American Journal of Medical and Biological Research, 2014, Vol. 2, No. 1, 18-25
Available online at http://pubs.sciepub.com/ajmbr/2/1/4

DOI:10.12691/ajmbr-2-1-4

Preliminary Results of Influence of Nonionizing Electromagnetic Radiation on Tumor and Healthy DNA and Role of Water

Vitali Kalantaryan[1, *], Radik Martirosyan[1], Yuri Babayan[2], Lusine Nersesyan[3], Hrachya Stepanyan[3]

[1]Microwave Radiophysics and Telecommunication, Yerevan State University, Yerevan, Armenia
[2]Medical Physics, Yerevan State Medical University, Yerevan, Armenia
[3]Fine Organic Chemistry Institute of National Academy Sciences of Armenia, Yerevan, Armenia
*Corresponding author: vkalantaryan@yandex.ru, vkalantaryan@ysu.am

Received January 21, 2014; Revised February 20, 2014; Accepted February 25, 2014

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 low-intensity EMFs can suppress tumoral cells growth *in vivo* without cytostatic agents. The course of influence of EMFs started 3 days before transplantation in order to raise activity of the animals' immune system. On the fourth day animals were injected by sarcoma-37 and daily exposure was continued during 15 days. For study of the effect of irradiation on the secondary structure of DNA, in the experiments DNA isolated from the liver of healthy mice (hDNA) as well as from the tumor sarcoma 37 (tDNA) was used. After 15 sessions of exposure without cytostatic drugs, at animals of the irradiated 0,5 hour was observed an inhibition of tumor growth by 33.5% compared with a control group and a sharp suppression of the level of DNA-methylation in 2.1 times. The tDNA has the high level of methylation (4.7 mol%), which after 0.5 hour daily exposure becomes (2.2 mol%) close to the corresponding value for hDNA (1.9 mol%). Differential melting curves (DMC) of tDNA are shifted relatively DMC of the hDNA to lower temperatures, and in the DMC of tDNA the additional peaks in the 52-60ºC range are appeared, which are absent for DMC of liver hDNA. The obtained results are correlated with the spectrophotometric data. Under the influence of EMFs the values of temperature and interval of melting of tDNA are changed and approach to the corresponding values of hDNA. Presented preliminary results have demonstrated the potential clinical application of low power EMFs for clinical oncology in the treatment of malignancies. The changes of physical-chemical properties of tumoral and healthy DNA under amplitude modulated radiation at 64.5 GHz and possible mechanisms of these changes have been investigated and discussed.

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

Cite This Article: Vitali Kalantaryan, Radik Martirosyan, Yuri Babayan, Lusine Nersesyan, and Hrachya Stepanyan, "Preliminary Results of Influence of Nonionizing Electromagnetic Radiation on Tumor and Healthy DNA and Role of Water." *American Journal of Medical and Biological Research*, vol. 2, no. 1 (2014): 18-25. doi: 10.12691/ajmbr-2-1-4.

1. Introduction

At present, there is a large number of experimental and theoretical material on the study of medical and biological effects of the low intensity coherent millimeter wave (MM-wave) electromagnetic radiation. However, the problem of the analysis of primary physical and chemical mechanisms underlying sensitivity of biological objects to this type of 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, etc [1,2,3]. According to the literature data, the Microwave therapy increases the level of immune resistance, influences on different stages of pathogenesis, changes enzymatic reaction activity and growth rate, destroys microorganisms and increases the thermostability of DNA [4,5,6,7]. It has been shown that millimeter waves 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 [8,9]. By penetrating into the organism (the penetration depth of MM-waves in tissues is very small, is about 1-1.5 mm due to high absorption by water molecules), this radiation is

transformed into information-carrying signals performing guidance and adaptation control or rehabilitation processes in the organism. The influence of electromagnetic fields (EMF) on various tumors has been investigated in [10-16].

In the case of malignant transformation the cells undergo changes, which lead to uncontrolled cellular proliferation and abnormal differentiation. Besides, the genesis is involved in all aspects of development and growth of the tumor. Studying the possible structural changes in DNA of tumor cells under the influence of MM-waves in the absence of cytostatics is useful, since the Microwave therapy used in complex antineoplastic treatment promotes the reduction of toxic effect of chemo- and radiotherapy and increasing of its antitumor effect. In the most of the literature data the tumor inhibition by means of Microwave therapy in the absence of cytostatic agents was studied in the experiments in vitro [17,18].

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 [19,20]. 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 low intensity MM waves.

Hypermethylation of tumor-DNA, the mechanism of which in many tumors is not clear, destabilizes the secondary structure of DNA as well, that may cause the selective sensitivity of malignant cells to the influence of millimeter electromagnetic radiation in the absence of chemo- and radiotherapy and allow receiving of pronounced antitumor effect. Therefore, in the present work the influence of non-thermal coherent electromagnetic radiation on the secondary structure of DNA sarcoma 37 tumor *in vivo* is investigated too.

2. Materials and Methods

2.1. Animals

Adult male mice (2 months of age, 20–22 g in body weight) of NMRI outbreed stock were used in all experiments. The animals were housed in an air-conditioned room under controlled temperature (22 ± 2ºC) and 12-h light/dark cycle conditions with standard chow and tap water freely available. All manipulations with the animals were conducted in accordance with experimental protocols approved by the Local Animal Care and Use Committee of the Fine Organic Chemistry Institute of Armenian National Academy of Sciences and the standards of the International Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). To reveal the features of influence of MM-wave therapy with different modes of an irradiation on DNA of tumor-bearing animals at the absence of cytostatic drugs, a study of effects of MM-waves on mice injected sarcoma 37 by a known method [21] has been carried out.

2.2. Exposure by MM–wave Electromagnetic Radiation (EMR)

The course of influence of MM-waves started 3 days before transplantation in order to raise activity of the animal's immune system [22]. 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.

The mice were exposed from the top in plastic containers with a size of 80 mm x 80 mm x 100 mm. The bottom square of the container for animals corresponded to the square of the exposed zone created by the major lobe of the antenna. To eliminate the interference in the plane of exposed object an effective multi-layer absorbent was placed between the animal container and the floor. Therefore, the conditions of exposure were close to the free field conditions. Animals of the control group were sham-exposed by placing the mice into the exposure zone when the generator was turned on but the output power was attenuated to zero. Duration of the sham-exposure was 30 minutes.

2.3. Source of Microwaves

As a source of millimeter wave radiation the generators of coherent Extremely High Frequency 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 μW/cm^2.

The output power of the generator was measured with the help of M5-49 or M5-50 thermistor heads and M3-10A wattmeter (Istok, Fryazino, Russia). The frequency of the output signal was controlled by CH2-25 or CH-2-26 wavemeters (Istok, Fryazino, Russia). The frequency of the output signal was controlled by CH2-25 wavemeter (Istok, Fryazino, Russia). To calculate the specific absorption rate (SAR) we used dielectric parameters of the skin of mice $\varepsilon' = 14$, $\varepsilon'' = 18$ and skin density ρ = 1.15 g/cm^3 [23]. The SAR on the surface of skin of the animals back was calculated by the formula [24].

$$SAR = \frac{\sigma\xi(1-R)P_0}{n\rho},$$

where $\sigma = \varepsilon_0\varepsilon''\omega = 42.3$ S/m is the electric conductivity of the skin at the frequency of 42.2 GHz, $\varepsilon_0 = 8.85 \cdot 10^{-12}$ F/m is the vacuum dielectric constant, ω is the circular frequency, ξ = 377 Ω is the vacuum wave impedance, P_0 is the incident power density, R=0.5 is the reflection coefficient, n = 4.2 is the refractive index of the skin. The calculated value for the rate of the specific absorption is approximately equal to 0.2 W/kg.

2.4. Definition of DNA Methylation Level

On the 16th day of the experiment all animals were decapitated under ether anesthesia and the tumors were extracted and weighed. The therapeutic effect of exposure was evaluated by inhibition of tumor growth. To determine the level of the DNA-methylation, DNA was extracted, after the slaughtering of animals, from tumor cells by phenol-chloroform method in the presence of 1, 5%-SDS [25]. Hydrolysis to the nitrogen bases was carried out in the sealed glass ampoules in 85%-formic acid at 176ºC for one hour (0.1 ml of acid per 1 mg of DNA). The separation of nitrogen bases: guanine (G), cytosine (C), 5-methylcytosine (5-MC), adenine (A), thymine (T) was produced by thin-layer chromatography (TLC) on DEAE-cellulose in the solvent n-butanol: water: ammonia. Spectrophotometry of eluates of all bases was made against eluates from the respective control areas of chromatograms.

2.5. Influence of Microwaves on DNA Second Structure

In the experiments on clarifying the influence of the electromagnetic radiation on the secondary structure of DNA, the DNA isolated from the liver of healthy mice (hDNA) as well as from the tumor sarcoma 37 (tDNA) was used. The method of isolation and characteristics are described in [26]. The melting of DNA was carried out in an aqueous solution containing 0.02 M NaCl, 0.5 mM EDTA, pH 7.3. The spectrophotometric melting curves were obtained on a spectrophotometer UNICAM SP8-100 (England) during the continuous heating of DNA solutions at the rate 0.28ºC/min. The accuracy of temperature is 0.1ºC, of the optical density 0.001 units. The melting curves for each DNA-sample were made 10 times. The parameters of fusion were calculated for each curve and whereupon where averaged. Since the peculiarities of primary and secondary structures of DNA on the spectrophotometric curves where revealed weakly, there was made the transition to the differential melting curves, which were obtained via numerical differentiation of the normalized melting curves by the method described in [27].

3. Results and Discussion

3.1. Correlation of Tumor Growth Delay with the DNA Methylation Level

In our experiments an increase in the level of DNA-methylation in the tumor without treatment was observed (Table 1), that in many cases are confirmed 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 [28,29]. 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 [30]. 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 mol%. The nucleotide composition of DNA corresponds to the rules of Chargaff. The difference in the level of methylation between the DNA samples obtained from the tumor without treatment (control group) and the DNA of tumor cells in the case of 15 minutes and half-hour exposure is clearly visible.

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%	ΔTºC	T_mºC
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-wave 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 MM-waves, 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 [31]. A similar effect was detected in our experiments. It is established that the duration of the procedure of 30 min MM-wave 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, in 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 [30,32].

In the case of MM-wave therapy with 15 min-duration of exposure an inhibition of DNA-methylation level of the tumor was not observed and delay in tumor growth was not marked. Data are shown in Table 2. As it is seen, the weight of tumors was identical with that in control group.

In our early work [33]results of Table 2 were presented.

3.2. Changes in Tumor DNA after Microwaves Exposure

In the works [27,34] have 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 (Figure 1). The effect of the MM radiation with a frequency of 42.2 GHz is investigated *in vivo* on the structure of DNA secondary structure of sarcoma 37.

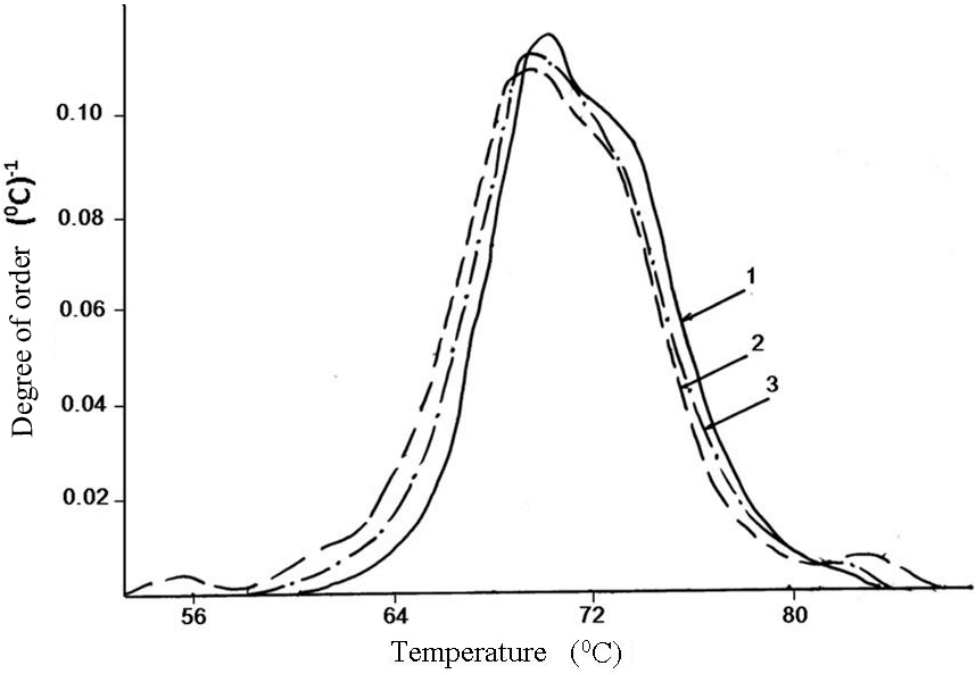

Figure 1. Differential melting curves (1) DNA of healthy mice, (2) DNA of sarcoma-37, and (3) DNA of sarcoma-37 under *in vivo* irradiation at 42.2 GHz for 30 min. On an axis Y degree of an order of DNA is given

It is necessary to note that the electromagnetic radiation of low intensity of sharp response of the human organism exists around the frequencies of 40 GHz that complies with resonance frequency of the tertiary structure DNA-spirals [35,36,37].

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 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-radiation 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 (tDNA) has the high level of methylation (4.7 mol%), which after 30 minutes influence of MM-wave radiation becomes (2.2 mol%) close to the corresponding value for health DNA (hDNA) (1.9 mol%). The obtained results are correlated with the spectrophotometric data (Table 1 and Figure 1). Under the influence of MM-wave radiation the values of T_mºC and ΔTºC of tDNA were changed and approached to the corresponding values of hDNA (Table 1 and Figure 1). The experimental data presented in Figure 1 and Table 1 show 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. Let us analyze the MDCs shown in Figure 1. The characteristic low-temperature peaks for MDC tDNA in the region of 54-62ºC and under the action of the MM-wave radiation almost disappear and

become close to those for MDC hDNA, but the curve is still shifted to lower temperatures compared to the MDC hDNA. The shift of MDC of tDNA towards MDC of hDNA as a result of exposure is apparently stipulated by the fact that due to the MM-wave radiation the fraction of tumor cells in the tumor decreases.

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. The MM-waves general toxic influence on the experimental animals with sarcoma 37 without cytostatics is negligible. Antitumor effect of coherent MM-waves obtained without drugs, shows promising development of millimeter therapy for clinical oncology in the treatment of malignancies.

3.3. The Change of the Thermostability of Irradiated DNA

The changes of the physical-chemical characteristics of the Tumoral 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 [38]. In this case 1 Hz amplitude modulation has been used.

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 tDNA (Table 3). Upon irradiation for 90 min T_m tDNA 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 64.5 GHz

	$_h$DNA		$_{S\text{-}45}$DNA	
Time of irradiation, min	T_m, ºC	ΔT, ºC	T_m, ºC	ΔT, ºC
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. Figure 2 shows the melting differential curves of irradiated for 90 min and non-irradiated tDNA. As it can be seen from the figure, MDC tDNA is shifted towards the high temperatures in comparison with non-irradiated tDNA. A similar increase of T_m was also obtained for hDNA, DNA of calf thymus, but this parameter is less (1,0°C) than at tDNA. With increasing of exposure duration (> 90 min) T_m and ΔT both of hDNA and tDNA practically do not change, which, in all probability, is due to the fact that the water structuring degree does not undergo further changes. The values of melting parameters for hDNA and tDNA are summarized in Table 3. As it can be seen from the table data, the dynamics of changing of T_m and ΔT for tDNA is more pronounced than for hDNA in the case of irradiation with low intensity MM-waves during the increasing of exposure duration.

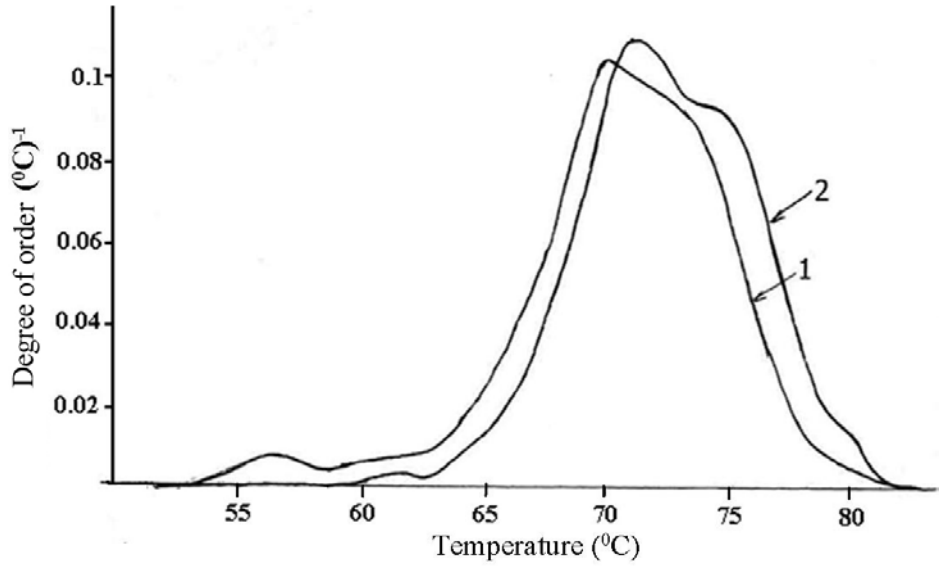

Figure 2. Differential melting curves for sarcoma-45 DNA under (1) non-irradiated and (2) irradiated at 64.5 GHz for 90 min. On an axis Y degree of order of DNA is shown (is given)

It should be noted that the T_m and ΔT of unexposed hDNA and tDNA do not coincide (Table 3): T_m tDNA is about 0,5°C lower than hDNA, while ΔT is higher for tDNA [34]. This is apparently due to the presence of "defective" parts in tDNA 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 [20,39]. As a result, the locally denatured regions are formed in DNA molecule, which leads to the reduction of T_m tDNA. The conformational transitions into the hypermethylated parts of DNA molecule are possible as well [40]. Due to the above mentioned structural differences there is more pronounced change in tDNA 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 [41]. Hence, we assume that at a frequency of 64.5 GHz the changes in the values of T_m and ΔT can not 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 were prepared in the irradiated only 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.

3.4. 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 bidistilled water and water-salt solution were also measured before and after irradiation. Density of water, 0.1×SSC 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 these 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 leads 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 MM-radiation at 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 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 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.

As it can be seen from the Figure 3, the dependence of $\Delta\rho$ on T ($\Delta\rho$ is the difference between the solution densities for irradiated and non-irradiated DNA) increases slightly in the range of temperature 20<T<40°C, and in the range of 40<T<70°C it is observed a sharp decrease in $\Delta\rho$. With further increase of temperature (T>70°C) $\Delta\rho$ sharply increases and as a result a minimum at a temperature of about 70°C on the curve is observed, that corresponds to the melting point of DNA. As it follows from the spectrophotometric melting curves, denaturation of DNA occurs in the interval of temperature changes 60<T<85°C. Therefore, this dependence may be caused by the fact that the hydration of the irradiated DNA with increasing temperature decreases to a greater extent than in the case of non-irradiated DNA, and at T=70°C, when a half of DNA is in a melted state, the reducing of hydration degree of the irradiated DNA is the maximal. The further increase in temperature leads to a sharp increase of $\Delta\rho$. The sharp increase of $\Delta\rho$ at T>70°C, to all appearances, is a consequence of the fact that in the single-stranded (ss) state the degree of hydration of the irradiated DNA-molecules is higher, than in the double-stranded (ds) state (~5 times), and, on the other hand, the single-stranded DNA-molecules probably become the "centers of crystallization" for the water molecules, so that the density of system "water-irradiated ss-DNA" is increased in comparison with non-irradiated ones. It is assumed that during irradiation some part of the "free" water molecules, which were involved in the hydrate structure of DNA, is released after exposure, leading to a sharp decrease in the density of the water-DNA system, while an analogous phenomenon does not occur in the case of non-irradiated DNA, and the density of the latter undergoes minor changes. Further, with an increase in temperature there is an increase of the lability both of macromolecule and hexagonal structures of water, so that in all likelihood

these structures are involved in the hydration shell of macromolecule, and the density of this system increases.

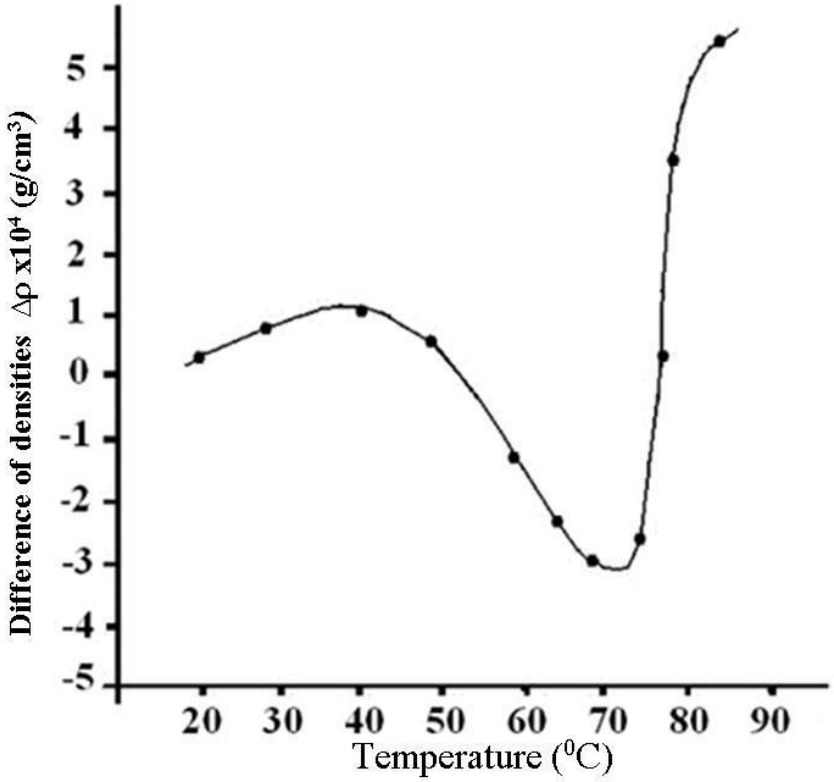

Figure 3. Curve of dependence of Δρ difference of density of solutions irradiated for 90 min and non-irradiated DNA on temperature

4. Conclusion

Thus, obtained results indicate that the influence of low-intensity MM-electromagnetic waves on the structure and functional characteristics of biomacromolecules can be determinative in various states of biological system. 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 EMFs 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.*

Thereby, on a question what is the primary target of the influence of the electromagnetic fields on the DNA water solutions, it is possible to answer with a good probability that it is 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.

Change of the physical-chemical properties of tumoral and healthy DNA under Extremely High Frequency EMR has been investigated on carrier frequency at 64.5 GHz which is a resonant frequency for oscillations of molecular hexagonal water structures.

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. Our researches once again confirm reliability of results and conclusions of works [14,15,16] about suppression of growth of tumoral cells by low-intensity electromagnetic fields. However, unlike these works where the modulated radiation was applied for achievement of antitumoral effect, in our work suppression of growth of tumoral cells took place without application of modulation of radiation, i.e. in a mode of continuous generation. It simplifies carrying out experiments and also application of this method for treatment.

These preliminary results open a very interesting research direction, which is connected to the possible use of a low-power MM-wave radiation against tumor cells without damaging other tissues and antitumoral drugs and without harmful ionizing radiotheraphy.

References

[1] Rojavin, M.A. and Ziskin, M.C., "Medical applications of millimeter waves," *QJ Med*, 91, 57-66, 1998.

[2] Pakhomov, A.G. and Murphy, M., "Low intensity millimeter waves as a novel therapeutic modality," *IEEE Trans Plasma Sci*, 28, 34-40, 2000.

[3] Pletnev, S.D., "The use of millimeter band electromagnetic waves in clinical oncology," *Crit Rev Biomed Eng*, 28, 573-87, 2000.

[4] Gapeyev, A.B. and Chemeris, N.K., "Model approach to the analyses of effects of modulated electromagnetic radiation on animal cells," *Biophysics*, 45, 299-312, 2000.

[5] Tadevosyan, H.H., Kalantaryan, V.P. and Trchounian, A.H., "Extremely high frequency electromagnetic radiation enforces bacterial effects of inhibitors and antiobiotics," *Cell Biochemestry & Biophysics*, 51(2-3), 97-103, Jul.2008.

[6] Kalantaryan, V.P., Vardevanyan, P.O., Babayan, Y.S., Gevorgyan, E.S., Hakobyan, S.N. and Antonyan, A.P., "Influence of low intensity coherent electromagnetic millimeter radiation (EMR) on aqua solution of DNA", *Progress In Electromagnetics Research Letters*, 13, 1-9, 2010.

[7] Babayan, Y.S., Tadevosyan, A.A. and Kalantaryan, V.P., "Influence of millimeter range coherent electromagnetic radiation on some properties of solutions of DNA", *Biomedical Radioelectronics*, No2, 52-57, Febr.2009.

[8] Shenberg, A.S., Uzbekov, M.G., Shihov, S.N., Bazyan, A.S., and Chernyakov, G.M., "Some neyrotrop effects of low intensity electromagnetic waves on the rats with different typological pecularities of the highest neural activity", *Journal of the Highest Neural Activity*, 50, 867-77, 2000.

[9] Minasyan, S.M., Grigoryan, G.Y., Saakyan, S.G., Akhumyan, A.A., and Kalantaryan, V.P., "Effects of the action of microwave-frequency electromagnetic radiation on the spike activity of neurons in the supraoptic nucleus of the Hypothalamus in rats", *Neuroscience and Behavioral Physiology*, 37(2), 175-80, 2007.

[10] Sitko SP, Mkrtchyan LN. *Introduction to Quantum Medicine*. Kiyev: Pattern;1994.

[11] Logani, M.K., Szabo, I., Makar, V.R., Bhanushali, A., Alekseev, S.I., and Ziskin, M.C., „Effect of Millimeter wave irradiation on tumor metastasis", *Bioelectromagnetics*, 27, 258-64, 2007.

[12] Kalantaryan, V.P., Babayan, Y.S., and Tadevosyan, A.A., "Investigation of the binding of antitumor compouds of Mitoxantrone and Amentantrone with the DNA-irradiated millimeter electromagnetic waves", in *UICC World Cancer Congress*, Geneva; p.82 POS- A288..2008a.

[13] Kalantaryan, V.P., Babayan, Y.S., and Gharibyan, J.V., "Cross influence of Doxorubicin antitumour drug and millimeter electromagnetic waves on structure of tumour DNA", in *UICC World Cancer Congress*, Geneva; PUB-306. 2008b.

[14] Barbault, A., Costa, F., Bottger, B., Munden, R., Bomholt, F., Kuster, N., et al. "Amplitude-modulated electromagnetic fields for the treatment of cancer: discovery of tumor-specific frequencies and assessment of a novel therapeutic approach", J Exp Clin Cancer Res, *28*(1), 51-60, Apr.2009.

[15] Costa, F.P., de Oliveira, A.C., Meirelles, R., Machado, M.C., Zanesco, T., Surjan, R., et al. "Treatment of advanced hepatocellular carcinoma with very low levels of amplitude-modulated electromagnetic fields", Br J Cancer, *105*(5), 640-48, Aug. 2011.

[16] Zimmerman JW, Pennison MJ, Brezovich I, Yi N, Yang CT, Ramaker R, et al. "Cancer cell proliferation is inhibited by specific modulation frequencies", *Br J Cancer*, 106(2), 307-13, Jan. 2012.

[17] Kuzmenko, A.P., Solovyev, E. and Bundyuk, L.S., "Features of course of tumoral process at influence low power microwave radiations on acupuncture points in experiment", *Experimental oncology*, 14(1), 72-6, 1992.

[18] Chidichimo, G., Beneduci, A., Nicoleta, M., Critelli, M., De Rose, R., Tkatchenco, Y., et al. "Selective inhibition of tumor cells growth by low power millimeter waves", *Anticancer Research* 22, 1681-8, 2002.

[19] Burnham, C.M., "Components of Listeria and Escherichia coli have been used to prime the immune system in a novel approach to fighting cancer", *Drug Discover Today*, 2(8), 54-8, 1992.

[20] Partha, M.D. and Rakesh, S., "DNA methylation and cancer", *J.Clinical Oncology*, 22(22), 4632-42, 2004.

[21] Chernov, V.A., Methods of experimental chemotherapy. Moscow: Medicine press; 1971.

[22] Makar, V.R., Logani, M.K., Bhanushali, A., Alekseev, S.I., and Ziskin, M.C., "Effect of Cyclophosphamide and 61.22GHz Millimeter Waves on T-Cell, B-Cell, and Macrophage Functions", *Bioelectromagnetics*, 27, 458-66, 2006.

[23] Gapeyev, A.B., Sokolov, P.A. and Chemeris, N.K., "A study of absorption of energy of the extremely high frequency electromagnetic radiation in the rat skin by various dosimetric methods and approaches", *Biophysics*, 47, 759-768, 2002.

[24] Gapeyev, A.B., Mikhailik, E.N. and Chemeris, N.K., "Anti-inflammatory effects of low-intensity extremely high-frequency electromagnetic radiation: Frequency and power dependence", *Bioelectromagnetics*, 29, 197-206, 2008.

[25] Vanyushin, B.F., Masin, A.H., Vasiliev, V.R. and Belozersky, A.N., "The content of 5-methylcytosine in animal DNA: the species ant tissue specificity", *Biochem. Et Biophys. Acta*, 299, 397-403, 1973.

[26] Babayan, YS, Khudaverdyan NV, Sngryan HE, Gharibyan JV. "Influence of Imidazen in a combination with RNA on structure of DNA sarcoma 45", *Biophysica*, 42(1), 125-8, 1997.

[27] Babayan, Y.S., Vardevanyan, P.O., and Gharibyan, J.V., "Application of a method of melting in revealing of distinctions between DNA from normal and tumoral tissues", *Biophysica*, 29(2), 313-4, 1984.

[28] Chih-Lin Hsieh, "The de novo methylation activity of Dnmt3a is distinctly different than that of Dnmt1", *BMC Biochemistry*, 6, 6, 2005.

[29] Li, E. and Bird, A., DNA methylation in mammals Epigenetics-Cold. Spring Harbor (NY): 2007.

[30] Barret, J.M., Salle, B., Provot, C. and Hill, B.T., "Evaluation of DNA repair inhibition by antitumor or antibiotic drugs using a chemiluminescense microplate assay", *Carcinogenesis*, 18, 2441-5, 2005.

[31] Teppone, M.V. and Avagyan, R.S., "Extremely high frequency (EHF) therapy in oncology", *Millimeter waves in biology and medicine*, 29(1), 3-19, 2003.

[32] Bhattacharya, S., Ramchandani, S., Cervoni, N. and Szyf, M., "A mammalian protein with specific demethylase activity for mCpG DNA", *Nature*, 397, 579-83, 1999.

[33] V., Martirosyan, R., Nersesyan, L., Aharonyan, A., Danielyan, I., Stepanyan, H. at al, "Effect on tumoral cells of low intensity electromagnetic waves", *Progress in Electromagnetics Research Letters*, 20, 97-105, 2011.

[34] Babayan, Y.S. and Gharibyan, J.V., "Structural features of DNA of sarcoma 45 tumor", *Biophysica*, 35(4), 592-6, 1990.

[35] Belyaev, I.Ya., Alipov, Y.D., Shcheglov, V.S. and Lystsov, V.N., "Resonance effect of microwaves on the genome conformational state of E. coli cells", *Z Naturforsch C*, 47(7-8), 621-7, 1992.

[36] Khabarova, O., "The influence of cosmic weather on the Earth.// International School of Space Science. Book of Proceedings of the 10th course on "Sun-Earth Connection and Space Weather" (L'Aquila 2000), Society Italiana di Fisica, 56-62, 2001.

[37] Weinsburg, S., "DNA Helix found to oscillate in resonance with microwaves", *Science News*, 125(16), 248-53, 1984.

[38] Petrosyan, V.I., Sinitsin, N.I., Elkin. V.A., Devyatkov, N.D., Gulyayev, Y.V., Betskii, O.V., et al."Role of resonance molecular-wave processes in the nature and their use for the control and corrections of a condition of ecological systems", *Biomeditsinskaya Radioelectronica*, No.5-6, 62-105, 2001.

[39] Adams, P.L. and Eason, R., "Increased GC content of DNA stabilizes methyl CpG dinucleotides", *Nucleic Asids Res*, 12(14), 5869-77, 1984.

[40] Behe, M. and Felsenfeld, G., "Effects of methylation on a synthetic polynucleotide: The B Z transition in poly(dG-m^5C)", *Proc. Nat. Acad. Sci. USA*, 78(3), 1619-23, 1981.

[41] Rodionov, B.N., "Energo-informational effect of low-energetic electromagnetic radiations on biological objects", *New Medical Technologies Report*, 6(3-4), 24-9, 199.