	Negative	Positive
	3	Mammary adenocarcinoma	Negative	Positive
	4	Mammary adenocarcinoma	Negative	Negative
	5	Mammary adenocarcinoma	Negative	Negative
	6	Mammary adenocarcinoma	Negative	Positive

of bleomycin inside the cell were sufficient to kill the cell (Poddevin *et al*., 1991).

Cisplatin was less potent in electrochemotherapy possibly because there was only seventy fold potentiation when electric pulses were applied when compared to several thousand folds in bleomycin. Cisplatin on the other hand had higher potential in clinical practice due to its effectiveness in many chemotherapy protocols (Sersa *et al.* 1995). Factors like survival time and disease-free interval have not been taken into the study like in the study by Karayannopoulou *et al.* (2001) and hence the efficacy of electrochemotherapy for mammary tumour in long term remains uncertain. In group I, cases 1, 2, 3, 5 and case 6 from Group II showed fewer clusters of neoplastic cells when compared to the pre-chemotherapy cytology slide. (Table 4 and 5) All the other cases did not show any signs of malignancy. This could also be misinterpreted as stable disease since these add-up to the bulk of the tumour.

There were no intra-therapeutic complications observed during the except muscle twitching during application of electric pulses in all cases.The adverse effects following electrochemotherapy were recorded and graded as per Veterinary Cooperative Oncology Group-Common Terminology Criteria for Adverse Events (VCOG-CTCAE). Except for cases 1, 2 and 3 in Group I, all the other cases showed tumour necrosis as a local reaction to electrochemotherapy. According to Snoj *et al.* (2005) and Shimizu *et al.* (2003), electrochemotherapy was considered

Table 2: TNM classification of the tumours under study.

Group	Case No.	Tumour size	Nodal involvement	Distant metastasis	Clinical stage
Group I	1	$T3_b$	N_0	M_0	III
	2	$T3_b$	N_0	M_0	III
	3	$T2_b$	N_0	M_0	II
	4	$T1_b$	N_0	M_0	I
	5	$T1_b$	N_0	M_0	I
	6	$T1_a$	N_0	M_0	I
Group II	1	$T1_b$	N_0	M_0	I
	2	$T1_b$	N_0	M_0	I
	3	$T2_b$	N_0	M_0	II
	4	$T1_a$	N_0	M_0	I
	5	$T1_b$	N_0	M_0	I
	6	$T2_b$	N_0	M_0	II

Table 3: Tumour volume, drug used and dosage.

Group	Case no.	Tumour volume (cm^3)	Drug used	Dosage
Group I	1	104.71	Cisplatin	8 mg
	2	39.40	Cisplatin	8 mg
	3	18.10	Cisplatin	8 mg
	4	5.92	Cisplatin	6 mg
	5	4.84	Cisplatin	5 mg
	6	1.74	Cisplatin	2 mg
Group II	1	4.97	Bleomycin	7.5 U
	2	3.28	Bleomycin	5 U
	3	14.84	Bleomycin	15 U
	4	1.17	Bleomycin	3 U
	5	0.73	Bleomycin	1.5 U
	6	31.73	Bleomycin	30 U

Table 4: Mean±SE values of tumour volume on different post-chemotherapy days (Group I).

Parameter	Day 0	Day 3	Day 7	Day 14	Day 21	Day 30
Mean volume of the tumour (in cm^3)	29.12±16.14	25.65±17.26	12.86±6.37	6.62±4.08	6.39±3.34	4.51±3.26

Table 5: Mean±SE values of tumour volume on different post-chemotherapy days (Group II).

Parameter	Day 0	Day 3	Day 7	Day 14	Day 21	Day 30
Mean volume of the tumour (in cm^3)	9.46±4.92	4.14±2.58	0.45±0.33	0.31±0.31	1.56±1.50	0.0000

as a cyto-reductive technique before surgical resection.This was true with the current study where three dogs from Group I and one dog from Group II which partially responded to the therapy underwent a simple mastectomy to remove the cyto-reduced mass. Simple mastectomy was preferred since the tumour size had reduced considerably and MacEwen *et al.* (1985) found no difference in recurrence rate and survival time between simple mastectomy and chain mastectomy. Reduction in tumour size was correlated to a better prognosis even after surgery according to Vail *et al*. (1990) who found that smaller sized tumours had a better prognosis.Though surgery was considered as the best treatment for mammary tumours, high mortality was still observed by Stratmann *et al.* (2008), proving that surgery alone might not be sufficient.

CONCLUSION

Both cisplatin and bleomycin can be effectively used to treat mammary tumours by electrochemotherapy.There were no significant changes in the haemato-biochemical parameters following electrochemotherapy using cisplatin or bleomycin when given intralesionally. Most cases in the cisplatin group produced partial response (3/6) according to the response evaluation criteria, whereas most cases in the bleomycin group produced complete response (100% regression). Bleomycin was comparatively more efficient (1.7 times) than cisplatin in electrochemotherapy for mammary tumours. The adverse effects were minimum and did not require clinical intervention and thereby comparatively safe when compared to systemic chemotherapy. The time under anaesthesia was less which makes this procedure a suitable alternative to geriatric dogs that cannot be anaesthetised for a longer duration to perform a surgery.

Conflict of interest: None.

REFERENCES

Bharathidasan, M., William, B.J., George, R.S., Arunprasad, A. and Sivasankar, R. (2019). Electrochemotherapy with intra-tumoral cisplatin for the treatment of fibrosarcoma in a horse. Indian Journal of Animal Research. 53(4): 541-544.

Desoize, B. and Madoulet, C. (2002). Particular aspects of platinum compounds used at present in cancer treatment. Critical Review in Oncology/ Hematology. 42(3): 317-325.

Dorn, C.R., Taylor, D.O.N., Schneider, R., Hibbard, H.H. and Klauber, M.R. (1968). Survey of animal neoplasms in Alameda and Contra Costa Counties, California. II. Cancer morbidity in dogs and cats from Alameda County. Journal of the National Cancer Institute. 40(2): 307-318.

Johnsion, S.D. (1993). Reproductive System (Mammary Neoplasia). In: Textbook of Small Animal Surgery. [Slatter, D.H. (Ed.)], Saunders, WB, Philadelphia. 2185-2192.

Karayannopoulou, M., Kaldrymidou, E., Constantinidis, T.C. and A. Dessiris. (2001). Adjuvant post operative chemotherapy in bitches with mammary cancer. Journal of Veterinary Medicine. A Physiology, Pathology, Clinical Medicine. 48(2): 85-96.

Lowe, R., Gavazza, A., Impellizeri, J.A., Soden, D.M. and Lubas, G. (2017). The treatment of canine mast cell tumours with electrochemotherapy with or without surgical excision. Veterinary and Comparative Oncology. 15(3): 775-784.

MacEwen, E.G., Harvey, H.J., Patnaik, A.K., Mooney, S., Hayes, A., Kurzman, I. and Hardy, J.W. (1985). Evaluation of effects of levamisole and surgery on canine mammary cancer. Journal of Biological Response Modifiers. 4(4): 418-426.

Mir, L.M. (2006). Bases and rationale of the electrochemotherapy. European Journal of Cancer Supplements. 4(11): 38-44.

Misdorp, W. and Hart, A.A.M. (2002). Canine mammary cancer: I. Prognosis. Journal of Small Animal Practice. 20(7): 385-394.

Moulton, J.E. (1990). Tumors of the Mammary Gland. In: Tumors in Domestic Animals. Moulton, J.E. [(Ed)]. University of California Press, London. 518-552.

Neumann, E., Schaefer Ridder, M., Wang, Y. and Hofschneider, P. (1982). Gene transfer into mouse lyoma cells by electroporation in high electric fields. The EMBO Journal. 1(7): 841-845.

Patrick J., Loehrer, M.D., Lawrence, H., Einhorn, M.D. (1984). Cisplatin: Diagnosis and treatment five years later. Annals of Internal Medicine. 100: 704-713.

Poddevin, B., Orlowski, S., Belehradek Jr, J. and Mir, L.M. (1991). Very high cytotoxicity of bleomycin introduced into the cytosol of cells in culture. Biochemical Pharmacology. 42: S67-S75.

Sersa, G. (1995). Electrochemotherapy. In Electrochemotherapy, Electrogenetherapy and Transdermal Drug Delivery. Humana Press. Pp: 119-136.

Shimizu, T., Nikaido, T., Gomyo, H., Yoshimura, Y., Horiuchi, A., Isobe, K., Ebara, S. and Takaoka, K. (2003). Electrochemotherapy for digital chondrosarcoma. J. Orthop. Sci. 8(2): 248-251.

Snoj, M., Cemazar, M., Slekovec Kolar, B. and Sersa, G. (2005). Effective treatment of multiple unresectable skin melanoma metastases by electrochemotherapy. Croatian Medical Journal. 48(3): 391-395.

Stratmann, N., Failing, K., Richter, A. and Wehrend, A. (2008). Mammary tumor recurrence in bitches after regional mastectomy. Veterinary Surgery. 37(1): 82-86.

Teissie, J., Golzio, M. and Rols, M.P. (2005). Mechanisms of cell membrane electropermeabilization: A minireview of our present (lack of?) knowledge. Biochimica Biophysica Acta (General Subjects). 1724(3): 270-280.

Vail, D.M., Withrow, S.J., Schwarz, P.D. and Powers, B.E. (1990). Perianal adenocarcinoma in the canine male: A retrospective study of 41 cases. Journal of the Amercian Animal Hospital Association. 26(3): 329-334.

Waldron, D.R. (2001). Diagnosis and surgical management of mammary neoplasia in dogs and cats. Veterinary Medicine. 96(12): 943-949.

Weaver, J.C. and Chizmadzhev, Y.A. (1996). Theory of electroporation: A review. Bioelectrochemistry and Bioenergetics. 41(2): 135-160.