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Perioperative desmopressin prolongs survival in surgically treated bitches with mammary gland tumours: A pilot study

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Abstract

Desmopressin (1-deamino-8-D-arginine vasopressin, also known as DDAVP) is a safe haemostatic compound capable of inhibiting lymph node and lung metastasis in a mouse model of mammary tumour manipulation and surgical excision. The aim of this study was to test the efficacy and safety of perioperative DDAVP (1 µg/kg) in surgically treated bitches with mammary gland tumours (MGT). Twenty-one, otherwise healthy, intact bitches, with malignant MGT stage III or IV were randomly allocated to DDAVP ($n = 11$) or placebo ($n = 10$) groups. En bloc mastectomy of the affected gland/s was performed. DDAVP had a significant beneficial effect on disease-free period ($P < 0.01$) and overall survival time ($P < 0.05$). No side effects were seen in any of the cases. Whatever the mechanism of action, it seems that DDAVP may have a novel use in cancer surgery to minimise spread or survival of residual malignant cells. Additional, large scale controlled trials are required to fully evaluate this adjuvant pharmacological protocol.

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Keywords: Desmopressin; Canine; Mammary tumours; Metastasis

Introduction

Mammary gland tumours (MGTs) are the most common neoplasm in the bitch (Moe, 2001; Egenvall et al., 2005), representing approximately 42% of all tumours and 82% of those of the female reproductive organs (Cotchin, 1954). Between 40% and 50% of MGTs are malignant and 50% of the malignant tumours may metastasise (Egenvall et al., 2005). The treatment of choice of MGTs is surgical excision, although surgery alone does not provide a cure in malignant cases (Johnston, 1993). The outcome of chemotherapy as an adjunct to surgery is uncertain in canine MGTs and its effect on survival has not been clearly documented (Karayannopoulou et al.,

2001; Loar, 1986; Ogilvie et al., 1989; Poirier et al., 2004; Rutteman, 1995; Theilen and Madewell, 1987).

Tamoxifen adjuvant therapy has been restricted in dogs owing to side effects in this species (Morris et al., 1993). Candidates for adjuvant therapy are dogs with clinical stage III malignancies (McEwen and Withrow, 1996) and dogs with any size of tumour with evidence of invasion to adjacent or lymphatic tissue (stage IV) (Loar, 1986; McEwen and Withrow, 1996). Currently, it is difficult to advise clients about the usefulness of adjuvant therapy after surgery as little information is published to support its use. Future studies should concentrate on the development of safe and effective adjuvant therapies.

Surgery and tissue trauma can enhance the growth and spread of malignant cells (Baker et al., 1989; Mutter et al., 1999). It has been suggested that surgical manipulation can produce release of viable cancer cells into the circulation.

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This fact has been confirmed by reverse transcription polymerase chain reaction in human patients undergoing breast cancer surgery (Brown et al., 1995; Galan et al., 2002). In addition, a series of axillary lymph node dissections taken after breast biopsy revealed the presence of epithelial cells in the draining lymph nodes that may be attributable to mechanical transport of tumour breast epithelium secondary to the previous surgical or needle manipulation (Carter et al., 2000).

Desmopressin (1-deamino-8-D-arginine vasopressin, also known as DDAVP) is a synthetic derivative of antidiuretic hormone with haemostatic properties that has been used in humans and dogs with diabetes insipidus and von Willebrand's disease (Authement et al., 1989; Papich, 2000; Richardson and Robison, 1985). DDAVP appears to be a safe and effective haemostatic agent for use during surgery in patients with bleeding disorders. The compound increases plasma levels of coagulation factor VIII, von Willebrand factor (VWF) and tissue-type plasminogen activator, and also enhances platelet adhesion to the vessel wall (Kaufmann and Vischer, 2003; Mannucci, 1997). Interestingly, DDAVP inhibited experimental lung colonization by aggressive breast cancer cells (Alonso et al., 1999) and dramatically decreased lymph node and distant metastasis in a mouse model of breast tumour manipulation and surgical excision (Giron et al., 2002).

Considering the anti-metastatic properties of DDAVP, as well as its well known haemostatic effect and safety, the compound could be an excellent candidate for adjuvant therapy to mammary tumour surgery in dogs. Therefore, the aim of this study was to test the efficacy of perioperative DDAVP on disease-free and overall survival in surgically treated bitches with stage III or IV MGTs.

Materials and methods

Animals

Twenty-one cross and pure bred, 8–15 year old, 5–45 kg, otherwise healthy, intact bitches, with malignant MGTs (diagnosed by deferred biopsy), stage III or IV (Owen, 1980), were included in the study (Table 1). Metastases were ruled out by latero-lateral and ventro-dorsal radiographies of the thorax and general health confirmed by routine blood chemical, cell counts and coagulation tests. Consent forms were signed by owners.

The study was reviewed and approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine of the National University of La Plata in accordance with international recommendations.

Therapeutic protocol

The bitches were randomly allocated to one of the following therapeutic protocols: DDAVP (Desmopressin, Ferring) at IV doses of 1 µg/kg in saline 30 min before and 24 h after surgery (DDAVP, $n = 11$) or pla-

Table 1
Clinical staging, histological features and disease progression in 21 bitches with mammary gland tumours

Group	Animal	Clinical stage ^{a,b}	Histological type ^{b,c}	Histological pattern ^c	Histological grade ^{d,e,f,g}	Disease progression
PLACEBO	1	T3aNoMo	SCC	N/A	II	Lung metastasis
PLACEBO	2	T3aNoMo	SC	TP	II	No
PLACEBO	3	T3aN1Mo	SC	S	II	Lung metastasis
PLACEBO	4	T3aNoMo	OS	N/A	II	Local relapse
PLACEBO	5	T3bNoMo	SC	TP	II	Local relapse, lung metastasis
PLACEBO	6	T3aNoMo	SC	TP	III	Local relapse, lung metastasis
PLACEBO	7	T3aNoMo	CS	N/A	III	Lung metastasis
PLACEBO	8	T3bN1Mo	SC	TP	III	Lung metastasis
PLACEBO	9	T3aNoMo	SC	TP	I	Lung metastasis
PLACEBO	10	T3aN1Mo	SC	TP	I	No
DDAVP	1	T3aNoMo	CC	TP	III	No
DDAVP	2	T3aNoMo	SC	S	I	No
DDAVP	3	T3bN1Mo	SC	TP	II	No
DDAVP	4	T3aNoMo	CC	TP	II	No
DDAVP	5	T3bNoMo	SC	TP	I	No
DDAVP	6	T3aNoMo	SC	TP	III	Lung metastasis
DDAVP	7	T3aNoMo	OS	N/A	I	Lung metastasis
DDAVP	8	T3aNoMo	SC	S	III	No
DDAVP	9	T3aNoMo	SC	TP	II	Lung metastasis
DDAVP	10	T3aNoMo	SC	TP	III	Local relapse
DDAVP	11	T3aN1Mo	OS	N/A	I	No

SC: Simple Carcinoma; SCC: Squamous Cell Carcinoma; CC: complex carcinoma; CS: carcinosarcoma; OS: Osteosarcoma; TP: Tubulo-Papillary; S: Solid; N/A: Not applicable.

^a Owen (1980).

^b Yamagami et al. (1996).

^c Misdorp et al. (1999).

^d Elston and Ellis (1991).

^e Karayannopoulou et al. (2005).

^f Sloane et al. (1999).

^g Kirpensteijn et al. (2002).

cebo (PLACEBO, $n = 10$) at equivalent volumes of isotonic saline solution 30 min before and 24 h after surgery.

Mastectomy

After premedication with atropine sulphate, (Atropina Sulfato, John Martin; 0.04 mg/kg, SC), acepromazine maleate (Acedan, Holliday; 0.03 mg/kg SC), and butorphanol (Torbutrol Plus, Fort Dodge; 0.2 mg/kg, IM) anaesthesia was induced with sodium thiopental (Pentovet TM, Richmond; 8 mg/kg, IV). Once intubated, anaesthesia was maintained with halothane and oxygen, delivered in a closed system. En bloc mastectomy of the affected gland(s), ipsilateral glands involved in the lymphatic drainage, as well as the glands cranial and caudal to the affected one, was performed (Bojrab et al., 1993; Loar, 1986; Wilkinson, 1971; Withrow, 1975). Regional lymph nodes were also excised.

Histopathological study

Representative fragments of each mammary tumour and the excised lymph nodes were fixed in 10% formalin, embedded in paraffin, sectioned at 5 μ m and stained with haematoxylin and eosin using routine procedures. Histopathological findings were recorded by a specialised pathologist and used to classify the tumours according to Misdorp et al. (1999). In cases with multiple tumours, the higher-grade malignant lesion was recorded. Tumour malignancy was graded histologically by the Nottingham method (Elston and Ellis, 1991; Sloane et al., 1999), which is the most commonly used method in human breast cancer and also in veterinary medicine (Castagnaro et al., 1998; Gilbertson et al., 1983; Karayannopoulou et al., 2001, 2005; Nieto et al., 2003; Reis et al., 2003).

Histological grade of carcinomas was derived from the assessment of three morphological features (tubule formation, nuclear pleomorphism and mitotic counts), each scored as 1–3. The scores were then added to obtain the tumour grade, as follows: 3–5 points = well-differentiated (grade I); 6–7 points = moderately differentiated (grade II); 8–9 points = poorly differentiated (grade III). Mammary tumours were defined as osteosarcoma when osteoid matrix was produced by the tumour cells, and lesions were then classified as low- (grade I), intermediate- (grade II) or high-grade (grade III), according to Kirpensteijn et al. (2002).

Follow-up

A clinical evaluation, including general health, examination of the remaining mammary glands, lymph nodes and surgical scar, and any side effects was carried out whenever there appeared any change in the patient or every 3 months for a minimum of 12 months after surgery. Thorax metastases were either diagnosed or ruled out by latero-lateral and ventro-dorsal radiographies at the same time points. Euthanasia was performed only in the terminal stage of the disease, and no post mortem examinations were performed.

Statistical analysis

Disease-free and overall survival times were defined as the time from surgery to recurrence (local, regional or distant) or death, respectively. Animals were statistically censored if the tumour had not recurred or no distant metastases had been detected at the time of death or data accrual closure. In the survival analysis, animals were statistically censored if they were alive at the time of data accrual closure. Survival probability curves were estimated using the Kaplan–Meier method (Kaplan and Meier, 1958), and treatment groups were compared by the log-rank test using the GraphPad Prism software. To verify the comparability of the treatment groups, comparisons with regard to tumour size, clinical stage and histological grade were performed by the Kruskal–Wallis test for continuous variables and Fisher's exact test for categorical variables using the GraphPad InStat software program. The influence of these variables was also examined on disease-free and overall survival times by multivariate

logistic regression analysis. For all statistical analyses, the level of significance was set at 0.05.

Results

Both groups of patients presented similar findings with regard to clinical stage, tumour size and histopathological features of the tumours (Table 1). Multivariate logistic regression revealed that none of the analysed variables significantly influenced disease-free and overall survival times. As expected, the most frequently diagnosed tumour type was simple carcinoma in both treatment groups. Three cases were diagnosed as having non-malignant lesions, including a patient with subacute mastitis in the DDAVP group and two patients with a fibroadenoma and a benign mixed tumour in the PLACEBO group, and were therefore excluded from the study.

Four (36%) and eight (80%) patients in the DDAVP and PLACEBO groups, respectively, presented either local relapses or lung metastasis during the study period. Rates of local recurrence and metastasis were 9% and 27% for the DDAVP group, and 30% and 70% for the PLACEBO group, respectively (see also Table 1). Kaplan–Meier analysis indicated that DDAVP had a positive effect on median disease-free survival time (DDAVP: 608 days versus PLACEBO: 85 days; $P < 0.01$; Fig. 1). Three (27%) and seven (70%) patients of the same groups died within the study period. Statistical analysis also demonstrated significant differences in median overall survival time between groups (DDAVP: >600 days versus PLACEBO: 333 days; $P < 0.05$; Fig. 2). We examined only the carcinomas as a separate subset, and we obtained similar results for median disease-free (DDAVP: >600 days versus PLACEBO: 97 days; $P < 0.05$) and overall survival times (DDAVP: >600 days versus PLACEBO: 351 days; $P < 0.05$).

Clinically, no side effects were seen in any of the cases. It was worth noting the decreased intraoperative bleeding in the DDAVP group, as assessed subjectively during surgery.

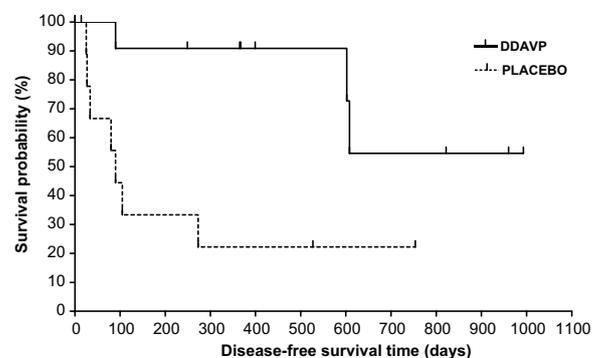


Fig. 1. Kaplan–Meier disease-free survival probability of 21 bitches with stage III or IV mammary gland tumours treated either with desmopressin (DDAVP, $n = 11$, median 608 days) IV, 1 μ g/kg, 30 min before and 24 h after surgery, or saline solution as a placebo (PLACEBO, $n = 10$, median 85 days). $P < 0.01$ (log-rank test). Vertical bars represent bitches censored at statistical analysis.

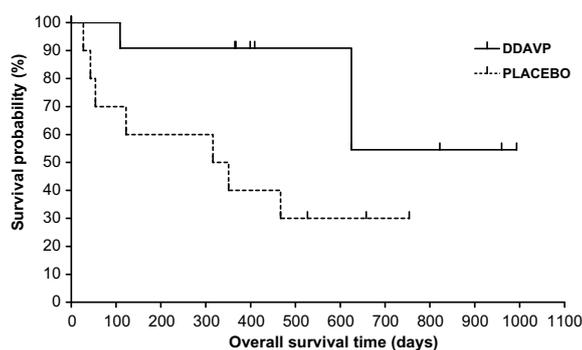


Fig. 2. Kaplan Meier overall survival probability of 21 bitches with stage III or IV mammary gland tumours treated either with desmopressin (DDAVP, $n = 11$, median >600 days) IV, 1 $\mu\text{g}/\text{kg}$, 30 min before and 24 h after surgery, or saline solution as a placebo (PLACEBO, $n = 10$, median 333 days). $P < 0.05$ (log-rank test). Vertical bars represent bitches censored at statistical analysis.

Discussion

Perioperative DDAVP significantly increased disease-free and overall survival in surgically treated bitches with stage III or IV MGT. The compound was administered 30 min before and 24 h after tumour surgery at a clinically relevant haemostatic dose (Mannucci, 1997; Papich, 2000). DDAVP appeared to be safe at this dose in canine cancer patients, and antitumour effects were obtained without overt toxic effects. As expected, the results obtained in the placebo group for the disease-free interval were comparable with those published for similar clinical stages of MGT, where 50% of bitches relapsed about 200 days after surgery (Kurzman and Gilbertson, 1986).

Surgical manipulation of a tumour may result in increased influx of tumour cells into the lymphatic and systemic circulation. Previous experimental studies in animal models have confirmed that manipulation of an aggressive mammary tumour is a relevant factor determining an enhanced local or regional as well as distant spread of malignant cells. In this regard, perioperative administration of DDAVP was able to decrease metastasis, therefore inhibiting tumour progression after surgery (Giron et al., 2002). Further investigations will now be conducted to determine the precise anti-metastatic mechanisms exerted by DDAVP in canine cancer patients. The haemostatic effect of DDAVP may represent a pivotal action, improving postoperative haemostasis. Enhanced coagulation after tumour surgery may contribute to a rapid encapsulation of residual tumour tissue, limiting extravasation of metastatic cells.

Recently, Terraube et al. (2006) showed that VWF plays a protective role against tumour cell dissemination in a VWF-deficient mutant mouse model. An increased metastatic potential of tumour cells was observed in VWF-null mice, and restoration of VWF plasma levels by injection of human recombinant VWF was capable of reducing lung metastasis. VWF is synthesised by both endothelial cells and megakaryocytes, but plasma VWF appears to be mainly of endothelial origin (Kaufmann and Vischer,

2003). Intravenous injection of DDAVP induces the release of highly multimeric forms of VWF, reaching peak levels at about 60 min and a plasma half-life of 8–10 h (Kaufmann and Vischer, 2003; Mannucci, 1997). VWF might participate in the interaction of tumour cells with the subendothelium, and appears to impede metastasis by reducing sustained adherence and/or survival of tumour cells in the microvasculature at the target organ. Moreover, as some tumour cells can form aggregates with platelets, VWF could modulate adhesion of such heterotypic aggregates via its dual effects on platelets and/or tumour cells (Terraube et al., 2006).

We cannot exclude other possible mechanisms for the antitumour action of DDAVP. The compound may modify tumour cell attachment by altering P-selectin expression on endothelial cells (Kanwar et al., 1995) or induce lysis of metastatic tumour cells through the production of nitric oxide from the vasculature (Hirano, 1997). In addition, it is known that DDAVP is a selective agonist for the vasopressin V2 membrane receptor, which is expressed in endothelial cells and several tumour variants, including breast cancer (North, 2000). Thus, DDAVP may mediate direct effects on tumour cell behaviour or modulate tumour-induced angiogenesis (Gomez et al., 2006).

In a small-scale study, Karayannopoulou et al. (2001) demonstrated the efficacy of chemotherapy in bitches with stage III and IV MGT. However, many authors have reported the recruitment of malignant cells into the peripheral blood after the first course of chemotherapy in patients with breast cancer (Sabbatini et al., 2000). Since DDAVP may decrease the implantation of metastatic cells, administration of the compound together with the courses of conventional chemotherapy may be also useful, considering the possible mobilising effect of chemotherapy on cancer cells.

Conclusions

Whatever the mechanism of action, it seems that a safe haemostatic agent, such as DDAVP, may be used to minimise spread and/or survival of residual malignant cells released during cancer surgery. Additional, large-scale controlled trials are required to fully evaluate this adjuvant pharmacological protocol. The results of this preliminary investigation suggest that perioperative administration of DDAVP may help to prolong disease-free and overall survival in bitches with MGT.

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