The pattern of the immune processes during activation therapy with the use of microwave electromagnetic radiation

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Abstract

The mechanisms of anti-tumor action of low intensity factors of activation therapy remain little investigated. The aim of the present paper is to study the changes in the immune system organs, the state of blood leukocytes and tumor area under effective activation therapy in tumor-bearing animals using EMR microwave range. The research is relevant due to the immune processes influence on cardiovascular system performance.

Materials and methods

In experiments on 77 albino outbred male rats with inoculated sarcoma-45 and Pliss lymphosarcoma tumors low intensity EMR of microwave and EHF ranges with bioeffective frequencies has been used as an activation therapy factor. The structural-functional changes in the immune system organs, some indices of blood leukocyte activity, composition of immune cells infiltrating tumors when inhibiting their growth and regression have been studied. Besides, the signs of intercellular interactions with participation of leukocytes in the tumor area have been considered.

Results

The pronounced anti-tumor effects in the studied EMRs are related to their anti-stressor action and, as a rule, accompanied by an increase in lymphoproliferative activity and intercellular interactions in thymus and spleen. Under the effect of microwave resonance radiation (1 GHz) noted are changes in blood lymphocytes activity, which precede the tumor regression beginning, as well as numerous contacts between malignant cells and leukocytes in regressing tumors. Peculiarities of large tumors regression and changes in thymus and state of blood lymphocytes, NK cells, monocytes and neutrophils influenced by modulated EHF EMR are described.

Conclusions

Immune mechanisms of anti-tumor effects in the studied EMR microwave ranges as activation therapy factors may be related to the following basic processes: increase in tumor cell differentiation under the effect of the immune cell factors, development of the antigen presentation processes and effective destruction of tumor cells by apoptosis induction, cytotoxic action of natural killer cells, macrophages and other immune system cells and their factors.

Keywords

Activation therapy, Microwave range electromagnetic radiation, Inoculated tumor, Anti-tumor effect, Immune processes, Thymus, Leukocytes

Introduction

Malignant process causes the multilevel system changes in the organism. The intensity of these changes depends on the stage of development and localization of a tumor. At the same time the earliest disorders occur in the neuroendocrine and immune systems later on influencing the performance of other organs and systems. A close relation between the cardiovascular and neuroendocrine systems is well-known. In recent years the immune processes’ influence on the cardiovascular system performance and pathology development becomes also evident [1, 2]. Thus, the type of changes in different parts of the immune system under malignant processes and anti-tumor actions may be very important for dynamics of state of cardiovascular and other systems in the tumor-bearing organism.

It has been demonstrated before that the activation electromagnetotherapy contributes to improvement
in the regulatory system state and effective mobilization of anti-tumor resistance mechanisms [3–5]. At the same time the irreversible malignant cell injury, often observed in the experiment, may above all be caused by the immune system factors. It denotes the necessity of investigation of the immune processes, which assist the anti-tumor effects of the activation electromagnetotherapy.

The aim of the present paper is to study the changes in the immune system organs, the state of blood leukocytes and tumor area under effective activation therapy in tumor-bearing animals using microwave electromagnetic radiation (EMR).

Materials and methods

Examination has been carried out in 77 albino out-bred male rats weighing 180-300g with inoculated sarcoma 45 (23), Pliss lymphosarcoma (47) as well as in animals without tumors (7). The used tumors differed in histological structure, development rate and invasion depth. The EMR of centimeter and millimeter (extremely high frequency, EHF) ranges have been applied. The experiments have been conducted in accordance with international ethical standards. The electromagnetotherapy course duration is 3.5–4 weeks. The actions have been carried out in accordance with the activation therapy principles [3–5].

In the experiments in 23 rats with inoculated sarcoma-45 applied has been resonance radiation (RR), low intensity (less than 50 µW/cm²) EMR of centimeter range, the frequency (1 GHz) of which coincides with the frequency of water-containing media self-radiation, generated by the EMR of millimeter range [6]. “Aquaton” device has been used for that. A head and tumor area have been sequentially exposed (double exposure) for 3–10 min.

Low intensity (not more than 10 mW/cm²) modulated by EHF EMR with 42.2 GHz frequency served as an additional factor. For modulation the 7.8 Hz frequency signal of Schumann resonance spectrum or the sequence of signals with pairwise multiple bioeffective frequencies of 1.7, 3.4, 7.8 and 15.6 Hz have been used [7]. The occipital region of head (hypothalamus projection area) has been influenced with changes during 15–30 min. depending on blood leukogram and animal's behaviour in the chamber. For that purpose the “Yav 1” device for EHF therapy with an additional ferrite isolator and the modulator developed in Rostov Research Institute of Radio Communication on the basis of the special form signal generator G6–37 have been used. The electromagnetotherapy course started with minimal exposure 3 days before tumor inoculation.

During the research studies the change in tumor size has been defined by calculating the volume according to Schreck formula for ellipsoids. The animals state has been evaluated according to dynamics in type and tension of general non-specific adaptation reactions (AR) of the organism, which is determined using the hematologic indicators, namely blood leukogram parameters, calculated for 200 cells [3, 8]. During the stages of the experiment in the peripheral blood lymphocytes activity of basic enzymes dehydrogenases, namely, succinate dehydrogenase (SDH) and alpha-glycerophosphate dehydrogenase (α-GPDH), which participate in significant energetic processes of cell metabolism [9], has been studied. The functional activity of natural killers (NK-cells) has been evaluated using the cytotoxic test [10]. Herewith the lymphocytes isolated in a ficoll-verografin density gradient (ρ 1,077–1,078) of rats blood have been used as effectors, and the cell culture of human erythromyeloleukosis K562, obtained by Research Institute of Clinical Immunology RAMS (Moscow), has been used as target.

Besides, evaluated have been the indicators characterizing activity of the phagocytic part of the immune system, namely, the phagocytic activity (PA – percentage of phagocytic cells), the phagocytic number (PN – average quantity of test objects absorbed per one phagocyte) and the intensity of oxygen dependent reactions of “respiratory explosion” which is evaluated in rats blood neutrophils and monocytes with the help of spontaneous and stimulated (by zymosan or latex) test of nitro blue tetrazolium reduction to dipharmaen (NBT test) with calculation of NBT stim./NBT spont. coefficient [11]. We would like to emphasize that the above indicators have been used with the aim of indirect, approximate evaluation of the blood neutrophils and monocytes functional state.

At the end of the experiments the animals were over-narcotized with ether; organs and blood were investigated. During morphologic and morphometric investigation of the immune system organs and tumor tissue the method of Brache staining modified by R.A. Simakova to identify nucleoproteins as well as the hematoxylin-eosin staining have been used. At the same time in thymus determined have been the indicators reflecting the lymphoproliferative processes activity, i.e. lobule size, width of cortical and medullary sub-
stances, as well as the stromal-parenchymatous coefficient. The prevalence of contacts between tissue basophils and thymocytes has been evaluated. In spleen defined have been the relative size of germinal centers and relative number of complexes (associates) of macrophages and lymphocytes.

The anti-tumor efficiency of activation electromagnetotherapy has been evaluated with the help of the following indicators: percentage of tumor growth inhibition, relative number of regression cases, intensity of regression, intensity of degenerative-dystrophic and necrotic changes in tumor cells, qualitative and quantitative composition of immune cells infiltrating the tumor. In the studies with resonance radiation (RR) the electron microscopic investigation of tumor tissue has been carried out with the help of the electron microscope Philips EM208.

For statistical processing of the obtained results the Student's t-criterion and Wilcoxon–Mann–Whitney criterion have been applied.

**Results and discussions**

The examined microwave range EMR had an evident anti-tumor effect on a number of animals, accompanied by rather significant change in central and peripheral parts of the immune system.

**Multilevel immunotrophic effects in case of resonance radiation exposure on rats with sarcoma-45**

Thus, the RR exposure proved to be effective for 77% of animals. Besides, complete or practically complete (85–95%) regression of sarcoma-45 has been observed in a third of animals. The remaining 6 rats showed in equal shares a partial regression of tumor (two times) or 40% inhibition of its growth. It is necessary to note that an anti-tumor effect of the given factor has been related to its anti-stressor action, i.e. development of anti-stressor AR of calm and elevated activation without evident signs of tension during the last stages of the experiment, when the AR of stress

**Fig. 1. Changes in thymus under the influence of resonance radiation (RR).** A. Growth in sarcoma-45 in the reference group: hypoplasia of lymphoid tissue with significant disorder in lobules structure and expansion of interlobular connective tissue segments, signs of hemostasis. AR of stress. B. Partial regression of sarcoma-45 influenced by RR: increase in lymphocellular density and expansion of cortical substance area in lobules. C. Complete regression of sarcoma-45 under the influence of RR: abundance of tissue basophils in interlobular connective tissue, their contacts with thymocytes. AR of elevated activation. Brache. Magn. X400.

prevailed in the reference group of animals (70–80% cases).

It should be noted that we have already considered the problem of greater efficiency of RR with double localization in comparison with the influence of this factor in case of its localization only on the head or tumor area as well as the problem of firstly detected possibility for low intensity EMRs to lead to an increase in a degree of differentiation of tumor cells, thus reducing their life cycle demonstrated exactly for RR [12, 13].

In the present research we are interested, first of all, in the changes occurring in the immune system of the tumor-bearing rats and accompanying the anti-tumor effect of RR. The results, obtained during studying micro pictures of thymus and spleen under the RR effective influence, corresponded to the information in literature concerning changes in the immune system organs in case of tumor regressions under the effect of activation therapy with ultra-low frequency EMRs [3, 4, 12]. As opposed to hypoplasia and degenerative disorders, typical for thymus and spleen under tumor stress (Fig.1A and 2A), in the cases of effective influence of resonance EMR in these organs high lymphoproliferative activity (Fig. 1B and 2B) and evident signs of intercellular interaction activation with participation of tissue basophils in thymus (Fig. 1C) and macrophages in red pulp of spleen (Fig. 2C) have been observed.

Changes in the immune system of the animals with the most evident effect of RR, i.e. the tumor regression, have been also observed in blood lymphocytes (Fig. 3). When examining the activity in basic enzyme-dehydrogenases of SDH and α-GPDH tricarboxylic acid cycle in mitochondria of these cells 10 days after starting the exposure, increase in SDH activity has been detected. The SDH activity increased by 1.4 times (p < 0.05, Fig. 3A) as compared with the data of the reference group. It has shown the activation of metabolic processes in blood lymphocytes under the effect of RR during the stages preceding tumor regression. After finishing the exposure sessions in animals with regressing tumors marked has been almost a 1.5 time increase in ratio between SDH and α-GPDH activity indicators as compared with rats in the reference group (р < 0,05, Fig. 3B).

Thus, the anti-tumor effect of RR is evidently related to the changes in central (thymus) and peripheral (spleen, blood) parts of the immune system. An effective influence of RR on central and peripheral parts of neuroendocrine and immune systems ended in mobilization of effector anti-tumor mechanisms and directly in the area of malignant process localization. In vessels, located close to tumor cells, the lymphocytes...
migrating through the tissue have been frequently observed. As a result of morphometric study of sarcoma-45 tissue with inhibition of growth demonstrated has been a significant (2.9 times, p < 0.05) increase in a number of lymphocytes and plasmocytes, infiltrating deep layers of tumor (Fig. 4).

In cases of effective RR exposure the immune cells infiltrating the tumor have been observed in the capsule, directly under it in the area of mature connective tissue, and in the form of lymphoplasmacytic barrier in the area of young connective tissue (width 145–165 μm) of the tumor boundary. At the same time immune cell infiltration under partial regression has been more intensive than under inhibition. The qualitative composition of cells also increased. In addition to lymphocytes and plasmocytes (Fig. 5A) the tissue basophils and numerous macrophages have also been observed (Fig. 5B).

In our opinion, the noted rise in a number of lymphoid and macrophage elements, as well as other immune system cells in the tumor area, is a result of the intercellular interactions activation with the participation of leukocytes and tumor cells under the influence of RR, which has led to malignant cells elimination by means of their direct destruction and increase in the degree of their differentiation, the signs of which have been described by us earlier [12, 13].

Thus, when investigating the tumor tissue under electron microscope numerous contacts between the activated lymphocytes cytoplasmic outgrowths and
the tumor cells surface (Fig. 6A) are observed. Besides, in some cases several lymphocytes contacted with one sarcoma-45 cell. Moreover, frequently observed are simultaneous contacts of the immune system cells with each other (lymphocyte-lymphocyte, lymphocyte-macrophage) and with the tumor cells. Thus, in the electron-diffraction pattern herein (Fig. 6B) we see the monocyte contacting simultaneously with several tumor cells and forming a cytoplasmic bridge with the lymphocyte.

The detected at the electron microscopic examination numerous intercellular contacts allow assuming the presence of the factors in immune cells which lead to the differentiation degree increase and, as a consequence, to decrease of the tumor cell life cycle. It corresponds to modern literature data which proves the leading role of lymphocytes (especially, T-lymphocytes) in the regulation of cellular growth and differentiation of not only immune but any somatic cells in the organism [14]. Quite probable are the other effector mechanisms of tumor cells elimination under the influence of RR, which are related to different variants of cytotoxicity in lymphocytes, macrophages and other immune system cells, as well as to production of cytokines by these cells and any other active factors directly or indirectly damaging the tumor cells [15].

Changes in the immune system and dynamics of regression of Pliss lymphosarcoma of different size under the effect of modulated EHF EMR

Another investigated acting factor, namely, the modulated EHF EMR, turned out less effective compared with the RR in rats with formed sarcoma-45 of 0.7–1.0 cm$^3$ volume, than the RR [7]. Noted has been an unstable 43 % inhibition of tumor growth ($p<0.05$) during the 2-nd week of exposures as well as 1.2–1.3 times increase in number and area of segments of degeneratively changed sarcoma-45 cells after finishing the exposures ($p<0.05$) as compared with the indices in the reference group of animals. Such an effect correlates to the improvement in the AR type and the lymphoproliferative process activation in the immune system organs. However, the EHF EMR effect in the studied mode of application turned out to be insufficient for mobilization of the effector anti-tumor immune mechanisms and tumor cells destruction.

It has been a success to obtain a significantly more evident effect of modulated EHF EMR on the mech-
anism of anti-tumor resistance with a part of male rats with inoculated Pliss lymphosarcoma in case of starting the exposure 3 days before the tumor inoculation. As it has already been mentioned, two modes of low frequency modulation, namely mono-frequency modulation by signal 7.8 Hz (group 1, n = 18) and poly-frequency modulation with the use of a sequence of signals of paired multiple frequencies of 1.7–3.4–7.8–15.6 Hz (group 2, n = 18) have been used in the experiment. Besides, conventionally used dilution (to 20 %) has been 10 times increased (to 2 %) in order to prolong the period of detectable involvement of the tumor to obtain more accurate evaluation of the acting factors influence. It is known that Pliss lymphosarcoma is characterized by rapid growth and active infiltration of the neighbouring tissues, as well as by practical absence of cases of spontaneous regression even in very small tumors (not exceeding 0.03 cm³ in volume, the so-called “pea”). We supposed that the low frequency modulation of the high-frequency signal could slow down the tumor formation and invasion processes that would lead to an increase in animals’ life expectancy. However, the result of this experiment turned out to be different and was related to not known earlier delayed reactions of the tumor-bearing organism on the modulated EHF EMR, which manifested themselves in complete or partial regression of the tumor.

The majority of animals in two main groups under exposure during the first five days after detectable involvement of the tumor had the same rate of the tumor growth as the reference group. Some cases in group 2 (EHF EMR with poly-frequency modulation) were an exception. In that group noted was presence of the smallest tumors not exceeding 1.3 cm³ volume whereas with all other animals the lymphosarcoma volume was not less than 2.4 cm³. More evident effect of the EHF EMR with poly-frequency modulation manifested itself on day 6 after inoculation, when 3 animals (17 %) had regression of the tumors with size up to 2 cm³. Since then, the EHF EMR effect has been noted in other animals: on the eighth day 4 males (22 %) from group 2 showed partial (40–50 %) regression of 11.3–13.5 cm³ tumors, 6 animals (33 %) from group 1 where the EHF EMR with mono-frequency modulation (7.8 Hz) had been used showed complete or 80 % (and more) evident regression of 4.9–6.1 cm³ tumors. Thus, a partial, evident or complete regression of tumors has been observed in approximately one third (6–7 of 18) male rats in each group where the EMR has been applied. At the same time, a partial, evident and complete regression of large tumors (4.9–13.5 cm³) occurred quite rapidly (4–7 days), and no signs of intoxication were noted. In other animals in main groups the tumor growth rate did not differ from the malignant process development in male rats in the reference group.

Visually different peculiarities of changes in tumors in case of their complete and partial regression under...
the effect of EHF EMR are demonstrated in Fig. 7. For cases of partial regression of Pliss lymphosarcoma with a size of more than 10 cm (group 2) typical was formation of “botryoidal” structure of regressing tumor, as well as formation of a capsule, which clearly marks the boundary between the tumor and the neighbouring tissue. Thus, mobility of formation and absence of its invasion into skin was provided (Fig. 7B). In cases of complete regression of Pliss lymphosarcoma some kind of tumor “drying” occurred, at the same time a little invasion to the skin in a form of local injure (wound) in microvessels of skin outer layers has been observed (Fig. 7C).

As in case with the RR application, the anti-tumor effect of the modulated EHF EMR manifested itself only against the background of anti-stressor action of these factors. 2 weeks after the detectable appearance of the tumor in all the reference group tumor-bearing rats the AR stress has been noted, whereas in the main groups the anti-stressor AR have been observed in more than a half of the cases.

Since then, the animals in main groups 1 and 2 have been divided into 3 subgroups, namely A, B, and C-subgroups depending on the EHF EMR effect intensity with different mode of modulation. 6 male rats with complete tumor regression have been included in subgroup A – 3 animals from group 1 and 3 animals from group 2. Subgroup B consisted of 7 animals, of which 3 female rats with evident tumor regression were from group 1 and 4 male rats with

Table 1. Correlation between some indicators of lymphocytes functional activity and total number of leucocytes in blood under different intensity of anti-tumor EHF EMR effect

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Subgroup A (n=6)</th>
<th>Subgroup B (n=7)</th>
<th>Control (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDH</td>
<td>9,7±0,6 ●</td>
<td>13,6±1,0</td>
<td>12,1±0,8</td>
</tr>
<tr>
<td>α-GPDH</td>
<td>11,0±0,2 ●</td>
<td>12,1±0,7 ●</td>
<td>15,5±1,3</td>
</tr>
<tr>
<td>SDH/α-GPDH</td>
<td>0,93±0,01</td>
<td>1,13±0,03</td>
<td>0,79±0,14 T●</td>
</tr>
<tr>
<td>L (mm³)</td>
<td>14775±2661 T●</td>
<td>7750±1143</td>
<td>10375±475 T●</td>
</tr>
</tbody>
</table>

Legend: SDH, α-GPDH and SDH/α-GPDH respectively, activity of SDH and α-GPDH enzymes and their relation; L – leucocyte content in blood

Note: deviation from the reference indicator – ●, in subgroup B – ●, p < 0,05. T – p<0.1

Table 2. Some indicators of blood leucocytes activity in rats with Pliss lymphosarcoma in case of different intensity of the EHF EMR anti-tumor effect

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Subgroup A (n=6)</th>
<th>Subgroup B (n=7)</th>
<th>Control (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NBT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>spont.</td>
<td>28,0±1,4 ●</td>
<td>21,5±3,1</td>
<td>20,1±1,1</td>
</tr>
<tr>
<td>stim.</td>
<td>33,4±0,7</td>
<td>30,0±0,6</td>
<td>28±3,2</td>
</tr>
<tr>
<td>AI</td>
<td>1,1±0,0 ●</td>
<td>1,4±0,1</td>
<td>1,3±0,1</td>
</tr>
<tr>
<td>phagocytosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>38,0±4,3 ●</td>
<td>19,0±0,7</td>
<td>20,3±1,4</td>
</tr>
<tr>
<td>PN</td>
<td>2,3±0,1 ●</td>
<td>1,5±0,1 ●</td>
<td>2,2±0,1 ●</td>
</tr>
<tr>
<td>Monocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NBT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>spont.</td>
<td>29,4±8,9</td>
<td>11,5±0,3 ●</td>
<td>19,9±1,0 T●</td>
</tr>
<tr>
<td>stim.</td>
<td>33,2±7,8</td>
<td>16,4±1,1 ●</td>
<td>22,5±0,3 T●</td>
</tr>
<tr>
<td>AI</td>
<td>1,1±0,1 ●</td>
<td>1,4±0,0 ●</td>
<td>1,1±0,1 T●</td>
</tr>
<tr>
<td>phagocytosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>28,1±1,2 ●</td>
<td>11,2±0,8</td>
<td>13,0±2,1</td>
</tr>
<tr>
<td>PN</td>
<td>3,9±0,2 ●</td>
<td>2,2±0,3</td>
<td>2,3±0,1</td>
</tr>
</tbody>
</table>

Legend: Spont., stim., AI are spontaneous, stimulated and activity index in NBT-test, respectively; PA is a phagocytic activity; PN is a phagocytic number;

Note: deviation from the reference indicator – ●, in subgroup B – ●, p < 0,05–0,01
partial lymphoscarcoma regression with a size of more than 10 cm<sup>3</sup> were from group 2. Subgroup C included 6 rats from group 1 and group 2. Those rats had no reduction in tumor size compared with the reference values. They were more active than the animals in the reference group having developed during the last 1.5 weeks of the experiment not only the AR stress, but also tensed anti-stressor ARs as well as absence of evident hypoplasia in thymus (Fig. 8).

In our opinion, rats with an evident EHF EMR effect, included into subgroup A and B, differed in degree of mobilization, type and phases of the immune anti-tumor processes development. In subgroup A the realization of anti-tumor effect modulated by EHF EMR, evidently, has been carried out to the full extent compared with subgroup B. Thus, rats in this subgroup differed from rats in subgroup B in significantly higher (2–4 times) activity of lymphoproliferative process in thymus (Fig. 8). Animals from subgroup B had an evident atrophy of some thymus lobules, whereas in other lobules the hypoplasia in the organ lymphoid parenchyma has been significantly less evident as compared with the reference group. At the same time the weighting factor of thymus has been significantly reduced in comparison with the rats in subgroups A and C (Fig. 8). The presence of evident contacts between tissue basophils and thymocytes (not less than 3–4 in majority of visual fields with magnification 400) has been observed in all the animals in subgroup A, whereas in subgroup B in some cases under partial tumor regression such contacts were single.

Differences between the animals in subgroup A and B were also noted when studying blood leukocytes functional activity (Table 1 and 2). The lowest activity of enzyme-dehydrogenases in lymphocytes (Table 1) and the highest phagocytic activity of neutrophils and monocytes (Table 2) have been noted in male rats in subgroup A. In Subgroup B the values of neutrophils phagocytic activity indicators have been the lowest, the decrease in monocytes phagocytic activity and leukocyte content in peripheral blood as compared with subgroup A (Table 1 and 2) have been observed. At the same time in both subgroups of animals with effective EMR action the indicator of the α-GPDH enzyme activity has been reduced by 22–29 % in comparison with the values in the reference group (Table 1).

In our opinion, the data on differences in the considered indicators in subgroup A and B animals, demonstrated in Table 1 and 2 and in Fig.8, may denote the phase changes in the blood thymocytes and leukocytes state, which accompany the anti-tumor effect of the modulated EHF EMR.

It is known that the accidental involution of thymus may be caused by not only lymphoproliferative processes inhibition but also tEMRorary migration of activated thymocytes from thymus due to the immune processes stimulation [16]. We can assume that on certain stages of the EHF EMR anti-tumor effect realization the migration of thymocytes occurred in tissue owing to their necessity to carry out systemic and/or local reactions.

Such an activation of the lymphocytes regulatory functions in tissues could evidently be the reason for decrease in weighting coefficient and thymus cortical substance width, reduction of total blood leukocytes content in animals of subgroup B, as well as a decrease in the α-GPDH enzyme activity in lymphocytes of the male rats in both considered subgroups, which is known for its close relation to realization of the regulatory functions [17]. After the tissue processes with participation of lymphocytes finished, there probably
occurred the restoration of thymocytes pool, thymus mass, and leukocytes level in blood (subgroup А). Herewith the restoration of the blood lymphocytes functional state could last longer than replacement of the lymphocytes pool in blood and thymus, that resulted in the lowest SDH activity in lymphocytes of the subgroup A animals, which experienced the complete realization of the considered potential.

A relatively high phagocytic activity of monocytes and neutrophils in blood of the subgroup A animals (Table 2) could be a reflection of mobilization of other, not lymphocytic, parts of the effector anti-tumor mechanisms influenced by EHF EMR. Probably, the activation of monocytes and neutrophils in subgroup A was close to its maximum, that could be proved by relatively low values of their activity indices in NBT-test.

Large size of most of regressing tumors, synchronism, delay in approach and dynamics of regression, as well as absence of intoxication signs in animals suggested activation of tumor-specific immunity with the help of modulated EHF EMR and elimination of tumor cells by apoptosis. This result corresponded to the most successful variants of immunotherapy of different malignant tumors [18]. At the same time complete regression of relatively small tumors (up to 1.7 cm³) under the effect of EHF EMR with poly-frequency modulation was, on the contrary, caused by the processes not related to the tumor antigen presentation. Thus, the results of test on cytotoxic activity of natural killer blood cells with the use of К562 tumor cells culture indicated the highest value of the given indice exactly in these cases (cytotoxic index 5.8–6.0 when maximum value of the given indicator in other examined animals 4.6, p < 0.05).

Probably, just due to the fact that the exposure with the modulated EHF EMR started before the Pliss lymphosarcoma inoculation, in the organism of at least some of the animals it was possible to activate the mechanisms inhibiting the development processes of tumor avoidance from immune recognition and cytotoxic factor effect.

Conclusions
The obtained results allow speaking about evident changes in different segments of the immune system, accompanying or anticipating the anti-tumor effects of microwave range EMR, used as factors of the activation therapy. Besides, the effect of the studied low intensity EMR could cause the immunological processes development in 3 main directions related to the following:

- increase in differentiation of malignant cells leading to partial restoration of their structural-functional properties typical for normal cells, and a sharp decrease in their life cycle,
- development of the antigen presentation processes and further effective destruction of tumor cells by apoptosis induction,
- cytotoxic action of natural killer cells, macrophages, other immune system cells and their factors (Fig. 9).

Thus, the anti-tumor influence of the microwave range EMR as an activation therapy factor may probably be caused by different immune mechanisms. The obtained results allow defining the direction of further improvement of the activation therapy methods and development of effective anti-tumor exposures. The fact that the activation of anti-tumor resistance mechanisms is provided by the studied EMR anti-stressor effect allows speaking about the absence of restrictions when using EMR for patients with cardiovascular pathology.

Statement on ethical issues
Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest
None declared.

Author contributions
The authors read the ICMJE criteria for authorship and approved the final manuscript.

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