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STUDIES ON MANAGEMENT OF CANINE MAMMARY TUMOURS WITH DENDRITIC CELL THERAPY

A. K. Vala	M. V. Sc. Scholar, Department of Veterinary Surgery & Radiology, College of Veterinary Science & Animal Husbandry
P. V. Parikh*	Professor & Head, Department of Veterinary Surgery & Radiology, College of Veterinary Science & Animal Husbandry *Corresponding Author
J. K. Mahla	Assistant Professor, Department of Veterinary Surgery & Radiology, College of Veterinary Science & Animal Husbandry
D. N. Kelawala	PhD Scholar, Department of Veterinary Surgery & Radiology, College of Veterinary Science & Animal Husbandry
S. Natesan	Chief Scientific Officer, Biomac Life Sciences, and Adjunct Faculty, Indian Institute of Public Health Gandhinagar.

ABSTRACT The present study was carried out with multiple objectives. Twelve cases of canine mammary tumours were detail examined in two groups (6 in each group). Radiography of thorax revealed absent of metastases in all the cases. Ultrasonography revealed size, irregularities of margins as well as echogenicity of mammary tumour masses with vasculature, helping to differentiate their nature and clinical significance. After one month of completion of Dendritic cells (DC) immunotherapy (Group 1), size of tumour mass was increased or remained constant in five case and in one case it disappeared. In surgery followed by DC immunotherapy (Group 2) protocol every case recovered uneventfully and no recurrence was observed. Data of MTT assay and IFNG+ indicated that DC therapy potentiate CMI response in this group of animals. Based on result, Surgery followed by DC therapy group was proved good compared to immunotherapy group. From the above study it can be concluded that DC therapy was observed to be safe and well tolerated in all animals without any adverse effects or toxicity. Study provided novel insights on the benefit of DC based cellular immunotherapy for managing mammary tumours in canines. Further studies to improve the therapeutic efficacy of DC therapy in canines are required in the future.

KEYWORDS : Dendritic cell therapy, CMI response, MTT assay, IFNG+, mammary tumour

Introduction

Mammary gland tumours are one of the most frequently diagnosed neoplasms in female dogs. The average age for the development of mammary gland neoplasia is 8 to 10 years. Approximately 50% of mammary tumors in dogs are benign and rest are carcinomas (MacEwen *et al*., 1996).

Dendritic cell (DC) based cellular immunotherapy for the treatment of cancers is a novel therapeutic vaccine approach using the autologous activated dendritic cells. Dendritic cell plays a central role in antigen capture, processing, presentation to induce the adaptive T cell mediated immune response. Dendritic cells are the professional antigen-presenting cells of the innate immune system with the potential to generate robust antigen-specific T cell immune responses (Mantia-Smaldone *et al*., 2013). Dendritic cells are playing key role in both immunity induction and tolerance maintenance. (Ballestrero *et al*., 2008). An autologous dendritic cell-canine mammary tumor hybrid cell fusion is another approach of DC vaccine and it is found more effective than other DC vaccines (Shu *et al.*, 2007 and Koido *et al*., 2009). This approach has been attempted for canine mammary tumor in dogs (Bird *et al*., 2011). Chemotherapy or radiation therapy after excision of tumour mass is commonly used in veterinary clinical practice to prevent reoccurrence. But both the therapies have several side effects. Hence, the present study was planned to evaluate the efficacy and scope of dendritic cell based therapy for mammary gland tumour in dogs with the following objectives.*viz*., Standardize the treatment protocol for dendritic cell therapy for mammary gland tumours, To study the safety and tolerability of dendritic cell based therapy for the treatment of mammary gland tumour in dogs, Evaluation of efficacy of dendritic cell therapy in dogs.

Materials and Methods

The present study was carried out on canine patients having mammary tumours which were presented at the Department of Veterinary Surgery and Radiology, Anand. The detailed information of 12 cases presented during the year October 2014 to February 2016 was meticulously recorded. Lateral plain radiography was done in all the animals to determine the presence of metastasis in lung. An ultrasound machine with a 7.5 MHz linear-array ultrasound transducer and 4.0 MHz convex ultrasound transducer were used to image the lesion and vascularization of mass. Approximately 20-30ml of blood was collected in sterile tubes with anticoagulant depending on the body weight of the animals to develop monocyte derived DC for therapeutic purposes. The final activated DC preparation in normal saline was administered to the same animal in which the blood was collected in an autologous mode through intravenous route from an infusion bag containing normal saline. If the tumor is assessable, the dose was divided and half was injected around the peripheral area of the tumour and the other half was given intravenously. The number of cells in each dose ranged from 2.6 to 29.3 million. Four doses were injected at an interval of 7-10 days. The primary objectives of this study was to evaluate the safety, tolerability, and efficacy of DC therapy in dogs. Following the administration of DC therapy, animals were observed for any adverse effect for assessing the safety and tolerability of proposed therapy. The animals were monitored for clinical response at different time points by measuring the size of tumour using a Vernier calliper and calculating the tumour volume, assessing the quality of life before and after therapy, progression of disease. Therapeutic protocol remained same in both group but in group 2 also performed surgery on first day.

Cell mediated immune response was measured following dendritic cell therapy by measuring proliferative potential of lymphocytes in response to antigenic stimulation through MTT assay and by measuring the interferon gamma positive cells in the peripheral blood mononuclear cells.

Results and discussion

Thoracic radiography was performed in 12 cases. Radiographic examinations revealed normal translucent lung. Not a single dog showed signs of tumour metastases in lung or thorax chest lesions. Sonography revealed out of 12 cases, four cases showed Hetrogenous hypoechoic pattern. Among them two have regular margin and two have irregular margins with high blood supply, three cases showed well defined hypoechoic pattern with anechoic multi lobules and regular margins, whereas three cases showed a well defined hypoechoic round regular margins and two have irregular margins.

Immunotherapy group: After administering 3 doses of DC therapy, the specific immune activation against tumor antigens was measured

by CMI response through MTT assay. The overall survival and size of tumor was observed at regular interval following the completion of 4 doses of DC therapy. In the present study naturally occurring canine mammary tumour, induction of the CMI response against the tumor antigens following dendritic cell therapy could be observed**. (Table 1 and 2)**

Table 1: Measuring CMI response using MTT assay of immunotherapy group (before and therapy)

Case	PBMC		PBMC- lysate+2µl		PBMC-lysate+4µl		PBMC-lysate+8µl		PBMC+PHA (2µl)	
	before	After	before	after	before	After	before	After	before	After
Case-1	1.02	3.16	0.96	3.29	1.12	3.47	1.09	3.29	3.46	4.86
Case-2	0.89	2.4	1.092	3.21	0.938	3.33	1.076	3.21	2.87	5.00
Case-3	1.45	0.83	1.64	0.56	1.82	1.25	1.62	0.56	2.64	1.29
Case-4	0.72	3.16	1.005	3.29	1.225	3.47	1.007	3.29	3.83	4.86
Case-5	1.08	2.84	1.322	3.41	1.52	2.86	1.31	3.41	3.23	5.8
Case-6	0.90	2.34	1.16	2.78	1.373	2.54	1.16	2.67	3.53	3.12

*the numerical value shown in table are mean of the optical density at 570nm (OD 570) of MTT assay triplicates.

Table 2: Measuring the IFNg positive cells in the canine PBMC stained with anti- IFNg antibody in immunotherapy group (before and after therapy)

Case	Case-1		Case-2		Case-3		Case-4		Case-5		Case-6	
	before	after	before	after	before	After	before	after	before	after	before	After
Total cells	126	68	155	82	96	63	113	91	114	114	121	87
IFNg+ cells	14	21	8	23	11	12	13	18	12	24	9	21
% of IFNg+ cells	11.11	30.88	5.16	28.05	11.46	19.05	15.04	19.78	13.68	21.05	10.89	24.13

*Number cells shown in the table are mean cell count of five fields counted on 400x magnification. Total cells were counted in phase contrast or bright field microscopy whereas IFNg+ cells were counted on fluorescent signal with TRITC filter.

In this group, DC immunotherapy was performed in eight cases. Size of tumour mass was measured on each therapeutic dose and after one month of completion of therapy. After one month of completion of therapy, size of tumour mass was found to be increased gradually and the disease progressed in four cases. Tumour mass remained in constant size with stable disease in three cases and one case was recovered with complete response and became tumor free.

In human cases of breast cancer, Svane (2007) and Park (2007) reported that DC therapy is safe, feasible, well tolerated and induced significant anti-tumour immune response.

Surgery followed by immunotherapy: In this group, eight cases were included. Surgery was performed in all cases initially, and then DC cells immunotherapy was given at 8-10 days interval. After completion of therapeutic protocol, all these cases recovered completely. The overall progression free survival is 100% during the current follow up period. We also observed a profound increase in the cell mediated immune response following DC therapy in this group **(Table 3 and 4).**

Table 3: Measuring CMI response using MTT assay of surgery followed by immunotherapy group (before and therapy)

Case	PBMC		PBMC- lysate+2µl		PBMC- lysate+4µl		PBMC-lysate+8µl		PBMC+ PHA (2µl)	
	before	After	before	after	before	after	before	After	before	After
Case-1	0.46	0.54	0.74	0.7	1.15	1.27	0.88	0.92	1.87	1.93
Case-2	0.85	2.06	0.99	2.2	1.27	2.52	1.08	1.8	2.72	2.6
Case-3	0.71	0.82	1.15	1.72	0.98	1.615	1.23	1.71	2.61	2.815
Case-4	0.84	1.22	0.92	1.34	1.07	1.43	1.14	1.39	1.19	1.45
Case-5	0.95	0.942	1.02	0.89	1.12	1.01	0.98	0.964	0.92	0.99
Case-6	0.49	1.46	0.76	1.56	0.91	1.62	0.85	1.55	1.82	1.63

Table 4: Measuring the IFNg positive cells in the canine PBMC stained with anti- IFNg antibody in surgery followed byimmunotherapy group (before and after therapy)

Case	Case-1		Case-2		Case-3		Case-4		Case-5		Case-6	
	before	after	Before	after	before	After	before	after	before	after	before	After
Total cells	145	103	95	86	134	58	121	108	119	89	102	96
IFNg+ cells	7	28	11	31	9	13	8	34	11	25	8	28
% of IFNg+ cells	4.76	27.18	11.57	36.05	6.71	22.41	9.68	31.48	13.09	28.09	8.16	29.16

In a group of human patients treated with DC cells immunotherapy following tumour reccurence after surgical excision of glioblastoma tumour, improved progression free survival of 3 months and overall survival of 9.6 months was observed by Steven (2008). Chang and Chen-Nen (2011) reported that patients with malignant glioma underwent surgery for maximal cytoreduction followed by a 6-month 10-injection course of autologous DC-tumor vaccine therapy showed tumour shrinkage, increased immune response, and increased overall survival.

In the present study observed that DC based therapy following our therapeutic protocol was safe and well tolerated in all the animal without any adverse effects or toxicity. Despite the strong induction of cell mediated immune response following DC therapy, major clinical benefit in terms of tumour regression was not achieved except in one case in the present study group where the animals were treated with only DC therapy. However, progression free survival or stable disease was achieved in many animals of the same group. Most DC based clinical studies in human and animal showed similar trend in terms of the clinical benefit without complete tumour regression. Further improvements in the design of DC therapeutic protocol for treating solid tumours are required to achieve maximum clinical benefit. Identification of tumor specific antigens, breaking immunological tolerance to cancer antigens, inducing strong T cell mediated immune response with immunomodulating agents, and in combination with other modes of cancer therapies including chemotherapy or radiotherapy would help to achieve major success in DC based immunotherapy through maximum clinical benefit in terms of bringing a cure through complete remission, overall survival and progression free survival. Canine species are very valuable to human beings as a companion, help in police and army force, and helping the disabled individuals. It is important to avail the advanced cell based therapeutic technologies to animals to save their lives. Studies in animals could also help to further improving potency and efficacy of DC based immunotherapy in animals as well as human beings. The present study provided novel insights on the benefit of DC based cellular immunotherapy for managing mammary tumours in canines. Further studies to improve the therapeutic efficacy of DC therapy in canines are required in the future.

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