Morphological Analysis of the Thymus of Albino Rats with Tumors for Experimental Chemotherapy Alone and in Combination with Activator Influences of an Alternating Magnetic Field

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A study of the histological structure and cell composition of the thymus in rats with ascitic ovarian tumors for chemotherapy alone and in combination with an alternating magnetic field, which induces an antistressor adaptive response in the organism, showed that an alternative magnetic field potentiates the antitumor effect of thioTEPA and protects the thymus from toxic damage caused by high doses of chemotherapy.

Key Words: *thymus; stress; activation*

The thymus is a crucial organ of the immune system that controls homeostasis and is responsible for the nonspecific resistance of the organism [3,6,14]. Destructive alterations have been observed in the thymus in various pathologies, particularly during tumorigenesis [10-13,15], impairing thymus function and acting as stressors [2,15,17]. Stimulation of the functional activity of the thymus and improvement of nonspecific resistance have been observed during the development of an antistressor adaptive activation response [7]. It was shown that an alternating magnetic field (AMF) stimulates proliferative processes in the thymus [5], which is associated with the development of an activation response in the organism under the influence of an AMF of certain biotropic parameters [8,9].

Our objective was to investigate the histological structure and cell composition of the thymus in rats with ascitic ovarian tumors.

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MATERIALS AND METHODS

Experiment were performed on 125 female Wistar rats weighing 180-200 g. Ovarian tumor cells were obtained from the Oncology Research Center (Russian Academy of Medcal Sciences) and were passaged by injection into the peritoneum (16×10^9 cells in 0.3 ml normal saline). The animals were divided into 5 equal groups. The rats of four groups were inoculated with tumor cells. Groups 1 and 5 served as controls. Group 2 rats were treated only with thioTEPA, group 3 rats received thioTEPA and were subjected to an AMF, and group 4 rats were just exposed to an AMF. The treatment was started on day 2 after inoculation. ThioTEPA was injected intramuscularly in a single dose of 15 mg/kg, which is close to the maximum tolerate dose. The animals were treated with an AMF as described elsewhere [8], the field being applied to the head, and the exposure time varying from 15 sec to 1 min. The AMF had an induction of 0.3 mT and a frequency of 50 Hz. Such a low AMF intensity was achieved by chang-

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ing the distance between the core of the apparatus and the rat. The animals were sacrificed on day 7. The thymus weight was calculated per 100 g body weight. Histological sections 5 μ thick were stained with methyl green-pyronin and azure-II-eosin. Morphometry was performed with the use of the Avtandilov ocular net [1] and an ocular micrometer. In sections stained with ethyl green-pyronin we determined the mean volume of the lobule, cortical, and medullar substance and the percentage of lobules according to their class [11], and calculated the number of thymic bodies in a lobule. In the sections stained with azure-II-eosin we studied the cell composition and calculated the number of cells and mitoses on a standard area at magnification 900. In each case 10-20 tests were performed and 5000-7000 cells were counted. The results were statistically analyzed using Student's t test.

RESULTS

Morphometric studies showed that pathomorphological alterations in the thymus occurred in all groups of animals with ovarian tumors, as evidenced by comparison with intact animals (Table 1). They intensity of these alterations was different. They were maximal in group 2 rats: the weight of the thymus, the width of the cortex, and the size of lobules were smaller ($p<0.001$), and there were more small lobules and much fewer large ones ($p<0.01$) In rats of groups 3 and 4 the alterations of the thymus were much less pronounced that in rats of groups 1 and 2. The thymus weight increased especially noticeably in group 4 ($p<0.001$), though remaining lower than normal, the mean area of the lobules reached the normal value, the cortex was considerably wider, particularly in group 4 animals ($p_{2\text{-}3,4}<0.01$), and the number of small lobules decreased, while the number of large lobules increased ($p_{2\text{-}3,4}<0.001$). In these groups the main mass of the thymus was represented by large and medium lobules, indicating that the thymus weight had increased due to hypertrophy of these lobules (Fig. 1). The number of thymic (Hassall's corpuscles) was the lowest in group 2 and pathologically increased in group 1 ($p<0.001$). Although a large number of thymic corpuscles was detected during tumor growth, these bodies were inactive (they were enlarged and sclerotic). In other groups the number of thymic corpuscles was somewhat higher than normal; however, they did not differ from those in the intact control, which indirectly indicates the normalization of thymic hormonal activity. The groups 3 and 4, the medulla-cortex ratio attests to the activation of both the lymphoid and epithelial components of the thymus.

Cell composition in the thymus varied depending on the experimental conditions. The most pronounced pathological alterations were observed in group 2 animals (Table 2). In the subcortical zone of the thymus in these animals, the total number of cells per unit area was the lowest, being 2-fold lower that in the other experimental groups ($p<0.001$) and almost 3-fold lower than in intact animals ($p<0.001$). In group 2 animals, the percentage of reticuloepitheliocytes and low-differentiated cells were the highest, the number of mitoses was the lowest, while the number of degenerating cells was the highest ($p<0.001$). The preserved lymphocytes were hyperchromatous and had pycnotic nuclei. The thymus contained large numbers of neutrophils, whose role under the chosen experimental conditions is ambiguous [6,11]. In

TABLE 1. Relative Weight of the Thymus and Morphometric Results for Different Variants of Antitumor Therapy ($M\pm m$)

Parameter	Group				
	1	2	3	4	5
Relative weight per 100 g body weight, mg	82±8.7*	46±4.2	67±3.4	97±6.3*	88±5.2
Volume, %					
entire lobule	82±3.7*	69±1.1	85±2.7*	89±2.7	81±3.4
cortex	27±2.5	24±2.6	71±3.6*	61±3.6	74±2.7
medulla	73±3.4	76±3.2	29±2.9	39±3.8	26±2.3
Content of lobules, %					
small	23	56	20	32	13
medium	44	41	51	55	48
large	33	3	29	13	39
Number of thymic corpuscles in a libule	7.8±0.2	7.2±0.3	4.8±0.3*	5.1±0.1*	4.6±0.6

Note. Here and in Table 2: one asterisk indicates $p>0.05$ according to Student's t test.

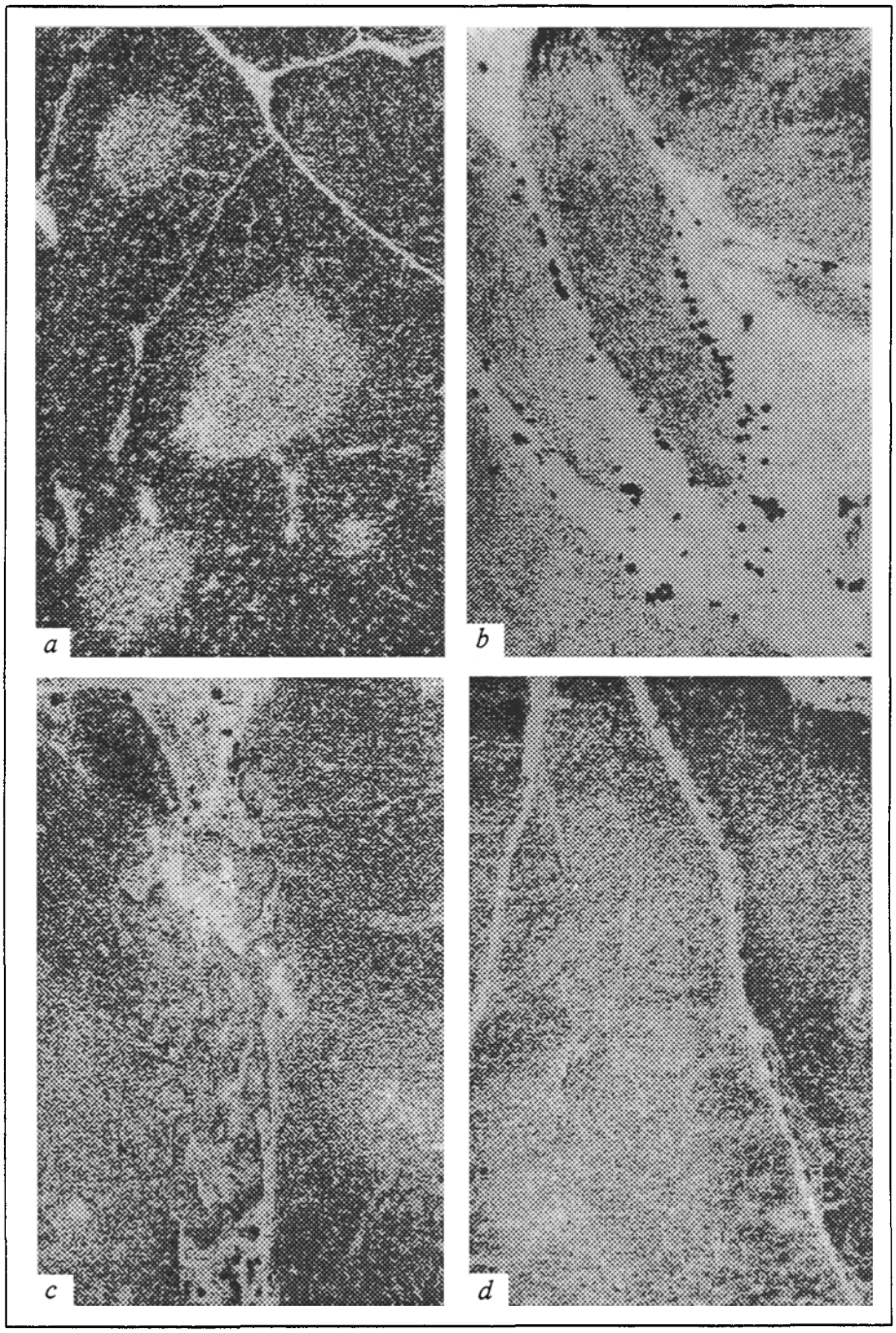

Fig. 1. Thymus under different experimental conditions. *a*) intact animals. High density of lymphocytes in the lobule. The cortex:medullar ratio is equal to 1. Activation reaction. *b*) administration of thioTEPA. Atrophy and dystrophy of the lobules. Stress reaction. *c*) combined influence of thioTEPA and AMF. Marked hyperplasia of lymphoid tissue in the lobular cortex. Activation reaction. *d*) tumor growth in normal saline. Pronounced hyperplasia of lymphoid tissue. Stress reaction. Staining with azure–II–eosin. ×140.

groups 3 and 4, these parameters were not so markedly altered, but were similar to those of intact animals (Table 2). For example, the total number of cells and the number of small lymphocytes were just as high, and the number of degenerating cells was just as moderate, as in group 1 rats ($p>0.05$). However, the number of mitoses was significantly higher ($p<0.01$). The macrophage count was markedly decreased in rats of both the 1st and the 3rd groups compared with the intact animals ($p<0.001$), whereas there were no significant differences in group 4.

In the central part of the thymic cortex, the number of cells per unit area was again the lowest in group 2. The number of reticuloepitheliocytes increased 8-fold compared with intact rats ($p<0.001$). The number of degenerating cells increased considerably, while the number of mitoses remained the lowest compared with the other experimental groups ($p<0.05$). The greater number of degenerating cells and macrophages than in other groups ($p<0.001$) pointed to the intensification of destructive processes in the thymus. In group 1 rats these processes were less pronounced, their intensity being the lowest in

groups 3 and 4. In group 3 rats, the overwhelming majority of cells in the thymus were represented by small lymphocytes, and the count of low-differentiated cells was somewhat reduced (Table 2). It should be noted that in group 4 rats the number of macrophages was higher than in all the other groups ($p<0.01$). The role of macrophages as a microenvironment of thymocytes is documented [4].

In the central zone of the medulla the number of cells per unit area was again lowest in the second group ($p<0.05$). The thymus of group 2 animals contained the greatest number of degenerating lymphocytes, while the number of mitoses was the lowest compared with the other groups ($p<0.001$). In group 3, cell density was high, small lymphocytes prevailed, and the number of reticuloepitheliocytes was the lowest ($p<0.001$). In group 4 rats these parameters were essentially similar to those in intact animals.

Comparison of the morphological alterations of the thymus and the antitumor effect showed that the magnitude of the later depends on the nonspecific antitumor resistance (which is increased at a high functional activity of the thymus) and on the specific action of chemical agents, although this has a negative effect on the thymus. For example, the total antitumor activity in group 3 animals 98% (11% tumor resorption and 87% tumor growth inhibition). In group 4, the total antitumor activity was 60% (4% resorption and 56% inhibition), but the thymus in these animals was more active. A pronounced accidental involution of the thymus was observed in group 2, and although the antitumor effect 50%, complete resorption of the tumor occurred only in 2% of the animals. In this group the antitumor effect was due to the specific activity of thioTEPA on tumor cells; however, the toxic activity of this drug was manifested in the inhibition of the morphofunctional activity of the thymus. When thioTEPA was combined with AMF, the nonspecific component participated in the development of an antitumor effect, and the nonspecific resistance of the organism increased due to the adaptive activator reaction, which was accompanied by a fairly high functional activity of the thymus.

Thus, the thymus responds in different ways to the maximum tolerated doses of the cytostatic thioTEPA applied alone or in combination with AMF. A pronounced inhibition of thymus function was observed after therapy with thioTEPA alone: the number of thymocytes was equally small both in the center and at the periphery of the lobule, which may indicate decreases and increases of mi-

TABLE 2. Content of Different Cells per Unit of Thymic Area for Different Variants of Antitumor Therapy ($M \pm m$)

Cells	Group				
	1	2	3	4	5
	Subcapsular zone of the cortex				
Total number	69±2.0	37±0.7	58±1.8	68±1.2	107±1.4
Small lymphocytes, %	63	38	64	73	70
Medium lymphocytes	13	30	14	9	8
Lymphoblasts	14	14	17	6	8
Reticuloepitheliocytes	8	15	4.5	8	10
Macrophages	1.9	3	0.5	4	2
Number of mitoses	1.6±0.32	0.8±0.03	4.1±0.2*	3.4±0.5	4.6±0.32
Number of degenerating cells	3.7±0.8	21.5±0.7	4.8±0.3	3.7±0.8	1.1±0.09
	Central zone of the cortex				
Total number	70±1.3	35±1.7	72±2.7	69±1.3	112±1.3
Small lymphocytes, %	54	13	62	68	86
Medium lymphocytes	11	9	6	5	9
Lymphoblasts	2	13	9	5	1
Reticuloepitheliocytes	27	52	18	25	2
Macrophages	6	13	5	7	2
Number of mitoses	0.33±0.08	0.26±0.06	0.88±0.07	0.32±0.08	1.6±0.2
Number of degenerating cells	20.0±0.3	29.3±0.5	5.7±0.9	4.4±0.7	1.7±0.2
	Central zone of the medulla				
Total number	36±1.5	35±1.6	43±0.1	47±1.1*	49±1.7
Small lymphocytes, %	18	23	27	32	23
Medium lymphocytes	5	6	7	1	8
Lymphoblasts	6	3	9	7	10
Reticuloepitheliocytes	59	48	40	46	49
Macrophages	12	20	10	14	10
Number of mitoses	0.26±0.03	0.18±0.03	0.56±0.1	0.6±0.1	0.87±0.1
Number of degenerating cells	9.9±0.1	24.0±4.1	3.2±0.7	4.7±0.3	1.7±0.4

gration of lymphocytes in and out of the thymus. The low number of mitoses with a high number of low-differentiated cells, denudation of the thymic stroma, and the large number of cells with hyperchromatous and pycnotic nuclei may be due to inhibited activity of the lymphoid and epithelial components with delymphatization and collapse of the lobules [10]. Similar alterations were reported to occur in the thymus after administration of 5-fluorouracil [15]. Presumably, since the structure of the thymus reflects the immune status [14], such alterations are manifestation of the secondary immunodeficiency that is observed for the influence of cytotoxic agents and during tumor growth and that can be characterized as stress [16]. The application of an AMF in doses inducing an antistress activation reaction result in stimulation of lymphocytes and epithelial components of the thymus, as is evidenced by the high number of lymphoblasts, elevated proliferative activity, and a certain increase in the number of Hassall's corpuscles.

REFERENCES

1. G. G. Avtandilov, *Medical Morphometry* [in Russian], Moscow (1990).
2. G. G. Aminova, L. M. Erofeeva, and E. B. Simakova, *Arkh. Anat.*, **44**, № 3, 66-69 (1986).
3. L. V. Beletskaya, E. V. Gnezditskii, and D. L. Belyaev, *Usp. Sovr. Biol.*, **102**, № 1, 82-96 (1986).
4. I. M. Belyakov, A. A. Yarilin, and O. I. Kuz'menok, *Immunobiologiya,* № 6, 3-12 (1992).
5. N. V. Vasil'ev, L. F. Boginich, and G. G. Simeeva, *Arkh. Patol.*, **23**, № 9, 47-51 (1971).
6. N. V. Vasil'ev and V. V. Yakovleva, in: *Role of the Thymus in the Regulation of the Organism's Functions* [in Russian], Tomsk (1985), pp. 14-16.
7. L. Kh. Garkavi, in: *Adoptive Reactions and Resistance of the Organism* [in Russian], Rostov-on-Don (1990).
8. L. Kh. Garkavi, E. B. Kvakina, and M. A. Ukolova, *Ibid.*
9. L. Kh. Garkavi and E. B. Kvakina, *Magnitologiya,* № 2, 3-11 (1991).
10. Yu. A. Grinevich and I. F. Labunets, in: *Stress and Immunity* [in Russian], Leningrad (1989), p. 62.
11. O. V. Zairat'aynts, Kh. V. Khavinson, and L. G. Kuz'menko, *Arkh. Patol.*, **52**, № 1, 25-28 (1990).
12. M. P. El'shanskaya, *Probl. Tub.*, № 5, 58-63 (1985).
13. T. E. Ivanovskaya and L. I. Katasonova, *Arkh. Patol.*, **48**, № 1, 3-9 (1986).
14. B. I. Kuznik, N. V. Vasil'ev, and N. N. Tsybikov, *Immunogenesis, Homeostasis, and Nonspecific Resistance* [in Russian], Moscow (1989).
15. N. A. Yurina, in: *Stress and Immunity* [in Russian], Leningrad (1989), p. 275.
16. Z. Kameneva, *The Thymus Gland* [in Russian, trans. from Bulgarian], Moscow (1984).
17. M. D. Kendall, *The Thymus Gland,* London (1981).