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Low dose metronomic chemotherapy with cyclophosphamide and piroxicam for the management of canine mammary neoplasms

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ABSTRACT

A clinical research was undertaken to evaluate the effect of low dose metronomic (LDM) chemotherapy in 18 clinical cases of canine mammary neoplasms (CMN). The cases were randomly divided into three groups each consisting of six animals. Group I was subjected to LDM therapy with cyclophosphamide @ 10 mg/m^2 and piroxicam @ 0.3 mg/kg per os for a period of eight weeks pre operatively on alternate days. Group II was subjected to mere surgical excision alone. Group III was subjected to LDM therapy from 10th day following tumour excision for a period of eight weeks as in group I. Effect of LDM therapy was evaluated based on regression of tumour size and the disease free interval. The haematological and biochemical values were evaluated. Among the three groups, Group I showed progressive regression in the disease free interval improving the quality of life.

Key words: Canine mammary neoplasms, Cyclophosphamide, Low dose metronomic chemotherapy, Piroxicam.

INTRODUCTION

Among the various types of neoplasm, mammary tumours were the most frequent and common type in the intact female dogs (Simon *et al.* 2009). Canine mammary neoplasms are found to be the second most commonly occurring tumours next to skin tumours accounting to approximately 50% of all tumours in female dogs of which 40 to 50% are malignant. Different factors like age, breed and genetic predisposition, hormones and growth factors, cyclooxygenase-2 expression and diet had an influence on the development of canine mammary tumours (Jain and Raghunath, 2007). The main concern in veterinary oncology is to maintain patient's quality of life. Treatment of canine mammary neoplasm is mainly based on surgical removal, which is carried out by different ways according to need but undetectable micro-metastasis remains the major cause of surgical failure.

Chemotherapy for canine mammary neoplasms is achieved by two ways – adjuvant chemotherapy given post-operatively to delay or prevent recurrence and metastasis and neoadjuvant chemotherapy in case of large, apparently inoperable tumours which may then be removed surgically (Brearly, 1989; Sleeckx *et al.* 2011). LDM refers to adminis tration of comparatively low doses of a chemotherapeutic or non-chemotherapeutic drug (compared with conventional doses) on a frequent (daily, several times a week, or weekly) or continuous schedule with no extended interruptions. Initially, it was proposed that this type of regimen exerts its effects absolutely by killing the rapidly dividing endothelial cells in tumours, thus preventing angiogenesis. As compared to conventional chemotherapy, metronomic chemotherapy has been shown to have an important stabilizing effect on human cancer resulting in prolonged clinical benefit. Positive effects are obtained without any sign of high grade toxicity. Moreover, low cost and oral administration were key characteristics of the schedule (Pierini *et al*. 2012).

Continuous administration of cyclophosphamide efficiently inhibits angiogenesis and exerts considerable positive effects on antitumour immunity. Cyclooxygenase-2 (COX-2), which may be overexposed by tumour cells or stromal cells, also can promote tumour growth by stimulating angiogenesis. Metronomic cyclophosphamide chemotherapy and COX-2 inhibitors decreased angiogenesis and suppressed regulatory T cells (Elmslie *et al*.2008) Hence, the present study was designed to evaluate the tumour regression and disease free interval in dogs suffering from mammary tumour administered with metronomic doses of cyclophosphamide and standard doses of piroxicam.

MATERIALS AND METHODS

The study was carried out in 18 canine cases with clinical signs of mammary tumours without any systemic diseases presented to the small animal surgery unit, TVCC, Veterinary College and Research Institute, TANUVAS, Namakkal. The cases were randomly divided into three groups of six animals each as follows:

Group I: Subjected to pre-operative (neoadjuvant) low dose metronomic chemotherapy with cyclophosphamide and piroxicam for a period of eight weeks.

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Group II: Subjected to mere surgical excision of mammary tumour only.

Group III: Subjected to surgical excision of mammary tumour followed by low dose metronomic (adjuvant) chemotherapy with cyclophosphamide and piroxicam from 10th post-operative day for a period of eight weeks.

In all the animals, signalment was obtained. Distant metastasis and the pair of mammary glands involved and morphology of the tumour including the size, appearance, consistency and any discharge and/or ulcer were evaluated. All animals were examined physically to record the symptoms. Blood samples were collected at day 0, 15th, 30th, 45th and 60th day during LDM therapy in group I animals. Blood collection from group I and II animals were done pre-operatively, post operatively at day 7 and at 30th, 60th, 90th, 120th, 150th and 180th days. In group III animals blood samples were collected pre-operatively, post-operatively and at 30th, 60th (after eight weeks of LDM therapy), 90th, 120th and 180th post-operative days to monitor any change in the haemato logical parameters. The blood samples were subjected for estimation of haemoglobin (Hb) (g/dl), packed cell volume (PCV) (%), total erythrocyte count (TEC) (10^6/µl), total leucocyte count (TLC) (10^3/µl), differential leucocyte count (DLC) (%), blood urea nitrogen (BUN) (mg/dl), creatinine (mg/dl), alanine aminotransferase (ALT) (I.U/l), aspartate aminotransferase (AST) (I.U/l) and serum alkaline phosphatase (SAP) (I.U/l). Chest X-ray (plain radiography) in lateral position was done to rule out lung metastasis. Fine needle aspiration cytology was done to differentiate benign and malignant tumours.

Cyclophosphamide (Tab. Endoxan™, Cadila Healthcare Ltd., Ponda, Goa) was administered orally at the dose rate of 10 mg/m^2 and Piroxicam (Tab. Mobicam-DT-Cipla Ltd., Nainital, Uttarakhand) was administered orally at the dose rate of 0.03 mg/kg body weight daily along with frusemide (Tab. Frusemide I.P.-Medibest Pharma Pvt. Ltd., Hosur, Tamil Nadu) at the dose rate of 2 to 4 mg/kg body weight. In addition, the animals were provided with ranitidine at the dose rate of 0.5mg/kg body weight respectively along with supportive therapy like liver tonic and haematinics as per the requirement in each case to minimize the post chemotherapeutic complications.

Simple mastectomy and regional mastectomy were performed depending on the invasiveness of the tumour and involvement of the regional lymph nodes. All the cases subjected to the study were assessed for clinical toxicity and were followed for a period of maximum six months to detect recurrence of neoplasms, death or survival rate, etc. to determine the efficacy of the treatment. The data obtained in the study were analysed statistically in SPSS software (version 15.0) as per the methods outlined by Snedecor and Cochran (1994). The significance between the treatment groups was analysed by one-way ANOVA test. P value statistical significance was declared at 5%.

RESULTS AND DISCUSSION

The highest incidence was noticed in Doberman pinscher followed by Spitz and non-descript, Labrador, German shepherd, Pug, Lhasa apso, Dachshund and Great Dane. Highest incidence of mammary tumour was seen in the age group of three to six years (40.63%), followed by six to nine years (28.13%) and 9-12 years (18.75%). The

Table 1: Signalment of animals of Group I, II and III

Group	Animal No.	Breed	Age (years)	Breeding History	Size (cm)
Group I	1	Pug	7	Intact	6.5
	2	Doberman	7	Intact	28.0
	3	Spitz	6	Intact	7.2
	4	Spitz	9	Intact	5.6
	5	Labrador	5	Intact	6.3
	6	Labrador	3.5	Intact	12.0
Mean ± S.E.			6.25 ± 0.77		14.17 ± 1.78
Group II	1	Spitz	5	Intact	3.5
	2	GSD	10	Intact	9.1
	3	Spitz	5	Intact	4.4
	4	Non-Descript	13	Intact	28.2
	5	GSD	11	Intact	9.2
	6	GSD	10	Intact	7.2
Mean ± S.E.			9 ± 1.34		10.27 ± 3.71
Group III	1	Doberman	3	Intact	11.6
	2	Doberman	6	Intact	15.7
	3	Doberman	9	Intact	12.1
	4	Daschund	5	Intact	22.4
	5	Doberman	8.5	Intact	10.8
	6	Non-Descript	3.5	Intact	12.4
Mean ± S.E.			5.83 ± 1.02		10.93 ± 3.54

Table 2: Regression of mammary tumour in group I animals (in cm)

Animal No.	Day 0	15th day	30th day	45th day	60th day
1	6.5	5.72	5.2	4.8	4.4
2	28	19.6	15.68	12.54	10.03
3	7.2	5.7	4.9	4.5	4.1
4	5.6	5	4.6	4.7	4.5
5	6.3	5.7	5.1	4.8	4.3
6	12	11	9	8.5	7.7

Table 3: Haematological values of Group I during LDM therapy

Parameter		Day 0	15th day	30th day	45th day	60th day
Hb (g/dL)		$14.50^{a} \pm 0.35$	$14.00^{a} \pm 0.38$	$14.02^{a} \pm 0.40$	$14.50^{a} \pm 0.44$	$14.60^{a} \pm 0.39$
PCV %		$42.41^{a} \pm 3.24$	$43.38^{a} \pm 3.32$	$41.43^{a} \pm 1.81$	$43.77^{a} \pm 2.12$	$44.28^{a} \pm 2.17$
TEC(10^6/cu.mm)		$7.03^{a} \pm 0.46$	$6.97^{a} \pm 0.59$	$6.93^{a} \pm 0.40$	$7.30^{a} \pm 0.4$	$7.46^{a} \pm 0.39$
TLC(10^3/cu.mm)		$9.20^{a} \pm 0.70$	$8.68^{a} \pm 0.44$	$8.72^{a} \pm 0.41$	$8.57^{a} \pm 0.39$	$9.12^{a} \pm 0.56$
	N (%)	$77.00^{a} \pm 1.63$	$75.67^{ab} \pm 1.38$	$72.50^{abc} \pm 1.20$	$70.83^{bc} \pm 0.95$	$70.50^{c} \pm 0.76$
	L (%)	$18.67^{a} \pm 1.61$	$20.00^{a} \pm 1.34$	$23.33^{ab} \pm 1.26$	$25.17^{b} \pm 0.79$	$26.67^{b} \pm 0.56$
DC	**M (%)**	$2.50^{a} \pm 0.43$	$1.83^{ab} \pm 0.40$	$1.50^{b} \pm 0.34$	$1.33^{ab} \pm 0.21$	$1.00^{b} \pm 0.00$
	E (%)	$1.67^{a} \pm 0.42$	$1.67^{a} \pm 0.21$	$2.17^{a} \pm 0.60$	$1.67^{a} \pm 0.33$	$1.67^{a} \pm 0.31$

Row wise mean (± SE) with different superscript (abc) differ significantly at ($p<0.05$)

Table 4: Serum biochemical values of Group I during LDM therapy

Parameter	Day 0	15th day	30th day	45th day	60th day
BUN (mg/dL)	$21.02^{a} \pm 1.43$	$22.05^{a} \pm 1.49$	$22.20^{a} \pm 1.53$	$21.70^{a} \pm 1.56$	$21.60^{a} \pm 1.42$
CREATININE (mg/dL)	$1.23^{a} \pm 0.12$	$1.27^{a} \pm 0.14$	$1.36^{a} \pm 0.18$	$1.35^{a} \pm 0.17$	$1.33^{a} \pm 0.14$
ALT (I.U/L)	$55.47^{b} \pm 1.71$	$68.00^{a} \pm 1.39$	$66.90^{a} \pm 2.12$	$65.62^{a} \pm 2.25$	$67.89^{a} \pm 1.75$
AST (I.U/L)	$40.26^{b} \pm 1.11$	$47.31^{a} \pm 1.04$	$45.62^{a} \pm 1.13$	$45.55^{a} \pm 1.15$	$47.25^{a} \pm 1.31$
SAP (I.U/L)	$90.32^{b} \pm 3.85$	$112.25^{a} \pm 5.11$	$101.41^{ab} \pm 3.75$	$106.72^{a} \pm 3.40$	$104.44^{ab} \pm 3.31$

Row wise mean (± SE) with different superscript (abc) differ significantly at ($p<0.05$)

least incidence was observed between zero to three years (6.25%) and 12-15 years (6.25%). All the animals reported were intact females. The site of occurrence of tumour was noticed as either solitary or multiple. The cranial abdominal and inguinal glands were found to be equally affected (36%) followed by caudal abdominal (24%) and caudal thoracic glands (4%). The mean ± S.E. value of the tumour size in centimeters in group I, group II and group III was 14.17±1.78, 10.27±3.71 and 10.93±3.54 respectively (Table 1). Chest radiography revealed no metastasis in all the animals.

Microscopic examination of the aspirated cytological sample showed a sheet of neoplastic glandular epithelial cells exhibiting pleomorphic cells with anisocytosis and anisokaryosis with prominent nucleoli. The cytological examination performed was very useful, reliable and it was correlated with the histopathological findings of malignancy (Sivashankar, 2003, Narayanan, 2010 and Gupta *et al.* 2014). It was found that the drug compounded in capsules in accordance with Leo *et al.* (2014) was easily dispensable and well accepted and 10mg/m^2 dose of cyclophosphamide (Elmslie *et al.* 2008) was well tolerated by the animals. The occurrence of Sterile Haemorrhagic Cystitis associated with metronomic low dose cyclophosphamide was found reduced and it was further prevented from the uroepithelial toxicity by treating with per oral furosemide administration before cyclophosphamide in the morning hours and by encouraging frequent drinking of fresh water and urination as reported by Warry *et al.* (2011).

In group I, there was non-significant regression in the size of the tumour in all the six cases (Table 2 and Fig. 1 and 2). Mastectomy was performed in all the animals following chemotherapy, as chemotherapy alone is not sufficient to treat malignant canine mammary neoplasms (Jain and Ragunath, 2007). Loven *et al.* (2013) observed tumour regrowth following termination of LDM therapy suggesting the tumour dormancy of the residual disease.

Pre-operatively in group I during LDM therapy, there was no significant difference in Hb and PCV values. A significant decrease in neutrophils and a compensatory increase in lymphocytes were observed (Table 3). The mean AST, ALT and SAP values were found increased significantly (Table 4) and this could be attributed to the metabolic activity of liver for detoxification of the drugs in accordance with Gupta *et al.* (2014).

Surgical excision of mammary tumour is considered as a routine therapy and is curative for benign tumours (Veena

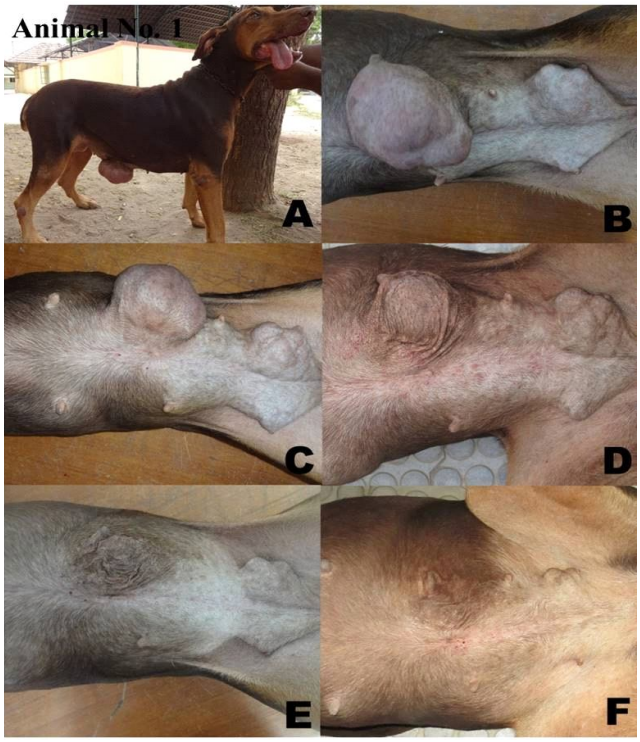

Fig 1: Tumour regression noticed in group I animal during LDM therapy as on day 0 (A & B), day 15 (C), day 30 (D), day 45 (E) and day 60 (F)

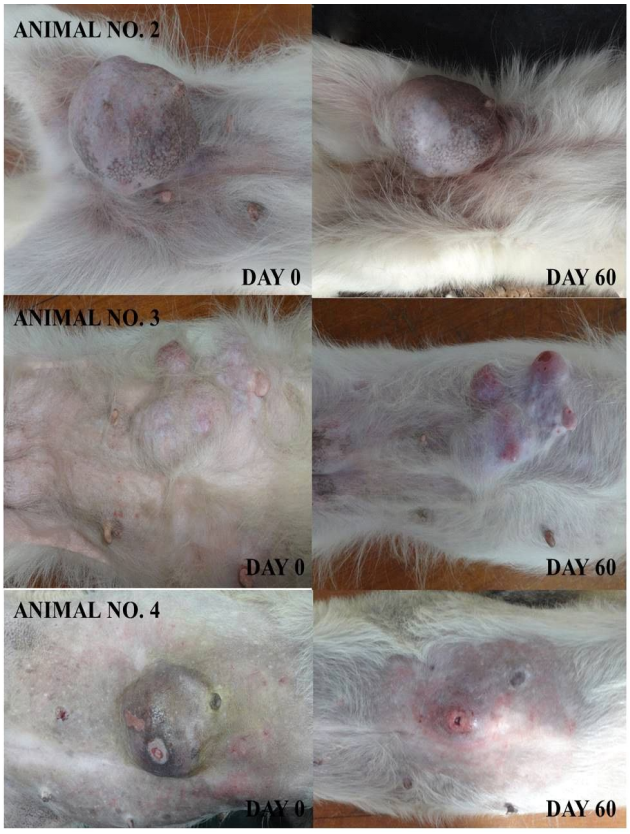

Fig 2: Tumour regression noticed in other animals of group I during LDM therapy as on day 0 and day 60

and Kumar, 2014). In this study, the tumours with no detectable metastasis responded well to mere surgical excision alone. Whereas, the value of surgery was reduced when it was used alone for certain tumours that have a high risk of distant metastasis (Riley and Riley, 1982).

Post-operatively, out of six animals in group I, the recurrence of tumour was noticed in one animal at the 120th day following LDM therapy and surgery. The mean ± S.E. value of tumour recurrence free period in days of group I was 170 ± 10.

In group II, one animal showed recurrence and died on the 34th day following surgery. The mean ± S.E. value of tumour recurrence free period of group II in days was155.67±24.3.

Post-operatively in group III, the haematological changes were similar to that of pre-operative LDM therapy. Out of six animals in group III, one animal had partial recurrence but survived and rest of the animals had disease free survival during the entire study period. In group III, the recurrence of tumour was noticed in one animal on 157th day following surgery and LDM therapy. The mean ± S.E. value of tumour recurrence free period in days of group III was 176.17 ± 3.83.

Histopathological examination of 18 canine mammary tumours revealed 4 as benign and 14 as malignant tumours.

All the animals under LDM therapy were monitored for complications and only a few animals had mild degree of anorexia and increased creatinine level and were well within the normal range.

Mild complications like inappetance and mild degree of cystitis were noticed in few animals and this is in accordance with Loven *et al.* (2013) who reported that the grade of toxicity related to LDM chemotherapy was relatively low and limited to a few percentages of cases that were grade3 or above, thus enabling the convenient administration even in the elderly or heavily pretreated patients.

CONCLUSIONS

The Low Dose Metronomic chemotherapy in pre operative dogs with mammary tumour caused progressive regression in the size of the tumour and they were more beneficial in geriatric animals that are at high anaesthetic risk. In post operative LDM therapy animals it caused a delay in recurrence of primary tumour with increase in disease free interval revealing that the chemotherapeutic protocol employed was safe, effective and improved the quality of life. From the present study, it is concluded that pre-operative LDM therapy along with surgical excision yielded the best result compared to other two treatment regimens and surgery was good in benign tumours.

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