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Desmopressin Inhibits Lung and Lymph Node Metastasis in a Mouse Mammary Carcinoma Model of Surgical Manipulation

SANTIAGO GIRON, MSc, AGUEDA M. TEJERA, MSc, GISELLE V. RIPOLL, MSc, DANIEL E. GOMEZ, MD, PhD, AND DANIEL F. ALONSO, MD, PhD*

Laboratory of Molecular Oncology, Quilmes National University, Bernal, Buenos Aires, Argentina

Background and Objectives: Desmopressin (DDAVP) is a synthetic derivative of vasopressin with hemostatic and fibrinolytic properties that has been used during surgery in patients with bleeding disorders. Our aim was to investigate the effect of DDAVP on lung and lymph node metastatic cell colonization using a preclinical mouse mammary carcinoma model of subcutaneous tumor manipulation and surgical excision.

Methods: Female BALB/c mice bearing the highly aggressive F3II mammary carcinoma were subjected to repeated manipulations of primary tumors (0.5 kg/cm^2 during 2 min), followed (or not) by surgical excision. DDAVP was administered intravenously 30 min before and 24 h after each manipulation or surgery, at a dose of 2 μg/kg. At the end of the experiment, mice were sacrificed and necropsied.

Results: Tumor manipulation induced dissemination to the axillary nodes and increased up to 6-fold the number of metastatic lung nodules. Perioperative treatment with DDAVP dramatically reduced regional metastasis. The incidence of lymph node involvement in manipulated animals was 12% with DDAVP and 87% without treatment ($P < 0.02$). Histopathological analysis of axillary nodes from DDAVP-treated animals showed sinusal histiocytosis and no evidence of cancer cells. Metastatic lung nodules were also reduced about 65% in animals treated with DDAVP ($P = 0.026$).

Conclusions: Our results suggest a potential clinical application of DDAVP in the management of breast cancer, as well as other aggressive solid tumors. DDAVP may be useful to reduce the risk of metastatic cell colonization both during and after surgical manipulation.

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KEY WORDS: **breast cancer; surgery; metastasis; treatment; vasopressin analogue**

Desmopressin (1-deamino-8-D-arginine vasopressin, also known as DDAVP) is a synthetic derivative of the antidiuretic hormone that has been used in patients with diabetes insipidus and in a variety of bleeding disorders [1,2]. DDAVP appears to be a safe and effective hemostatic agent for use during surgery in patients with hemophilia A and von Willebrand disease. The compound increases the plasma levels of coagulation factor VIII and von Willebrand factor [3] and also enhances platelet adhesion to the vessel wall [4]. In addition, it is known that DDAVP induces a rapid and marked increase of tissue-type plasminogen activator, the major effector of endogenous fibrinolysis [3,5].

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*Correspondence to: Daniel F. Alonso, MD, Laboratorio de Oncología Molecular, Universidad Nacional de Quilmes, R. Sáenz Peña 180, Bernal B1876BXD Buenos Aires, Argentina. Fax: +54 11 4365-7132.
E-mail: dfalonso@unq.edu.ar

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Previously, we reported for the first time that DDAVP strongly inhibited hematogenous lung colonization of metastatic mammary carcinoma cells in a mouse model [6]. Our results suggested that the effect of DDAVP was exerted in the early stages of metastasis, possibly limiting the formation of tumor cell emboli, as well as altering the interaction of cancer cells with the endothelium at the target organ. Interestingly, a direct cytotoxic effect of DDAVP on tumor cells was ruled out [6].

It has been suggested that surgical manipulation can provoke liberation of viable cancer cells into the circulation. This fact has been confirmed by reverse transcription-polymerase chain reaction (RT-PCR) in patients undergoing breast cancer surgery [7,8]. Recently, Carter et al. [9] reported the histological findings in a series of axillary lymph node dissections taken approximately 2 weeks after breast biopsy. These investigators described the presence of epithelial cells in the subcapsular sinus of draining lymph nodes that may be attributed to mechanical transport of tumor breast epithelium secondary to the previous surgical or needle manipulation.

Considering the antimetastic effect of DDAVP in animal studies, as well as its well-known hemostatic and fibrinolytic properties, the compound is an excellent candidate for adjuvant therapy both during and immediately after tumor surgery. In the present work, our aim was to investigate the effect of DDAVP on lung and lymph node metastatic cell colonization, using a preclinical mouse mammary carcinoma model of subcutaneous tumor manipulation and surgical excision.

MATERIALS AND METHODS

Cell Line and Culture Conditions

The sarcomatoid mammary carcinoma cell line F3II is a highly aggressive variant established from a clone of a spontaneous BALB/c mouse mammary tumor. Upon subcutaneous injection in the flank, F3II cells grow as invasive spindle cell carcinoma tumors with a high angiogenic response and a 90–100% incidence of lung metastases [10]. Stock F3II cells were maintained in minimal essential medium (MEM) 41500 from Gibco-BRL (Grand Island, NY) supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, and 80 μg/mL gentamycin in monolayer culture. For harvesting, cells were trypsinized using standard procedures and incubated in serum-free MEM for 30–60 min at 37°C for recovery.

Animals

Female BALB/c inbred mice were purchased from the Animal Care Division of the Institute of Oncology Angel H. Roffo (Buenos Aires, Argentina) and were housed in plastic cages under standard conditions with rodent chow and water ad libitum, according to an institutionally approved animal protocol [11]. Animals aged 12–16 weeks and with an average weight of 25 g were used.

Tumor Cell Inoculation

On day 0, groups of 6–10 mice received 2×10^5 viable F3II cells, resuspended in 0.2 mL of serum-free MEM. Animals were given inoculations in the subcutis of the right flank. The time of appearance of local tumors was monitored by palpation and was further confirmed by histopathology. In all cases, tumors were diagnosed as spindle cell carcinomas. Tumor size was measured with a caliper twice a week, and tumor volume was calculated by the formula: $\pi/6 \times \text{width}^2 \times \text{length}$.

Application of Pressure on Subcutaneous Tumors and Surgery

We have developed an experimental instrument, made of stainless steel, for the application of controlled pressures on subcutaneous tumors. It consists on a mobile platform that transmits pressure through an axis to a small surface of $\sim$6 cm^2. The platform is loaded with the appropriate weight and the instrument discharges a stable and controlled pressure (0.1–0.5 kg/cm^2) on the tumor mass (Fig. 1). Tumor-bearing mice were anesthetized with ketamine and xylacine (60 and 7 mg/kg, respectively). Then, subcutaneous tumors were subjected to experimental manipulations using pressures of $\sim$0.5 kg/cm^2 during 2 min, followed (or not) by surgical excision.

To examine the antimetastatic properties of DDAVP, subcutaneous tumors were subjected to experimental manipulations on days 14, 21, and 28 days or on days 21 and 28, and surgically excised on day 35. Animals were sacrificed by cervical dislocation and necropsied on day 60 after F3II cell inoculation. Tumors and axillary nodes were removed and fixed in 10% formalin, and paraffin-embedded sections were stained with hematoxylin and eosin or Mallory's aniline blue collagen staining. Liver, kidney, and spleen were also removed for histological examination. To investigate the presence of metastases, lungs were removed, fixed in Bouin's solution and the size and number of surface lung nodules were determined under a dissecting microscope, as described in detail [11].

DDAVP Treatment

DDAVP from Ferring Pharmaceuticals (Malmö, Sweden) was administered in 2 doses, one 30 min before and the second 24 h after each tumor manipulation or surgical excision. Mice received DDAVP by the intravenous route in physiological saline at a final dose of

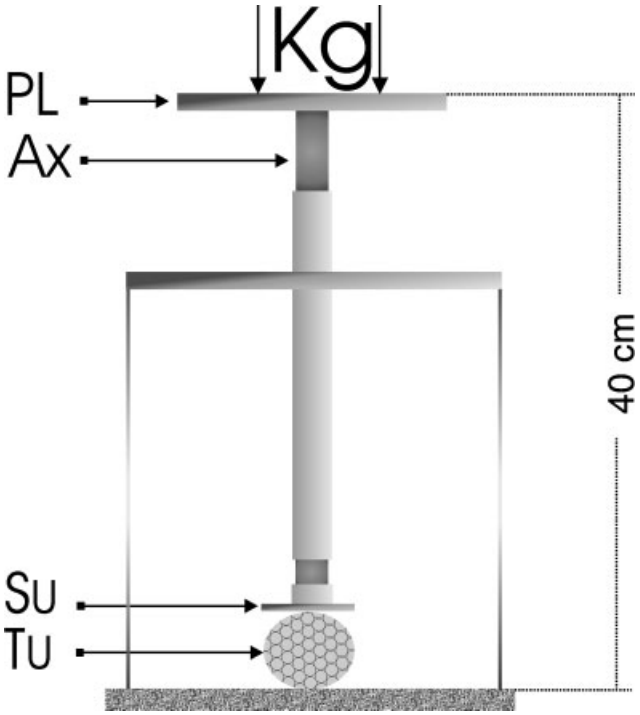

Fig. 1. Experimental instrument for the application of controlled pressures on tumor-bearing animals. The mobile platform (PL) is loaded with the appropriate weight (kg), and transmits pressure through an axis (AX) to a small surface (SU) in contact with the subcutaneous tumor mass (TU).

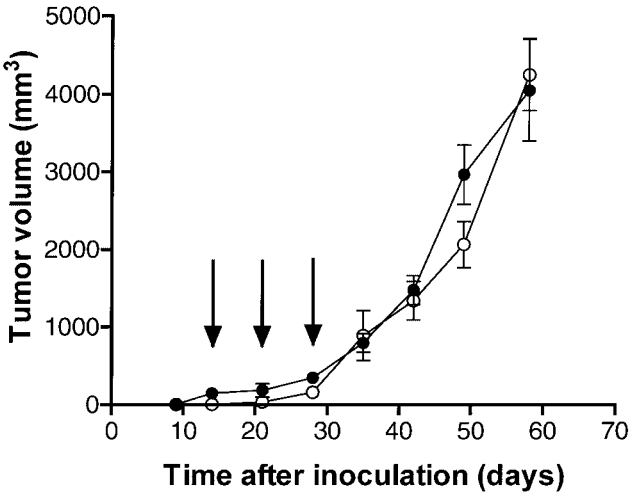

Fig. 2. Lack of effect of repeated experimental manipulation on tumor growth. Subcutaneous masses were exposed to 0.5 kg/cm^2 for 2 min (arrows) on days 14, 21, and 28 after inoculation of F3II mammary carcinoma cells in the manipulated group (●), and control tumors were not manipulated (○). Data represent means ±SEM of at least six mice.

2 μg/kg body weight (50 ng/0.3 mL saline/dose), as reported [6]. Control animals received only the saline vehicle.

Statistical Analysis

Tumor volumes were compared with Student's *t*-test, and the incidence of lymph node metastasis was evaluated by the chi-square test with the Yates correction. Statistical analysis of the number of metastatic lung nodules was done by the nonparametric Kruskal-Wallis or Mann-Whitney U-tests.

RESULTS

We first confirmed the effect of manipulation on the F3II mouse mammary carcinoma model. We applied controlled pressures (0.5 kg/cm^2 for 2 min) on subcutaneous tumors 14–28 days after tumor cell inoculation, when tumors averaged volumes of 200–400 mm^3. No significant differences were found in tumor growth rates in mice exposed to repeated weekly manipulations. Volumes of subcutaneous tumors that were not excised after manipulations were similar to control values until the end of the experiment (Fig. 2). Histopathological analysis of manipulated masses showed intratumor ruptures and microhemorrhages but preservation of the peripheral microvascular bed (Fig. 3).

Mice bearing nonmanipulated primary tumors showed spontaneous lung metastasis at day 60, with an average of six nodules per mouse, although no evidence of tumor-draining lymph node metastasis was found, as expected [10]. Manipulations induced an up to sixfold increase in the number of metastatic lung nodules, with higher values in animals subjected to at least two manipulations at 7-day intervals. In addition, 80–90% of manipulated mice demonstrated metastatic dissemination to the axillary lymph nodes.

We then tested the effect of DDAVP on lung and lymph node metastastic cell colonization using the present experimental model of tumor manipulation. Tumor-bearing animals were anesthetized and subcutaneous masses were subjected to controlled pressures on days 14, 21, and 28, and tumors were not surgically removed until the end of the experiment at day 60. Animals were treated (or not) with two intravenous doses of DDAVP (2 μg/kg/dose), 30 min before and 24 h after each manipulation. Treatment with DDAVP dramatically decreased dissemination to regional lymph nodes (Table I). As shown in Figure 4, histopathological examination of axillary nodes confirmed the presence of massive metastasis of a poorly differentiated carcinoma in mice receiving the saline vehicle. On the contrary, most lymph nodes from DDAVP-treated animals showed sinusal histiocytosis and no evidence of cancer cells. In the same way, DDAVP induced a reduction in the number of lung metastases, particularly in the small nodules of $\leq$2 mm in diameter (see also Table I).

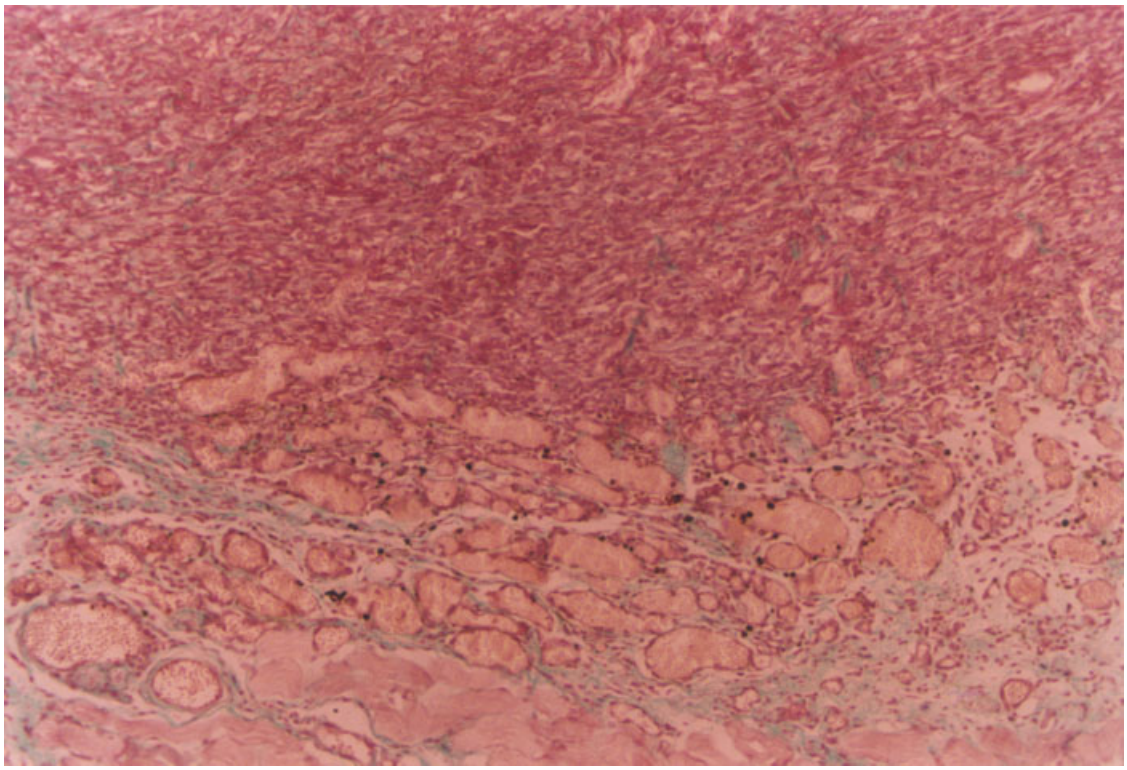

Fig. 3. Light micrograph of a paraffin section from a subcutaneous F3II sarcomatoid mammary carcinoma tumor. Preservation of the peripheral microvascular bed after repeated tumor manipulations. Mallory's aniline blue collagen stain. ×100.

In another set of experiments, subcutaneous tumors were manipulated on days 21 and 28, followed by surgical excision on day 35. Under these experimental conditions, the antimetastatic effect of DDAVP was clearly demonstrated by a significant reduction of total lung metastasis (Fig. 5).

In all cases, administration of DDAVP was not associated with overt toxic effects and treatment did not affect tumor growth (data not shown). Extrapulmonary tumor colonies were not found in any of the control mice or mice treated with DDAVP.

DISCUSSION

Surgical manipulation of a tumor may result in increased influx of tumor cells into the systemic and lymphatic

TABLE I. Effect of DDAVP on Axillary Lymph Node and Lung Metastasis in Mice Bearing Subcutaneous F3II Mammary Carcinoma Subjected to Repeated Experimental Manipulation

Treatment[a]	Incidence of axillary lymph node metastasis[b] (positive/total)	No. of lung metastases[c]		
		Small nodules (≤2 mm)	Large nodules (>2 mm)	Total
Control (saline)	87% (7/8)	9 (5–55)	20 (0–63)	27 (5–95)
DDAVP	12% (1/8)**	4 (0–24)*	5 (0–48)	9 (0–58)
No manipulation	0% (0/6)***	3 (0–6)*	3 (0–5)	6 (2–9)*

DDAVP, desmopressin.

[a]On day 0, 2×10^5 F3II cells were inoculated in the subcutis of the right flank of female BALB/c mice. Animals were anesthetized and subcutaneous tumors were manipulated (0.5 cm^2/kg for 2 min) on days 14, 21, and 28 after inoculation. DDAVP treatment consisted of two intravenous doses (2 μg/kg/dose), 30 min before and 24 h after each manipulation.

[b]Lymph node metastasis was confirmed by histopathology.

[c]The size and number of lung metastases were determined 60 days after tumor cell inoculation. Values represent medians, with range represented in parentheses.

*$P < 0.05$ versus control, Kruskal-Wallis test.

**$P < 0.02$ versus control, chi-square test.

***$P < 0.01$ versus control, chi-square test.

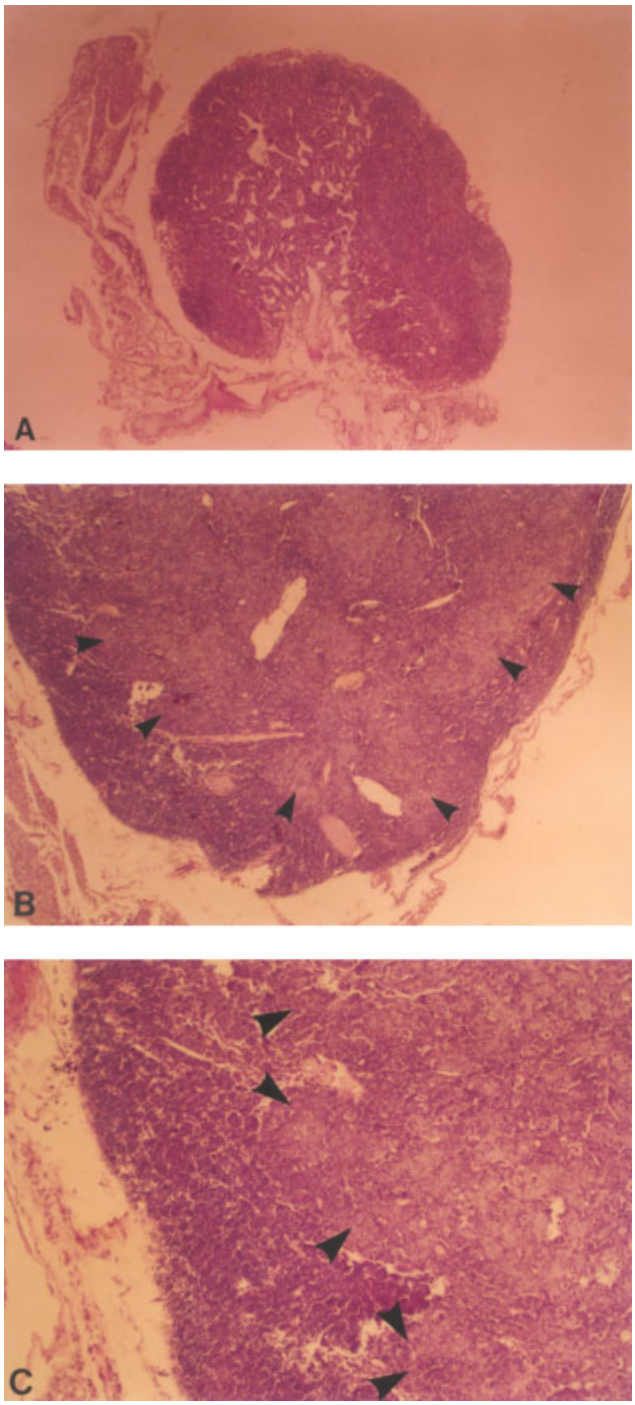

Fig. 4. Light micrographs of paraffin sections from axillary lymph nodes of F3II-bearing mice subjected to repeated tumor manipulations. **A:** Sinusal histiocytosis and no evidence of cancer cells in a desmopressin (DDAVP)-treated mouse. **B,C:** Massive metastasis of a poorly differentiated carcinoma (arrowheads) in a mouse receiving the saline vehicle. Hematoxylin and eosin stain. A,B: ×40; C: ×100.

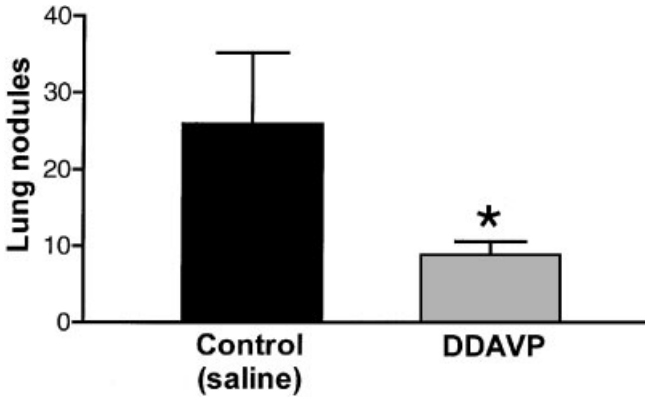

Fig. 5. Effect of desmopressin (DDAVP) on lung metastasis in F3II-bearing mice subjected to tumor manipulation and surgery. Subcutaneous masses were exposed to 0.5 kg/cm^2 for 2 min on days 21 and 28 after inoculation of F3II mammary cells, followed by surgical excision on day 35. DDAVP was administered intravenously in two doses (2 μg/kg/dose), 30 min before and 24 h after each manipulation or surgery. Surface lung nodules were counted at day 60. Data represent means ± SEM of at least six mice. $*P = 0.026$, Mann-Whitney U-test.

circulation. Several experimental studies with animal models have confirmed that intraabdominal tumor manipulation was the main factor acting on metastatic dissemination using conventional laparotomy or laparoscopy [12–15]. Recently, Lee et al. [16] showed that port site tumor recurrence rates decreased with increased surgical experience in a mouse adenocarcinoma model of laparoscopic splenectomy, suggesting that a poor surgical technique was the main cause of recurrence. In the same line, interesting results were obtained in an experimental model of breast cancer. Syngeneic mice were inoculated into the mammary fat pad with TA3Ha adenocarcinoma cells; the resulting tumors were surgically excised with a curative intent. Under these conditions, perioperative chemotherapy with doxorubicin reduced local recurrence, axillary metastasis, and lung metastasis, and also improved disease-free survival [17].

From our results presented above, it seems clear that DDAVP was capable of inhibiting lung and axillary lymph node metastasis in a mouse mammary carcinoma model of surgical manipulation. However, it is important to note that in these experimental conditions the compound caused a reduction in tumor cell colonization rather than an inhibition of the spontaneous metastatic process. Subcutaneous primary tumors were subjected to repeated controlled manipulations, whether followed by surgical excision or not. Thus, metastatic cells were experimentally induced to gain access into systemic or lymphatic circulation. As expected, tumor manipulation dramatically increased lung and axillary lymph node metastasis. A higher number of lung nodules were obtained in animals subjected to at least two manipulations at weekly intervals, suggesting that the persistence of wounded tumor tissue after manipulation was a critical determinant of metastasis. DDAVP was administered 30 min before and 24 h after each manipulation or surgery

at a clinically relevant hemostatic dose [3,6,18]. DDAVP appeared to be safe at this dosage, and antimetastatic effects were obtained without overt toxic effects.

Axillary lymph nodes from most DDAVP-treated animals showed sinusal histiocytosis. Interestingly, histiocytic reaction of the regional lymph nodes is considered a strong indicator of antitumor resistance in patients with breast cancer [19]. In contrast, axillary nodes from control mice bearing manipulated mammary tumors and administered with the saline vehicle evidenced metastasis and lacked sinusal histiocytosis.

Further investigations will determine the precise antimetastatic mechanisms exerted by DDAVP. Nevertheless, the hemostatic effect of DDAVP may improve the postoperative healing process. Enhanced coagulation after tumor manipulation may contribute to a rapid encapsulation of residual tumor tissue, limiting intravasation of metastatic cells. Besides, DDAVP increases intravascular fibrinolysis [3,5], helping dissolve the protective fibrin shield of circulating tumor cells and reducing tumor cell aggregation [6]. It is known that fibrin deposition around metastatic tumor cells entering into the blood stream enhances cell survival and facilitates trapping in target organ [20].

However, we cannot exclude other mechanisms of antimetastatic action of DDAVP. For instance, DDAVP may alter hemodynamics of blood flow or modify tumor cell attachment by altering P-selectin expression on endothelial cells [21,22]. DDAVP may also induce lysis of metastatic tumor cells through the production of nitric oxide from the vasculature [23,24].

Surgical manipulation and tissue trauma enhance the growth and dispersement of many types of malignant cells. Our results suggest a potential clinical application of DDAVP in the management of breast cancer, as well as other aggressive solid tumors, such as melanoma, prostate, ovary, colon, or lung cancer. DDAVP may decrease the implantation of metastatic cells released during and after surgical manipulation. Moreover, administration of DDAVP with the courses of conventional chemotherapy may be also useful, considering the possible mobilizing effect of chemotherapy on cancer cells. In this regard, Sabbatini et al. [25] reported the recruitment of tumor cells into the peripheral blood after the first courses of primary chemotherapy in patients with breast cancer enrolled in a prospective study.

Perioperative DDAVP inhibited lung and axillary lymph node metastasis in a mouse mammary carcinoma model of surgical manipulation. Whichever the mechanism of action involved, it seems that a safe hemostatic and profibrinolytic agent, such as DDAVP, may have a new use in cancer surgery to reduce the risk of metastatic colonization of malignant cells released during and after tumor manipulation.

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