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DEPRESSIVE-LIKE PSYCHOEMOTIONAL STATE VERSUS ACUTE STRESSES ENHANCES LEWIS LUNG CARCINOMA METASTASIS IN C57BL/6J MICE

A.V. Amikishieva, S.I. Ilnitskaya, V.P. Nikolin, N.A. Popova, V.I. Kaledin*
Institute of Cytology and Genetics of Siberian Department of the Russian Academy of Sciences, Lavrentiev avn. 10, Novosibirsk 630090, Russian Federation

Aim: **The effect of a depression-like status formed by chronic stress on the development of Lewis lung carcinoma metastases in C57Bl/6J mice was investigated. Two types of acute stress (restraint and social stress) were used for comparison.** ***Methods:*** **The depression-like status was induced by eight-week exposure to repeated but unpredictable stressors (chronic mild stress model) and was assessed in the forced swim test. Tumor cells were inoculated an hour after the onset of social stressor or immediately after physical or chronic stressor impacts. The number of metastases was counted 17 days after the inoculation.** ***Results:*** **Chronic mild stress provokes the development of a depression-like state in mice and causes a twofold increase in the number of metastases in the lungs, while both types of acute stress have no such effects.** ***Conclusion:*** **Depressive-like psychoemotional state of animals enhances the metastasis of Lewis lung carcinoma.**
Key Words**: depression, Lewis carcinoma, metastasis.**

By now this is a well-established fact that psyche is involved in neoplasm pathogenesis [1]. The psycho-neuro-endocrine-immune axis is known to act as a mediator between emotional state and cancer progression [2, 3], which is admitted *de facto* although our understanding of how the elements of the axis function and interact remains vague. Chronic stress and concomitant general tension erupting into deep anxiety and depression are thought to exert a marked influence on tumor outgrowth and metastasis.

Using experimental Lewis lung carcinoma (LLC) transplanted to C57Bl/6J mice it was shown that chronic stress induced by 20-day social confrontations enhances the outgrowth of solid tumors both in the winners and losers of inter-male confrontations [4]. The intensity of tumor progression correlates with the stress level: if tumor cells are inoculated immediately after social stressor impact, the tumor size doesn't differ too much from control, while inoculation during the stressor impact results in significant tumor growth enhancement (by 1.5-fold).

In the mainstream of experimental oncology, metastasis of malignant tumors presents a separate line of investigations, which is attributed much importance since metastasis is the primary cause of fatal outcome for patients with malignant diseases. In the experiments on metastasis of LLC the number of metastatic nodes increases in defeated animals only [4], which, in contrast to aggressive males, acquire negative psychoemotional state, anxiety and depression. Such state may provoke the so-called psychogenic immunodeficiency — a compromise of cytotoxic T lymphocytes and natural killer cells leading to the impairment of natural resistance to tumors [5]. Nowadays, the concept of stress-induced immunodeficiency to explain the mechanism underlying the stress-induced enhancement of carcinogenesis is actively discussed. At the same time, one has to bear in mind that the immune mechanism is not the only one involved in metastasis control [6, 7].

Besides, in the behavioral social stress paradigm used by the authors the immunodeficiency may be non-psychogenic in nature. As a consequence of daily fights the defeated males have skin bites, sores covered with coagulated blood etc. Such lesions are unavoidably accompanied by inflammation and could be the cause of immunity suppression. To exclude this possible way of influence on the metastasis, we investigated the effect of negative psychoemotional state on LLC metastasis using an animal behavioral model without physical lesions. Two types of acute stress not accompanied by changes in the emotional status of animals were used for comparison.

MATERIALS AND METHODS

Animals. Mice of C57Bl/6J strain maintained under the standard vivarium conditions at the Institute of Cytology and Genetics, Siberian Department of the Russian Academy of Sciences, were used. All experimental procedures with animals were in compliance with the European Community Council Directive (86/609/EEC).

Stress models.

Chronic mild stress (CMS). During the experiment the animals were housed in groups of 5–8 individuals per cage. The experimental protocol consisted of unpredictable randomized daily exposure to physical and social stressors (twice a day): cage skewing by 30 °C for 12 h, motion restraint for 1 h (see below), crowding animals from different cages into one cage for 1 h, placing of an individual with an aggressive partner until the first fighting, jolting in a vibrating unit for 1 h, removal of bedding for 1 day, housing on wet bedding for 12 h. Exposure to CMS for 6–9 weeks

Received: August 11, 2011.
*Correspondence: E-mail: amik@bionet.nsc.ru;
Fax: 7 383 333 12 78
Abbreviations used: CMS – chronic mild stress, LLC – Lewis lung carcinoma.

provokes the development of depression-like status in mice and rats [8].

Restraint stress (restriction). Animals were isolated in plastic cylinders with the diameter of 3 cm for 1 h to restrict the mobility.

Acute social stress. Strange males housed individually during a week were placed in groups of 5 animals per cage, thereby provoking fighting with subsequent (during 2 days) establishment of hierarchical relationships henceforth maintained by dominance-subordination rituals [9]. Control animals were housed in standard grouped housing vivarium conditions (5–8 individuals of the same sex per cage).

Behavioral methods.

Forced swimming test (Porsolt's test). This is the most commonly used test for assessment of depressive status in animals. The experimental setup is a transparent cylinder filled with water of room temperature. A mouse is placed in the water; its behavior is shot with a video camera for 5 min, then behavior parameters are registered using the *Mouse* software (Institute of Cytology and Genetics, Siberian Department of the Russian Academy of Sciences). The time of active swimming and the time of immobility (when a mouse just weakly moves the paws to keep afloat) are registered. The latter is interpreted as "hope loss behavior" and is longer in animals with depressive-like status. The test is conducted after 8 weeks from the beginning of the experiment.

Oncologic methods. LLC cells are inoculated into the tail vein (0.5 ml of suspension containing 170 thousand cells per mouse) a day after behavior assessment in case of CMS, after which stressor impacts are suspended. In case of restraint and social stress tests, tumor cells (0.5 ml of suspension containing 250 thousand cells per mouse) are inoculated an hour after the procedure completion (restraint) or, *vice versa*, an hour after animals grouping (social stress). Seventeen days after the inoculation the mice are decapitated. The lungs are fixed in 10% formalin solution. The number of metastases is counted using a microscope with eightfold magnification.

Statistical methods. STATISTIKA 6.0 software package and Student's t-criterion are used for the statistical processing of the results. There are 10–18 animals in each experimental group.

RESULTS

Exposure to unpredictable chronic mild stress for 8 weeks provoked a reduction in the active swimming time and an increase in the immobility time in the Porsolt's test (Fig. 1).

Inoculation with LLC cells resulted in the formation of lung metastases. Male mice with depression-like state induced by CMS had a maximal number of metastases, which differed significantly from that in control animals (Fig. 2, *a*). Chronic social and restraint stresses produced no effect on the intensity of metastasis (Fig. 2, *b*).

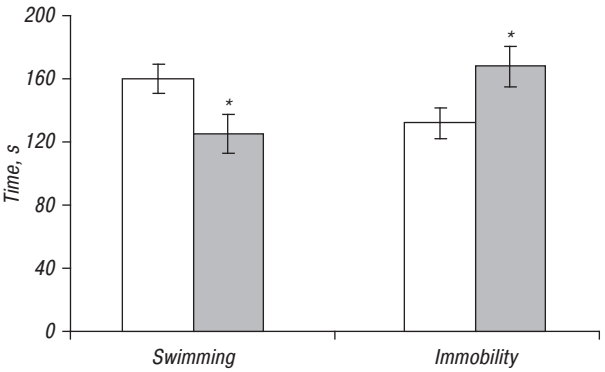

Fig. 1. The effect of chronic mild stress on forced swimming test's parameters.

Note: white columns — control animals, grey columns — mice after CMS; * $p < 0.05$ in comparison with control.

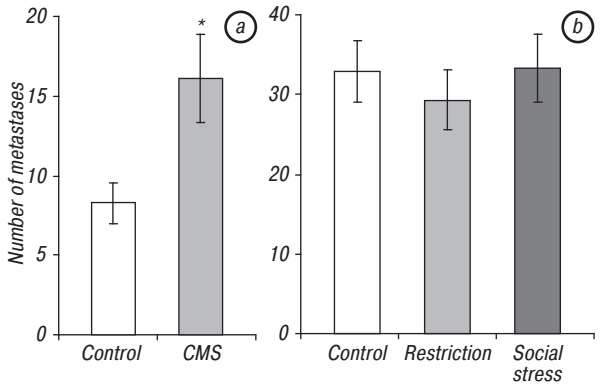

Fig. 2. The number of LLC metastases in the lungs of mice after different kinds of stress: *a* — chronic mild stress; *b* — acute stresses. Note: white columns — control animals, grey columns — mice after CMS; * $p < 0.05$ in comparison with control.

DISCUSSION

The effect of psycho-emotional state on the onset, progression and metastasis of malignant tumors is a focal issue of cancer research. Experimental data indicate that social affiliation and warmth of social relationships serve as a factor reducing the predisposition to breast cancer [10]. The density of animal population can also influence the resistance to neoplasm development [11, 12]. Clinicians and pathologists emphasize the importance of the mental factor in the development of malignant tumors. Positive emotions, optimism, confidence and dedication produce a modulating effect on the carcinogenesis and can even cause spontaneous cancer regression [13]. On the contrary, acute stress arising in conflict or desperate situations and accompanied by depression, despair or hopelessness with a high degree of significance produce a negative effect on resistance to tumor development [14, 15].

On the other hand, nonbrain neoplastic process provokes central neurohumoral alterations similar to the stress-induced responses of the body and under prolonged exposure can shift the emotional status of animals in a negative way [16, 17]. In humans the situation is aggravated by the fact that the cancer diagnosis alone is often a profuse mental damage that can affect the ability to control the emotional state [18], which further enhances carcinogenesis. Therefore,

there is a tangle of reciprocal influences between the functional state of the mind and neoplastic process.

Our experiments indicate that emotional state of an animal has a significant effect on both tumor growth and metastasis. Earlier it was shown that 10-day and 20-day social stress inducing anxiety-like and anxiety-depressive-like state, respectively, provokes faster outgrowth and metastasis of experimental lung [4, 19] and liver [20] tumors. Progression of the primary tumor and metastasis were shown not always to proceed in parallel. For example, LLC formation as a primary tumor node and lung metastases was enhanced only in animals which had long suffered defeats in inter-male confrontations and, as a result, developed depressive-like state. To confirm the direct effect of negative psychoemotional status on metastasis processes, the same results had to be confirmed on a different behavior paradigm inducing depressive-like status in animals without damaging cutaneous tissues to exclude the effect of possible immunodeficiency provoked by chronic inflammation [21].

The unpredictable chronic mild stress model has been used by experimenters for two decades for induction of depression-like state in rodents (mice, rats) [8, 22]. A variable set of daily impacts of physical and social stressors allowed choosing the procedures suitable for the current vivarium conditions. As a result, the negative psychoemotional state in the mice was formed practically without fighting, impairments and injuries. After 8 weeks of CMS impact the depressive-like status was assessed in the forced swimming test, which proved to be an adequate and valid tool for behavioral studies [23]. In particular the test is sensitive to all clinically effective antidepressants enhancing active swimming and reducing the time of animal immobility in water.

In our study an increase in the metastases number was observed only in the group of animals which were exposed for weeks to unpredictable stress inducing depressive-like status. For comparison, in the males exposed to two types of acute stress for an hour (restraint) and for about two days (period for establishment of dominance-subordination relationships) [9] the subsequent metastases development was not different from that in control mice. Therefore, a prolonged stress causing a steady shift of the psychoemotional status in the negative direction rather than a stressor impact itself produces a significant effect on the intensity of LLC metastasis. So two different behavior paradigms inducing depressive-like status in animals, transplanted with unequal doses of tumor cells (170 thousand cells per mouse in the current research and 250 thousand cells per mouse in earlier done work [20]), have shown an increase in the number of experimental metastases.

Therefore, the results implicate that psychotropic drugs may have a therapeutic potential in oncologic applications. In fact, the experiments on the mice exposed to 10-day social stress demonstrated the effectiveness of the anxiolytic diazepam for the inhibition of LLC metastasis, which was proven to be higher under chronic rather than single administration [19]. The psychotropic drugs are known to have a delayed onset of steady effect on the behavior. It may not be excluded that under chronic administration of anxiolytics the reduction in the number of metastases is not accidental and is caused by the biochemical mechanisms that regulate the psycho-emotional status and metastasis progression in animals. At the same time, a body of experimental data on the effect of psychotropic drugs on different types of tumors is quite contradictory. For example, anxiolytic drugs with similar mechanism of action in combination with cytostatics can produce opposite effects on the rate of experimental tumor growth [24]. The action of psychotropic drugs also depends on the phase of tumor development and administration regimen, implicating intimate involvement of these substances in tumor development. More studies are needed to elucidate the mechanisms whereby the psyche exerts influence on the neoplastic disease.

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