ON THE INFLUENCE OF THE BIORESONANCE TREATMENT ON TUMOR DEVELOPMENT IN EXPERIMENTAL ANIMALS

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Abstract
Every living cell produces a specific electronmagnetic field (EMF). The EMF can be accumulated modulated and amplificated by the BICOM instrument. The effect of these treatments were examined in different animal tumor models. In some cases (e.g., B16 melanoma) the BICOM's bioresonance therapy (BRT) inhibited the primary tumor growth and the development of metastases. The tumor growth of Heren carcinoma and Ehrlich carcinoma were not changed by the BRT. Some changes in the signals of paramagnetic metasbolites and free radicals were observed. Further experiments need to explain the mechanism of BRT. This treatment method may be useful for cancer therapy in the future.

Introduction

The cells of different organisms are assumed [1] to receive information regularly from their environment. This process may require not only a chemical substances but biophysical signals. All biochemical processes generate a wide range of frequencies of endogenous EMFs which correspond to the processes' characteristic relaxation times. Moreover, processes that occur at the biochemical level are then controlled by these fields or - say at the biophysical level. Any "biochemical disorder" sooner or later will affect the whole organism via the "reflection" and feedback from the biophysical level. Vice versa, biophysical level therapy will treat the organism as a whole by restoring the damaged links at the biochemical level rather than by only fixing a particular biochemical problem. The resonant intera
ctions between the endogenous electromagnetic fields of biological systems from subcellular structures, cells, organs, organisms to populations are usually referred to as bioresonant interactions. However, after the division, cells start acquiring positional information again. L. Wolpert [2] proposes that positional information is necessery during an organism's development and differentiation.
M. Terzi [3] suggests that positional information determines molecular differentiation, the biosynthesis of different biochemical pathways. Positional information may play an important role in cell-cell interactions, communication and recognition [4]. According to this theory, development is a sequence of gene expressions under the control of positional information. Some authors suggest that positional information is transferred by bioactive substances [5] or by biophysical mechanisms via autowaves [6, 7]. Weak electromagnetic fields are shown to alter genome activity, namely - gene transcripts [8]. Electromagnetic fields also alter membrane Na, K-ATPase activity [9], Plasmids (cell-free Transcription/Translation Systems) were exposed to a magnetic field which resulted in enhanced gene expression. [10]. According to A. Gurwitch [4, 11], homeostasis is controlled by a special anisotropic field of cells which is coupled to vectorial subcellular organization and ordering of molecular constellations. N. Koltsov considers that every cell has electromagnetic fields which are induced by gradients of physical and chemical potentials within the cells. Some experiments deal with the changes in the state of tumor cells under the influence of endogenous EMF. Sheldrake [12] suggested the morphogenetic field to be similar to known physical fields that can have an electromagnetic feature [8].
We suppose that the discrepancies between a cell's competence and positional information may cause the organism's control of transformed cells to be lessened. Tumor incidence may be due to an inappropriate balance between proliferation and differentiation, resulting in over-proliferation. Cells of a malignant phenotype have all genes that regulate normal proliferation [3, 13]. That is why it is plausible

to conclude that " malignancy can be suppressed by inducing differentiation either with or without genetic changes in the malignant cells; this suppression does not have to restore all normal controls." [14 - 16].

There may be various factors responsible for such an imbalance, however this imbalance may be restored by applying bioresonance therapy (BRT) [1, 13, 17-21]. Bioresonance is considered a general mechanism of maintaining the communication pathways within living matter via endogenous EMF interactions [18-20]. Although critics argue that bioresonance has no strict supporting theory, it has been successfully employed in medicine for the past two decades [13, 21-25]

Probably, the first attempts at treating cancer with electromagnetic fields (EMF) began in the 1930's when Nikola Tesla and Georges Lakhovsky constructed the Multiple Wave Oscillator: a generator of life-associated frequencies from 750 kHz to 3 MHz and numerous harmonics, which may extend as far as 300 GHz. They reported several successful applications of the device in cancer treatment and various metabolic disorders in plant, animal and human patients [26]. Another therapeutic device from that period was invented and applied for cancer treatment by Wilhelm Reich [27, 28]. Unlike Lakhovsky's Oscillator, it was not generating any EMFs but rather accumulating naturally occurring electromagnetic fields, which he termed "orgone energy". Both devices were intended to "harmonize" the endogenous electromagnetic oscillations of patients with the environmental EMFs by employing a wide range of EM frequencies from very high EMF frequencies to extremely low frequencies (including Schumann waves [29]) with the Multiple Wave Oscillator and the Orgone Accumulator, respectively. These works, as well as further achievements in various EMF applications in healing processes (starting from the late 50's [17]), made it possible to introduce the notion of bioresonance therapy (BRT) [1].

ESR spectroscopy is advantageous in this respect since during tumor development, all free radical sites and paramagnetic metal complexes alter substantially in quantity. These changes may be due to an excess production or metabolism of paramagnetic complexes as well as conformational alterations.

In order to estimate the non-specific response of animals with tumors to BRT, the content of various molecular weight fractions of the animal's blood serum was measured by high performance liquid chromatography (HPLC).

In vivo studies of tumor development by the interference from various metabolic changes in a tumor-bearing organism. In order to determine the differences in the metabolic response to different types of tumors: lung metastasizing melanoma B16, Heren carcinoma (both solid tumors) and ascitic Ehrlich carcinoma were studied.

To elucidate possible mechanisms of the influence of endogenous EMF on tumor cells, the study was divided into the following steps:

1. Tumor development in mice after BRT of inoculated cells *in vitro*;
   a) weight of primary tumor;
   b) quantity and volume of metastases;
   c) weight of immunocompetent organs;

2. Tumor development in mice after BRT of tumor-bearing animals.
   a) weight of primary tumor;
   b) quantity and volume of metastases;

c) weight of immunocompetent organs

MATERIALS AND METHODS

Cell culture maintainance
primary cell culture of B16 melanoma

Primary cell cultures of B16 melanoma were obtained by a sterile dissection of the fragments of a tumor, which was maintained in male mice of line C57Bl/6.

The tumor tissue was disaggregated with a 0.01 % solution of trypsin ("Sigma") according to a standard technique [14]. Cell suspensions (10^5 cells/ml) in sterile T-25 flasks ("Greiner") were incubated for 24 hours at 37 ^oC in a humidified atmosphere of 5% CO_2, 5% O_2 and 90% N_2. RMI1640 medium ("Sigma") with an additional 10% of fetal serum ("Sigma"), antibiotics (5 mg/ml of streptomicine and 5 mg/ml of penicillin) and 0.03 mg/100 ml L-glutamine medium was used for incubation. Hanks' balanced salt solution with the addition of antibiotics was used for disaggregation and subsequent washing of cells from trypsin [30].

24 hours after the incubation, the obtained tumor cell cultures were exposed to the influence of BRT.

Treatment modes and experimental arrangements

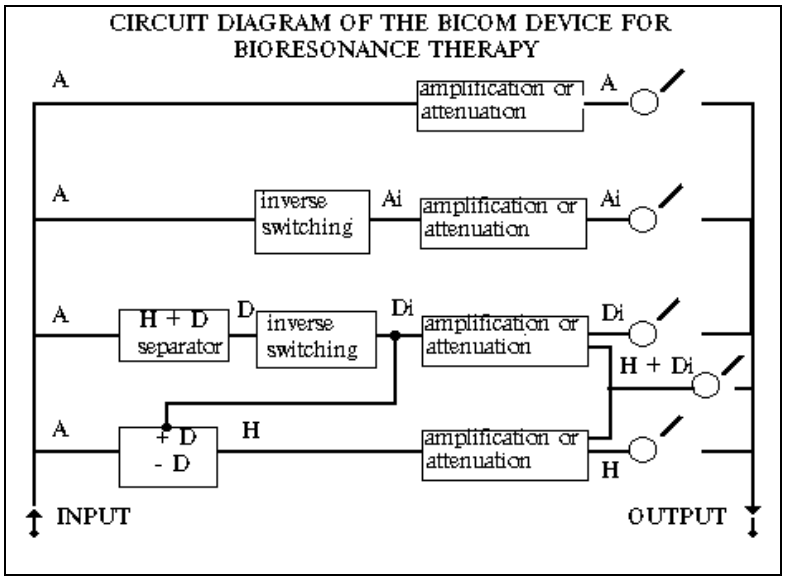

Fig. 1 Treatment types of the BICOM device :(Fig. 5 from [1], p.31):

A = pass all frequencies from the "input" system unchanged;
Ai = pass all frequencies from the "input" system, but inverted;
Di = pass only the disharmonious inverted portion of endogenous oscillations;
H = pass only the harmonious portion of endogenous oscillations of the "input" system;
H+Di = pass harmonious and disharmonious inverted frequencies

Depending on need, therapeutic oscillations can be amplified or attenuated and a certain frequency range (within 10 Hz - 150 kHz) can be filtered out.

In some experiments, primary melanoma cell cultures, adjacent-to-tumor tissue, or tumor pieces were used as a source of endogenous EMF and were placed into the input and output cup electrodes of the BICOM and treated as follows.

Maintainance of different tumor lines

The lung metastasizing melanoma B16, and Ascitic Ehrlich carcinoma were inoculated into mice and the Heren carcinoma was inoculated into rats.

a) B16 melanoma in mice

Line C57Bl/6 female mice (2 - 2.5 months old) from the vivarium of the Institute for Gerontology of the Natl. Acad. Sci. of the Ukraine were used in the experiments. Melanoma B16 was delivered from the pool of tumor cell lines at the Institute for Experimental Pathology, Oncology and Radiobiology of the Natl. Acad. Sci. of the Ukraine and stabilized in syngenic C57Bl/6 mice.

The fragments of tumor tissue were disaggregated in 0.01% tripsine solution according to a standard technique (Fresheney, 1983). The cells were washed free of the tripsine three times in an RPMI 1640 medium. The number of living cells was counted by a hemocytometer using the trypane blue exclusion method.

The suspension of tumor cells ($2.5 \cdot 10^5$ cells per 0.1 ml of medium) was inoculated into the right posterior leg of a mouse. On the 26th day after the inoculation, all mice were decapitated under ether anesthesia.

b) Ascitic Ehrlich carcinoma in mice

Outbred female mice (aged 1.5 - 2 months, 15 g) from the vivarium of the Institute for Gerontology of the Natl. Acad. Sci. of the Ukraine were used in the experiments. $5x10^6$ cells of Ehrlich carcinoma in 0.2 ml of Hanks' medium were inoculated subcutaneously. On the 10th day after the inoculation, all animals were decapitated under ether anesthesia.

c) Heren carcinoma in rats

White female rats (aged 2 - 2.5 months, 150 g) were used in the experiment. The cell suspension was prepared and counted as described above. 0.3 ml of cell suspension were transplanted subcutaneously. On the 14th day after inoculation, all rats were decapitated under ether anesthesia.

d) S180 carcinoma in mice

Swiss female mice (aged 2 - 2.5 months) from the vivarium of the Institute for Gerontology of the Natl. Acad. Sci. of the Ukraine were used in the experiments. The S180 was delivered from the pool of tumor cell lines at the Institute for Experimental Pathology, Oncology and Radiobiology of the Natl. Acad. Sci. of the Ukraine 5mm^3 fragments of tumor tissue were transplanted subcutaneously.

Parameters for evaluation of antitumor effects:

The weight of the primary tumor (g), spleen (mg), thymus (mg), the number and diameter of lung metastases (mm), the volume of the ascites (ml), the concentration of cells in the ascites and the total number of cells in the ascites were estimated. The number of cells in the ascites was counted by a hemocytometer.

The weight (mg) of the primary tumor was estimated according to the difference between the weight of healthy and tumor-bearing legs.

The weight [16] of pulmonary metastases is calculated according to the formula:

$$W = dr^3 p4/3$$

here, W - weight (mg), r - radius of a metastasis (mm), d - metastasis density, which is assumed to be approximately. 1 g/mm^3.

The quantity of resultant metastases was counted in the lung.

The influence of ELF-ELI EMF on the process of metastasis is characterized by the **metastasis inhibition index** (MII) [30]:

MII = [(Ac * Bc) - (A * B)/ Ac * Bc] x 100%

here, Ac and A are the incidences of metastases in lungs in intact and experimental groups of animals correspondingly; Bc and B - mean number of metastases in the lung of intact and experimental mice correspondingly.

The percentage difference between control and experimental values is calculated according to formula 2.

The **mitotic activity** of the tumor cells in mice was estimated by calculating the mitotic index (MI):
MI = (number of cells in mitosis)/(total number of cells) *100 %

The number of cells in mitosis was counted in chromosome dry-air preparations by analyzing about 1000 cells on each of the two glasses per sample.

Dry-air preparations were made from tissue dissociations with acetic acid [2]. Two fragments of tumor tissue without necrobiotic changes were fixed with a methylacetic fixing agent and then dry-air preparations were made, in which the number of dividing and non-dividing cells was counted. The preparations were stained by using the standard Romanovsky-Gymza technique.

Determinations of paramagnetic parameters

The content of paramagnetic metabolites was estimated by using EPR spectroscopy.

Liver and tumor tissues and the blood of the experimental animals were frozen in liquid nitrogen, and EPR spectra were obtained at 90 K by using an E-109 radiospectrometer (Varian, USA). Spectra were acquired under the following conditions:

* SHF power - 5 mW (0.2 mW for free radical registration);
* T magnetic field (0.1-0.2 T for Fe (3+) registration);
* SHF frequency 9.5-9.6 GHz;
* modulation frequency 100 kHz;
* modulation amplitude 0.8 mT (0.02 mT for free radical registration).

A ruby crystal was used as an internal standard of the signal amplitude. Amplitudes of the studied signals were measured in relative units (the actual amplitude was divided by the amplitude of the standard signal).

HPLC study of the blood serum of experimental animals with tumors after BRT:

7.8 mm x 30 cm columns filled with Toyopearl HW60 were used; a 0.1 M TrisHCl buffer solution (pH = 7.0) was used as an effluent: flow rate - 1.0 ml/min.; sample volume - 20 ml; serum was diluted ten times with a physiological solution before examination; and a UV (l_{max} 280 nm) detector was used. Standard samples of Blue Dextrane (MW 2000), b-Amylase (200 kD), Alcohol Dehydrogenase (150 kD) and Bovine Albumin (66 kD) were used for molecular weight calibration.

The chromatograms of the blood serum of mice contained three (sometimes four) peaks: p1 (elution time 10.6 min.) corresponds to the fraction with a molecular weight (MW) of about 20-25 kD; p2 (13 min.) corresponds to the serum albumin fraction, MW 60-70 kD; p3 (21-21.5 min.) - to high MW fraction, MW > 300 kD. The areas of these peaks were estimated (in % to the total area).

Statistical analysis

Since the obtained variables (data) were not normally distributed (skewness > 0) and Levene's test did not show a homogeneity of variance, statistical significances were estimated by using nonparametric alternatives to the t-test for independent variables: the Mann-Whitney U-test (which is computed based on rank sums rather than means) and the Kolmogorov-Smirnov test (which is sensitive to differences in the general shapes of the distributions in the two tested samples).

RESULTS AND DISCUSSION

The study of the BRT effects on tumorigenicity of melanoma B16 cells -

A suspension of the melanoma B16 cells was obtained as described above. Then 10 flasks of cell cultures were prepared, each containing 3 ml of the suspension (10^6 cells /1 ml). The flasks were divided

into 5 pairs and one of them (the control pair - group ¹ 5) was used as a source of ELF-ELI EMF. The first experimental pair of flasks contained cells which were treated with the 10 Hz- 150 kHz bandpass of acquired EMFs ("All frequencies"); the second - 50 Hz, Wobbling; the third - 10Hz, Wobbling; and the fourth - 125 kHz, Wobbling. The influencing EMFs (treatment mode A) are 14 and 20 times amplified for every 10 min of the treatment correspondingly. The cells are treated two times a day, for three days.

In order to be transplanted, the BICOM-treated cells were harvested from experimental vials and washed with HBSS. Then $2{,}5x10^5$ cells in 0.2 ml of 199 medium were injected intramuscularly, into the medial area of the right thigh.

After the transplantation, 5 groups of tumor-bearing mice were formed corresponding to the 5 groups of EMF-treated cell cultures.

Table 1. Growth rate and metastasis incidence in mice after the transplantation of melanoma B16 cells which are exposed to intrinsic EMFs before inoculation.

No	Treatment mode	primary tumor weight mg	Inhibition of growth (%)	METASTASIS number weight M.I. (mg) (%)		
1.	A 14,20; All frequencies, contin 2 day; 2*10 min.	2.8 ±0.23 p < 0.01	31	2.1 ±0.62 p < 0.01	1.4 ±1.51 p < 0.05	81.9
2.	A 14,20 50Hz, Wobbling, continuous 2 days, 2 times a day for 10 min.	2.8 ±0.36 p < 0.05	28	3.0 ±0.99	4.4 ±2.32	61.2
3.	A 14, 20; 10 Hz, Wobbling, contin 2 days, 2 X/day for 10 min.	4.5 ±0.52	15	6.5 ±0.68	9.6 ±2.62	-12.0
4.	A 14,20;125 kHz, Wobbling, continuous 2 days, 2 times a day for 10 min.	3.4 ±0.57	26	5.4 ±0.62	7.5 ±2.22	6.8
5.	CONTROL	3.9 ±0.33	0	5.8 ±1.25	7.7 ±2.26	0

The data in Table 1 show that the mean weights of primary tumors in the mice of groups 1 and 2 are substantially decreased as compared with those in the control group. Whereas in groups 3 and 4, changes in the primary tumor weight statistically were not significant. The decrease in the weight and number of metastases was characteristic only for the first experimental group. These results may testify that tumor cells possess an intrinsic EMF which may alter the functional state of target-cells.

A statistically significant decrease in the primary tumor weight were observed in case of treatment with frequency wobbling around 50 Hz, and 10 Hz - 150 kHz bandpass, whereas a significant decrease in the weight and number of metastases occurs in the latter wide range of frequencies. Meanwhile, the treatment of group 4 cells (frequency wobbling around 125 kHz) does not result in a significant decrease in the weight of metastases [15].

Considering the response of immunocompetent organs to the development of the EMF-treated cells, we should observe significant changes only in the mean weight of the spleen in the second experimental group (Table 1.).

The influence of *in vivo* BRT on tumor development in mice

30 adult C57BL/6 male mice (weight 20 - 21 g) were used in the experiment. Lung metastasizing 3LL carcinoma ($2.5x10^5$ cells) was inoculated into a posterior leg. Every second day after the inoculation, a tumor biopsia was taken and used at the "input" of the BICOM device to treat the other mice (treatment mode A_i, amplification 12). The posterior leg (without tumor) of a mouse was treated with an electrode connected to the BICOM "output". On the 25th day after inoculation, all mice were decapitated and the weight of the primary tumor was measured (Table 2.12.).

Animals are separated into 2 groups according to the administered BRT modulation:

1) Ai-mode amplification 12, 5 sec scans of 10Hz-150 kHz.
2) the control group of mice untreated by BRT

A similar experiment was also done by inoculating sarcoma 180 cells.

Sarcoma 180 was transferred 8 times into a fresh medium after being heating above its freezing point and then inoculated into non-bread mice. 40 female mice (age of 2 - 2.5 months, weight 20 - 25 g) were used. 0.3 ml of the tumor cell suspension in a physiological solution (1:1) was inoculated into a posterior leg. Animals were treated for 1 min. every second day. On the 14th day, animals were decapitated and the tumor weight was estimated (Table 2).

Animals are separated into 4 groups according to the administered BRT modulation:

1. Ai-mode, amplification 12, 5 sec scans of 10Hz-150 kHz
2. Treatment mode Ai, amplification 12, 5 sec scans within 10 Hz - 150 kHz + "Strahler" connected to the output of BICOM
3. Mice treated with "Strahler" only
4. The control group of mice (not treated with BICOM modulation of endogenous EMF)

A tumor biopsia (sarcoma 180 or carcinoma 3LL) at the corresponding stage of development was used as the source of EMF at the input of the device

Table 2. Tumor weight in mice after BRT treatment ($2.5x10^5$ cells are inoculated, treatment mode A*i*, amplification 12, 25^{th} day after inoculation)

Treatment mode	3LL carcinoma weight, mg	Sarcoma 180 weight, mg
No treatment (control group)	2.1 ± 0.5	1.5 ± 0.2
Ai, ampl. 12, 5' scan 10Hz-150 kHz	0.9 ± 0.1	1.2 ± 0.3
Ai, ampl. 12 + "Strahler"	-	1.7 ± 0.5
"Strahler"	-	0.4 ± 0.01

Table 2. shows that the "Ai-mode" of BRT of mice inhibited the 3LL carcinoma development but this changes was not statistically significant. However, treatment of sarcoma-bearing animals with "Strahler" induces a significant decrease in tumor progression. This may testify to a different sensitivity of tumors with BRT. Therefore, the experiment with 3LL carcinoma was repeated with a greater number of inoculated cells: the same treatment was administered after the inoculation of the 10^6 cells (results are summarized in Table 2.).

The data in Table 2. show that the effectivity of bioresonance treatment depends on the tumor progression. When the number of transplanted 3LL carcinoma cells were $2.5x10^5$ a tumor-inhibiting effect of bioresonance treatment was observed (Table 2.12). However, BRT was uneffective in the case when the number of transplanted tumor cells was $1x10^6$s.

The characteristics of the antitumor effect of BRT of experimental animals with tumors are summarized in Tables 3-6. Table 3 summarizes the results of the estimation of the mitotic index.

Dried melanoma cell cultures and adjacent-to-tumor tissue, preserved in glass ampules (prepared and delivered by Dr. H. Keymer), were used as the source of the endogenous EMFs.

BRT was administered to tumor bearing animals (mice and rats) every day starting from the second day after tumor inoculation. Animals were treated for 40 sec. by a 3 sec. scanning of the 10 Hz - 150 kHz band of the endogenous EMFs (amplification factor 10) acquired by the BICOM device (Regumed, Germany). Ampules with cell cultures were placed at the cup field sensor at the input of the device and the output field sensor was held less than 1 cm from the animals.

The animals were divided into four groups:

The intact group;

The control group: tumor bearing mice which did not undergo BRT;

Group "Ai": tumor bearing mice treated with the field of the adjacent-to-tumor tissue;

Group "A": tumor bearing mice treated with the field of the melanoma cell culture.

Table 3. Mean values of the data for the mice with melanoma B16.

Group of animals	Weight of primary tumor, g	Weight of thymus, mg	Weight of spleen, mg	Percentage of mice with lung metastases (%)
Group "Ai" n = 33	4.3 ± 1.8 $p < 0.05$	36 ± 21 $p < 0.01$	174 ± 44 $p < 0.01$	13.5
Group "A" n = 35	4.2 ± 2.4 $p < 0.05$	35 ± 23 $p < 0.01$	159 ± 50 $p < 0.05$	23.0
Control, n = 35	5.3 ± 1.9	21 ± 13	127 ± 55	31.0

Table 4. Mean values of the data for the mice with Ehrlich carcinoma.

Group of animals	Weight of animal, g	Volume of ascite, ml	Number of tumor cells per 1 ml of ascites	Total number of cells in ascites	Spleen weight, mg
Intact (n=25)	16.7 ± 2.0	-	-	-	99.8 ± 24.1
Control (n=38)	15.1 ± 2.1	4.8 ± 1.6	$(4.1 \pm 1.2)10^6$	$(1.9 \pm 0.7)10^9$	89.5 ± 24.1
"A" group (n=40)	15.7 ± 2.2	4.9 ± 1.6	$(3.5 \pm 0.9)10^6$# ($p < 0.01$)	$(1.7 \pm 0.6)10^9$	115.2 ± 41.4# ($p < 0.01$)
"Ai" group (n=39)	14.9 ± 1.8	4.4 ± 1.3	$(3.8 \pm 0.8)10^6$	$(1.6 \pm 0.5)10^9$# ($p < 0.05$)	92.5 ± 28.7

Statistically significant difference with the control group

Table 5. Mean values of data for rats with Heren carcinoma.

Group of animals	Weight of animal, g	Tumor weight, g	Spleen weight, mg	Thymus weight, mg
Intact (n=21)	146 ± 2.1		592 ± 191	207 ± 61
Control (n=30)	154 ± 24	10.3 ± 5.5	802 ± 117* $p_{int.} < 0.001$	201 ± 72
"A" group (n=27)	150 ±14	9.2 ±5.3	908 ± 127 *# $p_{int.} < 0.001$ $p_{contr.} < 0.01$	245 ± 46 * # $p_{int} < 0.01$ $p_{contr} < 0.05$
"Ai"group (n=28)	154 ± 17	8.6 ± 5.8	873 ± 180 * $p_{int.} < 0.001$	240 ± 62

* Statistically significant difference with the intact group
# Statistically significant difference with the control group

The data in Table 3 show that BRT inhibits the growth of primary melanoma B16 (within 20 % as compared with the control group of tumor bearing mice) and the number of metastases.

In the "Ai" group mice (tumor bearing mice treated with the field of the adjacent-to-tumor tissue), the primary tumor's weight is significantly lower ($p < 0.01$). This was accompanied by a more than double decrease in the number of metastases (13.5 % as compared with 31 % in the control group).

In the "A" group mice (treated with the endogenous field of the melanoma cell culture tumor tissue), the decrease in the primary tumor's weight is also statistically significant ($p < 0.05$), although the tumor growth was less inhibited than in the "Ai" group (the index is 21 %). The number of lung metastases in the "A" group also decreased less than in the "Ai" group (23 % of mice have lung metastases in the "A" group compared to 31 % in the control group).

Thus, BRT with the endogenous EMF of both adjacent-to-tumor tissue and tumor cell cultures shows a certain antitumor effect, with the growth of primary melanoma B-16 and lung metastases being more inhibited with the adjacent-to-tumor tissue field.

The obtained results (Table 4) show that BRT under the studied conditions modifies the development of Ehrlich carcinoma in mice. In the "Ai" group, a statistically significant decrease of the total number of cells in the ascites is observed. However, this is not accompanied by significant changes in the ascites volume. BRT with the field of the tumor tissue decreased the concentration of tumor cells in the ascites. This may testify to an antimitotic effect of the studied modes of BRT inhibit the Ehrlich tumor cell proliferation.

The treatment of Heren carcinoma (Table 5), with BRT did not result in a significant decrease of tumor growth. However, BRT with the field of the tumor cell culture yielded a significant increase in the weight of spleen and thymus as compared with intact and control animals (rats with tumors).

Table 6. Mitotic index in the tumors of experimental animals under BRT conditions.

Group of animals	Mean value of the MI, %	Standard deviation
Lung metastazing melanoma B16 in mice		
Control (with tumors)	5.31	2.19
"A" group	3.90 *	1.91
"Ai" group	3.98 *	1.88
Heren carcinoma in rats		
Control (with tumors)	6.81	3.83
"A" group	5.03 *	2.09 *
"Ai" group	4.45 *	2.73 *
Ascitic Ehrlich carcinoma in mice		
Control (with tumors)	8.26	1.94
"A" group	3.64 *	2.21 *
"Ai" group	4.10 *	1.48 *

* The difference is statistically significant ($p < 0.05$)

The data in Table 6 show a statistically significant decrease in the mitotic activity of tumor cells in animals after BRT (in all cases $p < 0.05$). BRT seems to affect the proliferation of tumor cells and reduce the pool of dividing cells.

It is noteworthy that the weight of the lymph organs (spleen and thymus) also significantly increased in mice after BRT, as compared with the control tumor-bearing mice (Tables 2-5). The suppression of tumor growth and lung metastasis formation in both experimental groups seems to be due to the activation of the lymphoid organs, exhibited as a substantial hyperplasia in these organs. Therefore, it should be interesting to analyze the reactions of immunocompetent organs in response to BRT.

The biochemical response of tumor-bearing mice to BRT was analyzed by measuring the content of paramagnetic sites in various organs of the studied mice by using EPR spectroscopy. The mean values are summarized in Tables 8, 9.

Table 7. The mean values (normed at the mean value for the control group) of the intensities of the EPR signals in the tissues of mice with Ehrlich carcinoma.

Liver				
	Intact	Control	"A" group	"Ai" group
Cytochrom p450	139 ± 20*	100 ± 13	112 ± 11	94 ± 10
Fe/S containing proteins	130 ± 22*	100 ± 12	111 ± 11	104 ± 12
Free radicals	134 ± 8*	100 ± 15	116 ± 14	110 ± 14
Tumor tissue				
Free radicals	-	100 ± 14	104 ± 16	107 ± 16
Fe/S containing proteins	-	100 ± 27	124 ± 26	118 ± 35
Fe(3+) in iron storing protein	-	100 ± 18	66 ± 15* p < 0.001	62 ± 9* p<0.001

* = Statistically significant difference from the control group

Table 8. The mean values of the intensities of EPR signals in the tissues of rats with Heren carcinoma.

Liver				
	Intact	Control	"A" group	"Ai" group
Cytochrom p450	147 ±19	100 ±16	105 ±12	93 ±13
Fe/S containing proteins	148 ±19	100 ±14	102 ±12	102 ±12
Free radicals	118 ±15	100 ±12	88 ±14	83 ±10
Mn(2+) containing groups	179 ±29	100 ±18	83 ±12* p < 0.05	75 ±15* p < 0.01
Cu(2+) containing groups	153 ±25	100 ±14	120 ±45* p < 0.05	83 ±15* p = 0.05
Mo(7+) containing groups	377 ±87	100 ±42	168 ±56* p < 0.01	120 ±38
Blood				
Transferrin	286 ±21	100 ±27	161 ±55* p < 0.01	122 ±26
Ceruloplasmin	97 ±10	100 ±18	135 ±32 p < 0.05	122 ±0.14
Free radicals	130 ±34	100 ±20	108 ±31	119 ±25
Tumor tissue				
Free radicals		100 ±15	85 ±14	71 ±6* p < 0.001
R-NO groups		100 ±16	94 ±16	78 ±14*

				$p < 0.01$
Fe(3+) in iron storing protein		100 ±18	67 ±12* $p < 0.001$	65 ±12 $p < 0.001$

The ESR studies of different wet tissues demonstrated the existence of different free radicals in various organs [7]. Spin concentrations were determined to range from $5x10^{14}$ to $5x10^{15}$ radicals/g in a series: liver > kidney > heart >> spleen, liver, muscle. Usually fewer free radicals were found in tumor tissues (however, this phenomenon is not universal).

The content of free radicals in the tissues of tumor-bearing mice decreased by comparison with that of intact animals. However, BRT resulted in an increase of free radical concentration in the liver and blood as well as a certain decrease in the free radical content in those tumors.(Table 7,8).

The content of all paramagnetic metal complexes decreased in the liver of tumorous animals. This shows the suppression of the functional activity of the liver during tumor progression. Bioresonance treatment of mice bearing Ehrlich carcinoma does not result in substantial changes in these parameters. However, a certain tendency to the normalization concerning the concentration of paramagnetic sites is observed in the "A" group. These results may suggest that, the BRT slightly intensifies the oxidative processes in hepatocytes and tumor cells on Ehrlich carcinoma bearing animals.

On the contrary, after the BRT treatment the concentration of paramagnetic sites in the tumor tissue of the rats with Heren carcinoma decreased. The most expressed changes are observed in the "Ai" group. This may be due to alterations in the process of oxidative phosphorylation within the tumor cells. However, a tendency toward normal level of the Cu- and Mo-containing protein content is observed in the "A" group of Heren carcinoma bearing rats.

The observed differences in the BRT effect on the content of paramagnetic sites in mice with Ehrlich carcinoma and rats with Heren carcinoma, reflect the dependence of the BRT effect on the peculiarities of the interaction of a tumor with the organism.

A statistically significant increase in the intensity of the oxidized iron signal (g=4.2) is observed in the tumor tissue of the treated animals (both mice and rats) as compared to the control. During tumor progression, the tumor cells can accumulate iron which is necessary for biosynthesis and fast proliferation. This is accompanied by a decrease in the level of unbounded iron contents in the organism. For example, the iron content of transferrin is 2.8 times lower in the control group by comparison with the intact ones. An Fe(3+) EPR signal in tumor cells is similar to the signal of transferrin.. In the blood of group "A" rats, the signal of transferrin increased 1.6 times and the signal of ceruloplasmin increased 1.35 times as compared with the control group. However, no transferrin destruction was registered.

The observed inhibiting in the iron metabolism in different tumors and the simultaneous activation of iron accumulation and binding to blood proteins, may be the result of the activated processes of lipid peroxidation and an accumulation of iron-containing proteins after the treatment of the studied modes of BRT.

The possible differencies in the molecular weight distribution in the blood serum fractions of tumorous animals was studied by high performance liquid chromatography (HPLC). The blood serum has three HPLC peaks: p_1 (10-11 min. in the experiment) corresponds to the low MW fraction (<30 kDa,); p_2 (13 min.) corresponds to the serum albumin fraction; p_3 (21-21.5 min.) corresponds to the high MW fraction (MW > 200 kDa,). The mean values of the peak areas are summarized in Tables 9, 10.

Table 9. The mean values of the peak areas in the HPL chromatograms of the blood serum of mice with Ehrlich carcinoma after BRT.

Elution time	Intact	Control	"A" group	"Ai" group
p_1 (10.6 min.)	6.2 ±1.3	10.2 ±2.7	10.1 ±2.5	10.8 ±2.4 # p < 0.01
p_2 (13 min.)	90.3 ±0.8	83.3 ±6.1	82.6 ±9.6	82.5 ±4.1# p < 0.001
p_3 (21-21.5 min.)	2.9 ±1.3	4.6 ±2.9	2.8 ±1.9	4.6 ±2.2

Statistically significant difference from the intact sample

Table 10. The mean values of the peak areas in the HPL chromatograms of the blood serum of rats with Heren carcinoma after BRT.

Elution time	Intact	Control	"A" group	"Ai" group
p_1 (10.6 min.)	6.3 ±2.7	5.5 ±0.8	5.2 ±0.9	6.4 ±2.5
p_2 (13 min.)	83.2 ±12.5	94.0 ±5.4 # p < 0.05	91.0 ±7.8	91.1 ±7.8
p_3 (21-21.5 min.)	2.7 ±1.1	1.1 ±0.6# p < 0.05	1.8 ±0.8	2.3 ±1.5

Statistically significant difference from the intact sample

The statistically significant increase in the content of albumin and the high MW fraction during tumor development is found in rats with Heren carcinoma, whereas the presence of Ehrlich carcinoma tumor in mice does not induce significant changes in the molecular weight distribution of different blood serum fractions. In this group, significant changes (as compared with intact blood serum) are induced by the BRT. This may testify to a certain complementarity between the changes induced in the blood serum molecular weight distribution by tumor development and BRT [31, 32].

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