MORPHOLOGY AND PATHOMORPHOLOGY

Morphofuctional Aspects of Antitumor Activity of Low-Intensity Microwave Resonance Radiation in Experiment

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Effects of low-intensity microwave radiation with bioactive frequency were studied in rats with transplanted sarcoma 45. After exposure to microwave radiation in the regimen of activation therapy, the antitumor effect was observed in 50% animals (marked inhibition of tumor growth or its partial regression). Light and electron microscopy revealed destructive changes in the tumor tissue, accumulation of activation of immune system cells, and signs of the increase in tumor cell differentiation.

Key Words: *microwave resonance radiation; activation therapy; antitumor effect; cell differentiation; electron microscopy*

Conventional methods of antitumor treatment, radiation and chemotherapy, are known for their immunosuppressive and toxic effects, therefore the search for complement and alternative treatments is still an urgent problem. Long-term studies showed the possibility of substantial improvement of antitumor resistance of the organism using low-intensity electromagnetic stimulation of brain structures inducing the development of nonspecific antistress adaptive responses [1,3]. These integral antistress responses promote activation of the immune and endocrine systems and improve nonspecific antitumor resistance. In this connection, methods of effective employment of low-intensity electromagnetic waves were developed; these methods are focused on creation of conditions for the development of antistress adaptive responses in the body (activation therapy regimen) and imply the use of radiation with bioactive frequencies [1], *e.g.* microwave resonance radiation (RR), recently discovered in connection with discovery of SPE-effect and associated with water component of biological fluids [4]. The frequency of this radiation generated by water media in response to exposure to bioactive millimeter radiation is close to 1 GHz. Biomedical effects of RR are poorly studied. Here we evaluate the potentialities of RR as a factor of antitumor therapy and present first results concerning the effects of RR used in activation therapy regimen on experimental primary tumors in animals.

MATERIALS AND METHODS

Experiments were performed on 18 white outbred mature male rats weighing 180-200 g with transplanted sarcoma 45. The tumor (0.5 ml 30% suspension of tumor cells in physiological solution) was transplanted subcutaneously into dorsal lateral right surface of the

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back. In the experimental group (n=12), exposure to RR with 1 GHz frequency and 50 μW/cm^2 power flux density was performed using Aquatone apparatus after the tumor reached 0.7-1.2 cm^3 in size. Animal head was exposed to radiation for 3-4 weeks (4 sessions per week). The exposure varied from 3 to 10 min in accordance to activation therapy regimens to attain the development of antistress adaptive reactions [1]. The state of adaptive reaction was assessed 1-2 times per week using white blood cell differential count [1]. Tumor volume was assessed 3 times per week using conventional procedure (using Shrek formula for ellipsoids: abcπ/6 cm^3, where a, b, and c – maximal diameters of the tumor node (in cm) in 3 perpendicular directions). Control group comprised 6 rats with sarcoma 45, which were not exposed to any influences. By the end of the experiment, the rats were decapitated under ether anesthesia. Tumors were separated and fixed in Carnoy solution and paraffin blocks were prepared according to conventional procedures. For light microscopy, the sections were stained with hematoxylin and eosin and van Geison's picrofuchsin. For electron microscopy, the material was taken layer-by-layer from the periphery to the center of the tumor, and correspondently labeled. Double fixation with buffered glutaraldehyde and OsO_4 solutions and sudsequent treatment were performed routinely. Ultrathin sections obtained using precision ultratomy on UNTP-4 ultramicrotome, were examined under Philips EM 208 electron microscope. Morphometric analysis employed automated measuring complex SAGA, based on personal computer. Tumor area was measured at ×20 (every third field of view) and tumor cell composition was measured under immersion lens ×100 (every 10th field of view).

Significance of differences for parameters with normal distribution was assessed using Student's t test.

RESULTS

Mean volume of sarcoma 45 in the control group by the end of experiment was 11.0±1.2 cm^3. Predominant adaptive reaction in this group was chronic stress according to Selye, which was characterized by marked immunosuppression judging from microscopy data of the immune system organs, thymus and spleen, significant lymphoid hypoplasia, numerous groups of degenerating lymphocytes, disturbances in lymphocyte maturation and differentiation, and normal microvascular bed in the studied organs.

In the experimental group, the course exposure to RR in activation therapy regimen resulted in antitumor effect in 50% of cases (n=6). It consisted in tumor growth inhibition by 83% (n=4; p<0.05) or its partial regression by 30-40% (from 1.5-1.6 to 0.9-1.0 cm^3 in 2 animals). In case of tumor growth inhibition, sarcoma 45 volume at the end of the experiment did not exceed 2 cm^3. In other experimental rats, tumor sizes did not differed from that in control animals. In addition, RR antitumor effect clearly correlated with the development of adaptive reactions of normal and advanced activation [1]. These antistress reactions (mainly reaction with advanced activation) are known to promote significant increase in nonspecific antitumor resistance. They possess common features and some systemic differences. In contrast to stress-reaction, they are characterized by activation of lymphoproliferative processes and cell-cell interactions in organs of the immune system [1]. Thus, the relative content of lymphocytes in the peripheral blood of animals with inhibition or partial regression of sarcoma 45 was 60-80%. In the thymus we observed enlargement of thymic lobes, prevalence of the cortex over medulla, pronounced layer of lymphoblast forms in the subcapsular zone (Brachet staining revealed cytoplasm-abundant pyroninophilic lymphoid cells), signs of activation of tissue basophilic leukocytes and intensification of their contact with thymocytes. In the spleen, increased number and sizes of germinative centers, signs of mitotic activity, widening of thymus-dependent zones, formation of complexes between splenocytes and macrophages in the red pulp were observed. In the adrenals, these reactions, unlike stress reactions, were not associated with pronounced hyperplasia of the cortical layer and signs of suppression of mineralocorticoid function. Changes in other endocrine glands, also with marked features characteristic for normal and advanced activation [1], were not studied here.

TABLE 1. Microimaging Changes in Sacroma 45 Tissue following Growth Inhibition and Partial Tumor Regression under the RR Infleuence (Light Microscopy)

Group	Capsule		Cell density	Collagen in intercellular space	Lymphocyte infiltration intensity
	thickness, μ	integrity			
Control	<45	Disrupted	High	Trace	Low
Experimental	200-300	Preserved	Low	Plenty in majority	Moderate and high

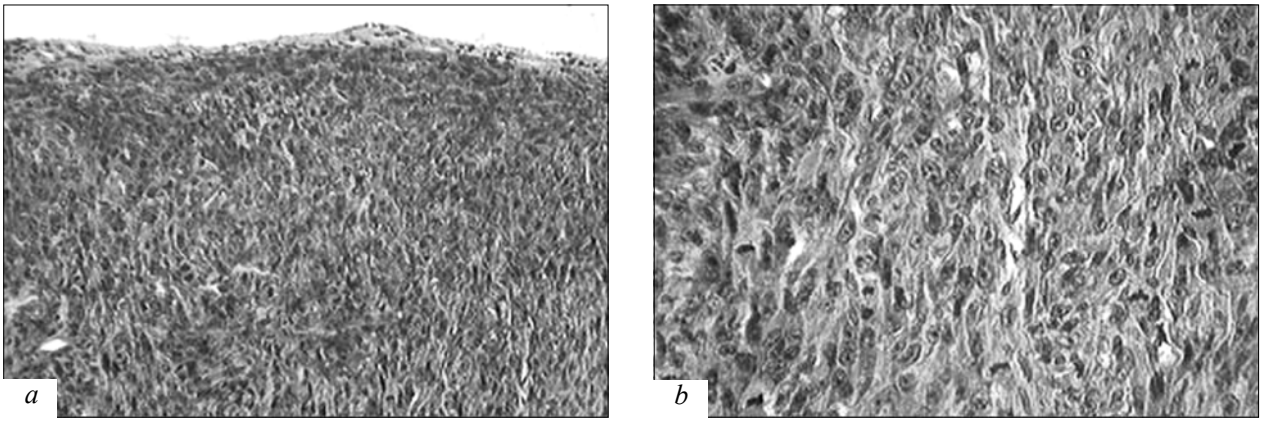

Fig. 1. Growth of sarcoma 45 in the control group. Hematoxylin and eosin staining. *a*) solid mass of tumor cell under thin capsule, ×200; *b*) spindle-shaped tumor cells are densely packed, intercellular spaces are small, numerous mitotic figures are seen (10 in this field of view), ×400.

Histological pattern of tumor node in control animals (Table 1, Fig. 1, *a, b*) was characterized by thin capsule <45 μ (Fig. 1, *a*) interrupted in sites of tumor cell invasion into the adipose tissue, dense distribution of chaotically oriented spindle-shaped cells with large nuclei, several nucleoli, numerous mitosis figures, and relatively low cytoplasm volume (Fig. 1, *b*). van Geison's staining revealed rare weak collagen traces. All that features were indicative of active proliferation and low differentiation degree in tumor cells. Lymphocytoplasmocytic infiltration was very uncommon among tumor cells. Vast hemorrhagic areas resulting from vessel erosion were observed in the tumor, which led to the development of cavities filled with hemorrhagic content and necrotic masses in the central part of the tumors.

Changes in the tumors of animals with marked inhibition and partial regression of the tumor after exposure to RR had some similar features (Table 1, Fig. 2, *a*, *b*). In both cases, the capsule was significantly thickened (Table 1, Fig. 2, *a*). Tumor cells contained more cytoplasm and intercellular spaces also increased (Fig. 2, *b*). Mitoses were rare (individual figures in cases of partial regression). Immune cells infiltrating the tumor were observed in the capsule, immediately below it in the mature connective tissue zone, and in

TABLE 2. Ratio of Main Structural Components of Tumor Node after Growth Inhibition and Partial Regression of Sarcoma 45 under the Effect of RR (%; *M*±*m*)

Group	Structural component			
	Connective tissue	Tumor cells	Necrosis zones	Hemorrhage
Control	1.4±0.1	69.5±3.8	19.1±2.2	10.0±1.5
Experimental	46.1±16.8*	44.1±19.6*	6.3±2.3*	3.4±2.6*

Note. Here and in Table 3: *p<0.05 in comparison with the control.

TABLE 3. Mitotic Activity and Cell Composition of Tumors after Growth Inhibition and Partial Regression of Sarcoma 45 under the Effect of RR

Group	Cell density per field of view	Mitotic activity, ‰	Per 1000 tumor cells	
			lymphocytes	plasmocytes
Control	42.0±6.5	14.4±9.3	50.6±5.3	0
Experimental	22.1±2.8*	9.3±0.9*	141.5±17.6*	6.2±1.1*

Note. Area of 1 field of view 6787 μ^2.

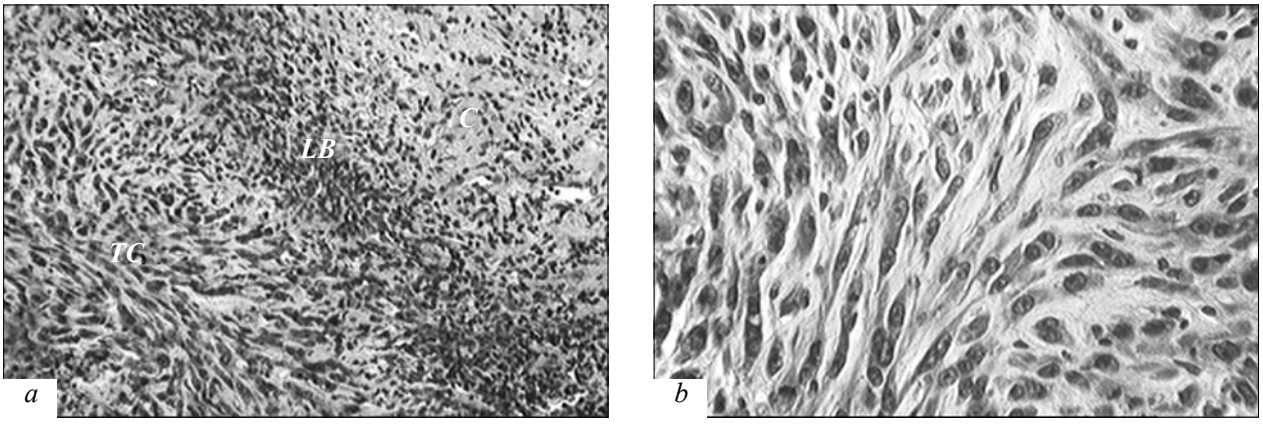

Fig. 2. Partial sarcoma 45 regression under the influence of microwave RR. Hematoxylin and eosin staining. *a*) thick capsule (C) infiltrated with lymphocytes covers lymphocytic barrier (LB) at the border of tumor cell layers (TC), ×200; *b*) spindle-shaped tumor cells with large nuclei and abundant cytoplasm, wide intercellular spaces filled with collagen are observed between them, ×400.

the immature connective tissue zone in the form of a lymphocytoplasmocytic barrier (145-165 μ wide) at the border with the tumor (Fig. 2, *b*). Lymphocytes and plasmacytes were also seen among tumor cells. Migrating lymphocytes were commonly observed in vessels located among tumor cells. The central part of the tumor had no necrotic cavities, hemorrhages and necroses were uncommon and were local.

Morphometry provided quantitative parameters of these changes for both cases with growth inhibition and tumor regression. Thus, we observed significant reduction in parenchyma area (by 1.6 times), density of tumor cells (by 1.9 times), mitosis number (by 1.5 times), sizes of necrosis and hemorrhage areas (by approximately 3 times), and dramatic increase in relative stroma area (by 32 times, Tables 2, 3). Exposure to RR

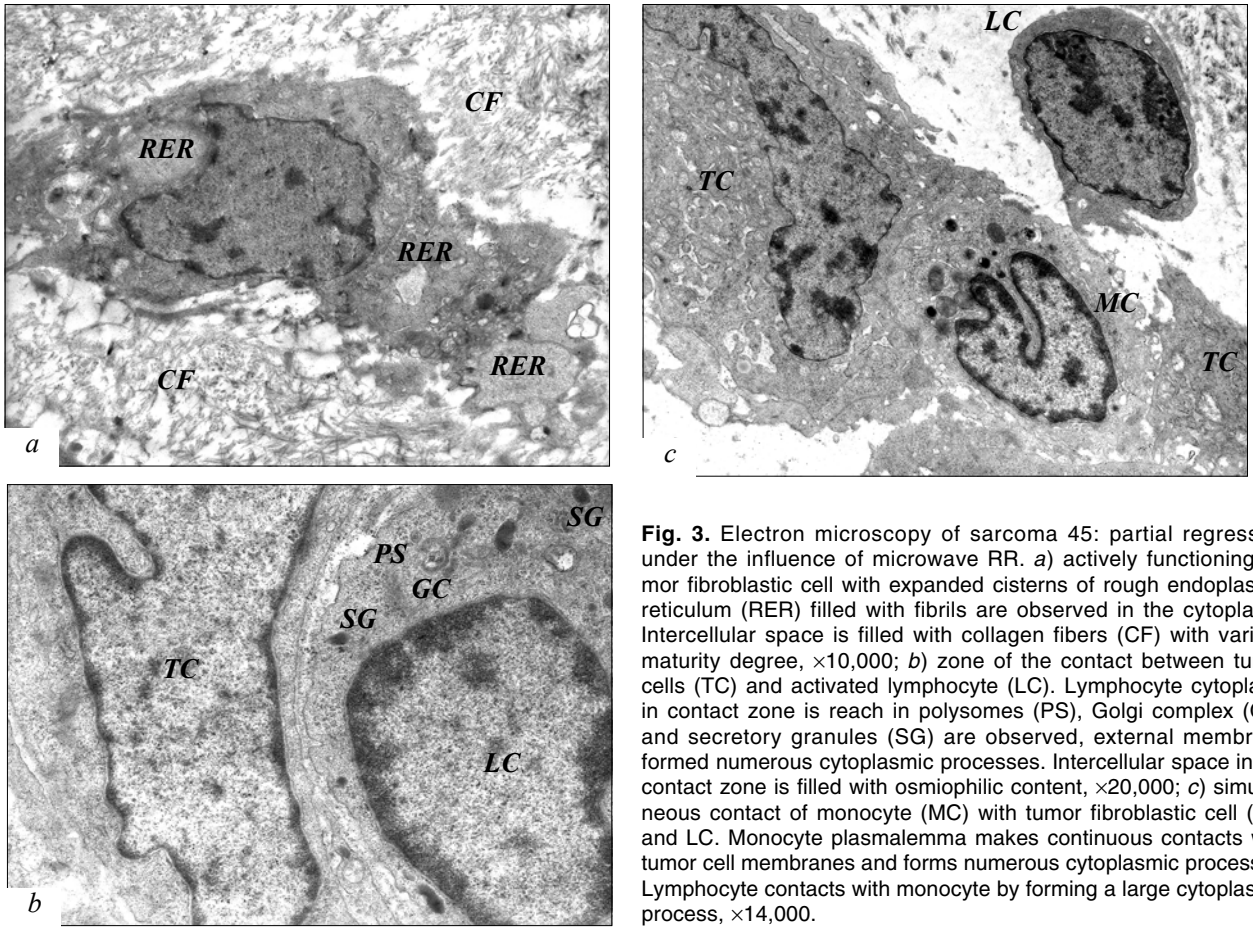

Fig. 3. Electron microscopy of sarcoma 45: partial regression under the influence of microwave RR. *a*) actively functioning tumor fibroblastic cell with expanded cisterns of rough endoplasmic reticulum (RER) filled with fibrils are observed in the cytoplasm. Intercellular space is filled with collagen fibers (CF) with various maturity degree, ×10,000; *b*) zone of the contact between tumor cells (TC) and activated lymphocyte (LC). Lymphocyte cytoplasm in contact zone is reach in polysomes (PS), Golgi complex (GC) and secretory granules (SG) are observed, external membrane formed numerous cytoplasmic processes. Intercellular space in the contact zone is filled with osmiophilic content, ×20,000; *c*) simultaneous contact of monocyte (MC) with tumor fibroblastic cell (TC) and LC. Monocyte plasmalemma makes continuous contacts with tumor cell membranes and forms numerous cytoplasmic processes. Lymphocyte contacts with monocyte by forming a large cytoplasmic process, ×14,000.

resulted in almost 3-fold increase in the number of immune cells infiltrating the tumor (Table 3).

It should be noted in animals with tumor growth inhibition and partial regression under the effect of RR, widened intercellular spaces were filled with collagen fibers (confirmed by van Geison's staining), which together with the data on the increase in cytoplasm volume and decrease in mitotic activity attested to higher degree of cell differentiation.

Electron microscopy confirmed this assumption. Thus, in control rats intercellular spaces appeared to be empty, sarcoma 45 cells had dense cytoplasm with low content of organelles and poorly developed endoplasmic reticulum. Growth inhibition and tumor regression were associated with the appearance of numerous cisterns of endoplasmic reticulum filled with fibrillar content, which indicates it active synthesis in the cell (Fig. 3, *a*). Exit of collagen protofilaments to the intercellular space was often observed at the borders of the cells. Dying cells with marked signs of dystrophy and degeneration were regularly observed, as well as fragments of destroyed cells embedded in previously synthetized and released collagen. Numerous contacts of cytoplasmic processes with tumor cell surfaces were noted (Fig. 3, *b*). Sometimes sarcoma 45 cells were in contact with several lymphocytes. Moreover, simultaneous immune cell contacts with each other (lymphocyte-lymphocyte, lymphocyte-macrophages) and with tumor cells were often observed (Fig. 3, *c*).

Tumor maturation under direct influences of various compounds or physical factors was noted both in cell culture and animal experiments and in clinical practice. Currently, tens of chemical compounds, biological products, and physical factors are known to affect differentiation of the cell of various genesis both *in vitro* and *in vivo* [5]. Interestingly, action on the tumor was indirect in this experiment and had no damaging character. Numerous intercellular contacts revealed by electron microscopy suggest that tumor cells contain certain factors stimulating differentiation of tumor cells. This completely agrees with published data on the key role of lymphocytes (particularly T lymphocytes) in the regulation of cell growth and differentiation in not only immune cells, but also all somatic cells [2].

Thus, obtained results suggest that exposure of some brain structures of rats with sarcoma 45 to RR in activation therapy regimen leading to the development of antistress adaptation reactions of normal and advanced activation, may result in immune cell activation and intensification of their interaction with tumor cells. This results in not only directs damaging of tumor cells, but also increases the degree of their differentiation and, consequently, reduction in their life span, which leads to inhibition of growth and partial regression of sarcoma 45.

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