

Impact of stress and levels of corticosterone on the development of breast cancer in rats

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Abstract: Stress is experienced during cancer, and impairs the immune system's ability to protect the body. Our aim was to investigate if isolation stress has an impact on the development of tumors in rats, and to measure the size and number of tumors and the levels of corticosterone. Breast cancer was induced in two groups of female rats (N=20) by administration of a single dose of *N*-methyl-*N*-nitrosourea 50 mg/kg. Rats in the control group (cancer induction condition) were allowed to remain together in a large cage, whereas in the second group, rats were also exposed to a stressful condition, that is, isolation (cancer induction and isolation condition, CIIC). The CIIC group displayed anxious behavior after 10 weeks of isolation. In the CIIC group, 16 tumors developed, compared with only eleven tumors in the control cancer induction condition group. In addition, compared with the control group, the volume of tumors in the CIIC group was greater, and more rats had more than one tumor and cells showed greater morphological damage. Levels of corticosterone were also significantly different between the two groups. This study supports the hypothesis that stress can influence the development of cancer, but that stress itself is not a sufficient factor for the development of cancer in rats. The study also provides new information for development of experimental studies and controlled environments.

Keywords: breast cancer, corticosterone, isolation condition, psychoneuroimmunology, stress

Introduction

Stress is not an external stimulus, but rather an internal reaction of an organism to its environment.¹ Stress can be positive (eustress), as in conditions of danger, when the body reacts with alert behavior. By contrast, negative stress or "distress" occurs when the activation, intensity, and persistence of the stress results in physical and psychological disorders.^{2,3}

Various studies have shown an association between stress and cancer and investigated the multiple effects exerted by stress on immune function; for example, during the diagnosis and treatment of the disease, some patients experience stress and depression that result in immune suppression.⁴⁻⁸ Similarly, patients with breast cancer have a decrease in their numbers of natural killer cells, and alterations in the secretion of cytokines⁹ and in DNA repair capacity.¹⁰⁻¹² It has been shown in vitro that stress is involved in pathways of cancer progression such as immunoregulation, angiogenesis, and invasion.¹³

During stress, the brain, endocrine system, and immune system form a circuit, communicating through systems such as neurotransmitters, neuropeptides, hormones, lymphoid tissue growth factors, cytokines, and eicosanoids.⁸ Psychoneuroimmunology studies have revealed how much the immune system is affected by the influence of stress. The reactions are complex, involving different tissues and body system responses.^{14,15}

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The target organ responding to stress is the brain, and it determines the activity of other tissues by the action of hormones and immune receptors on pathway that alter brain function and regulate stress response. The physiological changes caused by stress are crucial, and the major components in these changes are corticotropin-releasing factor and the locus coeruleus of the brain.¹⁶

The immune system is highly sensitive to changes in the body and the stimuli to which it is exposed. Immune function is often diminished by psychological stress,⁸ but increases as a result of significant and positive events.^{17,18} Social support has been shown to be a factor that improves the outcome of patients with cancer, and may produce a better immune response, as it reduces the level of cortisol and restores the natural killer cell number and production of cytokines,^{9,11,19,20} whereas social isolation increases the risk of death associated with several chronic diseases.⁶

The *N*-methyl-*N*-nitrosourea (NMU) rat model used in this study is considered to be one of the best animal models of cancer. Chemical induction of mammary cancer in rats has a number of features that make it very useful for investigating many aspects of breast cancer, for instance: 1) the time to tumor development is short; 2) tumors are generated mainly in the mammary gland (adenocarcinomas) as the cancer has limited metastatic potential; 3) the carcinogen causes little or no systemic toxic effect; and 4) the breast tumors produced have histologic origin and pathologic features that are similar to those of typical human breast cancer.^{21,22} In this model, we used a single intraperitoneal dose of 50 mg NMU/kg body weight.²¹ In addition, the stress induced by isolation condition has some theoretical support, applicable to studies of live rodents,^{5,23–25} and producing multiple physiological changes and behavioral effects.^{8,25} Finally, changes in tumor volume in animal models are often used to evaluate the effects of different factors on mammary gland carcinogenesis in female rats, because they may have important prognostic value for some malignancies, including breast cancer.²⁶

The objective of this study was to investigate whether stress caused by isolation of rats has an impact on the development of tumors, assessed by the volume and number of tumors, and the levels of corticosterone.

Methods

Animals

Breast cancer was induced by intraperitoneal administration of a single dose of 50 mg NMU/kg body weight in female Sprague Dawley rats aged 50±2 days, weighing 90±15 g (N=20).²⁷ This strain was chosen because studies have shown that it is an excellent model for the development

and progression of breast cancer, as the rats naturally develop a diagnostic range similar to humans.^{23,28}

Induction of cancer in rats

The carcinogen NMU (catalog number 684/93/5, product number N1517-isopac, PubChem Substance ID 24897498; Sigma, St Louis, MO, USA) is a model for chemical induction of mammary cancer in rats. It is an alkylating agent that acts directly on DNA, creating mutations.²⁹

Induction of stress in rats

A method for inducing stress by social isolation or deprivation was chosen. This model consists of isolating each of the rats in a cage, preventing any kind of social interaction with their peers, which leads to a pattern of apathy and chronic stress after approximately 10 weeks.^{24,25}

Procedure

Twenty Sprague Dawley rats were injected with the carcinogen NMU.²¹ At 51±2 days, the animals were divided into two groups of ten rats each. Rats belonging to the control group (cancer induction condition group) were allowed to remain together in a large cage, while the study group were exposed to both cancer induction and isolation stress (cancer induction and isolation condition, CIIC), so they were placed in individual cages, 12.5×14.5×26.5 cm in size, preventing social interaction with their peers. At 5 months of age, all rats were euthanized and the tumors removed. Tumor size was estimated using a Vernier caliper by two different methods: 1) taking into account only the larger diameter and 2) measuring three dimensions to give the approximate total volume. TNM classification was used (classification of the primary tumor size [T], node status or regional lymph nodes [N], and the presence of distant metastasis [M]).³⁰

Cancer type was confirmed by histopathological examination of paraffin wax-embedded sections from tumors of each subset of animals. Mammary tumors were classified according to Russo et al.³¹ Corticosterone quantification was performed by enzyme-linked immunosorbent assay using a commercial kit (Alpco, Salem, NH, USA, catalog number 55-CORMS-E01).

Statistical analysis

To assess the heterogeneity of the groups when comparing the number, diameter, and approximate total volume of the tumors that developed in the group, we used nonparametric analysis with the Mann–Whitney *U*-test for independent samples. We also used *t*-test for independent samples testing of corticosterone in rats.

Ethics

This study was approved by the Ethics Committee of the Department of Medical Sciences, University of Guanajuato. This study followed the criteria required by the Official Mexican Standard NOM-062-ZOO-1999 regarding animal husbandry (characteristics that comprise the laboratory animal research, the staff responsible for operational processes, the profile of technical staff involved in the care and use of the laboratory animals, obtaining of the animals, health and quality certificate, and the identification and registration of the thereof).³²

Results

It was observed that after 70 days, 100% of the rats that had received an injection of the carcinogen had developed at least one tumor. At the time of euthanasia, the control group had developed eleven tumors; nine rats (90%) had one tumor each, and one rat (10%) developed two tumors. By contrast, the rats in the experimental CIIC group had 16 tumors; six rats (60%) developed one tumor, two rats (20%) developed two tumors, and two rats (20%) developed three tumors. As shown in Table 1, the CIIC group tended to have a larger tumor volume, and more rats had more than one tumor, compared with the control group.

The between-group comparison groups revealed no significant differences in: 1) number of tumors or 2) the two methods of estimating tumor size used: i) larger diameter and ii) approximate volume of the tumors (Table 2).

Level of corticosterone was significantly different between the CIIC and control groups ($t=2.50$; $P=0.02$). Higher corticosterone levels were found in the CIIC (1058.57 ng/mL \pm standard error [SE] 1.96) than in the control (599.7 ng/mL \pm SE 1.96) group (Figure 1). The normal range of serum corticosterone in female rats is 292.5–819.0 ng/mL.³³

Table 1 Number, diameter, and volume of tumors in both rat groups

Parameter	Cancer (control) group (mean \pm SD)	Cancer + stress group (mean \pm SD)
Number of tumors	1.1 \pm 0.31	1.6 \pm 0.84
Number of tumors with larger than average diameter	2.62 \pm 2.27	2.56 \pm 1.96
Number of tumors with larger than average diameter without outliers	1.99 \pm 0.88	2.19 \pm 1.30
Approximate average total volume of tumors	7.06 \pm 7.07	24.65 \pm 67.85
Approximate average total volume of tumors without outliers	5.56 \pm 5.30	7.93 \pm 11.72

Abbreviation: SD, standard deviation.

Table 2 Comparison between both groups with the Mann–Whitney *U*-test, significant difference $P<0.05$

Parameter	U-value	P-value*
Number of tumors	34	0.226477
Larger diameter	87	0.960643
Approximate total volume	43	0.596702

Notes: *Mann–Whitney *U*-test; significant difference $P<0.05$.

Figure 2 shows the histopathologic characteristics of representative tumors from each of the groups. The CIIC group exhibited cells with increased nuclear/cytoplasmic ratio, indicating a more active process of division, which is a morphological criteria of malignancy. Further, to demonstrate the capacity of mammary tumor cells to respond to corticosterone, we performed immunohistochemistry and found that rat mammary tumors, including benign fibroadenomas, ductal carcinoma in situ, and invasive ductal carcinoma all expressed glucocorticoid receptors.

Rats in the CIIC group also showed apathetic behavior (eg, stopped eating) and appeared fearful.

Discussion

The main objective of this research was to investigate whether stress caused by isolation could affect the development and size of tumors and the levels of corticosterone in rats. There were statistically significant differences in corticosterone levels between the two groups. Although both groups showed tumor development, the CIIC group had greater morphological damage, with larger tumor size and a higher number of multicentric tumors in each rat, and also more aggressive activity in invasive cancer as indicated by immunohistochemical studies. Moreover, anxious behavior was evident in the group with insulation. The lack of statistically significant difference may be due to the small size of the sample, causing a possible type II error.

The CIIC group also had higher levels of corticosterone. Similar findings were reported by Hermes et al,²³ who observed that mice kept in isolation developed significant dysregulation of corticosterone responses, while cancer recovery was markedly delayed, and this was associated with increased mammary tumor progression. Further, to demonstrate the capacity of mammary tumor cells to respond to corticosterone, they performed immunohistochemistry, and found that, rat mammary tumors, including benign fibroadenomas, ductal carcinoma in situ, and invasive ductal carcinoma all expressed glucocorticoid receptors.

Studies have evaluated multifocal and multicentric breast cancer tumors, and using (in addition to the TNM method) the aggregate tumor volume (ie, the summation of the diameter

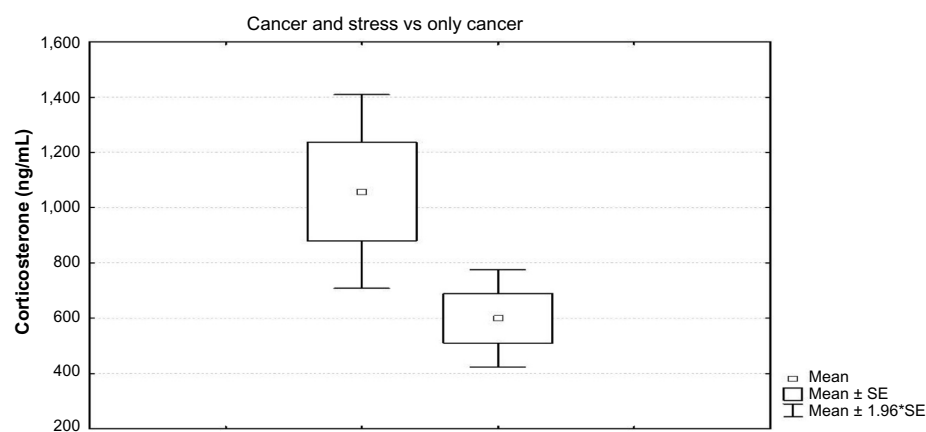


Figure 1 Comparison between the level of corticosterone in the group with CIC and CIIC.

Abbreviations: CIC, cancer induction condition; CIIC, cancer induction and isolation condition; SE, standard error; vs, versus.

of all tumors found in a single quadrant or in different quadrants of the breast) have found that tumor size is a variable that can predict the existence of metastases in lymph axillary nodes.^{34,35} One research, however, suggests that the estimate of the approximate total tumor volume is a more successful predictor of metastatic potential than the use of: 1) the largest tumor diameter as the only estimate or 2) the aggregate tumor volume.³⁶ However, to obtain approximate total tumor volume, it is necessary to measure the tumor in three dimensions (length, width, and depth), and the calculation of the total volume is only possible in objects having regular surfaces. In the current study, we used both the TNM method and measurement of the estimated approximate total volume of the tumor.

During the study, we observed that the behavior of rats in response to isolation stress was different in individual rats;

some resisted and struggled, trying to get out of the cage, whereas others showed apathetic behavior (such as ceasing to eat) and appeared fearful. Other studies have observed similar behavior in rats, associated with early exposure to stressors, glucocorticoid dynamics, and subsequent development of mammary tumors and cancer progression.^{37,38}

It is likely that the behavioral responses seen in rats in this study are similar to those expressed by humans, although it is easier to control for variables within the laboratory, whereas studies on the influence of stress on cancer in humans relies on subjective evaluation of the event, performed by the individual patient and according to which the patient responds to stress.³⁹ However, these findings indicate a necessity to provide psychological and social support, along with strategies for stress reduction, for patients who are given a diagnosis of cancer.

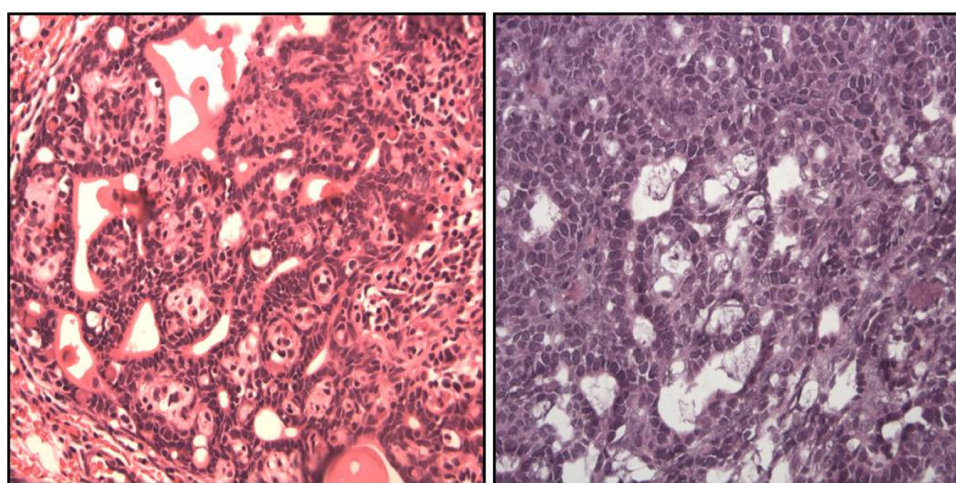


Figure 2 Histopathologic characteristics.

Notes: In the tumor of CIC group, the neoplastic cells have smaller nucleus and increased tubule formation. Mast cells infiltrating the tumor are observed ($\times 40$). Tumor of CIIC group shows abundant cellularity ($40\times$), lower degree of histological differentiation, areas more solid without inflammation, cells with larger nucleus and less cytoplasm; indicating a process of division more active.

Abbreviations: CIC, cancer induction condition; CIIC, cancer induction and isolation condition.

The significant differences in the level of corticosterone seen in the two groups in this study support the hypothesis that stress can influence the development of cancer, but we cannot conclude that stress itself is a sufficient factor for the development of cancer in rats. Therefore, further research is needed to clarify the relationship between stress, the immune system, and cancer, using reliable and innovative experimental methods. Previous findings from various investigations that have studied the relationship between stress, the effects on the immune system, and how both of these influence the progression of cancer are controversial, owing to the complexity of studying these variables, because many factors may converge, and these must be weighed with extreme care.

The results of our study support those of previous studies^{5,6,23} showing that stress influences tumor development, and directly influences tumor size and corticosterone level. They also provide new information for development of controlled environments to measure experimental stress in cancer models and reduce interference from other variables.

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Disclosure

The authors report no conflicts of interest in this work.

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