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Survival Time in Mice Bearing TLX5 Lymphoma Subjected to Rotational Stress and Chemotherapy with CCNU

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Abstract. *The effects of a psychological stress model rotational stress were examined in mice bearing TLX5 lymphoma. The survival time of the animals was determined as a function of tumor inoculum size and treatment with the antitumor drug, CCNU. Rotational stress significantly decreased the mean survival time of mice implanted with 10 or 10^2 tumor cells, and significantly increased tumor takes in mice implanted with 10 cells. Treatment with CCNU significantly prolonged the survival time of the treated animals; the application of rotational stress significantly attenuated the increase in survival time caused by CCNU. These results indicate that in mice with a limited tumor burden, psychological stress favors the progression of TLX5 lymphoma, and reduces the effectiveness of the antitumor drug, CCNU. Moreover, the experimental model employed may provide a tool useful for investigating the mechanisms involved in the sensitivity of lymphoma to psychosocial stress.*

The relatively vast range of literature indicates that the application of experimental stressors facilitates the incidence and progression of solid tumors in laboratory rodents (1). The physical stress produced by therapeutic procedures, such as radiotherapy (2-4), chemotherapy (5-7), anesthesia (8) and surgery (4,9,10) increases tumor metastasis in experimental animal tumor systems. Psychological stressors, including isolation or overcrowding during animal housing, have also been shown to influence the incidence and growth of tumors in laboratory rodents (11-13).

On the other hand, fewer data are available on the effects of stress on the progression of hematological malignancies in laboratory animals. Auditory stress was found to be without effects on spontaneous leukemia incidence in mice (14). The growth of P815 mastocytoma in mice was facilitated by the application of inescapable footshock sessions (15), although the application of footshock (16) and electroshock (17) was ineffective in modifying survival in mice implanted with L1210 leukemia. Physical restraint decreased the incidence of leukemia in male mice injected with Friend erythroleukemia virus (18). No report on the effects of experimental stressors on the progression of lymphomas in laboratory rodents appears to be available in the literature.

Consequently, the aim of the present study was to determine the effects of the application of a stress paradigm, rotational stress, in mice implanted with TLX5 lymphoma as a function of the size of tumor inoculum. Rotational stress was chosen since it is a well defined paradigm of mild psychological stress, which includes the control of housing stress (19). Since malignant lymphomas are almost invariably treated with antitumor agents in the clinic, the effects of rotational stress have been also examined in mice bearing TLX5 lymphoma, which were treated with the cytotoxic antitumor drug CCNU. The effects of the treatments were recorded in terms of the survival time of the treated mice, and are hereafter reported.

Materials and Methods

Animals and tumor transplantation. The animals used are female CBA/LAC mice weighing 18-20 g, belonging to a conventional local breeding colony. TLX5 lymphoma was originally provided by the Chester Beatty Research Institute, London, England. Tumor implantation was performed by injecting 0.1ml per mouse of a tumor cell suspension containing the number of tumor cells indicated in Tables I and II (10^5 for tumor line maintenance). Tumor cells were obtained from donors inoculated 8 days before, were washed by centrifugation at 500 *g* and were resuspended in PBS after counting for trypan blue exclusion.

Animal housing and rotational stress. The mice were kept 5 per cage in order to avoid the effects of overcrowding or isolation on tumor progression. The cages were placed in a low stress housing for 2 weeks before tumor inoculation, in order to allow the animals to recover from the stress of shipment, and to adapt them to the new housing conditions. Temperature and humidity in the protected housing were constant and equal to 20°C and 60 % respectively. The cycle of illumination was 12/12 hours (lights on from 8 a.m. to 8 p.m.) and the intensity of illumination in the cages was 5 lux. Rotational stress was applied by spinning the cages at 45 rpm for 10 minutes every hour from time of tumor inoculation for a maximum time of 21 days. Further details on the experimental setting have been reported in detail elsewhere (19).

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Table I. *Survival time of CBA mice implanted with a different inoculum size of TLX5 lymphoma and subjected to rotational stress.*

Inoculum size (cells/mouse)	Rotational stress	Mean survival time (days) #	p§	N° of mice with tumor/total N°
10	-	15.5	<0.0001	6/20[a]
	+	14.1		16/20[a]
10^2	-	14.8	<0.0001	10/10
	+	12.9		10/10
10^3	-	12.8	0.379	10/10
	+	13.7		10/10
10^4	-	12.4	0.961	10/10
	+	12.3		10/10

Groups of 10-20 female CBA mice were implanted i.p. with the indicated number of viable TLX5 lymphoma cells; the animals were subjected to rotational stress starting from tumor inoculation for 21 days, or until death if occurring earlier.

#: as determined with Kaplan-Meyer analysis
§: as determined with log-rank test
a: fractions of mice significantly different, Pearson chi-square test, $p<0.0001$.

Drug treatment and survival time. 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU, Belustine®) was provided by Rhone-Poulenc Rorer S.p.A., Milan, Italy. To avoid the stress of repeated intraperitoneal injection, the drug was administered orally admixed in powdered food for 7 days following tumor implantation. The drug concentration in the food was 50 or 100μg/g, corresponding to a daily dose of CCNU taken up with the food of 12.5 or 25 mg/kg/day, respectively; the measured average daily food consumption was 5.0±0.1 g per mouse and remained constant throughout the experiments. The survival time of the control and treated animals was recorded, and their survival was analysed using Kaplan-Meyer and logrank statistics (20).

Results

Rotational stress significantly decreased the mean survival time of mice implanted with 10 TLX5 lymphoma cells, and increased the fraction of mice with tumor from 6/20 to 16/20. With an inoculum of 10^2 tumor cells, rotational stress still significantly decreased the mean survival time, and tumors develop in all of the mice. When the inoculum size was further increased to 10^3 or 10^4 cells, tumor takes occurred in all of the mice and no significant effect on survival was caused by rotational stress (Table I).

The treatment with CCNU (12.5 mg/Kg/day) of mice implanted with 10 tumor cells increased their mean survival time, and the fraction of mice with a tumor was increased from 1/10 to 10/20. In mice treated with the same dosages of CCNU rotational stress significantly decreased mean survival time and increased the fraction of mice with a tumor from 10/20 to 19/20. In mice implanted with 10^2 tumor cells the increase in survival time caused by CCNU (25 mg/Kg/day) was significantly attenuated by rotational stress; tumor take occurred in all of the mice.

Table II. *Effect of CCNU on survival time of CBA mice implanted with a different inoculum size of TLX5 lymphoma and subjected to rotational stress.*

Inoculum size (cells/mouse)	Rotational stress	CCNU (MG/KG/DAY)	Mean survival time (days) #	N° of mice with tumor/total N°
10	-	-	16.0[ab]	1/10
	-	12.5	24.2[ac]	10/20
	+	-	14.7[bd]	9/10
	+	12.5	18.6[cd]	19/20
10^2	-	-	13.5[ef]	20/20
	-	25	20.5[eh]	10/10
	+	-	12.2[fg]	20/20
	+	25	18.6[gh]	10/10

Groups of 10-20 female CBA mice were implanted i.p. with the indicated number of viable TLX5 lymphoma cells; the animals were subjected to rotational stress starting from tumor inoculation for 21 days (or until death if occurring earlier).

#: as determined with Kaplan-Meyer analysis
a: $p=0.05$; b,c,e,g,h: $p<0.0001$; d: $p=0.010$ and f: 0.002 as determined using log-rank test.

The fractions of mice inoculated with 10 cells and subjected to rotational stress and to CCNU treatment are significantly different, Pearson chi-square $p<0.0001$.

Discussion

The results obtained are in accord with reports showing that the application of stress paradigms facilitates the tumor take and metastasis of solid malignant tumors. In syngeneic mice which were maintained in a (low stress) protected housing, tumor takes which did not occur upon implantation s.c with 10^5 cells of Lewis lung carcinoma, but occurred either after adminstration of rotational stress or increase of tumor inoculum size to 10^6 cells. Pulmonay metastasis was increased when the animals received a larger inoculum size and were subjected to rotational stress (21). Moreover, in these animals the magnitude of the cytotoxic effects of cyclophosphamide, and that of the antimetastatic action of razoxane, were significantly reduced upon application of rotational stress (21). Similarly, the reduced effectiveness of combined treatment with adriamycin plus cyclophosphamide has been observed, depending on the social housing conditions of mice bearing Shiongi mammary carcinoma SC115 (22). These findings might be interpreted as showing that the antitumor immune responses of the host directed against tumor cells, are amenable to modulation by stress *via* neuroendocrine citcuits (23), and participate in determining the overall magnitude of the action of antitumor drugs. This view is also supported by data showing that low dosages of

cyclophosphamide and melphalan were equally or more effective than higher dose levels of the same drugs in mice bearing MOPC-315 plasmacytoma (24-26). Mice cured with the low dosage treatment schedules showed a strong acquired immune resistance to further tumor challenges, which did not occur when using high dosages of these antitumor drugs (24,26-28). Furthermore, this acquired immune resistance was shown to depend on the appearance of Lyt 2+ T-cells (28,29). Findings consistent with a dosage-dependent immunoregulatory action have also been found in rats bearing KMT-17 fibrosarcoma upon treatment with bleomycin (30).

In conclusion, these data indicate that in mice with a limited tumor burden psychological stress favors the progression of TLX5 lymphoma, and attenuates the effectiveness of CCNU. These results may be of significance in relation to clinical data showing that psychological factors contributing to prognosis for Hodgkin lymphoma (31); the experimental model presently employed may provide an tool useful for investigating the mechanisms involved in the sensitivity of lymphoma to psychosocial stress.

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