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Tumor bearing decreases systemic acute inflammation in rats – role of mast cell degranulation

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Abstract. *Objective and design:* To investigate the effect of experimental tumor bearing on acute inflammation models in rats.
Methods: Four and 7 days after Walker tumor implantation in the right armpit, carrageenan or dextran– induced edema in the contralateral paw, carrageenan induced neutrophil migration into peritoneal cavities, cutaneous vascular permeability induced by bradykinin, histamine, serotonin, substance P, capsaicin or compound 48/80, and mesenteric mast cell degranulation induced by compound 48/80 were evaluated. The control group did not receive tumor implantation. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by the Bonferroni test.
Results: On the 7th day after tumor inoculation, there were significant decreases in both carrageenan and dextran- induced paw edema. Tumor bearing did not change the neutrophil infiltration induced by carrageenan. There were decreases in cutaneous vascular permeability induced by compound 48/80, serotonin or bradykinin, but not that induced by histamine, substance P. A significant inhibition of mesenteric mast cell degranulation induced by compound 48/80 was observed, on the 4th and 7th days after tumor inoculation.
Conclusion: Tumor bearing can limit mast cell function and vascular events in acute systemic inflammation in rats, without changes in neutrophil migration.

Key words: Tumour bearing – Acute inflammation – Mesenteric mast cell degranulation

Introduction

Several inflammatory mediators, such as cytokines, metalloproteinases, lipoxygenase and cyclooxygenase products, are part of the tumor microenvironment, and are important factors in tumoral growth [1–2]. It has been recently suggested that cancer can induce immunosuppression, which evolves in an immunosuppressive network extending from the primary tumor site to secondary lymphoid organs and peripheral vessels, and is affected by several mediators, such as interleukin-10 (IL-10), transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF) [3].

Mast cell accumulation in peritumoral inflammatory infiltrate contributes to a permissive microenvironment during tumor growth [4–6]. There is no evidence that a decrease in mast cell function can be associated with cancer imunossupression. [7]

Walker 256 tumor is widely utilized in experimental cancer research, due to its easy transplantation, lack of regression or strain specificity and rarity of spontaneous metastases, as well as its successful transposition to tissue culture [8]. Brozna & Ward demonstrated in 1979 that both acute and chronic cellular inflammatory reactions were suppressed in rats bearing Walker 256 tumor [9]. However, the mechanisms by which Walker 256 tumor induces a decrease in rat systemic inflammation were not elucidated.

The main goal of the present study was to investigate the effects of experimental tumor bearing on classical acute inflammation models in rats, and evaluate the role of mast cell degranulation inhibition in these events.

Materials and methods

Animals

Wistar rats weighing 180–200 g were housed in temperature-controlled rooms and received water and food ad libitum until use. All experiments were conducted in accordance to NIH standards and approved by the Committee of Ethics in Animal Research and Care of the Federal University of Ceará.

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Drugs

Carrageenan, dextran, bradykinin, serotonin, substance P, histamine, capsaicin and compound 48/80 were purchased from Sigma Chemicals (St. Louis, MO, USA). Vehicle solutions consisted of PBS buffer or saline.

Tumor inoculation

Tumor nodules from tumor bearing rats were excised, gently homogenized in a hand-operated tissue grinder, suspended in sterile actated ringer's with gentamicin and adjusted to a concentration of 10^6 viable tumor cells/mL. The tumor was implanted by intramuscular injections of 10^6 tumor cells into the right armpit [10]. Animals were housed in cages with food/mineral water *ad libitum*. In preliminary experiments, tumor growth was observed, starting from the 4th day ($329.3 \pm 86.1\,mm^3$), continuing to the 7th day ($2892.0 \pm 614.8\,mm^3$), and reaching maximum growth on the 15th day ($16390.0 \pm 3050.0\,mm^3$) after tumor inoculation. We also observed that after 15 days of tumor inoculation, animal mortality reached 100 %. In this study we evaluated tumor bearing effects after the 4th and 7th days of tumor implantation.

Carrageenan or dextran-induced paw edema

Paw edema was induced by subplantar injection of carrageenan (300 µg/paw; 0.1 mL) or dextran (500 µg/paw; 0.1 mL). This edema was evaluated in the tumor contralateral hind paw. Animals with tumor (4th, 7th days after tumor inoculation) or without tumor (control group) received the same inflammatory stimulus. Paw volume was measured by plesthysmometry (Ugo-Basile 7140 Plesthysmometer) immediately before (basal volume) and at 1,2, 3 and 4 h after carrageenan administration or 30',1 2, 3 and 4 h after dextran administration. Results were expressed as paw volume variation (mL), calculated by subtracting paw basal volume from the hydroplesthymometer.

Stimulation of neutrophil migration into peritoneal cavities

Carrageenan (300 µg/1,0 mL) or saline (1,0 mL) were injected intraperitoneally (i. p.) into rats with or without tumor (4th, 7th after tumor inoculation). Three hours after carrageenan injection, animals were sacrificed and peritoneal fluid was collected. Total and differential cell counts were performed as described elsewhere [11].

Myeloperoxidase activity assays

Carrageenan (Cg; 300 µg/paw) was injected into the left hind paw in animals with or without tumor (4th and 7th and day after tumor inoculation). Four hours after carrageenan administration, animals were sacrificed and the whole plantar region left paw skin was harvested. After homogenization and centrifugation (4500 rpm, 20 min), myeloperoxidase activity, an enzyme found in azurophil neutrophil granules, was measured with a previously described colorimetric method and expressed as units of myeloperoxidase activity per mg of tissue [12].

Cutaneous vascular permeability

In the 4th and 7th day after tumor inoculation, rats with or without tumor were divided into six groups and stimulus injections (bradykinin, histamine, serotonin, substance P, capsaicin or 48/80 compound) were administered intradermically into shaved dorsal skin. Immediately after stimulus, Evans blue dye was administered (25 mg/kg, i. v., 100 µl/100 g body weight). Thirty minutes later, the blue area was collected and soaked with 1 ml of formamide for 48 h at 37 °C. Supernatant absorbance was measured by spectrophotometry (630 nm). Supernatant dye content was calculated according to the Evans Blue standard curve and values were expressed as µg of Evans blue per mg of tissue [13].

Mast cell degranulation in mesenteric tissue

Animals with or without tumor (4th, 7th day after tumor inoculation) were sacrificed. Mesenteric tissues were collected from the respective groups and placed into each of the Petri dishes containing Ringer Locks fluid (10 ml). Mast cell degranulation was induced by incubation of dishes containing mesenteric tissue collected from animals with or without tumor with compound 48/80 (final concentration, 0.8 µg/mL) for 30 min and placed on microscopic slides. Hydrated tissue sections were immersed in a 0.1 % toluidine blue solution (in 0.9 % sodium chloride) for 60 s, followed by extensive rinsing in deionised water as previously described [14]. The percentage of degranulated mast cells was determined by counting one hundred stained cells per tissue section.

Statistical analysis

Results are presented as means and standard errors of the mean for groups of six animals each. Differences between experimental groups were compared by Analysis of variance (ANOVA) followed by Bonferroni's t-test. Significance level was set at $p < 0.05$ and $p < 0.001$.

Results

Effects of tumor bearing on carrageenan or dextran – induced paw edema

In Figure 1, it can observed that from the 7th day after tumor inoculation onwards, there was a significant decrease in both carrageenan (panel A) and dextran (panel B) – induced paw edema at all intervals. However, on the 4th day after tumor inoculation, we observed a decrease in paw edema with carrageenan (panel A), but not with dextran (panel B).

Effect of tumor bearing on carrageenan – induced neutrophil infiltration

Figure 2 shows that neither on the 4th nor on the 7th day after tumor inoculation were there any changes in carrageenan – induced peritoneal neutrophil infiltration. Furthermore, in Figure 3, it can be seen that tumor bearing did not change MPO activity induced by carrageenan in the paw.

Effect of tumor bearing on the increase in cutaneous vascular permeability induced by bradykinin, serotonin, substance P, histamine, capsaicin and compound 48/80.

In Figure 4, it can observed that on the 4th and 7th day after tumor inoculation, there was a decrease in cutaneous vascular permeability induced by compound 48/80 (panel A), serotonin (panel B) or bradykinin (panel D), but not by histamine (panel C). Tumor bearing did not change cutaneous vascular permeability induced by capsaicin (panel E) or substance P (panel F).

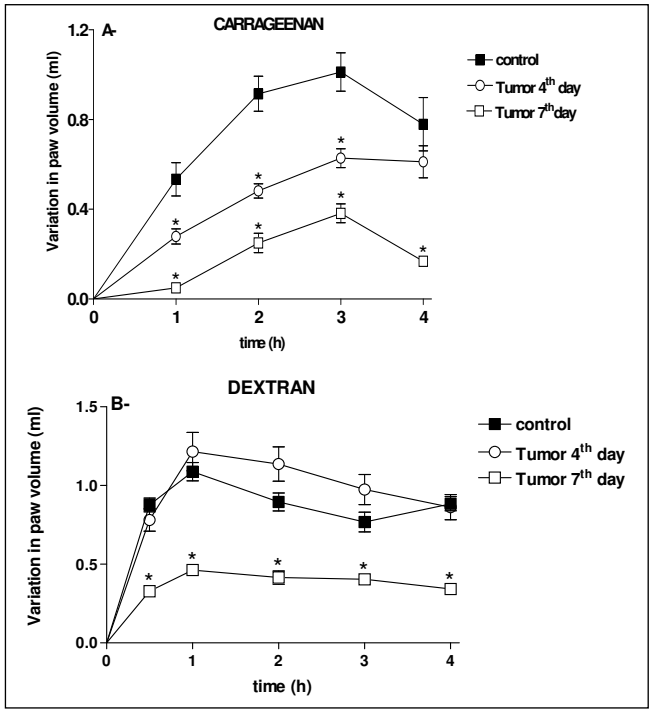

Fig. 1. Effect of tumor bearing on carrageenan or dextran – induced paw edema. Carrageenan (300 µg/paw) and dextran (500 µg/paw) were injected and paw volume was measured after 0, 1, 2, 3 and 4 h for Carrageenan and after 30 min, 1, 2, 3 and 4 h for dextran. A: Evaluation of paw edema induced by Carrageenan after 1 h, 2 h, 3 h and 4 h on the 4th and 7th day after tumor inoculation. B: Evaluation of paw edema induced by dextran, after 30 min, 1, 2, 3 and 4 h on the 4th and 7th day after tumor inoculation. (*) $p<0.05$ compared to control group (ANOVA/ Bonferroni).

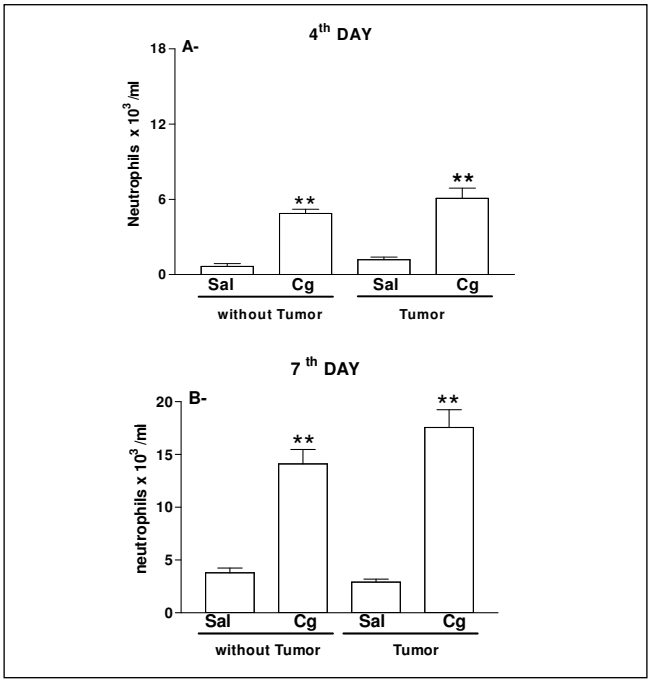

Fig. 2. Effect of tumor bearing on carrageenan - induced neutrophil infiltration. On the 4th (panel A) or 7th day (panel B) after tumor inoculation, carrageenan (300 µg) or saline solution was injected into the rat peritoneal cavity with or without Walker tumor. Three hours after saline or carrageenan injection, rats were sacrificed and neutrophil migration was evaluated. Bars represent the mean ± SEM of 10^3×neutrophil/mL for 6 rats of each group. Statistically significant increases were retained in all the groups Cg (without or with tumor). (**) $p<0.01$ compared to saline group (ANOVA/Bonferroni).

Effects of tumor bearing on compound 48/80-induced mesenteric mast cell degranulation

Figure 5 shows a significant inhibition of mesenteric mast cell degranulation induced by compound 48/80, on the 4th and 7th day after tumor inoculation when compared to the control group (saline).

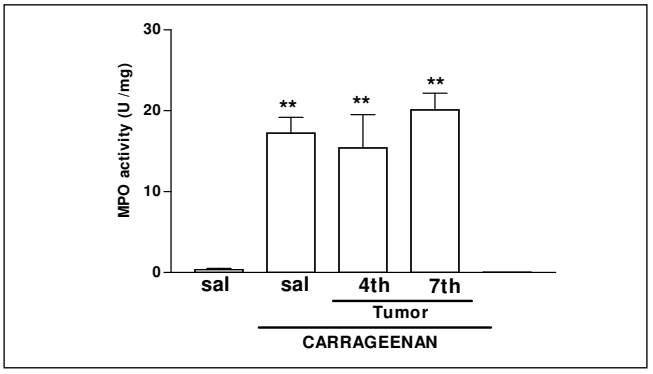

Fig. 3. Effect of tumor bearing in MPO activity. Carrageenan - induced growth in MPO activity did not change during tumor bearing (4th, 7th day after inoculation). Columns represent the mean ± SEM of MPO group units. Saline (sal, n = 6), Carrageenan (n = 6), Carrageenan + Tumor (n = 6). (**) $p<0.001$ compared to saline (sal) group. (ANOVA/ Bonferroni).

Discussion

Only a few findings in the literature have shown the interference of tumoral microenvironment with the acute systemic inflammatory response. Previous studies had shown that Walker tumor, when inoculated into rats, reduces inflammatory parameters, including vascular permeability induced by histamine and neutrophil migration induced by polyvinyl sponges [9]. However, this study did not define mechanisms by which Walker tumor decreases the systemic acute inflammatory process. Our results demonstrated that tumor bearing reduces carrageenan and dextran paw edema; serotonin, bradykinin and compound 48/80 provoked cutaneous vascular permeability, and decreases mesenteric mast cell degranulation induced by compound 48/80, with no changes in neutrophil migration induced by carrageenan. Therefore, we can infer that tumor bearing can decrease mast cell function and vascular events in acute systemic inflammation.

Our results demonstrated that Walker tumor inoculation decreases paw edema induced by carrageenan and dextran. Evidence found in the literature demonstrates that carrageenan-induced paw edema has two phases. The early phase is the

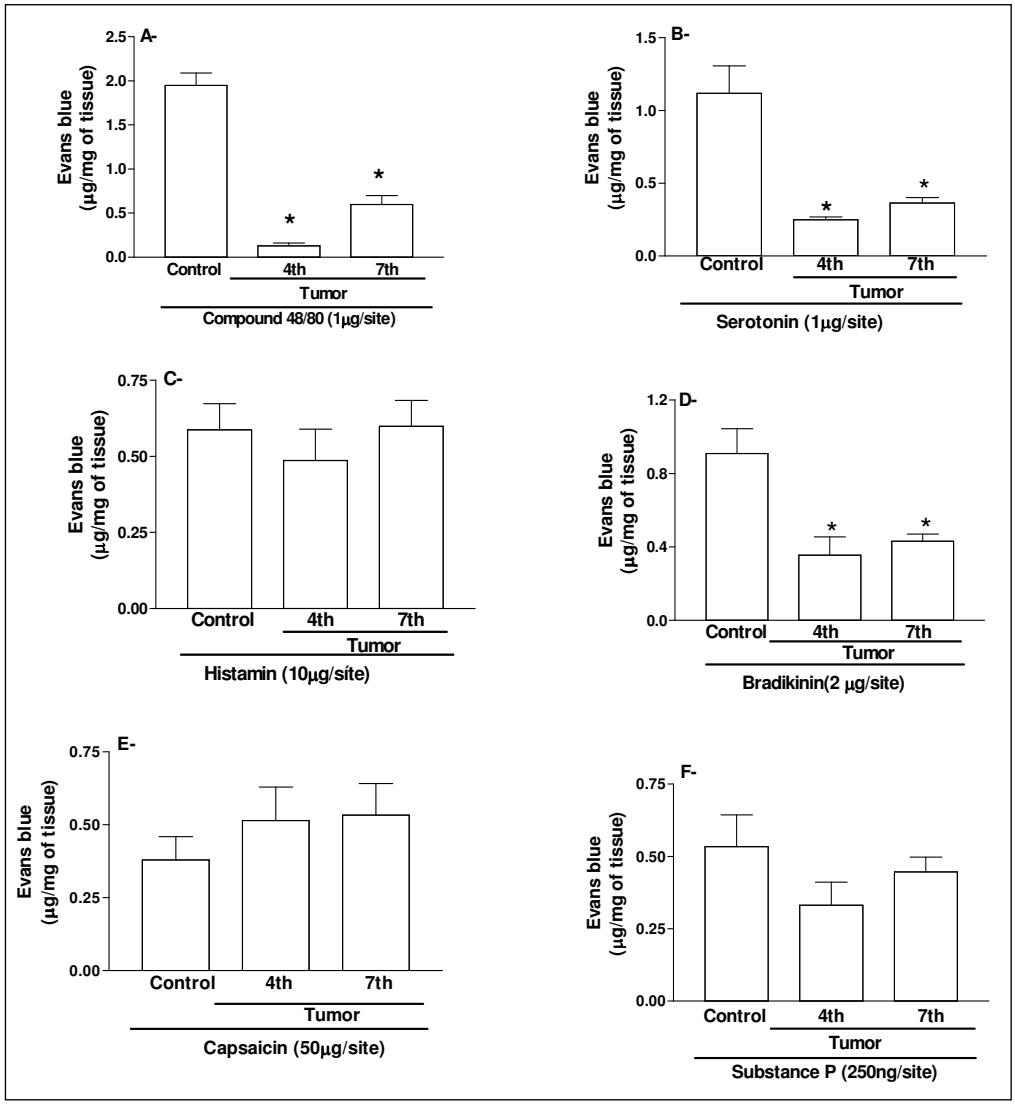

Fig. 4. Effect of tumor bearing on the increase in cutaneous vascular permeability induced by compound 48/80, serotonin, histamine, bradykinin, capsaicin or substance P. The 48/80 compound (panel A), serotonin (panel B), histamine (panel C), bradykinin (panel D), Capsaicin (panel E) and substance P (panel F) were injected into rats with tumor (4^{th}, 7^{th} day after inoculation). Control group was composed of rats without tumor (control). After 1 h, animals were sacrificed and skins removed. Skin permeability was evaluated by the amount of Evans blue extravasated as measured by spectrophotometry at 600nm. Bars represent the mean ± SEM µg of Evans blue per mg of tissue. (*) $p<0,05$ compared to control group. (ANOVA/Bonferroni).

result of the increased histamine and serotonin concentration in the extracellular space [15]. The late edema phase is known to be dependent on cytokine production by resident cells and neutrophil infiltration [16–18]. On the other hand, paw edema induced by dextran is only mediated by an increase in vascular permeability, through fluid accumulation and mast cell degranulation [19]. Our results show that tumor bearing reduced both the early and late phases of carrageenan-induced paw edema, and also decreased dextran-induced paw edema. Therefore, we can infer that at least the vascular component of the edema is reduced during tumor bearing.

In order to study the cellular component participating in systemic acute inflammation reduction during tumor bearing, we evaluated neutrophil infiltration in two classical models. Contrary to the literature, our results showed that neither peritoneal nor paw tumor bearing changed neutrophil infiltration induced by carrageenan. Brozna & Ward demonstrated that 2×10^7 Walker tumor inoculation cells reduced neutrophil migration induced by implanted polyvinyl sponges after 16–24 hours [9]. Compared to our model, there are some important differences. Firstly, Brozna & Ward inoculated more tumor cells; secondly, polyvinyl sponges induced chronic inflammation.

In an attempt to identify possible mediators involved in Walker tumor anti- edematogenic activity, we evaluated cutaneous vascular permeability for some important inflammatory stimuli. Our results demonstrated that tumor bearing diminished bradykinin and serotonin- induced vascular permeability, but not histamine- induced vascular permeability. Bradykinin is formed in the plasma in response to inflammatory processes and improves vascular permeability and edema formation by NO production, and it also activates potassium channels [20–23]. Serotonin is a mediator found in large amounts in mast cells, participates in the genesis of acute inflammatory vascular events and in various stages of the immune response [24–26]. Serotonin receptors 5-HT1 and 5-HT3 have also been associated with mast cell degranulation modulation [27–28]. The effect of Walker tumor inoculation in serotonin-induced vascular permeability could be explained at least in two ways: (1) Walker tumor could have produced serotonin receptor antagonists located in the peripheral microvasculature, (2) Walker tumor could have interfered with serotonin's

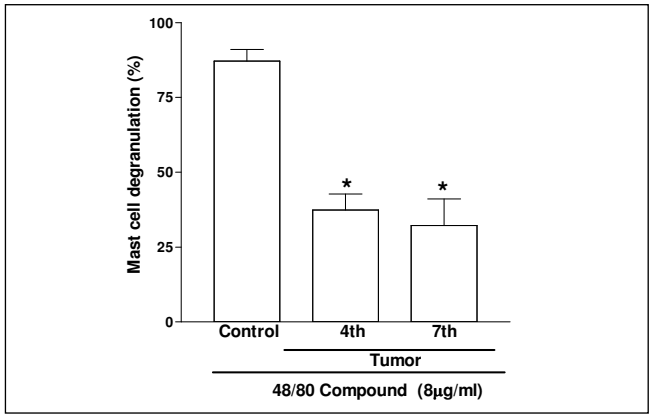

Fig. 5. Effect of tumor bearing in compound 48/80 compound induced mesenteric mast cell degranulation. Animals with tumor (4^{th}, 7^{th} day after tumor inoculation) or without it (control group) were sacrificed. Mesenteric tissue was harvested and fixed. Tissue samples were rinsed in PBS or 48/80 compound (0.8 µg/ml). Afterwards, tissue fixed sections were immersed in a solution of 0.1 % toluidine blue. Percentages of degranulated mast cells were determined by counting one hundred stained cells in different fields (×400). Bars represent the mean ± SEM of mast cell degranulation percentage. (*) $p<0.001$ compared to control group (ANOVA/Bonferroni).

autocrine effect in mast cells, thus preventing degranulation. However our results do not role out the possibility that other mechanisms could be involved, and other experiments need to be made to confirm our hyphoteses.

Our results have demonstrated that tumor bearing does not change capsaicin and substance P – induced vascular permeability. Capsaicin and substance P stimulate peripheral terminals of C-fibers, causing the release of neuropeptides (such as tachykinins), thus initiating the cascade of neurogenic inflammation with a growth in vascular cutaneous permeability [29–31]. According to these data, we can infer that Walker tumor doesn't inhibit vascular permeability induced by neurogenic inflammation.

In order to study whether tumor bearing prevents mast cell degranulation, we utilized a pharmacological approach using compound 48/80, a substance that degranulates mast cells and promotes content release. Our results demonstrated that vascular permeability and mast cell degranulation induced by compound 48/80 decreases tumor bearing. These findings indicate that tumor bearing interferes with mast cell function, which is responsible for the inflammatory vascular permeability and the edema [18].

In rodent mast cells, serotonin is found in significant quantities [19]. Thus, the fact that vascular permeabilty induced by compound 48/80, which results from mast cell degranulation, was decreased during tumor bearing could be explained only by the effect of serotonin.

To summarize, our results demonstrate that tumor bearing reduces carrageenan and dextran paw edema, serotonin, bradykinin and compound 48/80-induced cutaneous vascular permeability, and also diminishes mesenteric mast cell degranulation induced by compound 48/80, without changes in neutrophil migration induced by carrageenan. Therefore, we can infer that tumor bearing can limit mast cell function and vascular events in acute systemic inflammation.

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