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Research report

Effects of antalarmin and nadolol on the relationship between social stress and pulmonary metastasis development in male OF1 mice

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ABSTRACT

It has been found that acute social stress in male OF1 mice produced a general immunosuppression and increased B16F10 tumor development. This study examined the effects of blocking either the hypothalamic–pituitary–adrenocortical (HPA) axis or the sympathetic adrenomedullary (SAM) system on the impact of such stress on tumor development. Naive male OF1 mice were individually housed for 12 days before being inoculated with tumor cells or vehicle. Six days later, tumor-bearing mice were inoculated with antalarmin (a corticotropin-releasing factor receptor antagonist), nadolol (a beta-adrenergic antagonist) or vehicle. All these mice were subjected to social stress by pairing them for 24 h with counterparts selected for their high and homogeneous levels of aggressiveness. The pairs were only in physical contact for three 5-min periods, being in sensory contact for the rest of this period. One hour after social stress, serum corticosterone and IFN-*gamma* levels were analyzed in each experimental group. Fifteen days later, lungs were removed to determine the number of metastatic foci with their areas, and blood samples were taken to assess serum titers of corticosterone and IFN-*gamma*. Both antalarmin and nadolol-treated mice developed significantly fewer metastatic foci with smaller areas than vehicle-treated subjects although only the group treated with antalarmin had reduced corticosterone levels. This study confirms that social stress has complex effects on immune system and tumor development that are not simply linked to corticosterone titers.

1. Introduction

An understanding of the general neurobiology of stress and the specific alterations associated with an imbalance in the hypothalamic–pituitary–adrenal (HPA) and sympathetic–adrenal medullary (SAM) axes will likely lead to a clarification of the role of stress in disease, including neoplastic processes [2,38,48]. Abundant evidence suggests that the HPA and SAM axes are the two major pathways through which the immune function can be modulated, providing a finely tuned regulatory system required for health. Immune system cells express receptors for glucocorticoids and catecholamines [47,63,74], which are secreted during the response to stress by the activation of the HPA and SAM axes. These hormones can regulate a wide variety of immune-cell functions including cellular activation, cytokine production and cell trafficking [23,24,26,56].

Although increasing evidence is emerging of the adverse effect of the activation of the HPA and SAM axes on different parameters of the immune activity, very few studies have examined the role of these pathways in the modulation of tumor growth. While some studies have shown the anti-tumor effect of glucocorticoids [4,7,13,14,68], Arbiser et al. [1], using both *in vitro* and *in vivo* assays, have found that corticotropin-releasing factor (CRF) is able to enhance angiogenesis and stimulate epithelial tumor growth in the skin. Also, the administration of Z-100, an immunomodulating agent, increases interferon *gamma* (IFN-*gamma*) levels and reduces the development of B16F10 melanoma tumor metastases via the suppression of glucocorticoid-genesis [53]. Nevertheless, Ben-Eliyahu and Shakhar [5] found a negative effect of stress on natural killer (NK) cell activity and tumor development, which was independent from the reactivity of the HPA axis, since the administration of corticosterone was found to have no effect on tumor development.

Evidence also exists of a possible involvement of the SAM axis in tumor development. A number of studies have shown that sympathetic ganglionic blockade, adrenal demedullation or the administration of a nonselective beta-blocker either ameliorated or attenuated increases in MADB106 tumor metastases induced by swim stress or social stress. Although treatment with nadolol blocked the effects of acute stress in MADB106 metastases, this treatment had no effect on NK-depleted rats, suggesting that adrenergic effects on tumor development are mediated by NK cell activity [6,58,61]. Similarly, Hasegawa and Saiki have shown that oral administration of propranolol completely abrogated social isola-

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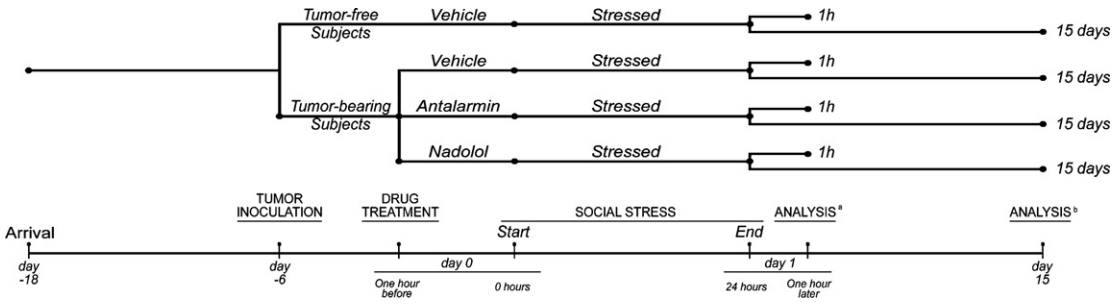

Fig. 1. Schematic representation of the experimental design. The stress model was applied 6 days after tumor inoculation. During the first analysis (a), serum corticosterone and IFN-*gamma* levels were measured; during the second analysis (b), the development of B16F10 melanoma metastases was measured along with the levels of serum corticosterone and IFN-*gamma*.

tion stress-induced reduction in host resistance to B16 melanoma and Meth A fibrosarcoma growth in syngeneic C57Bl/6 and BALB/c mice, suggesting an interrelationship between psychosocial stress, tumor growth and beta-adrenergic activation [32].

Previous studies in our laboratory show that social stress triggers an imbalance in cellular immunity and increases the pulmonary metastatic development of B16 melanoma cells [3,67]. Furthermore, tumor development differs in accordance with the coping strategies manifested by the subjects, which themselves have been associated with different types of HPA and SAM axis activity [19,20,41,55,57]. Bearing these results in mind, the principal objective of this study is to analyze the involvement of the HPA and SAM axes in the negative effect of social stress on tumor development and the immune activity. To this end, the sensorial contact social stress model [43] was used, along with B16F10 melanoma cells, as an experimental tumor model which is particularly immunogenic and commonly used in studies focusing on tumor immunology [33,35]. In order to assess immune activity, IFN-*gamma* levels were measured. IFN-*gamma* is an important cytokine produced by activated lymphocytes that has immunomodulatory activities such as the augmentation of Th1 cell activity and NK cell cytotoxicity, thus helping to suppress tumor growth and pulmonary metastasis [8,10]. Two drugs were used to block HPA and SAM axis activity: antalarmin, a CRF1 receptor antagonist, and nadolol, a beta-adrenergic antagonist which cannot cross the blood–brain barrier.

Furthermore, the cytokines released in response to the activation of the immune system induced by tumor development, have an effect on the central nervous system (CNS), influencing both physiological parameters and behavior [17,66]. A number of different studies involving the peripheral or central administration of these cytokines have shown that mainly interleukin 1 (IL-1α and IL-1β), but also interleukin 6 (IL-6) and the tumor necrosis factor (TNF-α) [50], are responsible (either directly or indirectly) for sickness behavior [9,36,40]. From this perspective, another aim of this study is to determine the possible changes that tumor development itself may provoke in social behavior, and whether or not these effects are reversed by drug treatment.

2. Materials and methods

2.1. Subjects and husbandry

Six-week-old male OF1 mice (CRIFFA, Barcelona, Spain) were individually housed for 17 days in transparent plastic cages measuring 24.5 cm × 45.5 cm × 15 cm. Food and water were available *ad libitum*. The holding room was maintained at a constant temperature of 20 °C with a 12 h light/dark cycle (white lights on from 20:00 to 08:00 h). The light cycle was reversed to facilitate behavior assessment during the animal's active (dark) phase. All experimental procedures were conducted under dim red light conditions in a room adjacent to the holding facility. All procedures involving mice were carried out according to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 18 March, 1986) as well as to related secondary and supplementary legislation.

2.2. Experimental design

On the 12th day of individual housing, animals (n = 144) were randomly allocated to two groups (Fig. 1). One group was inoculated with tumor cells (n = 108) and the other with vehicle. Six days after inoculation with tumor cells, tumor-bearing subjects were then randomly allocated to three subgroups in accordance with the drug treatment to be received: antalarmin (TUM-ANT, n = 36), nadolol (TUM-NAD, n = 36) or vehicle (TUM-VEH, n = 36). At this time, tumor-free subjects were inoculated with vehicle (VEH-VEH, n = 36). One hour after the drug treatment, all the animals (tumor-free and tumor-bearing subjects) were exposed to the sensory contact social stress model [43] for 24-h. Following exposure to social stress, all groups (group factor) were divided into two subgroups (time factor). One subgroup was terminated 1 h after the social stress, in order to analyze serum corticosterone and IFN-*gamma* levels (6 animals per group; first analysis) and the other 15 days after the end of the social stress in order to analyze corticosterone and IFN-*gamma* levels, and tumor development (30 animals per group; second analysis).

2.3. Experimental tumor induction

Tumors were induced by B16F10 melanoma murine cells. The B16F10 cells were maintained *in vitro* by subculturing the tumor cells at 37 °C in a humidified atmosphere of 5% CO_2 at a concentration of 10^5/ml in 75 cm^2 cell culture flasks (Corning, New York) in RPMI-1640 culture medium (Sigma–Aldrich, Madrid, Spain), supplemented with 10% fetal calf serum (Gibco, Life Technologies, MD, USA), 25 mM HEPES, 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol (Gibco, Life Technologies, MD, USA), and 2 g/L sodium bicarbonate (Sigma–Aldrich, Madrid, Spain). Melanoma adherent cells were detached by exposure to 0.02% EDTA for 5–8 min and washed three times in RPMI-1640 medium. The mice, pre-anesthetized with intraperitoneal (ip) Nembutal (sodium pentobarbital; 60 mg/kg), were injected with 5×10^4 of viable B16F10 cells in 0.1 ml of medium into the lateral tail vein with a 30½ gauge. Mice tails were previously heated with a thermal pillow. In order to ensure the success of the tumor inoculation, all subjects which did not receive the complete dose of 0.1 ml following the first injection were eliminated.

2.4. Drug treatments

Both antalarmin and nadolol were purchased from Sigma (St. Louis, MO), and were dissolved in sterile saline containing 5% dimethyl sulfoxide and 5% Cremophor EL., which was also used for vehicle injections. The two drugs were administered ip according to body weight. Antalarmin was administered at 30 mg/kg and nadolol was administered at 20 mg/kg. These doses were chosen on the basis of previous studies [12] and our preliminary experiments (data not shown). No evidence of toxic effects was observed after any drug treatments.

2.5. Social stress model

Dyadic resident–intruder interactions were carried out using the sensorial contact model [43]. Resident subjects had been specially trained and selected for their high aggressiveness levels, and the stressed animals were exposed to these subjects during a 24-h period [66]. During this time, animals were only subjected to direct physical interaction for three periods of 5 min (08:00–08:05 h, 20:00–20:05 h, and the next day 08:00–08:05 h). The rest of the time, the intruders were separated from residents by special perforated methacrylate structures, which enabled the continuation of indirect-sensory confrontation in the same cage, at the end of the direct confrontation periods. The separator prevented injuries that may have triggered the immune system and distorted the results. Although during the direct interaction period, subordinate subjects received some bites, no wounds were evident. The handled control group remained in isolation during the entire 24 h period, but a methacrylate separator was introduced into the cages in order to monitor the effect of the separator itself and the resulting reduction in space.