SEASONAL DEPENDENCY OF THE EFFECTS OF EXPERIMENTAL STRESSORS ON TUMOR METASTASIS IN MICE BEARING LEWIS LUNG CARCINOMA*

LAURA PERISSIN[1], SONIA ZORZET[2], VALENTINA RAPOZZI[1]
DIEGO PAOLETTI[1], TULLIO GIRALDI[1]

Dipartimento di Scienze e Tecnologie Biomediche, Università di Udine, Italy [1]
Istituto di Farmacologia e Farmacognosia, Università di Trieste, Italy [2]

INTRODUCTION

Models of laboratory animals subjected to experimental stressors have been widely used to investigate the action of stress on tumor growth [4]. When the relevant reports are critically reviewed, a high heterogeneity appeared both for the animal-tumor systems used and for the characteristics of the stressor employed. The occurrence and magnitude of the effects of the stressors on tumor growth was correspondingly variable. Moreover, tumor metastasis was marginally considered, in spite of its outstanding clinical relevance [2].

Among the numerous experimental stressors studied, the application of rotational stress (RS) to mice housed in a stress-protected environment, appears to be a carefully and widely characterized mild psychological stressor [5]. The effects of the application of RS have been examined in syngeneic mice implanted with Lewis lung carcinoma, in order to specifically determine the action of the stressor upon tumor dissemination in addition to tumor growth. Rotational stress was shown to significantly modify metastasis, independently from its effects on the primary tumor growth. However, a high variability in the results obtained was observed, which could not be reduced by the accurate control of housing conditions and experimental procedures. When a retrospective analysis of the results obtained in numerous experiments repeated in different periods of the year was performed, a seasonal dependency could be recognized, consisting of an increase in metastasis by the stressor in summer, and of a decrease in winter months.

Key-words: Experimental stressors; Lewis lung carcinoma; Melatonin; Metastasis; Mice.
Received September 30, 1993.
Chronobiologia *21,* 99-103, 1994.

*Presented at the 3rd National Meeting of the Italian Society for Chronobiology, Florence (Italy) June 11th 1993.

The aim of the present investigation has been therefore to examine the possible seasonal dependency of the effects of experimental stressors on tumor growth and metastasis in mice bearing Lewis lung carcinoma. The stressor paradigms used are RS, forced immobilization and electric foot shock. The possible participation of pineal gland and of melatonin have also been examined including in the experimental protocol the measurement of melatonin urinary excretion.

MATERIALS AND METHODS

Animals, tumor transplantation and evaluation

The animals used are female C57BL/6 x DBA/2F_1 (BD2F1) mice weighing 18-20 g, purchased from Charles River, Calco, Como, Italy. Lewis lung carcinoma, originally provided by the National Cancer Institute, Bethesda Md, U.S.A. was propagated in BD2F1 mice by subcutaneous or intramuscular injection, as indicated, using a tumor cell suspension containing 10^6 viable tumor cells [1]. Measurement of metastasis formation was performed at sacrifice of the animals on day 22 from tumor inoculation, as already described [1].

Experimental stressors

Rotational stress was applied to the animals maintained in a low stress environment, as indicated, by spinning the cages at 45 rpm for 10 min every hour from the time of tumor inoculation until sacrifice [1]. Forced immobilization was imposed in the low stress environment by tying the animal's legs with strings fixed to small plastic boards, for 1h daily on days 1-6 from tumor inoculation. Electric foot shock (1mA) was delivered through the grid floor lasting 5 sec. Sessions, consisting of the repetition of this cycle every 10 min for 3h, were repeated for 21 days following tumor inoculation.

Urinary melatonin excretion

For urine collection, the mice were housed individually in conventional cage (21×27×14 cm), each containing a stainless steel grid. Urine was collected on chromatographic paper (Whataman 3MM) placed below the stainless steel grid. Melatonin extraction and assay were performed as already described [3].

RESULTS AND DISCUSSION

The effects of experimental stressors on tumor progression in mice are being examined in the laboratory of the authors since several years. In spite of the most accurate control of the experimental condition, a high variability in the result obtained with RS was evident, which could not be reduced by a further control of the experimental setting. A similar high variability was encountered during repeated experiments with electric foot shock. A retrospective analysis of the data collected with RS indicated that the results obtained could have a seasonal dependence. Indeed, data in Table 1 illustrate the results of representative experiments performed in winter or summer. The effects of the application of RS on primary tumors are not reported since they are not significantly different in any of the experimental groups. On the contrary, metastasis weight is significantly increased in April, whereas in December a significant decrease is noted. Similar results are obtained in unreported experiments using a light intensity of 2000 lux. The effects of application of electric foot shock on metastasis

month	RS	metastasis (mg)	weight %[2]	melatonin[1] day[3]	melatonin[1] night[4]
April	–	183 ± 41[a, b]		58 ± 9	81 ± 17[c]
	+	332 ± 62[a]	+81	18 ± 16	750 ± 104[c]
December	–	209 ± 28[f]		12 ± 4	177 ± 38[g]
	+	101 ± 17[f]	−52	10 ± 3	8 ± 1[g]

[1]: urinary excretion (pg); [2]: variation over controls; [3]: 8 a.m.-8 p.m. (light on); [4]: 8 p.m.-8 a.m. (light off). The light-dark cycle is 12/12 hr and intensity of illumination measured in the cages 5 lux. Each value is the mean ± S.E.M. obtained using groups of 10 mice implanted subcutaneously with the tumor. Melatonin assay was performed in the 24 hr period before the day of sacrifice. Means marked with the same letter are significantly different, Mann Whitney test (6), $p < 0.05$.

Table 1 - Effect of rotation stress (RS) on tumor metastasis and melatonin urinary excretion, as a function of the month of evaluation.

weight also display a seasonal variation, consisting of a significant increase in summer, accompanied by no significant variation in winter. On the contrary, the application of forced immobilization does not cause season-dependent effects on metastasis.

The stressor paradigms examined are widely different, particularly as far as the magnitude of their physical component is concerned. The neuro-vegetative and neuro-endocrine pathways determining the reported effects on tumor progression are known to be correspondingly different (e.g. opiod dependent vs independent). These differences might be related to the differences in seasonal dependency of the effects of only some of the stressors.

experimental stressor		month	metastasis (mg)	weight %[1]
electric foot shock	–	December	54 ± 7	
	+		38 ± 8	− 30
	–	July	17 ± 6[a]	
	+		82 ± 27[a]	+382
forced immobilization	–	January	59 ± 7[b]	
	+		121 ± 11[b]	+105
	–	August	202 ± 29[c]	
	+		328 ± 30[c]	+ 62

[1]: variation over the controls.
Each value is the mean ± S.E.M. obtained using groups of 10 mice. The light-dark cycle is 12/12 hr and intensity of illumination measured in the cages 5 lux. Tumor implantation was subcutaneous (electric foot shock) or intramuscular (forced immobilization). Means marked with the same letter are significantly different, Mann Whitney test (6), $p < 0.05$.

Table 2 - Effects of the application of different experimental stressors on the weight of lung metastasis, as a function of the month of evaluation.

The above reported seasonal effects might be related to pineal gland function, and melatonin urinary excretion has been measured in relation to the seasonal effects of RS. Nocturnal melatonin excretion is markedly increased by RS in April, and is remarkably decreased in December. These variations in endogenous melatonin levels caused by RS appear to directly correlate with the effects of the stressor on metastasis. These observations appear of relevance for their experimental implications. Indeed, seasonal effects, in addition to housing stress, appear of relevance when the effects of experimental stressors are investigated in laboratory animals. Moreover, the role of pineal gland and of its indoleamine hormone, melatonin, appear significant in relation to cancer progression, stress sensitivity and chronobiological aspects as illustrated in the present report. These data further indicate the relevance of chronobiology in experimental cancer research and neuro-immuno-modulation.

SUMMARY

Increasing evidence indicates that the application of stressor paradigms in experimental animals affects tumor incidence and progression. However, a high heterogeneity appears both for the animal-tumor system used and for the characteristics of the stressor employed. A high variability was observed also with the application of rotational stress, a carefully and widely characterized mild psychological stressor, to mice bearing Lewis lung carcinoma. The aim of this work has been therefore to examine the possible seasonal dependency of the effects of experimental stressors (rotational stress, forced immobilization and electric foot shock) on spontaneous lung metastasis formation in mice bearing Lewis lung carcinoma. The possible participation of pineal gland and of melatonin have also been examined including in the experimental protocol the measurement of melatonin urinary excretion. The stressor paradigms used significantly increased metastasis weight in spring, in comparison with non-stressed animals. When examined in winter, rotational stress and foot shock significantly decreased metastasis formation, in comparison with non-stressed mice. The effects of forced immobilization were not season-dependent. The melatonin urinary excretion has been measured in relation to the seasonal effects of rotational stress. Nocturnal melatonin excretion is markedly increased by rotational stress in spring and is remarkably decreased in winter. These variations in endogenous melatonin levels caused by rotational stress appear to directly correlate with the effects of the stressor or metastasis. These results lend support to the view that the mechanisms underlying the tumor enhancing action of stressors involve the psycho-neuroendocrine network, and indicate the relevance of chronobiology in experimental cancer research and neuro-immuno-modulation.

ACKNOWLEDGMENT

This work was supported by the Italian National Research Council (CNR) Special Project "ACRO" contract n. 92.2192.PF39, and by MURST 40 and 60% - Prof. T. Giraldi - U.S.U. 1991. The generosity of Fondazione C. e D. Callerio in providing the protected environmental facility is gratefully appreciated.

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Requests for reprints should be addressed to:

LAURA PERISSIN
Dipartimento di Scienze e Tecnologie Biomediche,
Sez. Farmacologia
Università di Udine
Piazzale M. Kolbe, 4
33100 Udine, Italy