Pathogenetic substantiation for anti-tumor effectiveness of experimental electromagnetic therapy

Oleg I. Kit1, Ekaterina F. Komarova1, Alla I. Shikhlyarova2, Elena P. Korobeinikova1, Ludmila V. Vanzha1, Viktoria V. Pozdnyakova1, Andrey V. Dashkov1, Elena M. Frantsiyants1

1 Rostov-on-Don Oncology Research Institute, Ministry of Healthcare of the Russian Federation, Russia, 344037, Rostov-on-Don, 14th Line st. 63

Corresponding author: phone: +7 (928) 777-75-23, e-mail: katitako@gmail.com

Aim
The objectives to be pursued by our studies are to assess some effects made by experimental actions of ultra-low frequencies electromagnetic field (ULF EMF) in combination with the SCENAR therapy on morphological changes in lung tissues and the regulation structures considering tumor growth dynamics.

Materials and methods
We investigated sex hormone and prolactin concentrations in 156 male rates of outbred strains in the tumor growth dynamics in tissues of the lungs and the hypophysis, and we also studied morphological changes in the lung tissue. All animals were divided into two groups: the reference group, covering the rodents not affected by any exposures, and the experimental group of the rats, who were subjected to ULF EMF exposure in combination with the SCENAR therapy. Both therapeutic exposures were applied upon expiration of 1 week after tumor cell inoculation in a lung.

Results
The applied combination of the experimental therapy, involving the central and local electromagnetic actions, has resulted in the reliably established prolongation of life spans in tumor-bearing animals as well as in the negative dynamics in tumor growing; it has produced effects both on the hormonal homeostasis subsystems: the hypophysis and the lung tissue affected by the cancer tumor. Histological examination of the prepared tissue samples taken from the regressing tumor in the lungs has demonstrated that there has been a certain amount of the tumor cells, which have been excluded from proliferation with taking an opportunity to differentiate.

Conclusions
The central action produced by ULF EMF is a trigger responsible for the neuro-hormonal regulation that promotes a temporal manifestation of an anti-tumor effect and that in combination with the SCENAR therapy provides for the compensatory-adaptational re-setting of the respiratory system and prolonged life maintenance in the animals.

Keywords
ULF EMF, SCENAR, Experimental tumor in lungs, Anti-tumor effect, Morphology, Sex hormones, Prolactin, Hypophysis

Imprint

Introduction
The pathogenetic mechanisms of progression of a tumor disease are determined by the possibility of coordination and integration of re-setting of homeostasis in a tumor-bearing organism which is governed by the central regulation of the processes at the organ-, cell- and molecule-related levels [1, 2]. Therefore, designing therapy strategies based on these assumptions is most promising in applied oncology.

A biological groundwork for the phenomenon of high sensitivity of an organism to low-intensity factors of electromagnetic and electrical nature is that their deficient influence intensity is compensated by gaining a signal character by them; in doing so, a re-coding of the information carrier takes place at the level of the neuro-humoral mechanisms [3, 4]. Much evidence in reference literature shows that there is an anti-tumor effect detectable that is made by electromagnetic influences applied in experimental studies, along with their use for complex therapy in oncology treatment practice [5–8]. So, some research work has demonstrated that the effects produced by ULF EMF on the central structures in the brain in the post-surgery period in lung cancer patients contribute to a reduction in the complication occurrence and to a re-
liably established prolongation of the 3-year survival span [9]. In this case, we observe normalization of the regulatory function of the hypophysis and the epiphysis that is supported by indicators of the hormonal activity of the thyroid glands and the androgenic areas in the adrenal glands and gonads.

Applications of ULF EMF in complex treatment of colorectal cancer patients have made it possible to considerably improve the two-year survival rates after radical surgery and prolong an average life span in cases of palliative surgical interventions for localized tumor processes [10].

Use of the adjuvant chemotherapy supported by ULF EMF focusing on the brain for treatment of malignant glial tumors in our practice allowed us to obtain an immediate clinical effect for 93.3% of the patients, reliably establish the two year recurrence-free survival rate and minimize symptoms of neurological toxicity in them [11].

Previously we mentioned that we had revealed the role of a change in the hormonal homeostasis indicators of the lungs and the hypophysis in the dynamics of progression of inoculated malignant tumor in the lungs, along with the morphological correlates of development of the experimental tumor in the lung tissue [12, 13]. The pathogenetic significance of the said factors may be confirmed by a pronounced efficacy due to their anti-tumor effect potentiality.

In this context, it should be stated that the aim of our offered research work was to assess effects produced by experimental ultra-low frequency magnetic fields in combination with the SCENAR therapy, considering morphofunctional changes in the lung tissue and in the regulatory structures in the tumor growth dynamics.

Materials and methods

In sets of our experiments used were 156 male albino rats of outbred strains. Malignant tumors developed in the animals after they had been given a single intravenous injection of sarcoma 45 cells into their subclavian veins. Taking into account the tumor growth dynamics (upon expiration of 1, 3, 5 and 45 weeks from inoculation), we examined the morphofunctional changes in the lung tissue. With the use of the method of an immunoferment analysis we quantified progesterone, testosterone, estradiol and prolactin in the lung tissue as well as gonadotropins: luteinizing and follicle stimulating hormones (LSH and FSH, respectively) plus prolactin in the hypophysis tissue.

All animals were divided into two groups as follows: Group 1 (the reference group, n = 54) showing tumor growth without any influences or exposures applied; Group 2 (the test group, n = 102) to cover the rodents exposed to ULF EMF in combination with SCENAR.

The experimental therapy was carried out by ULF EMF acting on the brain (with the use of device Gradient-2 manufactured by R&D Company PULSE at All-Russia Research Institution GRADIENT, Rostov-on-Don, Russia), in a discrete sequence of frequencies 0.03 Hz – 0.3 Hz – 9 Hz with the a signal-related exposure of 5 minutes – 1 minute – 1 minute, respectively, and at an intensity of 5 mT – 3.5 mT – 2.8 mT, respectively, the values of which were decreased exponentially by a factor of 0.7. Upon expiration of 15-20 minutes after completion of each magnetotherapy session, the skin areas in the projection of the lungs and the sternum were treated with a pulse electric current supplied by the SCENAR equipment (manufactured by Closed-End Joint Stock Company “R&D Department RHYTHM”, Taganrog, Russia) producing varying frequencies from 15.3 Hz to 33.6 Hz. Both treatment actions were started in 1 week after the S 45 tumor inoculation into the lungs, and all treatment sessions, performed usually at 9 or 10 am., covered 5 weeks (4 times a week).

All manipulations in animals in experimentation, including sacrifice procedures, were in accordance with applicable rules, guidelines and regulations prescribed by European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Strasbourg, 18.III.1986. Statistics data were processed with the use of our original Software Statistica 10.0 with performing Student’s t-test (p < 0.05).

Results

The results of our analysis show that the applied influence has led to a reliably established prolongation of a life span in the animals affected by the growing malignant tumor in the lung (see Table 1 herein). The animals within the applied therapy test group, belonging to the cohort with a favorable effect, demonstrated a life span 9 times longer than that recorded in the reference group and 3 times longer than that found in a part of the reference group showing no therapy effect.

Differences between the reference group and the individual subgroups within the experimental test group were also detected in the sizes, the number of
the regional nodes involved and timing of the node detectability. We found that the detectable involvement of the regional nodes in 33.3% of the animals in the test group took place 2–3 weeks later as compared to the time in question in the rodents in the reference group. It should be mentioned that the tumor-affected nodes in the majority of the test group animals could not be visually identified over the course of the entire experiment.

When carrying out morphological examinations of the lung tissue upon expiration of 1 week from the time of inoculation with Sarcoma 45, i.e. prior to the therapy action (the ULF EMF and SCENAR therapy), it was found that the primary alterations in the tissue in the upper and bottom lobes in the right and left lungs are the same as it was the case with the micro-picture identified in the animals in the reference group. A first triggering mechanism of a pathologic influence exerted by tumor cells on the lung tissue might be a reduced blood flow rate and ample large-focal hemorrhages. Actually, we observed that the entire tissue of the lung was completely soaked with blood, and the interalveolar spaces were filled with a large body of erythrocytes and lymphocyte clusters (see Figure 1 herein). As a result, due to such “an invasion attack” some large-focal dystelectasis areas, interstitial pneumonia and microabscess areas in alveolar spaces were made evident.

In week 2 upon inoculation with the tumor cells (the beginning of the ULF EMF and SCENAR treatment), in the upper and bottom lobes observed was a degradation of the bronchiol and alveolar structures not only due to large-scale blood cell aggregations, but mainly due to an egress of tumor cells, leaving the blood vessels, and their growing as thin prolonged filamentous extensions (see Figure 1b herein). Formation of large tumor cell clusters took place, which demonstrated a curved boundary line separating them from the blood-soaked lung tissue (see Figure 1c herein). Formation of a tumor node was characterized by a compression and destruction of the lung tissue structures, closely packed tumor cells, which retained the typical S-45 spindle shape and maintained their high proliferative potential that was evidenced by abundant pathological mitotic figures (see Figure 1d given herein).

The morphological evidence for the fact that the true tumor process was in progress in the lungs in the rats came from the successive short-time going of the tumor cells spread by way of the blood hematogenous route to formation of tumor cell populations in the form of thin extensions and, finally, in the form of a completely formed tumor node.

When continuing the experimental treatment, as designed by us, we observed some destructive alterations in the tumor node structures in the lungs. Starting with week 3 upon the inoculation with S-45, we observed the mobilization of the immune system at the cellular level and formation of a condensed circumferential structure made by matured lymphocytes along the periphery of the tumor node (see Figure 2a herein). Lymphoid cells in concert with histiocytes, macrophages and fibroblasts penetrated into spacing between some individual clusters of the tumor cells, which had their common boundary line. In other
a)

b)

c)

d)

Fig. 1 а-d. Lung tissue in a rat inoculated with Sarcoma 45 upon the ULF EMF and SCENAR treatment: a) a large-focal hemorrhage, interstitial pneumonia in progress, microabscesses and dypelectasis, upon expiration of 1–2 weeks from the date of inoculation; b) degradation of bronchiol-alveolar structures, egress of tumor cells leaving the blood vessels and formation by them of thin prolonged filamentous extensions; c) formation of large-sized tumor cell clusters with a curved boundary line; d) formation of a tumor node with compressed lung structures. Hematoxylin and eosin staining. Magnification x40.

In weeks 5–6, we were able to identify some amorphous, optically homogenous, areas produced from materials due to lysing of the tumor malignant cells as well as due to growing of the new connective tissue within the lung tissue in the rats, who demonstrated a pronounced anti-tumor effect.

Under microscopic examination, identified were some individual groups of the tumor cells with signs of loss of the malignant character as follows: loss of predominance of the typical spindle-shaping and attaining a more round contour profile, with ordering the cells to form single-layer structures of linear- or follicular-type design (please, refer to Figure 3a, b herein).

An intensification of the processes of cell regeneration was noted in the lung tissue with a marked anti-tumor effect. The cellular sources of these processes were fibroblasts, alveolocytes of type II, macrophages, and endotheliocytes of blood capillaries. In our field of...
vision we detected sizable areas filled with fibroblasts (see Figure 3c herein). In addition, we identified accumulations of alveolocytes of type II (pneumocytes of the IIInd order) representing an intensively secrete releasing, epithelial, and proliferating surface cell, the main component of which is dipalmitoylphosphatidylcholine (see Figure 3d herein).

The condition of some connective-tissue related elements like macrophages, mast cells, elastic, collagen and reticular fibers, suggested that some protection and compensation processes were being formed in the rat lungs affected by the tumor, under the effective treatment with ULF EMF and SCENAR.

When examining our specimens, we detected sometimes elastic structures of peri- and interlobular, intervascular, perivascular and peribronchial localization. The fibers demonstrated a layered order and exhibited their clear-cut excessive curvature pattern (see Figure 4a herein). An increase in the content of the reticular fibers indicated that metabolism processes in the lungs were intensified. Under a relative stabilization of the lung condition, the relationship among the fiber types was maintained due to an increase in the
number of the collagen fibers referred to the elastic and reticular ones.

The stroma tissue was formed by fibers grouped into bundles due to the lateral aggregation at the bronchiolar structures (see Figure 4b). Following the run of the fibers, we observed some isthmi and flask-shaped protrusions: they were the false bronchi surrounded by accumulations of the bronchi-associated lymphoid elements (see Figure 4c). In the bronchi of the rat lung, there ciliated epithelium lined with the smooth muscle fibers forming the typical sublayer, basal cells layer and cylindrical epithelium (see Figure 4d) was retained.

An examination of the level of steroid hormones and prolactin concentrations demonstrated pronounced dependence of these parameters on the effectiveness of the experimental therapy as well as on the tumor growth time (see Table 2 herein).

Upon expiration of 3 weeks from the time of tumor inoculation, the prolactin concentration in the lungs in the rats without effect upon completed combined electromagnetic treatment showed no statistically significant difference from the same parameter, recorded for the reference group, and exceeded the normal values by 40 %. At the same time, while the prolactin level in the tissues of the lungs of the animals demonstrating an effect upon the experimental anti-tumor therapy was higher than the normal values by 28 %, it remained by 28,2 % lower than that determined in the lung tissues in the reference group rats. During the said test period, there was no significant difference in the levels of testosterone and estradiol concentrations in the lung tissue in the rats of the “no-effect” group from those in the reference group identified, and the said parameters were reduced by 57 % and 32.6 %, respectively, as compared to the normal values. We
Fig. 4a-d. Lung tissue inoculated with Sarcoma 45 in a rat, upon the ULF EMF and SCENAR effective treatment, status in week 5-6 from the time of inoculation: a) growing of elastic, collagen and reticular fibers with a excessive curvature pattern; b) lateral peribronchial aggregation of stromal elements like bundles; c) accumulations of bronchus associated lymphoid elements – BALT structure; d) preservation of ciliated epithelium in bronchi, lined by smooth muscle fibers, basal cells and cylindrical epithelium. Magnification x100. Hematoxylin and eosin staining.

had a completely different situation with the parameters recorded in the lung tissue in the rats responding effectively to the applied treatment: the levels of testosterone and estradiol concentrations remained within the range of the values identified in the intact animals.

Let us analyze the concentrations of progesterone in the two groups upon expiration of 3 weeks from the date of inoculation. In the lung tissue in rats, demonstrating the experimental therapy effect, the said parameter increased practically by 33 % referred to the values recorded in the intact animals and exceeded the same value identified in the reference group by a factor of 4,2. The progesterone concentration in the lung tissue in the “no-effect” group rats decreased by a factor of 1,9 referred to the normal values, but it remained by 62,9 % higher than that recorded in the reference group rodents.

The concentrations of prolactin and estradiol in the animals, demonstrated a favorable response to the anti-tumor treatment, within 5 weeks upon the inoculation, remained unchanged as compared to the previous test period, and the concentrations of testosterone and progesterone were reduced by a factor of 1,9 and by a factor of 1,8, respectively. As to the lung tissue in the “no-effect” group rats, it was the concentration of progesterone only, which changed as compared to the period examined before: it decreased by a factor of 1,9. It should be noted that in the period 5 weeks upon the inoculation with the tumor, the differences in the parameters under examination between the “effect-demonstrating” group rats and those in the “no-effect” group referred to the concentration levels of prolactin and progesterone only, whereas the concentrations of testosterone and estradiol identified in the lung tissue in either group were at the same levels.
Table 2. Concentrations of sex hormones in the lung tissue in male rats: the normal values vs. actual values in dynamics in rats upon inoculation with S-45.

<table>
<thead>
<tr>
<th>Test period</th>
<th>Prolactin (ng/g tissue)</th>
<th>Progesteron (ng/g tissue)</th>
<th>Testosterone (ng/g tissue)</th>
<th>Estradiol (ng/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The background data (intact animals)</td>
<td>206.8±14.3</td>
<td>165.2±11.5</td>
<td>170.7±13.2</td>
<td>114.9±10.5</td>
</tr>
<tr>
<td>Reference group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 3 upon inoculation</td>
<td>315.8±18.8</td>
<td>52.3±3.0</td>
<td>68.9±4.7</td>
<td>71.9±5.8</td>
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<tr>
<td>Week 5 upon inoculation</td>
<td>391.7±23.6</td>
<td>46.3±2.8</td>
<td>46.4±4.3</td>
<td>46.1±3.7</td>
</tr>
<tr>
<td>&quot;Effect-demonstrating&quot; group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 3 upon inoculation</td>
<td>265.2±11.7</td>
<td>219.5±17.2</td>
<td>152.5±13.2</td>
<td>93.0±8.8</td>
</tr>
<tr>
<td>Week 5 upon inoculation</td>
<td>267.3±18.1</td>
<td>120.1±9.7</td>
<td>81.6±7.3</td>
<td>83.4±7.3</td>
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<tr>
<td>&quot;No-effect&quot; group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 3 upon inoculation</td>
<td>289.4±9.3</td>
<td>85.2±4.5</td>
<td>73.4±1.1</td>
<td>77.4±5.4</td>
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<tr>
<td>Week 5 upon inoculation</td>
<td>328.3±26.1</td>
<td>45.6±1.9</td>
<td>74.1±6.3</td>
<td>77.1±3.2</td>
</tr>
</tbody>
</table>

Notes: 1 – statistically significant in relation to the background data (p<0.01); 2 – statistically significant in relation to the reference group data (p<0.01); 3 – statistically significant in relation to the data of the previous period of experiment (p<0.01); 4 – statistically significant in relation to the data of the “effect-demonstrating” group (p<0.01).

Table 3. Concentrations of hormones in hypophysis tissue in male and female rats within the norms and those identified for dynamics of tumor growth upon S-45 inoculation.

<table>
<thead>
<tr>
<th>Test period</th>
<th>Prolactin (%)</th>
<th>LH (%)</th>
<th>FSH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background data (intact animals)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Reference group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 3 upon inoculation</td>
<td>217.4±11.9</td>
<td>173.7±12.2</td>
<td>35.4±2.1</td>
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<tr>
<td>Week 5 upon inoculation</td>
<td>63.6±7.7</td>
<td>233.3±17.5</td>
<td>16.8±1.2</td>
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<tr>
<td>&quot;Effect-demonstrating&quot; group</td>
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<td></td>
<td></td>
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<tr>
<td>Week 3 upon inoculation</td>
<td>132.4±9.2</td>
<td>90.8±8.7</td>
<td>80.7±5.6</td>
</tr>
<tr>
<td>Week 5 upon inoculation</td>
<td>135.1±4.5</td>
<td>85.4±1.2</td>
<td>43.6±1.2</td>
</tr>
<tr>
<td>&quot;No-effect&quot; group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 3 upon inoculation</td>
<td>179.4±15.8</td>
<td>142.9±10.3</td>
<td>59.6±2.2</td>
</tr>
<tr>
<td>Week 5 upon inoculation</td>
<td>79.2±5.6</td>
<td>173.3±13.4</td>
<td>30.1±2.7</td>
</tr>
</tbody>
</table>

Notes: 1 – statistically significant in relation to the background data (p<0.01); 2 – statistically significant in relation to the reference group data (p<0.01); 3 – statistically significant in relation to the data of the previous period of experiment (p<0.01); 4 – statistically significant in relation to the data of the “effect-demonstrating” group (p<0.01).
Another intriguing fact was that we observed some alterations in the hypophysis in rats upon inoculation of a malignant tumor into the lung under the experimental therapy (see Table 3 therein).

So, upon expiration of 3 weeks after the tumor inoculation, the concentration level of prolactin in the hypophysis in rats, demonstrated a favorable response to the anti-tumor treatment, increased by 32.4% as compared to the same parameter in the intact animals, and in the reference group it was recorded to be more than doubled. A statistically significant change in the LH concentration within the indicated period of time was not detected, whereas it exceeded the normal values by 73.7% in the reference group. The concentration level of FSH in the hypophysis in the “effect-demonstrating” group rats, recorded 3 weeks after the inoculation, lowered by 19.3% as against 64.6% in the reference group animals. Upon expiration of 5 weeks after the inoculation, it was established that it was the concentration of FSH in the hypophysis in the “effect-demonstrating” group rats upon completion of the anti-tumor experimental therapy that changed: it was by 46% lower than that found in the previous period; but at the same time, the value remained higher by a factor of 2.6 as compared to that in the reference group.

The animals in the “no-effect” group demonstrated the concentrations of prolactin, LH and FSH intermediate between the values recorded in the reference group and those in the group with a pronounced anti-tumor effect.

Conclusions

The proposed combined application of the experimental therapy based on the central and local electromagnetic action has exerted an effect on both of the constituents of homeostasis: the hypophysis and the tumor-affected lung tissue. It is likely that this has created such conditions of the hormonal “environment”, when and where the tumor has not been able to grow and has experienced a structural involution. Under the histological examinations of the prepared samples, taken from the regressing tumor in the lung upon completion of the treatment, the fact has been noticed that a number of the tumor cells terminate proliferation and acquire a differentiated state. The mechanism of this transformation remains unclear, but it is known that in order to induce differentiation it is required to provide a pre-specified normal hormonal status. When compared the prolactin concentration level in the tumor-affected lungs in the rats of the “effect-demonstrating” group to that in the “no-effect” group, it is easy to note the normalization thereof in the first case considered and an essential increase in the level in the second case.

It is probably that the action adequate in its intensity and frequency, applied to the projection of the hypophysis, is capable of providing the stable character of its command issuing system responsible for the regulation of the level of prolactin and sex-related hormone concentrations, and the local treatment with SCENAR facilitates retarding of pathological stimulation of hormones in the tissue-related homeostasis.

The central action produced by ULF EMF is a trigger of the neuro-humoral regulation with increasing of the general and local anti-stress potential and the associated level of the organism resistance that promotes the temporal manifestation of the anti-cancer effect, and in combination with the SCENAR therapy it provides the compensatory-adaptational re-setting of the respiratory system and prolongation of a life span in general.

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.

References.