NMU-induced mammary carcinogenesis in female rats is influenced by repeated psychoemotional stress*

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Epidemiological and experimental studies indicate psychoemotional stress as an important factor in carcinogenesis.

The aim of this study was to evaluate the effect of restraint stress on N-nitroso-N-methylurea (NMU)-induced mammary carcinogenesis. Female Sprague-Dawley rats were injected with two intraperitoneal NMU doses each per 50 mg/kg b.w. between 39–49 postnatal days. Three experimental groups were created: 1. NMU (without restraint – control group, 12 animals), 2. NMU+1IMS (group with single restraint, IMS – immobilization stress, 12 animals), 3. NMU+7IMS (group restrained 7 times during aweek, 12 animals). Animals were immobilized daily in special boxes for 120 minutes or 7x120 minutes, respectively from third day after carcinogen administration. The observation lasted for 20 weeks. The incidence, frequency, latency and volume of mammary tumors were evaluated.

In repeatedly immobilized group NMU+7IMS increase in tumor incidence by 57% ($p<0.05$), marked increase in frequency per group by 153% ($p<0.01$), increase in frequency per animal by 61% and shortened latency period by 7 days were recorded. The effect of single restraint was not seen.

In this experiment repeated immobilization carried out early after carcinogen administration had a remarkable stimulatory effect on chemically-induced mammary carcinogenesis in female rats.

Key words: Mammary carcinogenesis, psychoemotional stress, restraint, rat, NMU.

Incidence of breast cancer remains high but the effect of well-documented risk factors (early menarche and late menopause or first pregnancy in higher age) was proved only in a part of women afflicted with cancer. The mammary carcinogenesis is supposed to be related to life style factors as dietary fat intake, alcohol consumption as well as to various kinds of psychoemotional stress. It is very important to determine the role of these factors in manifestation and progression of this disease particularly in genetically predisposed women. Numerous studies proved that familial breast cancer is a chronic life stressor [4]. Threatened women were aware of higher risk and lived in permanent fear of possible breast cancer manifestation [1, 27]. The results of Lerman's and Schwartz's [15] study proved that women with positive familial breast cancer history are exposed to almost the same degree of stress as breast cancer patients. The hormonal and immune system are known to be generally involved in the promotion and progression of mammary tumors. As the stress influences both of these systems, a presumption exists that psychosocial factors act through immune and/or endocrine system changes. The communication among nervous, immune and endocrine system is mediated by cytokines, neurotransmitters, neuropeptides and hormones which can be produced in all three systems and via special receptor they function as endogenous mediators in intersystematic communication.

Studies carried out on laboratory animals proved tumor-inhibitory as well as tumor-stimulatory effect of psychoe-

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motional factors. The differences in experimental results are assigned to experimental conditions i.e. kind, intensity, duration, time of application as well as to experimental animal species and tumor type. The main advantage of experimental studies is the ability to control the environmental changes which is not possible in human studies.

In this study we evaluated effect of the psychoemotional stress, specifically the immobilization (IMS) on NMU-induced mammary neoplasia growth in female Sprague-Dawley rats.

Material and methods

Female Sprague-Dawley rats (Central vivarium, Faculty of Medicine, P. J. Šafárik University, Košice, Slovak Republic) aged 31–34 days adapted to standard vivarium conditions with temperature 23 ± 2 °C, relative air humidity 60–70%, light:dark 12:12h light regimen (light on 7.00 a.m.) were used. During the experiment animals were fed MP diet (Top-Dovo, Dobrá Voda, Slovak Republic) and drank tap water *ad libitum.* N-methyl-N-nitrosourea (Sigma, Deisenhofen, Germany) was administered intraperitoneally in two doses (with 7-day interval between doses) each of 50 mg/kg b.w. between 39–49 postnatal days. Three experimental groups were created:

1. NMU control group without immobilization (12 animals)
2. NMU+1IMS – group with single immobilization (12 animals)
3. NMU+7IMS – group immobilized 7 times during one week (12 animals).

Three days after second carcinogen application animals were immobilized in special boxes for 120 minutes either in one or in 7 consecutive days. Each week the animals were weighed and palpated, the occurrence, location and size of palpable mammary tumors were recorded. Twenty weeks after first NMU dose administration rats were sacrificed by quick decapitation, tumors were removed and their size, weight and location were recorded. The tumor volume was calculated according to following formula:

$V = \pi x(S_1)^2 x S_2/12;\ S_1<S_2$

Statistical significance of differences between groups was evaluated by Mann-Whitney U-test (incidence), Kruskal-Wallis test (latency period) and one-way analysis of variance (frequency per group and animal, average tumor volume gain).

Results

Tumor incidence, frequency per group and per animal, latency period, and average tumor volume gain in the last week of experiment are given in Table 1. The values of tumor incidence and frequency per animal during the experiment are outlined in Figures 1 and 2.

Table 1. Effects of immobilization stress on NMU-induced mammary carcinogenesis in female Sprague-Dawley rats at final analysis

Group	NMU	NMU + 1IMS	NMU + 7IMS
Tumor bearing animals	7	7	12
Number of tumors	25.00	19.00	69.00
Tumor incidence (%) (0%) (+57%)	63.60	63.60	100
Tumor frequency per group[&] (–24%) (+153%)	2.27 ± 0.78	1.73 ± 0.57	$5.75 \pm 0.69^{**}$
Tumor frequency per animal[&]	3.57 ± 0.90	2.71 ± 0.64 (–24%)	5.75 ± 0.69 (+61%)
Tumor latency[&] (days) (+5%) (–10%)	72.14 ± 9.77	75.57 ± 8.01	64.92 ± 4.77
Tumor volume gain[&#] (cm^3) (+14%) (–51%)	4.07 ± 2.60	4.63 ± 2.01	2.01 ± 0.45
Cumulative tumor volume • (cm^3)		(+26%)	(+54%)

[&]Data are expressed as means ± SEM; [#]data are expressed as means of tumor volume gains evaluated during the last 8 weeks of experiment (all mammary tumors appeared in experimental groups until the 12th week post NMU were included) – number of evaluated tumors: *n = 12 (NMU), n = 14 (NMU + 1IMS), n = 44 (NMU + 7IMS)*; • data are expressed as a sum of volumes. Values in brackets are calculated as % deviation from the 100% of non-influenced control group (NMU). Significant difference: $^{*}p<0.05$ vs NMU, $^{**}p<0.01$ vs NMU; IMS – immobilization stress.

In the final analysis of individual tumor growth parameters significant increase in incidence by 57% ($p<0.05$) and marked increase of frequency per group by 153% ($p<0.01$) in NMU+7IMS group were recorded. Repeated restraint resulted in increase of frequency per animal by 61% and shortened latency period (by 7 days). On the other hand, in NMU+7IMS group a 51% decrease in average tumor volume gain in comparison with controls was registered. Single restraint in NMU+1IMS group decreased non-significantly the frequency per animal and per group by 24%, when compared to controls. Other mammary carcinogenesis parameters in this group were not influenced by immobilization.

Discussion

Results of experimental studies investigating the effect of psychosocial factors on breast cancer manifestation are not consistent.

In our previous experiment we exposed female Sprague-Dawley rats to immobilization prior to carcinogen adminis-

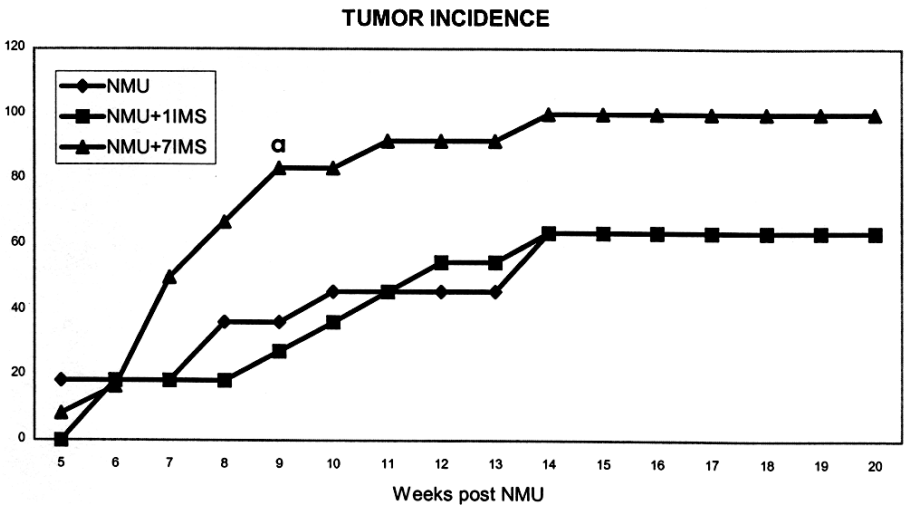

Figure 1. Percentage of animals with mammary tumors in all three groups during the experiment. a – start of significant difference ($p<0.05$) in NMU+7IMS vs NMU.

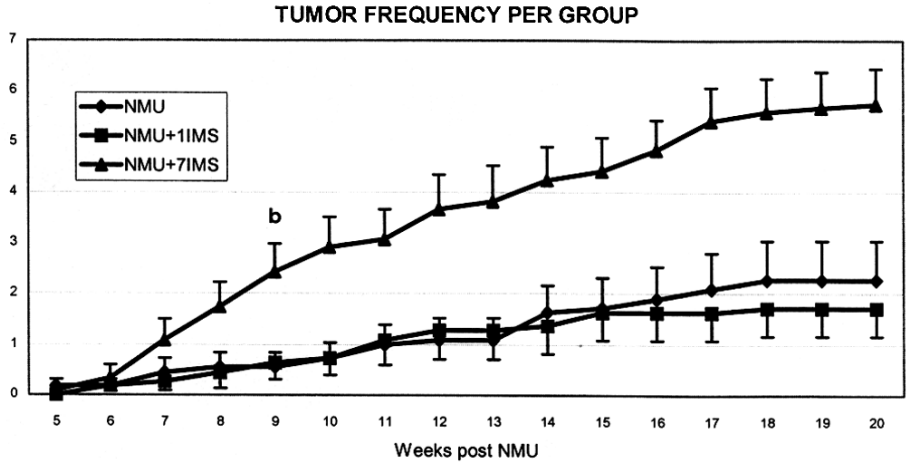

Figure 2. Number of mammary tumors per group in all three groups during the experiment. b – start of significant difference ($p<0.01$) in NMU+7IMS vs NMU.

tration (NMU). One group of animals was restrainted only once for 120 minutes, the other group was restrainted in 5 consecutive days for 120 minutes per day. In the final evaluation we recorded increase in incidence, frequency per animal and per group as well as in average tumor volume in animals immobilized 5 times. The most remarkable change in single stress application was the shortening of latency period by 23 days. Other mammary carcinogenesis parameters in this group were not influenced. Despite the fact that the achieved results were not statistically significant, the immobilization stress was thought to stimulate mammary tumors growth (E. Adámeková et al, prepared for publication). In the present work we applied stress after NMU administration. Increase in incidence by 57%, increase in frequency per group by 153% and increase in frequency per animal by 61% indicate that repeated application of immobilization stress after carcinogen administration remarkably stimulates NMU-induced mammary tumors formation in female rats. In both our experiments immobilization stress increased mammary tumor induction, no matter whether the animals were restrained before or after NMU induction.

So far, we have not found any study dealing with the effect of single or repeated stress on mammary gland tumor induction, whereas the effect of chronic stress in mammary carcinogenesis was evaluated in several studies (Tab. 2). Experiments mentioned in Table 2 rather represent the model of adaptation to stress what is not comparable to our experiments.

The relation between psychoemotional stress and subsequent breast cancer occurrence was analyzed in humans as well. Results of Priestman et al [21], Schonfield [23] and Cooper et al [7] did not prove relation between stress experience and higher risk of breast cancer incidence. Investigation of critical life events on breast cancer recurrence did not reveal the same results. Ramirez et al [22] observed positive relation between the risk factors and cancer onset, meanwhile Barraclough et al [2] did not find the relation between stressful life events and higher risk of breast cancer recurrence. Stress-induced changes in immune system can be related to impaired prognosis in breast cancer afflicted women [16]. Psychosocial stressors as divorce or bereavement reduced the therapy efficacy in breast cancer [25], on the other hand reduction of life stressors through social intervention or social support may extend survival time and reduce the toxic side effects of chemotherapy [6, 11].

The influence of stressors on tumor induction is probably executed through neuroendocrine and immune system changes. The fact that stress hormones, particularly glucocorticoids inhibit proliferation, migration and cytotoxicity of lymphocytes as well as secretion of certain cytokines such as IL-2 and interferon γ (IFN-γ), is known for more than 20 years. These findings led to general conclusion that stress mainly acts immunosuppressively: this point of view is, how-

Table 2. Effect on tumor formation by various types of experimental stressors applied chronically

References	Carcinogenesis induction (rat)	Tumor type	Stressor	Stressor application	Effect on mammary carcinogenesis
Burchfield et al (1978)	transplant	Moloney-murine sarcoma	cold stress	before inoculation after inoculation	↓ none effect
Newberry et al (1972)	3x5 mg/g DMBA iv.	mammary tumors	electric footshocks	before and after DMBA administration (continuously)	↓
Newberry et al (1976)	3x5 mg/g DMBA iv.	mammary tumors	restraint	before and after DMBA administration (continuously)	↓
Nieburgs et al (1979)	40 mg DMBA single bolus (diet)	mammary tumors	electric tail shocks forced swimming in 2 ˚C water handling	 after DMBA administration	↓ ↑ ↑
Tejwani et al (1991)	15 mg DMBA intragastric	mammary tumors	restraint	after DMBA administration	↑

DMBA – dimethylbenz/a/anthracene, iv – intravenous route, ↑ – increase, ↓ – decrease.

ever, too simplified. Stressful events increasing level of circulating adrenal hormones may not unavoidably suppress immune responses of T and/or B cells [10, 20]. Moreover, continuous glucocorticoid increase during chronic stress does not always lead to immune system impairment [14]. These data indicate that the link between glucocorticoids elevation and immune changes is not so clear as it was supposed. This fact is evidenced also by KELLER et al [13] who found stress-induced suppression of mitogenic blood lymphocytes response of adrenalectomized rats *in vitro*. WEISS et al [26] recorded marked suppression of cellular immune functions of splenic lymphocytes in stressed animals irrespective of the kind of animals used – intact, adrenalectomized, sham-operated or adrenalectomized with an implant producing constant low level of circulating corticosterone.

The studies carried out in the last ten years demonstrated that glucocorticoids and catecholamines differentially regulate Th1/Th2 lymphocyte patterns. Th1 cells produce particularly IFN-γ, IL-2 and TNF-β (proinflammatory cytokines), while Th2 cells produce mainly IL-4, IL-10 and IL-13 (antiinflammatory cytokines). Very important cytokine inducing Th1 lymphocytes differentiation is IL-12. This can induce expression of IFN-γ and inhibit expression of IL-4. Glucocorticoids as well as catecholamines suppress IL-12 production [9, 3] what is probably the main mechanism by which these hormones mediate the Th1-Th2 shift towards Th2 [8]. IL-10 and TGF-β overproduction mediated by IL-12 and TNF-α production inhibition as well as by NK and Tc cells cytotoxicity reduction probably lead to undesirable immunosuppressivity resulting in increased tumor growth [12].

The results of studies investigating the relation between psychosocial factors and breast cancer are inconsistent. In order to find out whether such a relation exists, the determination of the role of these factors in the carcinogenesis seems to be important. Further studies investigating possible mechanisms of psychoemotional stress impact on tumor formation as well as on their progression and metastasis are needed. Evaluation of the role of psychoemotional stress in experimental carcinogenesis as well as in clinical practice in the discipline called "Psychooncology" can provide useful information.

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