Antitumor Effects of Desmopressin in Combination with Chemotherapeutic Agents in a Mouse Model of Breast Cancer

GISELLE V. RIPOLL, SANTIAGO GIRON, MARTIN J. KRZYMUSKI, GUILLERMO A. HERMO, DANIEL E. GOMEZ and DANIEL F. ALONSO

Laboratory of Molecular Oncology, Department of Science and Technology, Quilmes National University, Buenos Aires, Argentina

Abstract. *The vasopressin peptide analog desmopressin has been used during surgery to prevent bleeding in patients with coagulation defects. Recent experimental and clinical data revealed that perioperative desmopressin therapy can minimize the spread and survival of residual cancer cells. Here, we explored the antitumor effects of desmopressin in combination with chemotherapeutic agents using the F3II mammary carcinoma in syngeneic Balb/c mice. Intravenous administration of desmopressin at a dose of 2 μg/kg together with weekly cycles of carmustine (20 mg/kg) prevented primary tumor infiltration of the skin. Combination of desmopressin with paclitaxel (25 mg/kg) significantly reduced metastatic progression to the lung. Although desmopressin had an antiproliferative effect on F3II cells, in vitro studies did not demonstrate an enhanced cytotoxicity with chemotherapy. Our results suggest that desmopressin may contribute to impair aggressiveness of residual mammary tumors during chemotherapy.*

The synthetic peptide analog of the antidiuretic hormone, desmopressin (DDAVP, 1-deamino-8-D-arginine vasopressin), is a well-tolerated and convenient hemostatic drug that can be used during surgery in patients with bleeding diathesis (1, 2). The compound increases the plasma levels of coagulation factor VIII, von Willebrand factor (VWF) and tissue-type plasminogen activator, and also enhances platelet adhesion to the vessel wall (3).

Previously, we reported that DDAVP inhibited lung colonization by blood-borne breast cancer (4) and melanoma cells (5) in experimental mouse models. Antimetastatic properties of DDAVP were not associated with direct cytotoxicity on tumor cells, suggesting that the compound modulates a complex biological mechanism on the host which influences tumor spread. We further demonstrated that perioperative administration of DDAVP dramatically reduced lymph node and lung metastasis in a model of mammary tumor manipulation and surgical excision in mice (6). More recently, a veterinary clinical study showed that perioperative DDAVP prolonged disease-free survival in surgically treated bitches with locally advanced mammary cancer (7).

Breast cancer is one of the most commonly diagnosed malignancies in women and mortality for the disease is related to the capacity of breast tumor cells to invade and metastasize. A multidisciplinary approach to the management of breast cancer and the introduction of novel systemic therapies have improved the quality of life and survival of patients (8, 9). In this regard, the potential combination of standard chemotherapy with novel biological or cytotoxic agents is exciting. The aim of the present study was to explore, for the first time, the potential antitumor effects of DDAVP in combination with chemotherapeutic agents using a clinically relevant mammary carcinoma model in syngeneic mice.

Materials and Methods

Tumor cell line and culture conditions. The mammary carcinoma cell line F3II is a highly invasive and metastatic variant derived from a clone of a spontaneous Balb/c mouse mammary tumor (10). F3II cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco, Grand Island, NY, USA) supplemented with 5% fetal bovine serum (FBS), 2 mM glutamine and 80 μg/ml gentamycin in monolayer culture.

Tumor cell inoculation. Syngeneic adult female Balb/c mice were injected in the subcutis of the right flank with F3II cells (2×10^5 viable cells per animal). The time of appearance of local mammary tumors was monitored by palpation and further confirmed by histopathology. In all cases, tumors were diagnosed as spindle-cell carcinomas, as reported elsewhere (11). Tumor size was measured periodically with a caliper and tumor volume was calculated by the formula: $\pi/6$ x width^2 x length. Animals were sacrificed by cervical dislocation and necropsied on day 50-60 after F3II cell inoculation. To investigate the presence of spontaneous metastases, lungs were

Correspondence to: Dr. Daniel Alonso, Laboratorio de Oncología Molecular, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, R. Sáenz Peña 352, Bernal B1876BXD Buenos Aires, Argentina. Tel: +54 1143657100, e-mail: dfalonso@unq.edu.ar

Key Words: Desmopressin, vasopressin, peptide analog, chemotherapy, breast cancer.

removed, fixed in Bouin's solution and the number of surface lung nodules were determined under a dissecting microscope.

Administration of chemotherapy and DDAVP. Mice were administered intraperitoneally 3-4 weekly cycles of carmustine or paclitaxel (Bristol-Myers Squibb, Princeton, NJ, USA) at doses of 20 or 25 mg/kg, respectively. Chemotherapy was initiated 14-21 days after F3II cell inoculation, when subcutaneous mammary tumors reached volumes of about 50-100 mm^3. DDAVP from Ferring Pharmaceuticals (Malmö, Sweden) was administered in 2 doses, 30 min before and 24 h after each chemotherapy cycle. Mice received DDAVP intravenously in physiological saline at a final dose of 2 μg/kg body weight (50 ng/0.3 ml saline/dose), as reported elsewhere (4). Control animals received only the saline vehicle.

In vitro studies. F3II cells were seeded on 96-well plates (2.5×10^3 cells/well) in DMEM plus 5% FBS. After 24 h, a range of concentration of carmustine (50-200 μM), paclitaxel (1-20 μM) and DDAVP (50-1,000 ng/ml) was added and culture was continued for 72 h at 37˚C in a humidified 5% CO_2 atmosphere. The effect of compounds was tested by the MTT assay and the concentration producing 50% inhibition (IC_{50}) was determined by plotting percentage of cell survival *versus* drug concentration.

Results

We first tested the effects of DDAVP alone in Balb/c mice bearing the aggressive F3II mammary tumors. No relevant effects on subcutaneous tumor growth or metastatic progression were obtained with weekly intravenous administration of DDAVP (2-6 μg/kg) in the present experimental conditions (data not shown).

As shown in Figure 1, weekly treatment with carmustine significantly reduced F3II tumor volume, either alone or with DDAVP. Interestingly, combination of chemotherapy cycles with DDAVP clearly inhibited tumor infiltration in the skin. In control mice and mice treated with carmustine alone, tumors grew by invading the subcutis and dermis, causing necrosis in the epidermal layer and a visible ulceration on top of the tumors (Figure 2 A-D). In contrast, most animals receiving chemotherapy plus DDAVP showed preservation of superficial layers of skin (Figure 2 E-F).

Although treatment with paclitaxel caused a modest effect on tumor growth in the present breast cancer model, combination of chemotherapy cycles with DDAVP produced a significant inhibition of lung metastatic progression (Figure 3). Similar antimetastatic effects were obtained administering DDAVP both during the first 24 h and 48-96 h after each weekly paclitaxel cycle (data not shown).

DDAVP significantly reduced proliferation in F3II cell cultures approximately 20% at doses higher than 250 ng/ml (Figure 4). The IC_{50} values ranged from 116 to 135 μM for carmustine and from 9 to 14 μM for paclitaxel after a 72-h exposure of log-phase growing F3II cells. However, addition of DDAVP did not modify the *in vitro* cytotoxic activity of chemotherapy agents at the doses employed.

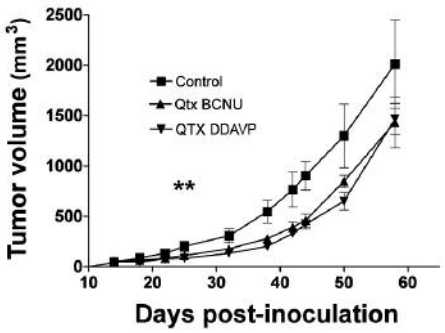

Figure 1. *Effect of carmustine plus DDAVP on subcutaneous mammary tumor growth in vivo. F3II cells were inoculated in the flank of syngeneic Balb/c mice and 14 days later animals were treated with 4 weekly cycles of carmustine in combination or not with DDAVP (for details see Materials and Methods). No effects were obtained with DDAVP alone in the present experimental conditions. Values represent means ± standard deviation of 10 animals per group. **p<0.01 versus control from day 25 onwards (ANOVA test).*

Discussion

Previous studies have demonstrated the antimetastatic properties of DDAVP in mouse models of breast cancer. The compound inhibited experimental lung colonization when co-injected intravenously with metastatic tumor cells (4) and also reduced locoregional spread when administered perioperatively at the time of primary tumor surgery (6).

To the best of our knowledge, this is the first experimental evidence indicating that the addition of a vasopressin peptide analog can improve chemotherapy results in solid tumors. Many authors have reported the recruitment of malignant cells into the peripheral blood after the first course of chemotherapy in patients with breast cancer (12). Since DDAVP was able to decrease implantation of residual breast cancer cells (6, 7), administration of the compound together with chemotherapy cycles may affect the biological mechanisms associated with metastatic progression.

During invasion and metastasis, tumor cells gain access to the target site *via* a series of processes involving invasion through basement membranes and adhesion to endothelium. Plasminogen activation, platelet aggregation and some factors involved in coagulation may influence cancer metastasis (13). It was demonstrated that VWF plays a protective role against tumor cell dissemination in a VWF-deficient mutant mouse model. Restoration of VWF plasma levels by administration of recombinant VWF reduced lung metastasis (14). VWF is synthesized by both endothelial cells and megakaryocytes, but plasma VWF appears to be mainly of endothelial origin. It is known that intravenous injection of DDAVP induces the release of VWF, with a time to peak levels of about 60 minutes and a plasma half-life of 8-10 hours (1, 3). VWF might participate in the interaction

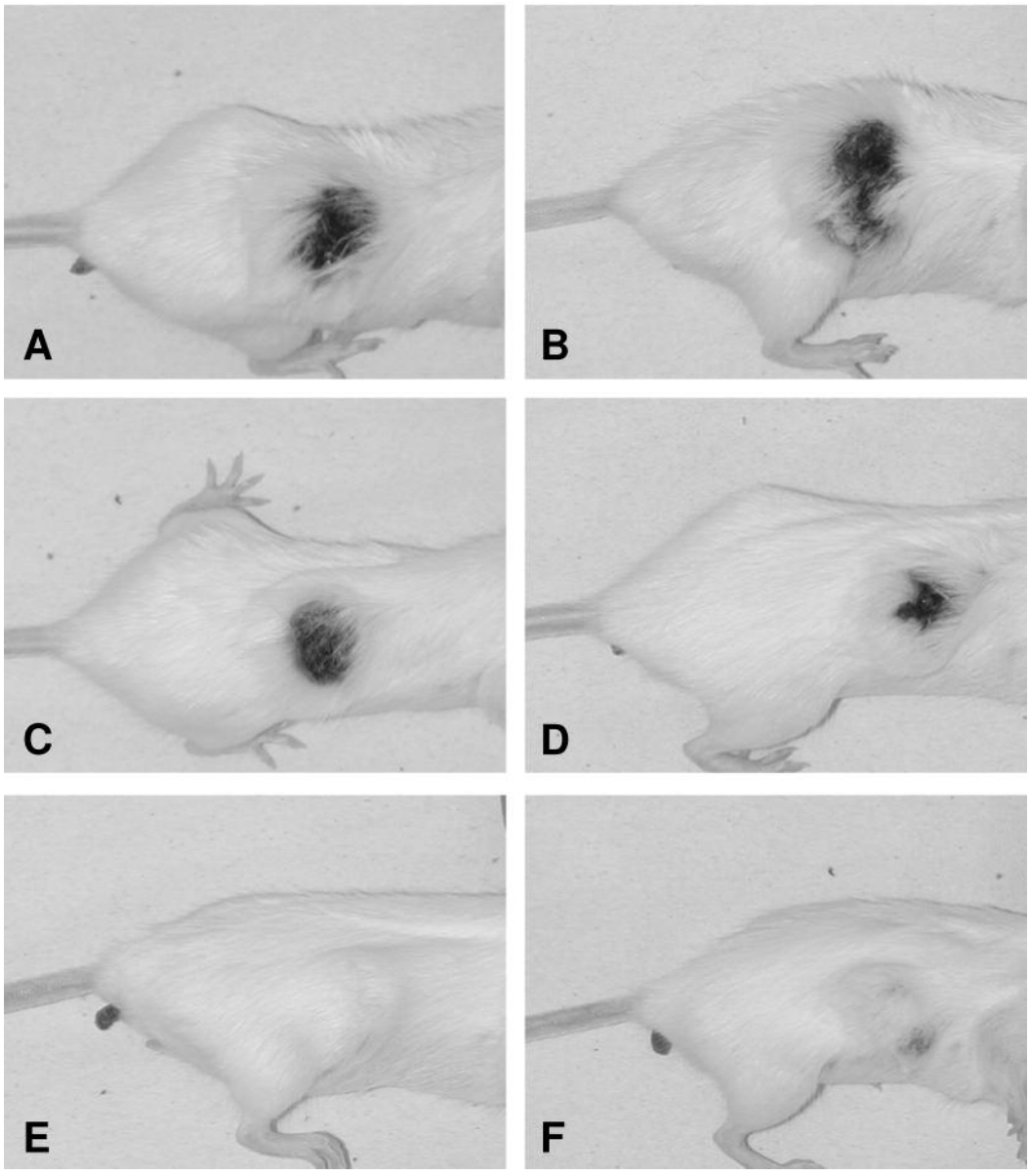

Figure 2. *Effect of carmustine plus DDAVP on local mammary tumor infiltration of the skin. F3II tumor-bearing mice were treated with weekly cycles of carmustine in combination or not with DDAVP (for details see Materials and Methods). No effects were obtained with DDAVP alone in the present experimental conditions. Images were taken 30-35 days after tumor inoculation and are representative of 10 animals per group. A and B: control; C and D: carmustine; E and F: carmustine + DDAVP.*

of tumor cells with platelets and subendothelium, and it appears to impede metastasis by reducing the sustained adherence and survival of tumor cells in lung microvasculature (14).

DDAVP is a selective agonist for the vasopressin V2 membrane receptor, which is expressed in endothelial cells and in the kidney collecting duct, mediating hemostatic and antidiuretic effects of the peptide, respectively (3). Interestingly, the presence of vasopressin receptors was also documented in several tumors and cell lines, including human breast cancer (15, 16). DDAVP reduced the *in vitro* growth of F3II cells, although no increase in cytotoxicity could be observed in combination with chemotherapy. Similarly, a mild antiproliferative effect of DDAVP was previously reported on the human MCF-7 mammary carcinoma cell line (17). Such action is likely to be mediated through V2 receptor signaling and involves activation of adenylate cyclase followed by intracellular cAMP elevation (15, 17). Taylor *et al*. (18) also showed that natural vasopressin can inhibit the growth of MCF-7 cells at high concentrations.

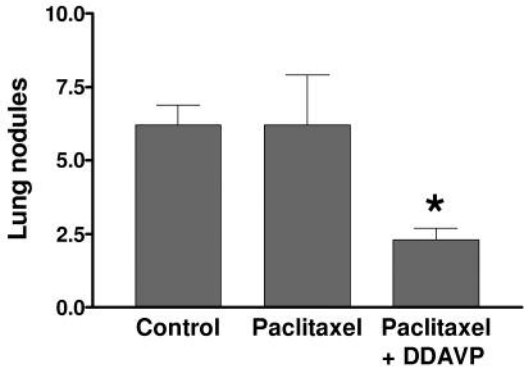

Figure 3. *Effect of paclitaxel plus DDAVP on metastatic progression of mammary tumors. F3II cells were inoculated in the flank of syngeneic Balb/c mice and 21 days later treated with 3 weekly cycles of paclitaxel in combination or not with DDAVP. At day 50, animals were sacrificed and lung nodules were counted (for details see Materials and Methods). No effects were obtained with DDAVP alone in the present experimental conditions. Values represent means ± standard error of the mean of at least 6 animals per group; *p<0.05 versus control (Kruskal-Wallis test).*

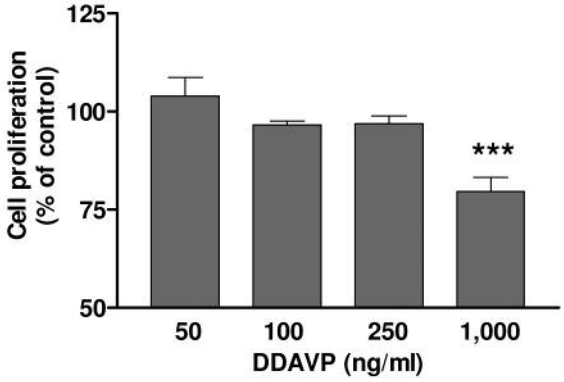

Figure 4. *Effect of DDAVP on in vitro growth of mammary tumor cells. F3II cells were grown on 96-well plates in the presence of appropriate concentrations of DDAVP for 72 h and then tested by the MTT assay (for details see Materials and Methods). Values represent means ± standard error of the mean from two independent experiments; ***p<0.001 versus control (ANOVA test).*

Nevertheless, we cannot exclude other possible mechanisms for the antitumor activity of DDAVP in combination with chemotherapy. The compound may facilitate lysis of malignant cells through the production of nitric oxide from the vasculature (19, 20), or modify tumor cell attachment by altering P-selectin expression on endothelial cells (21). In addition, DDAVP may mediate *in vivo* effects on the interaction of tumor cells with the microenvironment or modulate tumour-induced angiogenesis (3).

We conclude that our experimental findings suggest desmopressin may contribute to impair aggressiveness of residual mammary tumors during chemotherapy. With recent advances showing the need for combined administration of therapeutic agents in breast cancer management, our results support the notion that synthetic vasopressin peptide analogs may complement conventional cytotoxic drugs.

Acknowledgements

This work was supported by the R&D Grant Program 53-1004 from Quilmes National University and by the grant PICT 14255/03 from ANPCyT (Argentina). The support of the company Romikin S.A. is also acknowledged. G.V.R., D.E.G. and D.F.A. are members of the National Research Council (CONICET).

References

1 Mannucci PM: Desmopressin (DDAVP) in the treatment of bleeding disorders, the first 20 years. Blood *90*: 2515-2521, 1997.

2 Gomez DE, Ripoll GV, Giron S and Alonso DF: Desmopressin and other synthetic vasopressin analogues in cancer treatment. Bull Cancer *93*: E7-12, 2006.

3 Kaufmann JE and Vischer UM: Cellular mechanisms of the hemostatic effects of desmopressin (DDAVP). J Thromb Haemos *1*: 6682-689, 2003.

4 Alonso DF, Skilton G, Farias EF, Bal de Kier Joffe E and Gomez DE: Antimetastatic effect of desmopressin in a mouse mammary tumor model. Breast Cancer Res Treat *57*: 271-275, 1999.

5 Ripoll GV, Farina HG, Yoshiji H, Gomez DE and Alonso DF: Desmopressin reduces melanoma lung metastasis in transgenic mice overexpressing tissue inhibitor of metalloproteinases-1. In Vivo *20*: 881-885, 2006.

6 Giron S, Tejera AM, Ripoll GV, Gomez DE and Alonso DF: Desmopressin inhibits lung and lymph node metastasis in a mouse mammary carcinoma model of surgical manipulation. J Surg Oncol *81*: 38-44, 2002.

7 Hermo GA, Torres P, Ripoll GV, Scursoni AM, Gomez DE, Alonso DF and Gobello C: Perioperative desmopressin prolongs survival in surgically treated bitches with mammary gland tumours: A pilot study. Vet J. 2007 Aug 23 [Epub ahead of print].

8 Moulder S and Hortobagyi GN: Advances in the treatment of breast cancer. Clin Pharmacol Ther *83*: 26-36, 2008.

9 McSherry EA, Donatello S, Hopkins AM and McDonnell S: Molecular basis of invasion in breast cancer. Cell Mol Life Sci *64*: 3201-3218, 2007.

10 Alonso DF, Farias EF, Urtreger A, Ladeda V, Vidal MCC and Bal de Kier Joffe E: Characterization of F3II, a sarcomatoid mammary carcinoma cell line originated from a clonal subpopulation of a mouse adenocarcinoma. J Surg Oncol *62*: 288-297, 1996.

11 Alonso DF, Tejera AM, Farias EF, Bal de Kier Joffe E and Gomez DE: Inhibition of mammary tumor cell adhesion, migration, and invasion by the selective synthetic urokinase inhibitor B428. Anticancer Res *18*: 4499-4504, 1998.

12 Sabbatini RF, Morselli M, Depenni M, Cagossi R, Luppi K, Torelli M and Silingardi G: Detection of circulating tumor cells by reverse transcriptase polymerase chain reaction of maspin in patients with breast cancer undergoing conventional-dose chemotherapy. J Clin Oncol *18*: 3196-3197, 2000.

13 Korte W: Changes of the coagulation and fibrinolysis system in malignancy: their possible impact on future diagnostic and therapeutic procedures. Clin Chem Lab Med *38*: 679-692, 2000.
14 Terraube V, Pendu R, Baruch D, Gebbink MF, Meyer D, Lenting PJ and Denis CV: Increased metastatic potential of tumor cells in von Willebrand factor-deficient mice. J Thromb Haemost *4*: 519-526, 2006.
15 North WG, Fay MJ and Du J: MCF-7 breast cancer cells express normal forms of all vasopressin receptors plus an abnormal V2R. Peptides *20*: 837-842, 1999.
16 North WG: Gene regulation of vasopressin and vasopressin receptors in cancer. Exp Physiol *85*: 27S-40S, 2000.
17 Keegan BP, Akerman BL, Péqueux C and North WG: Provasopressin expression by breast cancer cells: implications for growth and novel treatment strategies. Breast Cancer Res Treat *95*: 265-277, 2006.
18 Taylor AH, Ang VT, Jenkins JS, Silverlight JJ, Coombes RC and Luqmani YA: Interaction of vasopressin and oxytocin with human breast carcinoma cells. Cancer Res *50*: 7882-7886, 1990.
19 Kaufmann JE, Iezzi M and Vischer UM: Desmopressin (DDAVP) induces NO production in human endothelial cells *via* V2 receptor- and cAMP-mediated signaling. J Thromb Haemost *1*: 821-828, 2003.
20 Hirano S: *In vitro* and *in vivo* cytotoxic effects of nitric oxide on metastatic cells. Cancer Lett *115*: 57-62, 1997.
21 Kanwar S, Woodman RC, Poon MC, Murohara T, Lefer AM, Davenpeck KL and Kubes P: Desmopressin induces endothelial P-selectin expression and leukocyte rolling in postcapillary venules. Blood *86*: 2760-2766, 1995.

Received April 7, 2008
Revised June 13, 2008
Accepted June 18, 2008