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Action of Non-Ionizing Radiation on Tumor and Healthy DNA

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Abstract. Unlike now widely used traditional methods of treatment of tumors by means of ionizing radiation and the chemotherapy, the method of the use of low intensity electromagnetic fields (EMF) is non-ionizing and non-invasive and hence is completely deprived of any harmful side effects. The present study was undertaken to investigate whether millimeter range EMFs can suppress tumor cells growth in vivo without cytostatic agents. Presented preliminary results have demonstrated the potential clinical application of low power EMFs for clinical oncology in the treatment of malignancies.

Keywords: weak electromagnetic fields, antitumor effect *in vivo*, demethylation DNA

1. Introduction

At present, the problem of the analysis of primary physical and chemical mechanisms underlying sensitivity of biological objects to the non-ionizing electromagnetic radiation remains unclear, that determines the importance of such investigations. Revealing the effects which electromagnetic radiation at millimeter wavelengths has on the organism and its biological significance serve as a basis for using microwave exposure as a physiotherapeutic procedure for treating various diseases. They include cancer of different organs, cardiovascular diseases, diabetic angioneuroropathies, peptic ulcers, leucopenia, pain relief, skin disorders, infantile cerebral palsy, bronchial asthma, wound healing, diseases of immune system, etc[1-3]. It has been shown thatEMFs of millimeter range have strong effect on the process and bioelectric activity of neurochemical functions of the brain, increase the cortical tension, influence on the spike activity of neurons in the supraoptic nucleus of the Hypothalamus of rats [4,5]. The process of DNA-methylation is closely related to the appearance of tumors. Imbalance of DNA-methylation is observed in all, without exception, studied neoplasias. The infringement of methylation process is observed at the early stages of malignant transformation of cells, and the content of 5-methylcytosine (5-MC), which is the only methyl base in DNA of animals and humans, could serve as a diagnostic test for tumor genesis. This opens the possibility for **early diagnostics and treatment** of disease [6,7]. The present study has been performed to investigate the influence of non-ionizing electromagnetic radiation on tumor and healthy DNA *in vivo* without cytostatic agents, to reveal the target of the radiation impact and to study the changes of some physical-chemical properties of aqueous salt solutions of DNA under weak EMFs.

2. Materials and Methods

Adult male mice (2 months of age, 20–22 g in body weight) of NMRI outbreed stock were used in all experiments. The course of influence of MM-waves started 3 days before transplantation in order to raise activity of the animal's immune system [8]. On the fourth day, animals were injected by sarcoma-37 and daily exposure was continued during 15 days. Several groups of mice with five animals in each group were used in each experiment. The animals were randomly distributed among the groups. In each experiment, there was a group of animals, which were not exposed (cage-

control) and a group of control animals, which were sham-exposed (sham-control). Animals of other groups were exposed to the extremely high frequency (EHF) EMR with different duration of exposure – 15 minutes and 0.5 hour. The experiments were independently repeated three times. All experiments were conducted by the "blind" experimental protocol, when an investigator making the measurements did not know which treatments the animals received. The statistical significance of differences in the means for each experimental group was calculated with the Student's t-test. All statistical tests were performed at the 0.05 level of significance.

Duration of the sham-exposure was 30 minutes. As a source of MM wave radiation the generators of coherent EHF oscillations G4-141 and G4-142 (Russian made) were used, operating in a range of frequencies of 38.5÷78.8 GHz. A whole-body exposure of mice to Microwaves was conducted in the far-field zone of cone-shaped antenna at a distance of 400 mm from the radiating plane of the antenna in the mode of continuous generation with incident power density (IPD) at the location of the object about 10 $\mu W/cm^2$.The calculated value for the rate of the specific absorption is approximately equals to 0.2 W/kg. On the 16^{th}day of the experiment all animals were decapitated and the tumors were extracted and weighted. The therapeutic effect of exposure was evaluated by inhibition of tumor growth.

3. Results and Discussion

In our experiments an increase in the level of DNA-methylation in the tumor without treatment was observed (Table 1), that are confirmed in many cases by literature data, since there is a significant interaction between chromatin modification and DNA-methylation and accessibility of DNA in it for the corresponding methylases and their activation [9,10]. It is also assumed that single-stranded DNA formed during replication and repair may be a subject for *de novo* methylation by DNA-methyltransferase that often occurs in tumors [11]. The content of the main pairs of bases in the studied DNA is almost identical. The isolated DNA belongs to the AT-type. Quantity (G+C+5MC) in them is 42.2-44.9 mole %. The nucleotide composition of DNA corresponds to the rules of Chargaff.

Table 1. The content of 5-methylcytosine and DNA melting parameters under the influence of MM-radiation at 42.2 GHz. Melting range is defined as the temperature difference at the points where the optical density of DNA solution varies from 17 to 83%

Experimental condition	Source of DNA	5-MC mol%	ΔT°C	T_m°C
Healthy animals	liver	1.9 ± 0.1	6.6 ± 0.1	71.8 ± 0.2
Animals with sarcoma 37	tumor	4.7 ± 0.1	7.6 ± 0.1	70.6 ± 0.1
Animals with sarcoma 37 + MM-radiation effect 15 min	tumor	4.7 ± 0.1	7.5 ± 0.1	70.5 ± 0.1
Animals with sarcoma 37 + MM-radiation effect 30 min	tumor	2.2 ± 0.1	6.9 ± 0.1	71.7 ± 0.2

As it can be seen from the Table 1, the pronounced effect of MM EMF therapy appears in the group with a half-hour continuous irradiation. A strong suppression of the level of DNA-methylation of sarcoma-37 is observed, which can be explained as follows: low intensity EMFs, impacting on the growth and proliferation of cells, the enzyme activity, the genetic apparatus of cells and not accelerating tumor growth, exert an inhibitory influence on the development of the grafted sarcoma and increase the lifetime of experimental animals [12]. A similar effect was detected in our experiments. It is established that the duration of the procedure of 30 min EMF exposure caused inhibition of tumor growth by 33.5%, and 15-minute exposure did not exert an inhibitory effect on the tumor. General toxic effect of MM-wave therapy on the organism of animals in both groups (15 and 30 min) exposure is insignificant Kp = (-1.2) ÷ (-1.5). Correlation

between tumor growth delay and the level of the DNA-methylation is obvious. After 15 sessions of MM-wave therapy without cytostatic drugs, for animals of the third group (30 min exposure) an inhibition of tumor growth by 33.5% was observed compared with a control group and a sharp suppression of DNA-methylation level of 2.1 times at the most. DNA-demethylation in the tumor tissue under the influence of a half-hour exposure of MM-waves can be explained by enzymatic demethylation of remains of 5-MC, i.e. the mechanism of impact of the studied waves basically involves demethylation of tumor DNA, which in its turn could sensitize the damage of chromatin, inhibit an efficient repair of DNA, providing genomic instability, which can bring to apoptosis of tumor cells leading to inhibition of tumor growth[11,13].

3.1. Changes in Tumor DNA after Microwaves Exposure

In the work [14] has been shown that with the help of melting differential curves (MDC) DNA tumor sarcoma can be distinguished from DNA isolated from the liver of healthy rats. MDC of tumor DNA are shifted relative to MDC of the liver DNA towards lower temperatures, and in the MDC of tumor DNA the additional peaks appear in the 52-60ºC range, which are absent for MDC of liver DNA of healthy animals. The following table shows the values of temperature (T_mºC) and interval (ΔTºC) of melting and content of 5-MC in the studied samples of DNA. The interesting for us parameters characterizing the primary and secondary structures of DNA, under the action of MM in 30 minutes exposure undergo certain changes (see Table 2). We have examined the effect of MM-wave radiation on the structure of DNA *in vivo*, based on the nature of the changes in the parameters of melting and the content of 5-MC.

Table 2. MM-wave influence on growth sarcoma-37 at 42.2 GHz. In each group were 10 animals. Numbers of definitions were 9. These changes were reliable ($p < 0.05$) compared with control

	Antitumor activity					
Time of influence of MM-radiation	**Number of animals**		**Tumor weight in grams**			
	Control group	Investigated group	Control group	Investigated group	T%	P
15 min	10	10	1.49±0.12	1.47±0.13	0	-
30 min	10	10	1.49±0.12	0.99±0.1	33.5	=0.05

As it can be seen from Table 1, the tumor DNA (**t**DNA) has the high level of methylation (4.7 mole %), which after 30 minutes influence of MM-wave radiation becomes (2.2 mole%) close to the corresponding value for health DNA (**h**DNA) (1.9 mol%). The obtained results are correlated with the spectrophotometric data (Table 2). Under the influence of MM-wave radiation, the values of T_m ºC and ΔT ºC of **t**DNA were changed and approached to the corresponding values of **h**DNA (Table 2). The experimental data presented in Table 2show that it is quite possible, 30 min MM-wave radiation leads to the activation of specific molecular mechanisms of cells, resulting in decreased undesirable structural changes in the tumor DNA, resulting in inhibition of tumor growth. Thus, the correlation data between the ability of MM-wave radiation to modify the structure and content of 5-MC in tumor DNA *in vivo* and inhibition of tumor growth, allow assuming that the MM-radiation with a frequency of 42.2 GHz has antitumor activity.

3.2. The Change of the Thermostability of Irradiated DNA

The changes of the physical-chemical characteristics of the tumor and healthy DNAs' have been investigated under radiation at frequency 64.5 GHz, in correspondence with a resonance frequency of oscillations of hexagonal molecular structures of water [15]. We used 1 Hz amplitude modulation.

The studies have shown that the form of the melting curves, the values of T_m and ΔT, do not exhibit a certain dependence on the duration of post-irradiated term, since after irradiation both about 12 and 24 h, these parameters are within experimental error. It is found that depending on the

duration of exposure, the thermostability of DNA increases, which is more pronounced for **t**DNA (Table 3). Upon irradiation for 90 min T_m **t**DNA is increased by about 1.5°C, while the ΔT is decreased. Perhaps, the irradiation leads to the ordering of water molecules associated with the macromolecule, especially in AT-rich regions, which in turn affects the compaction of the macromolecule, and this, in turn, affects the T_m and ΔT.

Table 3. Temperature and range of DNA melting obtained from of healthy rats liver and tumor sarcoma - 45 at 64.5GHz

	hDNA		s-45DNA	
Time of irradiation, min	T_m, °C	ΔT, °C	T_m, °C	ΔT, °C
0	69.4 ± 0.1	7.2 ± 0.2	68.8 ± 0.2	7.9 ± 0.2
30	69.4 ± 0.1	7.2 ± 0.2	68.9 ± 0.1	7.9 ± 0.2
40	69.5 ± 0.2	7.1 ± 0.2	69.0 ± 0.1	7.8 ± 0.2
60	69.9 ± 0.1	7.0 ± 0.2	69.8 ± 0.1	7.8 ± 0.2
90	70.3 ± 0.2	7.0 ± 0.2	70.2 ± 0.2	7.6 ± 0.2
120	70.4 ± 0.2	6.9 ± 0.2	70.2 ± 0.2	7.5 ± 0.2

To confirm this fact, the MDC of irradiated and non-irradiated DNA are obtained. The values of melting parameters for **h**DNA and **t**DNA are summarized in Table 3. As it can be seen from the table data, the dynamics of changing of T_m and ΔT for **t**DNA is more pronounced than for **h**DNA in the case of irradiation with low intensity MM-waves during the increasing of exposure duration.

It should be noted that the T_m and ΔT of unexposed **h**DNA and **t**DNA do not coincide (Table 3): T_m **t**DNA is about 0.5°C lower than **h**DNA, while ΔT is higher for **t**DNA. This is apparently due to the presence of "defective" parts in **t**DNA molecule arising as a result of methylation and subsequent enzymatic dezamination of cytosine and its transformation to thymine, which leads to the formation of unstable guanine–thymine pair [7,16]. As a result, the locally denatured regions are formed in DNA molecule, which leads to the reduction of T_m **t**DNA. The conformational transitions into the hypermethylated parts of DNA molecule are possible as well [17]. Due to the above mentioned structural differences there is more pronounced change in **t**DNA hydration during the irradiation resulting in the increase of the melting temperature.

The assumption that changes in the DNA melting parameters under the influence of low intensity MM-waves stipulated by the structure of water, is based on the fact that the resonant absorption frequencies of DNA are in the region of 2 to 9 GHz [18]. Hence, we assume that at a frequency of 64.5 GHz, the changes in the values of T_m and ΔT cannot be due to the resonance absorption of DNA, i.e. the radiation not directly influences on the DNA. Consequently, the increase in the thermostability of DNA during the irradiation by MM-waves with a frequency 64.5 GHz can be caused by their mediated influence through the water. DNA-samples only were prepared in the irradiated water-salt solution (buffer) for the confirmation of the mentioned fact. Melting curves obtained for them do not practically differ from the curves obtained by irradiation of DNA solutions within the experimental error. Therefore, it can be assumed that the observed changes in the parameters of DNA-denaturation are caused just by changes in the structure of water arising due to exposure.

3.3. The Change under Radiation Density of Aqueous Salt Solutions of DNA

This is also indicated by the results on the measurement of the density of aqueous salt solutions of DNA in the case of irradiating by MM-waves. For a control, the densities of bi-distilled water and water-salt solution were also measured before and after irradiation. Density of water, 0.1SSC and DNA solutions was determined on densitometer DMA 4500 Anton Paar (USA), with resolutions 10^{-5}g/cm^3. The studies have shown that in the case of irradiation by pure water with a frequency of 64.5 GHz, its density does not practically change, while the density of the buffer and the DNA-solution increases. This indicates that the structural state of pure water does not change due to irradiation, since under those medium conditions the water molecules form a most stable,

from a thermodynamic point of view, structure, and an increase in ordering after exposure becomes thermodynamically non-profit. Therefore, the density of water under these conditions should not be changed. In contrast, in the case of irradiation of the buffer and the DNA-solution some of the free water molecules ("not included" in composition of the most common hexagonal structures) are structured around the dissolved ions or macromolecules (increasing the hydration degree). Moreover, most probably, the water molecules are involved in the formation of additional bonds with the salt ions or with functional and atomic groups of macromolecules, which lead to an increase in size of the ions or macromolecules, and the latter is the cause of density increase.

Table 4. Magnitude of solution density (g/cm^3) before and after exposure of EMFsat 64.5 GHz

Time of irradiation, min	Buffer	Buffer + DNA
0	0.999201 ± 0.000005	0.999232 ± 0.000004
30	0.999220 ± 0.000005	0.999242 ± 0.000005
60	0.999241 ± 0.000004	0.999269 ± 0.000004
90	0.999253 ± 0.000004	0.999291 ± 0.000005

The results of measurements of the density of buffer and the DNA-solution are summarized in Table 4. As it can be seen from the table, there is almost the same dynamics of changing of the buffer and the DNA-solution densities and that the obtained data are in a good agreement with the results of DNA-melting. The dependence of the density of the DNA-solution on the temperature has been also studied, in the case of irradiation by duration of 90 and 120 min, to detect changes in the structure of water by irradiation, depending on temperature. It is found that with increasing temperature the density of the irradiated and non-irradiated DNA is reduced, but there is a significant difference between the solution of the irradiated and non-irradiated DNA.

Conclusion

Thus, it has been experimentally shown that the low-intensity electromagnetic fields do not act directly on DNA molecules, and the influence takes place through a **mediated** influence of the EMWs on the water, stimulating structural change of the water shell surrounding the DNA. Thereby, we may conclude that the primary targets of the influence of the electromagnetic fields on the DNA water solutions are the water molecules. Because the therapeutic effect of coherent MM-waves was estimated by inhibition of tumor growth and changes in the level of methylation, our studies revealed a correlation *in vivo* between antitumor activity of non-ionizing MM-wave therapy and inhibition of methylation level of tumor DNA. Hypermethylation of tumor-DNA may cause a selective sensitivity of malignant cells toward the influence of the MMWs, which allows an expressed antitumor effect in the absence of chemo- and radiotherapy. It is possible, that 30 min daily exposure leads to the activation of specific molecular mechanisms of cells, resulting in a decrease of undesirable structural changes in the tumor DNA and inhibition of tumor growth. The antitumor effect of the MM-waves obtained without cytostatics shows promising development of the MM-wave therapy for clinical oncology in the treatment of malignant neoplasms. In our studies, suppression of growth of tumor cells took place without application of modulation of radiation, i.e. in a mode of **continuous** generation. It simplifies experiments and also application of this method for treatment. These preliminary results open-up a very interesting research direction, which is connected with the possible use of a low-power MM-wave radiation against tumor cells without damaging other tissues and antitumor drugs and without harmful ionizing radiotheraphy.

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