Effect of Adjuvant Perioperative Desmopressin in Locally-Advanced Canine Mammary Carcinoma and its Relation to Histologic Grade

Guillermo A. Hermo, Esteban Turic, DVM, Daniel Angelico, DVM, Alejandra M. Scursoni, MD, Daniel E. Gomez, MD, PhD, Q:2 Cristina Gobello, DVM, PhD, Daniel F. Alonso, MD, PhD

ABSTRACT

Desmopressin (DDAVP) is a vasopressin peptide analog with hemostatic properties that has been successfully used during surgery in patients with bleeding disorders. Recently published experimental and clinical data indicate that perioperative administration of DDAVP can minimize spread and survival of residual mammary cancer cells. The central aim of this study was to explore the effect of perioperative DDAVP and its relation to histologic grade in bitches with locally-advanced mammary carcinoma. Of the 32 dogs initially recruited, 28 intact bitches with mammary carcinoma tumors stage III or IV were ultimately Q:4 included. These dogs were randomized to receive DDAVP at intravenous doses of 1 μg/kg (n=18) or saline solution as placebo (n=10). En bloc mastectomy of the affected gland(s) was performed. Tumor malignancy was graded by the method of Elston and Ellis into well-differentiated (grade 1), moderately differentiated (grade 2), or poorly differentiated (grade 3). DDAVP therapy significantly prolonged the disease-free survival ($P<0.001$) and overall survival ($P<0.01$) in bitches with grade 2 or 3 carcinomas compared with bitches in the control group. No significant difference in disease-free period or overall survival was found between treatment groups in bitches with grade 1 tumors. The present data suggest that DDAVP may be an excellent candidate as a surgical adjuvant in the management of aggressive cancers in small animals. More research in this field is warranted. (*J Am Anim Hosp Assoc* 2011; 47:■■■–■■■. DOI 10.5326/JAAHA-MS-5509)

Introduction

Mammary tumors are the most frequent neoplasm in female dogs.[1,2] Occurrence of malignant forms varies from 40% to 50% with carcinoma being the most common histopathological type.[2,3] The treatment of choice of mammary tumors is surgical excision; however, surgery alone does not provide a cure in advanced or aggressive cancers.[4] The outcome of postoperative chemotherapy is uncertain in canine mammary cancer and its effect on survival has not been clearly documented.[5–8] Candidates for adjuvant therapy are dogs with locally-advanced disease, including primary tumors >5 cm in maximum diameter (clinical stage III), and tumors of any size with loco-regional lymph node involvement (clinical stage IV).[9] Dogs with aggressive cancers (i.e., those with a high histologic grade) are also good candidates for adjuvancy. In this regard, histologic grading of canine mammary carcinomas is strongly related to prognosis and its routine use appears to be helpful in suggesting appropriate adjuvant therapy.[10]

In a pilot study, desmopressin (1-deamino-8-D-arginine vasopressin, DDAVP) prolonged survival in bitches with mammary cancer when administered during surgical excision of the primary tumor.[11] In addition, DDAVP inhibited lymph node and lung metastasis in murine models of mammary cancer.[12,13] DDAVP is a synthetic peptide compound with hemostatic properties that has been used in humans and dogs with diabetes insipidus and von Willebrand disease.[14–16] DDAVP is a safe and effective hemostatic agent for use during surgery in patients with bleeding disorders.

From the Laboratory of Molecular Oncology, Department of Science and Technology, Quilmes National University (G.H., D.G); Biogenesis-Bago S.A. (E.T., D.A.); Laboratory of Pathology, Bernal Medical Offices (A.S.); and Animal Physiology, Faculty of Veterinary Medicine, La Plata National University, Buenos Aires, Argentina (G.H., C.G.).

Q:3 *Correspondence: ghermo@unq.edu.ar (G.H.)*

DDAVP *desmopressin;* IM *intramuscular;* IV *intravenous;* SC *subcutaneous;* VWF *von Willebrand factor*

The compound increases plasma levels of coagulation factor VIII, von Willebrand factor (VWF) and tissue-type plasminogen activator.[17,18]

Taking into account the pilot study on the antimetastatic properties of DDAVP and its well known effect on hemostasis, our research group conducted an extended study including a larger number of bitches. The central aim was to investigate the effect of perioperative DDAVP and its relation to histologic grade in bitches with locally-advanced mammary carcinoma.

Materials and Methods

Animals

Between September 2003 and January 2008, 32 purebreed and mixed-breed otherwise healthy intact bitches, 5–13 yr old, and weighing 4–55 kg with stage III or IV mammary carcinoma (confirmed by deferred biopsy) were included in the study.[9] Metastases were ruled out by lateral and ventrodorsal thoracic radiographs. General health was confirmed by routine complete blood cell counts, blood biochemistry, and coagulation tests. The study was conducted in accordance with the Institutional Animal Care and Use Committee guidelines and consent forms were signed by owners.

All cases of any histologic variant of mammary carcinoma were included. Animals diagnosed as having noncarcinoma malignant tumors, benign tumors, or nontumor lesions were excluded from the study.

Therapeutic Protocol

This single-blind study was conducted to investigate the effects of adjuvant perioperative DDAVP. The bitches were randomized 2:1 to receive a veterinary formulation of DDAVP[a]. Dogs received either DDAVP at a final dose of 1 μg/kg of body weight IV 30 min before and 24 hr after surgery (DDAVP group, n=21) or a placebo consisting of equivalent volumes of isotonic saline solution[b], 30 min before and 24 hr after surgery (placebo group, n=11).

Mastectomy

A complete physical examination was conducted prior to surgery to detect the presence of multiple tumor nodules within the mammary glands.[4,5] After premedication with atropine sulfate[c] (0.04 mg/kg subcutaneously [SC]), acepromazine maleate[d] (0.03 mg/kg SC), and butorphanol[e] (0.2 mg/kg intramuscularly [IM]), anesthesia was induced with sodium thiopental[f] (8 mg/kg IV). Once intubated, anesthesia was maintained with halothane[g] 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 cranial and caudal glands to the affected gland was performed.[19,20] A wide surgical margin of at least 3 cm around the tumor was performed in all cases. Skin margins were checked by histopathology in some cases and regional lymph nodes were excised in all cases.

Histopathology and Grading of Mammary Carcinoma

Representative fragments of each mammary tumor and the excised lymph nodes were fixed in 10% formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin using routine procedures. Histopathological findings were recorded by a specialized pathologist and tumors were classified according to Misdorp et al. (1999).[21] In cases with multiple tumors, the higher-grade malignant lesion was recorded.

Tumor malignancy was graded histologically by the method reported by Elston and Ellis which is the most commonly used method in human breast cancer as well as in veterinary medicine.[10,23–25] Histologic grade of carcinomas was derived from the assessment of three morphologic features (tubule formation, nuclear pleomorphism, and mitotic counts). Each feature was scored on a scale from 1 to 3. The scores were then added together to obtain an overall tumor grade as follows: 3–5 points=well-differentiated (grade 1); 6–7 points=moderately-differentiated (grade 2); 8–9 points=poorly-differentiated (grade 3).

Follow-up

Clinical evaluation including general health, remaining mammary glands and lymph nodes, surgical scarring, and side effects were performed whenever there appeared to be any change in the patient or every 3 mo for a minimum of 12 mo postsurgically. Thoracic metastases were either diagnosed or ruled out by lateral and ventrodorsal radiographs at the time of each evaluation. Euthanasia was performed only in the terminal stage of disease and no post mortem examinations were performed.

Statistical Analysis

Median (range) and number (percent) were calculated where appropriate. Overall and disease-free survival were defined as the time from surgery to death or recurrence (local, regional, or distant), respectively. Animals were statistically censored if their tumor 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 and treatment groups were compared by the log-rank test using the GraphPad Prism software[h].[26] To verify the comparability of the treatment groups,

comparisons with regard to age, animal weight, tumor size, clinical stage, and histologic features were performed by the Mann-Whitney test for continuous variables and the χ^2 test for categorical variables using the GraphPad InStat software program[h]. The influence of these variables was also examined on disease-free and overall survival by uni- and multivariate logistic regression analysis. For all statistical analyses, the level of significance was set at 0.05.

Results

Treatment groups presented similar findings with regard to dog age and weight, size, and number of tumor lesions, clinical stage, and histopathological features of mammary carcinoma (**Table 1**). The most frequently diagnosed tumor type was simple carcinoma (n=22). Other diagnosed types included complex carcinoma (n=2), squamous cell carcinoma (n=1), lipid-rich carcinoma (n=1), carcinosarcoma (n=1), and anaplastic carcinoma (n=1). The different histologic grades were represented in both groups, although moderately differentiated (grade 2) tumors were the most frequent in the placebo group and poorly differentiated (grade 3) tumors were the most frequent in the DDAVP group. Four cases were diagnosed as having noncarcinoma lesions, including two cases with osteosarcoma, one case with a mixed benign tumor in the DDAVP group, and one case with lobular hyperplasia and adenosis in the placebo group. These four cases were therefore excluded from the study. Uni- and multivariate logistic regression revealed that none of the analyzed variables significantly influenced disease-free and overall survival. DDAVP was well tolerated at the dose used in the study and overt toxic effects were not documented in any of the included cases.

Independent of the histologic grade, five (28%) and eight (80%) patients in the DDAVP and placebo groups, respectively, presented either local relapses or lung metastasis during the study period (**Table 2**). Kaplan-Meier analysis indicated that DDAVP had a positive effect on median disease-free survival. Median disease-free survival was 608 days in the DDAVP group versus 88 days in the placebo group ($P<0.001$) (**Figure 1A**). Three (17%) and eight (80%) cases in the DDAVP and placebo groups, respectively, died during the study period. Median overall survival was 809 days in the DDAVP and only 237 days in the placebo group ($P<0.01$) (**Figure 1B**).

As shown in **Figures 2A and B**, disease-free and overall survival were both significantly related to histologic grade in the bitches included in the placebo group. Most bitches with grade 2 or 3 carcinomas receiving the placebo relapsed within 100–200 days after surgery. Median disease-free and overall survival in the placebo group were only 26 and 35 days, respectively, for grade 3 cases, 85 and 351 days for grade 2, and median values were not reached in cases with grade 1 carcinomas. This same trend was observed in the DDAVP group but survival rates were higher (**Figures 3 and 4**).

Perioperative DDAVP treatment significantly prolonged disease-free (Figures 3B and C) and overall survival (Figures 4B and C) in bitches with grade 2 carcinomas ($P<0.001$ and $P<0.01$, respectively) or grade 2 carcinomas (both $P<0.001$). Median disease-free and overall survival in the DDAVP group were both

TABLE 1

Clinical and Histopathological Features of 28 Cases of Locally-Advanced Canine Mammary Carcinoma Included in this Study

	Placebo group (n=10)	DDAVP group (n=18)	*P*
Age (yr)	10 (7–13)	10 (5–13)	0.772*
Animal weight (kg)	32 (15–55)	22 (4–45)	0.088*
Number of tumors per animal	2 (1–7)	2 (1–7)	0.827*
Main tumor diameter (cm)	7 (5–37)	6 (5–13)	0.527*
Clinical stage			
Stage III	8/10 (80%)	16/18 (89%)	0.935†
Stage IV	2/10 (20%)	2/18 (11%)	
Histologic type			
Simple carcinoma	7/10 (70%)	15/18 (83%)	0.731†
Other carcinomas	3/10 (30%)	3/18 (17%)	
Histologic grade			
Grade 1	3/10 (30%)	3/18 (17%)	0.407†
Grade 2	5/10 (50%)	7/18 (39%)	
Grade 3	2/10 (20%)	8/18 (44%)	

*Mann-Whitney test; †χ^2 test
Data are presented as median (range) or number (percent).

TABLE 2

Histologic Type, Histologic Grade, and Outcome of 28 Cases of Locally-Advanced Canine Mammary Carcinoma Treated with Either Placebo or Perioperative DDAVP

Treatment and case number	Histologic type	Grade*	Outcome
Placebo #1	Simple carcinoma	1	Lung metastasis
Placebo #2	Simple carcinoma	1	Disease-free
Placebo #3	Simple carcinoma	1	Disease-free
Placebo #4	Simple carcinoma	2	Local relapse and lung metastasis
Placebo #5	Simple carcinoma	2	Local relapse and lung metastasis
Placebo #6	Simple carcinoma	2	Lung metastasis
Placebo #7	Squamous cell carcinoma	2	Lung metastasis
Placebo #8	Lipid-rich carcinoma	2	Local relapse and lung metastasis
Placebo #9	Simple carcinoma	3	Lung metastasis
Placebo #10	Carcinosarcoma	3	Lung metastasis
DDAVP #1	Simple carcinoma	1	Disease-free
DDAVP #2	Simple carcinoma	1	Disease-free
DDAVP #3	Simple carcinoma	1	Disease-free
DDAVP #4	Simple carcinoma	2	Lung metastasis
DDAVP #5	Simple carcinoma	2	Disease-free
DDAVP #6	Simple carcinoma	2	Disease-free
DDAVP #7	Simple carcinoma	2	Disease-free
DDAVP #8	Simple carcinoma	2	Disease-free
DDAVP #9	Simple carcinoma	2	Disease-free
DDAVP #10	Complex carcinoma	2	Disease-free
DDAVP #11	Simple carcinoma	3	Local relapse and lung metastasis
DDAVP #12	Simple carcinoma	3	Lung metastasis
DDAVP #13	Simple carcinoma	3	Lung metastasis
DDAVP #14	Anaplastic carcinoma	3	Lung metastasis
DDAVP #15	Complex carcinoma	3	Disease-free
DDAVP #16	Simple carcinoma	3	Disease-free
DDAVP #17	Simple carcinoma	3	Disease-free
DDAVP #18	Simple carcinoma	3	Disease-free

*Histologic grade was assessed by the method of Elston and Ellis.[22]

>600 days for cases with grade 2 and 3 tumors. No significant difference in survival (Figures 3A and 4A) was found between dogs in the treatment and placebo group ($P>0.05$) diagnosed with grade 1 tumors. Animals with grade 1 tumors administered DDAVP neither presented with local relapses nor lung metastasis and no animals in this group died during the study period.

Discussion

The present work confirmed the beneficial effect of perioperative DDAVP on disease-free and overall survival in bitches with locally-advanced mammary carcinoma, as suggested by a previously reported pilot study.[11] The compound was administered as a surgical adjuvant at a clinically relevant hemostatic dose of 1 μg/kg IV 30 min presurgically and again 24 hr postsurgically.[15,18] DDAVP appeared to be safe at this dose in canine cancer patients and antitumor effects were obtained without overt toxicity.

Previous studies in a murine model demonstrated that aggressive manipulation of an experimental mammary carcinoma could contribute to locoregional and distant spread of malignant cells. In such a model, perioperative administration of DDAVP decreased metastatic foci by 70%.[13] In the present canine study, DDAVP did not eliminated residual tumors in all cases; however, only 28% of animals receiving perioperative treatment developed a late local relapse or lung metastasis whereas most bitches in the placebo group experienced a rapid disease progression. DDAVP seemed to improve perioperative hemostasis and may have contributed to a rapid encapsulation of residual tumor tissue limiting extravasation of metastatic cells.[11] In this regard, intravenous DDAVP prevented aggregation of mammary carcinoma cells thereby reducing the efficiency of the metastatic process.[12]

It is known that the IV administration of DDAVP induces a rapid release of highly multimeric forms of VWF from microvascular

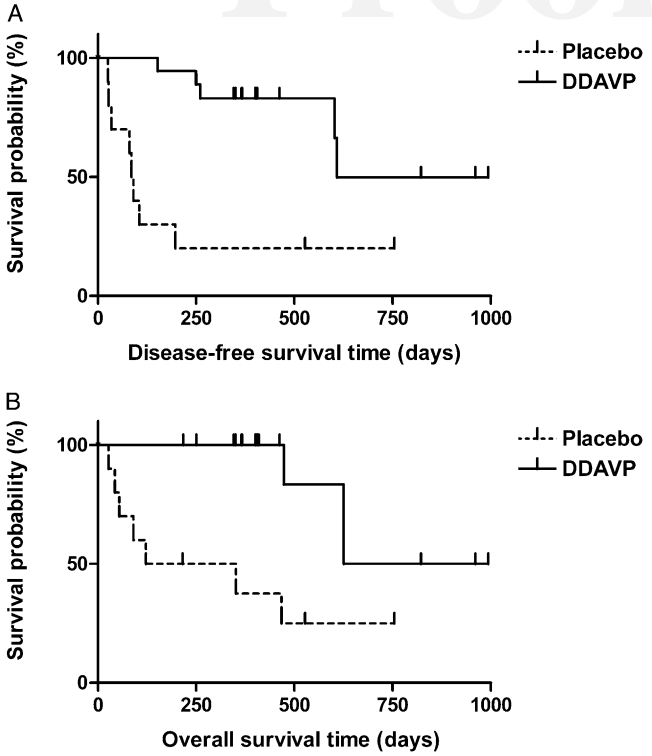

FIGURE 1 *Kaplan-Meier disease-free* (A) *and overall survival* (B) *probability of bitches with locally-advanced mammary carcinoma treated with perioperative DDVAP or placebo. Vertical bars represent bitches censored at statistical analysis. Significance:* $P<0.001$ *for disease-free and* $P<0.01$ *for overall survival (log-rank test).*

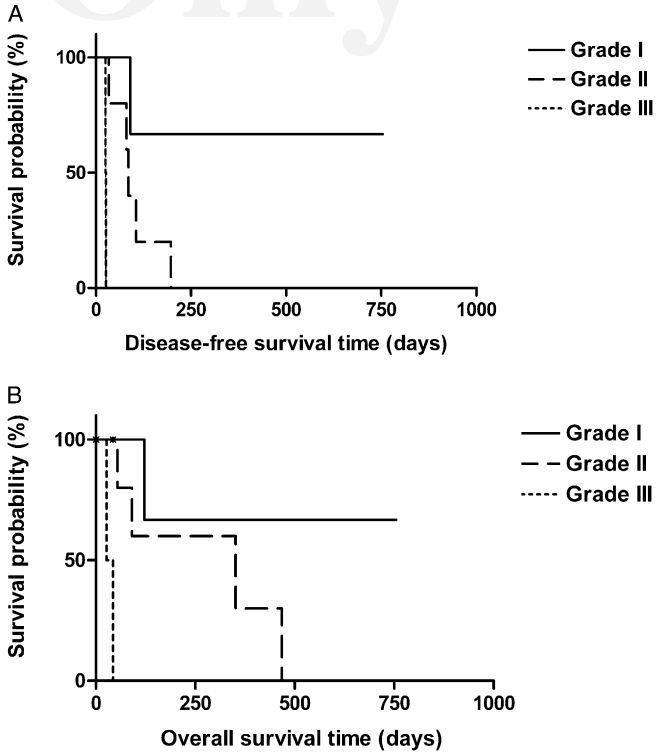

FIGURE 2 *Kaplan-Meier disease-free* (A) *and overall survival* (B) *probability of bitches included in the control group with locally-advanced mammary carcinoma based on histologic grade of tumors. Significance:* $P<0.05$ *for disease-free and* $P<0.01$ *for overall survival (log-rank test for trend).*

endothelial cells reaches peak levels approximately 60 min after injection, and has a plasma half-life of 8–10 hr.[17,18] Terraube et al. (2006) showed that VWF plays a protective role against tumor cell dissemination in a mouse model.[27] VWF might participate in the interaction of tumor cells with the subendothelium and appears to obstruct metastasis by reducing sustained adherence of malignant cells in the microvasculature at the target organ. Furthermore, VWF was shown to directly induce apoptosis of tumor cells in vitro and caused death of metastatic cells arrested in the lungs.[28] Together, these observations suggest that abrupt release of VWF from the microvasculature may favor the collapse of early metastatic foci. Thus, it is likely that DDAVP injection not only inhibits perioperative metastatic events but also combats spontaneous micrometastases that occurred before surgery.

In addition, DDAVP is a selective agonist for the vasopressin V2 membrane receptor that is expressed in endothelial cells and also in several tumor variants, including mammary cancer.[29] A mild antiproliferative effect of DDAVP was previously reported on mammary carcinoma cell lines.[30,31] Such action is likely to be mediated through V2 receptor signaling and involves activation of adenylate cyclase followed by intracellular cyclic adenosine monophosphate elevation.[31] Other authors have shown that the natural hormone vasopressin can inhibit the in vitro growth of MCF-7 human breast carcinoma cells at high concentrations.[32]

Histologic grading of canine mammary carcinomas was significantly related to prognosis, especially in cases of simple carcinoma.[9] The improved method of Elston and Ellis for histologic grading of carcinoma provides powerful prognostic information. The measurement of three parameters (i.e., tubule formation, nuclear pleomorphism, and mitotic count) markedly reduced problems of consistency and reproducibility.[22] In the current study, adjuvant perioperative treatment with DDAVP prolonged survival in bitches with moderately- or poorly-differentiated (grade 2 or 3, respectively) carcinomas. The effect was not significant in bitches with well-differentiated (grade 1) carcinomas. These observations indicate that perioperative DDAVP can provide a beneficial effect in patients with aggressive disease at high risk of recurrence or metastasis.

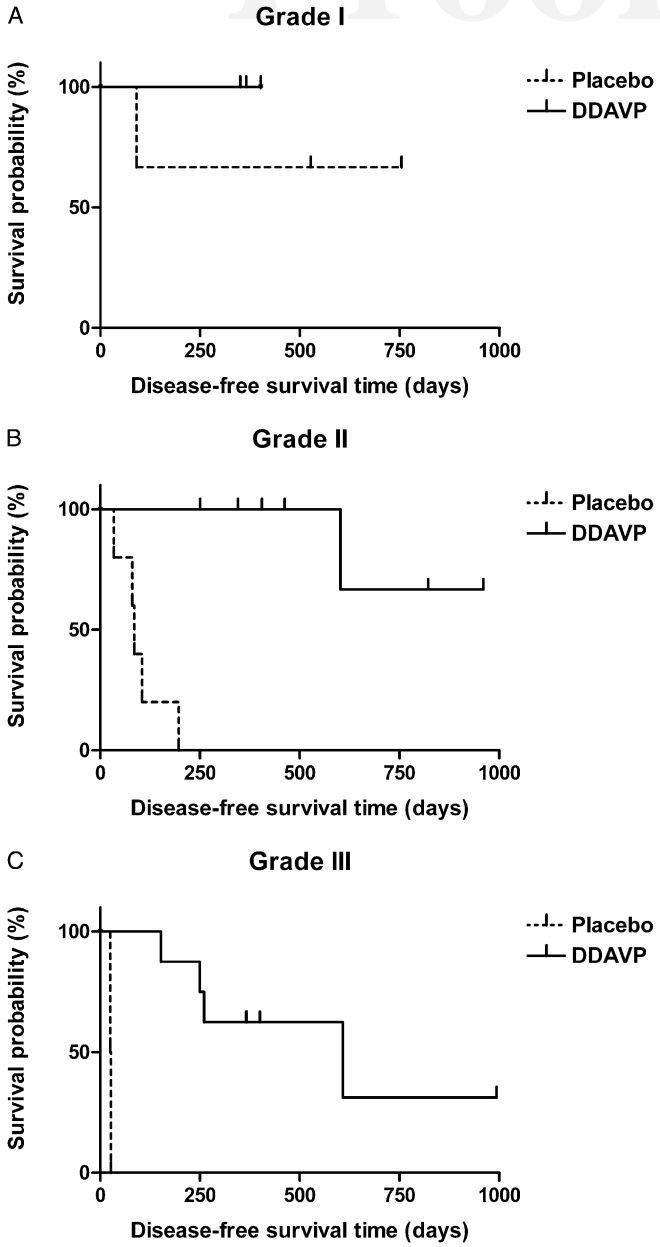

FIGURE 3 *Kaplan-Meier disease-free survival probability of bitches with locally-advanced mammary carcinoma treated with perioperative DDVAP or placebo described according to histologic grade of tumors.* (A) *Well-differentiated (grade 1) tumors;* (B) *moderately-differentiated (grade 2) tumors;* (C) *poorly-differentiated (grade 3) tumors. Vertical bars represent bitches censored at statistical analysis. Nonsignificant difference for grade 1 and* $P<0.001$ *for grade 2 and 3 carcinomas (log-rank test).*

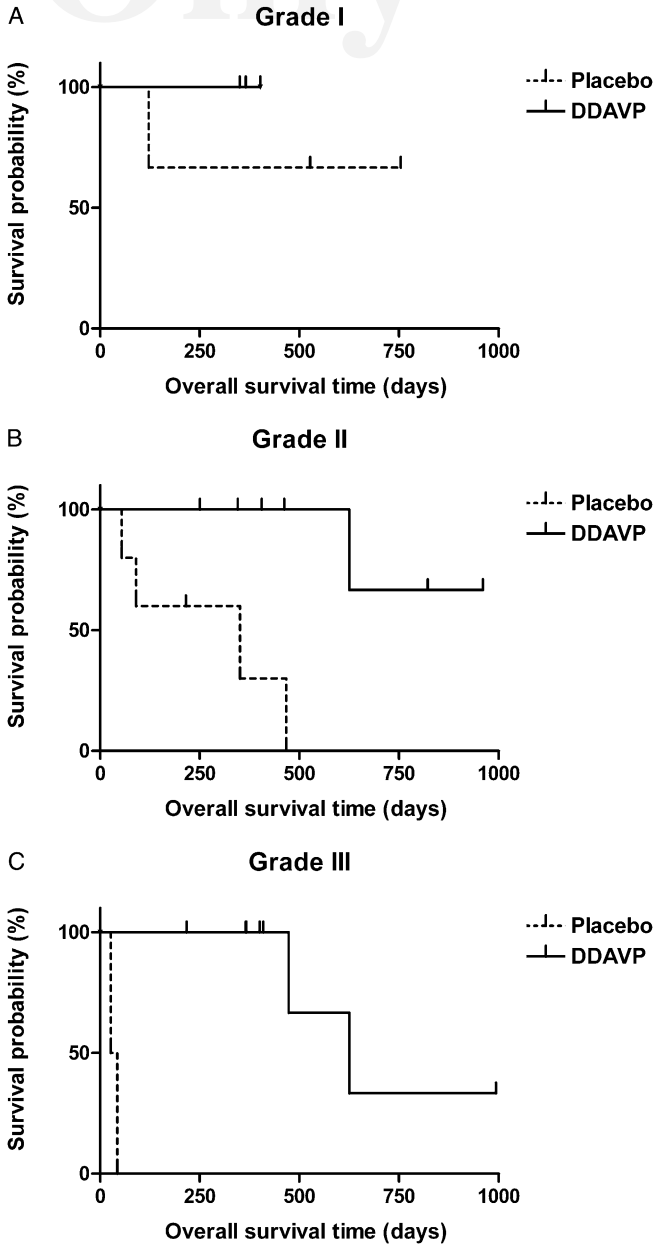

FIGURE 4 *Kaplan-Meier overall survival probability of bitches with locally-advanced mammary carcinoma treated with perioperative DDAVP or placebo, according to histologic grade of tumors.* (A) *Well-differentiated (grade 1) tumors;* (B) *moderately-differentiated (grade 2) tumors;* (C) *poorly-differentiated (grade 3) tumors. Vertical bars represent bitches censored at statistical analysis. Nonsignificant difference for grade 1,* $P<0.01$ *for grade 2 and* $P<0.001$ *for grade 3 carcinomas (log-rank test).*

Conclusion

The routine use of the hemostatic and antitumor peptide DDAVP could be helpful in minimizing spread or survival of residual malignant cells postsurgically in bitches with grade 2 or 3 locally-advanced mammary tumors. Perioperative therapy could prolong disease-free and overall survival, particularly in bitches with high-grade mammary carcinomas. Future large-scale trials will need

to confirm the role of DDAVP as a safe surgical adjuvant in the management of different solid cancers.

This study was partially funded by the National Agency for Scientific and Technological Promotion (Argentina), Quilmes National University, and the Morris Animal Foundation (USA). The support of the company Romikin S.A. is also acknowledged. G.A. Hermo is a Research Fellow of CONICET (Argentina).

FOOTNOTES

[a] DDAVP (containing 5 μg/ml of DDAVP); Biogenesis-Bago, Buenos Aires, Argentina

[b] Isotonic saline; Laboratorios Rivero, Buenos Aires, Argentina

[c] Atropine sulphate; John Martin, Buenos Aires, Argentina

[d] Acepromazine maleate; Acedan TM, Holliday, Buenos Aires, Argentina

[e] Butorphanol (Torbutrol Plus TM); Fort Dodge, Buenos Aires, Argentina

[f] Sodium thiopental (Pentovet TM), Richmond, Buenos Aires, Argentina

[g] Halothane; Laboratorios Rivero, Buenos Aires, Argentina

[h] GraphPad Prism software; GraphPad Software Inc., CA

REFERENCES

1. Egenvall A, Bonnett BN, Ohagen P, et al. Incidence of and survival after mammary tumors in a population of over 80,000 insured female dogs in Sweden from 1995 to 2002. *Prev Vet Med* 2005;69(1-2):109–27.
2. Moe L. Population-based incidence of mammary tumours in some dog breeds. *J Reprod Fertil Suppl* 2001;57:439–43.
3. Morrison WB. Canine and feline mammary tumors. In: Morrison WB, editor. *Cancer in dogs and cats: medical and surgical management*. Baltimore, MD: Williams and Wilkins; 1998:591–6.
4. White RA. Mammary gland surgery. In: Slatter DH, ed. *Textbook of small animal surgery*. 3rd ed. Philadelphia: WB Saunders; 2002:349–51.
5. Rutteman GR. Mammary tumors in dog. In: Kirk RW, ed. *Current veterinary therapy XIII. Small animal practice*. Philadelphia: WB Saunders; 1999:518–22.
6. Ogilvie GK, Reynolds HA, Richardson RC, et al. Phase II evaluation of doxorubicin for treatment of various canine neoplasms. *J Am Vet Med Assoc* 1989;195(11):1580–3.
7. Karayannopoulou M, Kaldrymidou E, Constantinidis TC, et al. Adjuvant post-operative chemotherapy in bitches with mammary cancer. *J Vet Med A Physiol Pathol Clin Med* 2001;48(2):85–96.
8. Poirier VJ, Hershey AE, Burgess KE, et al. Efficacy and toxicity of paclitaxel (Taxol) for the treatment of canine malignant tumors. *J Vet Intern Med* 2004;18(2):219–22.
9. Rutteman GR, Withrow SJ, McEwen EG. Tumors of the mammary gland. In: Withrow SJ, Mc Ewen EG, eds. *Small animal clinical oncology*. 3rd ed. Philadelphia: WB Saunders; 2001:455–77.
10. Karayannopoulou M, Kaldrymidou E, Constantinidis TC, et al. Histological grading and prognosis in dogs with mammary carcinomas: application of a human grading method. *J Comp Pathol* 2005;133(4):246–52.
11. Hermo GA, Torres P, Ripoll GV, et al. Perioperative desmopressin prolongs survival in surgically treated bitches with mammary gland tumours: a pilot study. *Vet J* 2008;178(1):103–8.
12. Alonso DF, Skilton G, Farías EF, et al. Antimetastatic effect of desmopressin in a mouse mammary tumor model. *Breast Cancer Res Treat* 1999;57(3):271–5.
13. Giron S, Tejera AM, Ripoll GV, et al. Desmopressin inhibits lung and lymph node metastasis in a mouse mammary carcinoma model of surgical manipulation. *J Surg Oncol* 2002;81(1):38–44.
14. Authement JM, Boudrieau RJ, Kaplan PM. Transient, traumatically induced, central diabetes insipidus in a dog. *J Am Vet Med Assoc* 1989;194(5):683–5.
15. Papich MG. Table of common drugs: approximate dosages. In: Bonagura JD, ed. *Kirk's current veterinary therapy XIII. Small animal practice*. Philadelphia: WB Saunders; 2000:1241–64.
16. Richardson DW, Robinson AG. Desmopressin. *Ann Intern Med* 1985;103(2):228–39.
17. Kaufmann JE, Vischer UM. Cellular mechanisms of the hemostatic effects of desmopressin (DDAVP). *J Thromb Haemost* 2003;1(4):682–9.
18. Mannucci PM. Desmopressin (DDAVP) in the treatment of bleeding disorders: the first 20 years. *Blood* 1997;90(7):2515–21.
19. Wilkinson GT. The treatment of mammary tumors in the bitch and a comparison with the cat. *Vet Rec* 1971;89(1):13–6.
20. Withrow SJ. Symposium on surgical techniques in small animal practice. Surgical management of canine mammary tumors. *Vet Clin North Am* 1975;5(3):495–506.
21. Misdorp W, Else RW, Hellmen E, et al. *Histological classification of mammary tumor of the dog and the cat*. Vol VII. Washington, DC: Armed Forces Institute of Pathology and American Registry of Pathology and the World Health Organization Collaborating Center for Worldwide Reference on Comparative Oncology; 1999:58–9.
22. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathol* 1991;19(5):403–10.
23. Gilbertson SR, Kurzman ID, Zachrau RE, et al. Canine mammary epithelial neoplasms: biologic implications of morphologic characteristics assessed in 232 dogs. *Vet Pathol* 1983;20(2):127–42.
24. Castagnaro M, Casalone C, Bozzetta E, et al. Tumour grading and the one-year post-surgical prognosis in feline mammary carcinomas. *J Comp Pathol* 1998;119(3):263–75.
25. Reis AL, Carvalheira J, Schmitt FC, et al. Immunohistochemical study of the expression of E-cadherin in canine mammary tumours. *Vet Rec* 2003;152(20):621–4.
26. Kaplan EL, Meier E. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958;53:457–81.
27. Terraube V, Pendu R, Baruch D, et al. Increased metastatic potential of tumor cells in von Willebrand factor-deficient mice. *J Thromb Haemost* 2006;4(3):519–26.
28. Terraube V, Marx I, Denis CV. Role of von Willebrand factor in tumor metastasis. *Thromb Res* 2007;120(Suppl 2):S64–70.
29. North WG. Gene regulation of vasopressin and vasopressin receptors in cancer. *Exp Physiol* 2000;85:27S–40S.
30. Ripoll GV, Giron S, Krzymuski MJ, et al. Antitumor effects of desmopressin in combination with chemotherapeutic agents in a mouse model of breast cancer. *Anticancer Res* 2008;28(5A):2607–11.
31. Keegan BP, Akerman BL, Péqueux C, et al. Provasopressin expression by breast cancer cells: implications for growth and novel treatment strategies. *Breast Cancer Res Treat* 2006;95(3):265–77.
32. Taylor AH, Ang VT, Jenkins JS, et al. Interaction of vasopressin and oxytocin with human breast carcinoma cells. *Cancer Res* 1990; 50(24):7882–6.

Proof Only

Author Queries jaaha5509q

[AQ1] Please ensure this running head is suitable or please provide an alternate.

[AQ2] Please include academic degree(s) for first author.

[AQ3] Please confirm all affiliations are correct.

[AQ4] Is this accurate? The abstract needs to reflect that 4 dogs were ultimately not included because of nontumor lesions.