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Effect of Thymalin on the Tumor and Thymus under Conditions of Activation Therapy *In Vivo*

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Pronounced antitumor effect of Thymalin in doses lower than the therapeutic doses was shown in experiments on albino outbred male rats with transplanted sarcoma 45. Tumor growth arrest and its regression were observed in more than half of animals and in other cases, the growth was suppressed by 78%. Microstructural changes in the thymus were analyzed. Significant increase in lymphoproliferative activity and the content of tissue basophils and plasmocytes in the thymus lobules was observed. Tumor regression was accompanied by the development of stable antistress adaptation reactions of calm and elevated activation. High efficiency of Thymalin can be attributed to the use of lower doses of the substance and their modulation during the treatment course in accordance with the regimes of *activation therapy*.

Key Words: *Thymalin; transplanted tumors; antistress adaptive responses; thymus, immune system cells*

Previous experiments have demonstrated the important role of peptide bioregulation in the maintenance of body resistance to various damaging factors, prevention of rapid aging, and development of various pathologies including tumor growth [8,9]. Peptide substances isolated from the thymus exhibit also immunocorrecting and stress-limiting properties [7,9,10]. At the same time, published data on the efficiency of thymus-derived preparations in complex antitumor therapy are ambiguous. Despite preventive effects of these preparations on tumor development, metastasizing, and toxic effects of chemoradiotherapy were reported [1,4,5], the results of large-scale randomized clinical trials do not decisively proved their significant effect on lifespan of tumor patients and efficiency of traditional antitumor therapy [14]. It is suggested that uncertainty about the influence of thymus peptides on antitumor resistance is determined by poor development of methods of their application methods in combined antitumor treatment [5]. Of specific interest in this context are algorithms of activation therapy aimed at induction of general nonspecific adaptation responses (AR) of the antistress type contributing to the increase in the efficiency of various treatment schemes during malignant processes under experimental and clinical conditions [3,12,15].

Here we studied the effects of a thymus preparation Thymalin in regimen of activation therapy in animals with developed transplanted tumors.

MATERIALS AND METHODS

Experiments were performed on outbred male rats (n=49) weighing 200-280 g with transplanted sarcoma 45. The experiments were conducted in accordance to international principles of humane treatment of animals. The rats were divided into 4 groups: intact animals (n=9), tumor-bearing animals (control, n=10), and two groups of tumor-bearing animals receiving Thymalin in different doses (n=15 in each group).

The tumor was inoculated subcutaneously in the lateral surface of the back by the standard method. After the tumor reached the size of 0.7-1.2 cm^3, 3-week treatment course was started. Thymalin was dissolved in

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physiological saline to a concentration of 0.05 mg/ml. The solution was injected intramuscularly in the external surface of the leg once in 3 days over 3 weeks using a tuberculin syringe. In the treatment group 1, single dose of 0.07 mg/kg was used, which corresponded to single minimal therapeutic dose for humans [2]. However, this dose for rats was below the therapeutic dose due to their lower sensitivity in comparison with humans. In treatment group 2, principles of activation therapy implying minimization of exposure intensity and modulation of the dosage during the course according to exponential dependence were used [3,12,15]. Thymalin doses varied from 0.014 to 0.040 mg/kg. These doses were lower than in the treatment group 1 and significantly lower than the doses used in clinical practice [2,6] and known experiments on Thymalin effects on spontaneous and induced carcinogenesis in rats [1]. Control group received physiological saline.

The status of animals was estimated by the type and intensity of AR during the experiment. The relative number of lymphocytes per 200 blood cells served as a signal indicator of AR character (the blood was taken from the subcutaneous vein of the medial thigh surface) [3,15]. The tension of the antistress AR reflecting the body reactivity level was evaluated by deviations from the normal values of relative content of neutrophils, monocytes, eosinophils and basophils in differential blood count, as well as total leukocyte count [3]. Deviations from the normal values could vary in different rats.

The animals were decapitated under ether narcosis in 3 days after completion of the treatment course. Histological analysis of the thymus was performed after calculation of its weight coefficient as the ratio of organ weight (mg) to body weight (g). Microstructure of the thymus was analyzed by the Brachet method. The stromal-parenchymal coefficient reflecting activity of lymphoproliferative processes in the organ was determined morphometrically, and the number of tissue basophils and plasma cells in the thymus lobules was assessed. Each sample was analyzed in 10 fields of view on 3-5 thymus sections at ×400.

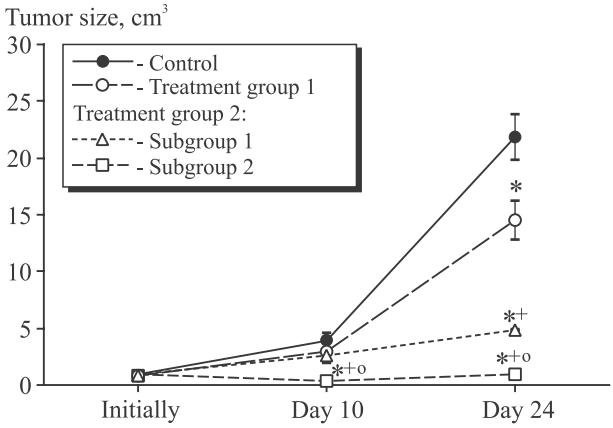

Fig. 1. Changes in the size of sarcoma 45 after Thymalin treatment. *p<0.05-0.01 in comparison with *control, +treatment group 1, °subgroup 1 of treatment group 2.

Statistical analysis of results was performed using Mann—Whitney test and Student's t test.

RESULTS

The effects of Thymalin on the growth of sarcoma 45 depended on the dose and administration regimen (Fig. 1). By the end of experiment, the mean tumor size in the control and in treatment groups 1 and 2 was 21.9±2.0, 14.5±1.7, and 2.6±0.3cm^3, respectively. Suppression of tumor growth by 34% (p<0.05) was observed in group 1. In group 2, the mean size of the tumors was by 8.4 times lower than in the control group and by 5.6 times lower than in group 1 (p<0.001).

TABLE 1. Parameters of the Thymus in Rats with Different Thymalin Efficiency

Parameter	Control	Treatment group 1	Treatment group 2		Intact animals
			subgroup 1	subgroup 2	
Tumor volume, cm^3	21.9±2.0	14.5±1.7*	4.9±0.1*+	1.0±0.1*+o	—
Peripheral blood lymphocytes, %	34.5±5.7	61.5±4.7*	64.6±8.9*	65.3±3.4*	67.2±3.5*
Stress	100	7*	0*	0*	0*
Tension antistress AR, %	0	60*	33*+	0*+o	0*+o
Weight coefficient of the thymus, ×10	2.1±0.4	4.8±0.9*	6.5±0.9*	7.9±0.6*+	7.5±1.2*+
Stroma/parenchyma coefficient, ×100	6.1±0.6	5.0±0.5	5.2±0.5	2.0±0.2*+o	2.7±0.7*+o
Number of plasma cells	0.7±0.1	0.3±0.1	0	2.1±0.1*+ox	1.3±0.3+o
Number of tissue basophils	3.7±0.5	4.0±0.4	3.5±0.7	5.6±0.4*+ox	4.0±0.3

Note. *p<0.05-0.01 in comparison with the control; p<0.05 in comparison with +treatment group 1, °subgroup 1, ×intact animals.

Treatment group 2 can be divided into two subgroups by the intensity of Thymalin effects (Fig. 1). In subgroup 1 rats (n=6), inhibition of sarcoma 45 growth by 78% was noted. In subgroup 2 rats (n=9), this effect was more pronounced: tumor growth arrest at early stages (4 rats) or partial or complete regression of tumor growth after a short period of active growth (5 rats; 55%). Tumor regression was proven histologically (Fig. 2, *a*).

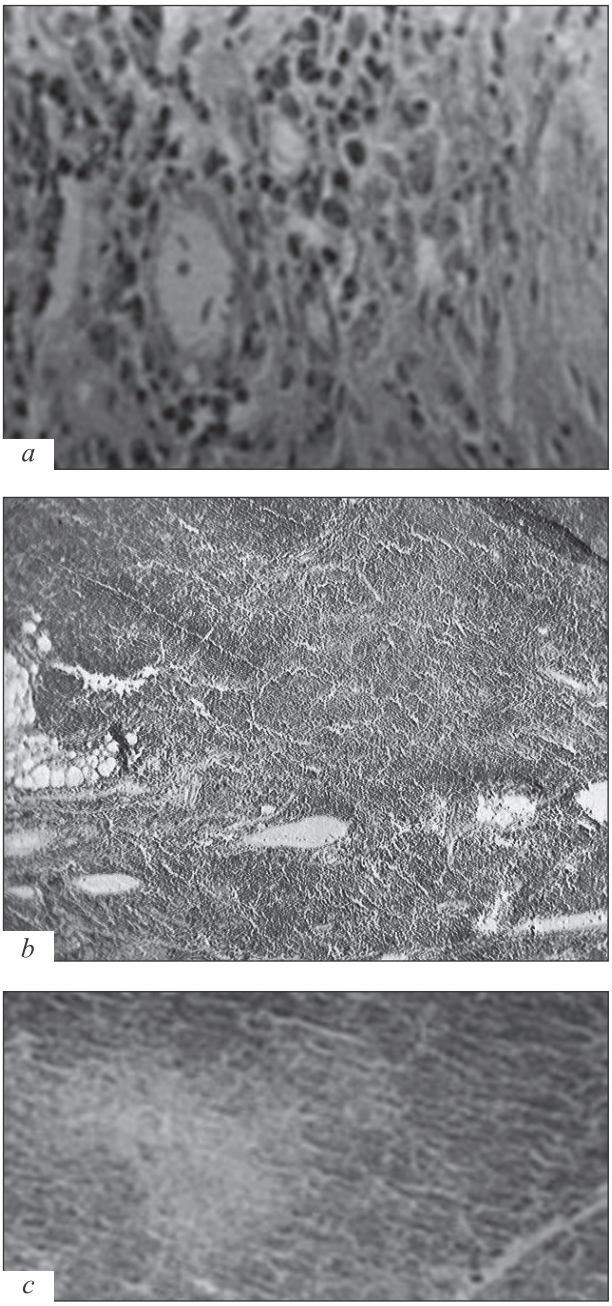

Fig. 2. Changes in the tumor and thymus structure after effective Thymalin treatment. Brachet method, ×400 (*a*), ×100 (*b*, *c*). *a*) Sarcoma 45 regression. Broad connective tissue roll. Replacement of the regressed tumor tissue by the connective tissue. Intensive infiltration by lymphoid and plasmocyte elements and macrophages. *b*) Thymus in a rat with sarcoma 45. Control group (tumor growth). AR — stress. The absence of a borderline between the cortical and medullary substance, hypoplasia of the lymphoid tissue, thymocyte degeneration, and atrophy of lobules. *c*) Thymus of a rat with complete regression of the tumor. Adaptational reaction of elevated activation. Large lobules. The area of the cortical substance significantly surpassed the area of the medullary substance.

Mean tumor volume in subgroups 1 and 2 by the end of the experiment was 4.9±0.1 and 1.0±0.08 cm^3, respectively (p<0.01). Antitumor effects of Thymalin correlated with its antistress properties in the treatment group 2 and all other tumor-bearing rats (Table 1). Chronic stress was observed in the control group rats at the end of the experiment. Tension antistress AR prevailed in group 1 (60% rats) and stress was observed in only 1 animal (7%). Antistress AR of calm and elevated activation without tension signs prevailed in group 2 (87% cases), no stress reactions were observed. Tension antistress AR of elevated activation and training were observed in only subgroup 1 in animals with tumors >3 cm^3 (33%). Thus, treatment with Thymalin in the regimen of activation therapy enhanced its antistress effect, which manifested in a significant increase in the frequency of physiological antistress AR of calm and increased activation associated with the activation of the neuroendocrine and immune systems, which obviously led to the mobilization of systemic and effector (local) antitumor mechanisms. Between-group differences in the intensity of these changes can be stipulated by differences in individual sensitivity of animals in the study group.

Structural and functional changes in the central immune organ in animals with different tumor response to Thymalin were never studied. We found that the weight coefficient of thymus of treatment group rats was by 2.3 and 3.8 times higher than the control level (p<0.05-0.01, Table 1). This parameter in the group of animals receiving Thymalin in regimen of activation therapy was higher than in treatment group 1 (p<0.05) and did not differ from that in intact males (Table 1).

At the same time, thymus microstructure and stroma/parenchyma coefficient showed that the lymphoproliferative processes in the thymus were most markedly activated in subgroup 2 animals of treatment group 2 (Fig. 2, *b*, *c*; Table 1). The stroma/parenchyma coefficient in these rats was significantly lower (by 2 times and more) than in other tumor-bearing animals. Moreover, rats with most pronounced antitumor effect of Thymalin were characterized by increased content of tissue basophils and plasma cells in the thymus lobes in comparison with all other animals including rats without tumors (Fig. 2, *c*; Table 1). The increase in the number of degranulating basophils acting as regulators of tissue homeostasis [11] can reflect enhancement of cell—cell interactions

followed by thymocyte activation. The functional role of plasmocytes in the thymus lobules of animals with the most pronounced treatment effect should be further investigated. It can be hypothesized that they belong to plasmacytoid dendritic cells producing interferons (α and β) and activating various pathways of cell immunity [13].

Our results demonstrate pronounced *in vivo* antitumor effects of Thymalin administered in regimen of activation therapy (up to complete regression of the inoculated tumors). The changes observed in the thymus after effective Thymalin treatment supplement our understanding of systemic immune processes related to strengthening of the antitumor resistance of the organism.

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