

On the Possibility of Using Non-Ionizing Electromagnetic Radiation (Millimeter Waves) in Oncology

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Abstract—A study on the use of non-ionizing and non-thermal millimeter electromagnetic radiation in tumor chemotherapy was conducted. DNA released from sarcoma 45 tumor (tDNA) and healthy rats (hDNA) in water-saline solution was irradiated during 90 min by frequencies at both resonances for oscillations of water molecular structures (at 64.5 GHz and 50.3 GHz) and non-resonance (48.3 GHz). Non-irradiated and irradiated tDNA and hDNA binding constants with anti-tumorous drugs doxorubicin (DX) and netropsin (NT) were studied. The absorption spectra of non-irradiated and irradiated complexes of DNA with DX and NT were obtained by spectroscopic method. From the absorption spectra, binding constants at 290 K, 300 K, and 310 K temperatures have been determined. According to our calculations, doxorubicin and netropsin with irradiated DNA form were more stable complexes and much stronger with tDNA irradiated at resonant frequencies: selective binding of doxorubicin and netropsin was observed. For a DNA irradiation at resonant frequencies of 64.5 GHz and 50.3 GHz, the binding constant K to DX and to NT is almost an order of magnitude higher than for the non-irradiated DNA. The obtained data suggest that the irradiation of malignant tumors by non-thermal (ultra-weak intensity) millimeter electromagnetic waves in combination with anticancer drugs may be promising for clinical oncology. The same antitumor effect can be achieved at much lower doses of medicines (considerable dose reduction). This is essential from the point of view of the application of gentle therapies for patients and the reduction of expenses associated with acquisition of expensive medicines.

1. INTRODUCTION

At present, there are a large number of experimental and theoretical data on the study of medical and biological effects of the low intensity non-ionizing 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, which determines the importance of such investigations.

Revealing of influence of electromagnetic radiation of millimeter wavelengths on an organism and its biological significance is the basis for using the microwave effect as a physiotherapeutic procedure in the treatment of various diseases such as the cancer of different organs, cardiovascular diseases, diabetic angione uropathies, peptic ulcers, leucopenia, pain relief, skin disorders, infantile cerebral palsy, bronchial asthma, wound healing [1–3]. According to literature data, MM-wave therapy increases the level of immune resistance, influences different stages of pathogenesis, changes enzymatic reaction activity and growth rate, destroys microorganisms, and increases the thermostability of DNA [4–7]. It has been shown that MM-waves have strong effect on the process and bioelectric activity of neurochemical functions of the brain, increase the cortical tension, and influence the spike activity of

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neurons in the supraoptic nucleus of the Hypothalamus of rats [8, 9]. Penetrating into the organism (the penetration depth of millimeter waves in tissues is about 1–1.5 mm due to high absorption by water molecules), MM-wave radiation is transformed into information-carrying signals performing guidance and adaptation control or rehabilitation processes in the organism. The influence of low-intensity millimeter range electromagnetic radiation on tumors has triggered great interest among researchers mostly due to the absence of harmful side-effects opposed to the widely used X-rays or γ -rays therapy. Unlike ionizing X- and γ -rays, the method of MM-therapy is non-ionizing, hence is completely deprived of any harmful side effects. The influence of electromagnetic fields on various tumors has been investigated in [10–16]. The microwave therapy used in complex antineoplastic treatment promotes the reduction of toxic effect of chemo- and radiotherapy and increase of its antitumor effect. In recent years, due to the combined use of MM-waves with anticancer drugs, it became possible during chemotherapy of experimental animals to significantly reduce toxic side effects of anticancer drugs without reducing their antitumor activity [17, 18]. Doxorubicin and netropsin are widely used in chemotherapy due to their efficacy in fight against a wide range of cancers such as carcinomas, sarcomas, and hematological cancers [19]. Despite extensive clinical use, the mechanisms of DX and NT action remain under intense debate [20–23]. Although the molecular mechanisms of DX and NT action have not yet been completely revealed, it can be considered proven that DX and NT molecules, penetrating the cell, bind to DNA. This binding takes place mainly by intercalation and groove binding mechanism [21]. At high DX concentrations, exceeding the concentration of base-pairs of DNA it is also the possible formation of an external aggregate complex with DNA [21]. Recent years studies carried out in experimental animals have shown [24] that when DX is injected into tumor-bearing animals with sarcoma-45 almost by 40% reduction of the size of tumors takes place, and significant (about 2–3 times) decrease in content of 5-methylcytosine isolated from sarcoma-45 DNA is observed, which is known [25] as a molecular indicator of the malignancy process in solid tumors. The aim of this work is to identify the features of the interaction of anticancer drugs doxorubicin and netropsin with DNA isolated from the tumor sarcoma-45, pre-irradiated at resonant frequencies of oscillations of molecular water structures (hexagons and triads) and to establish the effect of non-thermal MM-wave radiation on the degree of binding of anticancer drugs to DNA.

2. MATERIALS AND METHODS

In our experiments, DNA samples were used, which were isolated from liver of healthy white rats (hDNA) and from the tumor of the animals affected with sarcoma-45 (tDNA).

As a source of MM-wave radiation, the generators of coherent extremely high frequency oscillations G4-141 and G4-142 (Russian made) were used, operating in a range of 38.5–78.8 GHz. Exposure to electromagnetic waves was carried out in the far-field zone of a cone-shaped antenna at a distance of 300 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 $10 \mu\text{W}/\text{cm}^2$ (Figure 1). The output power of the generator was measured by the 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 and CH2-26 wavemeters (Istok, Fryazino, Russia).

In the case of ionizing radiation, the amount of energy (dose) received by the sample is very important. In the case of non-ionizing ultra-weak radiation, as in our case, the energy given to the sample is so small that it ceases to play an essential role, and the information phenomena become decisive (predominate). The conditions of manifestation of the effect of “information” actions are determined by the competition of energy (ΔU) and entropy (ΔS) expressed at a given a temperature (T) formula for the free energy of Helmholtz: $\Delta F = \Delta U - T\Delta S$. Hence the criterion of distinction between informational and energetic influences, which can also be interpreted as the boundary between strong and weak stresses, is determined by the ratio: $f = \Delta U/T\Delta S$. Thus, with strong effects $f > 1$, energy processes prevail, and the accompanying “entropy-information” effects are secondary, with weak effects $f < 1$. The processes are controlled by “entropy-information” mechanisms, and energy is assigned the role of a translator of “information”.

The solutions were exposed from the top in plastic containers with a diameter of 70 mm. The bottom square of the container corresponded to the square of the exposed zone created by the major

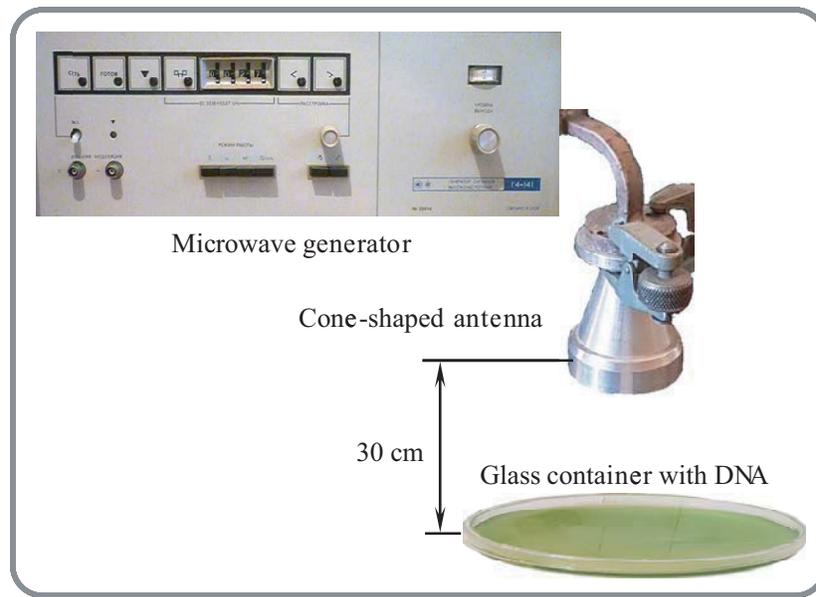


Figure 1. The generator of coherent extremely high frequency oscillations with cone-shaped antenna. Measurements were done at far-field zone (30 cm).

lobe of the antenna. To eliminate the interference in the plane of exposed object an effective multi-layer absorbent was placed between the solution container and the floor. Thus the conditions of exposure were close to the free field conditions. hDNA and tDNA solutions were irradiated for 90 min at frequencies of 50.3 GHz and 64.5 GHz (which coincide with the resonant frequencies of oscillations of the water molecular structures-hexagons and triads) and at a frequency of 48.3 GHz, which does not coincide with resonant frequencies. In [4], it was shown that irradiation at resonant frequencies of 50.3 GHz and 64.5 GHz for 90 minutes for in-vitro, the greatest change in the DNA melting parameters was observed. Therefore, in further experiments hDNA and tDNA solutions were irradiated for 90 min.

3. RESULTS AND DISCUSSION

By the method of thermal denaturation of DNA in-vitro effect of non-ionizing and non-thermal millimeter electromagnetic radiation on healthy and tumor DNA was investigated [26, 28]. As a result of irradiation, the melting parameters of tDNA undergo change more than hDNA. In all likelihood, these changes of the melting parameters of tDNA compared with hDNA are due to the structural features of tDNA (caused by 5-methylcytosine) [25, 27, 28], owing to which the degree of tDNA hydration in hypermethylated sites may significantly differ from hydration in other parts [25, 27, 28]. It is known that the resonant frequencies of DNA absorption are within 2–9 GHz range [29]. Therefore, summarizing the literature and our experimental data, it can be assumed that DNA thermostability growth by irradiating at resonant frequencies of oscillations of the water molecular structures probably is caused by indirect influence of millimeter waves on DNA, namely, by affecting the water, waves cause quantitative and qualitative changes of water associated with DNA and NaCl [26, 30]. It is known that in the process of malignization (neoplastic transformation) the content of 5-methylcytosine significantly increases in DNA extracted from solid tumors [25]. Relatively recently it has been shown [31] that the cytosine methylation contributes to the binding of a number of anthracycline antibiotics to DNA. Because a combination of anticancer drugs with radiation increases the effectiveness of drugs action, we can assume that the interaction of DX and NT with tDNA can, to some extent, be changed and be selective if tDNA was prior irradiated with resonant frequencies of oscillations of the water molecular structures (triads and hexagons). By spectrophotometric method, we investigated the changes of the thermodynamic parameters values of the system due to the interaction with DX and NT of irradiated and non-irradiated

hDNA and tDNA. We studied the behavior of the change in the absorption spectra of DX and NT in the visible region, due to DNA interaction with them. Since in the visible region DNA does not absorb light, so the changes of the absorption spectra of DX and NT in the visible region are caused only by complex with DNA. Consequently, in these environments there is only one type of a bound state of DX-DNA, distinguishable by spectrophotometric absorption spectra. The experimental data show that starting from a certain value of the relative concentration of C_p/C_0 (where C_p is a molar concentration of DNA for base pairs, and C_0 is a molar concentration of DX), absorption spectra of the complexes DX-DNA in the visible region are no longer changed, which means that all DX molecules in the solution are in bound state. From the absorption spectra of DX-DNA complexes, the values of the basic quantitative parameters characterizing the complexation were determined: binding constant (K) and a parameter determining the complex stoichiometry at saturation of the interaction (n). The absorption spectra were obtained for non-irradiated and irradiated complexes of hDNA-DX and tDNA-DX at three different temperatures. From these spectra the concentrations of free (C_f) and bound (C_b) DX in the solution were determined

$$C_f/C_o = A - A_b/A_f - A_b, \quad C_b = C_o - C_f, \quad (1)$$

where A_f and A_b are respectively free and bound DX absorption values at 538 nm, which corresponds to the maximum of the absorption spectrum, and A is the absorption value of DX-DNA complexes in intermediate states. A_b is determined by linear extrapolation of $A = f(1/c_p)$ dependence, when $1/c_p \rightarrow 0$. Taking into account the values of C_f and C_b calculated by Eq. (1), the binding isotherms of DX with non-irradiated and irradiated hDNA and tDNA in Scatchard coordinates were drawn (r/C_f dependence on r , where $r = C_b/C_p$). The binding isotherms were described by Eq. (2), which more precisely describes the binding of biologically active compounds with nucleic acids [32]

$$r/C_f = K(1 - nr)^n [1 - (n - 1)r]^{1-n}, \quad (2)$$

where n is equal to the number of base-pairs with which one DX molecule binds at the saturation.

Figures 2, 3, and 4 show the binding isotherm at 290 K, 300 K, and 310 K for non-irradiated and irradiated DX-tDNA and NT-DNA complexes at a frequency of 64.5 GHz (resonant frequency of oscillations of water triad structures). The solid line is the theoretical curve drawn through the experimental points, according to Eq. (2) by the method of least squares, where the values of K and n parameters were determined.

Table 1 shows the values of K and n complexation parameters of DX and NT for non-irradiated and irradiated hDNA and tDNA at three different resonant frequencies, calculated by using Eq. (2). As

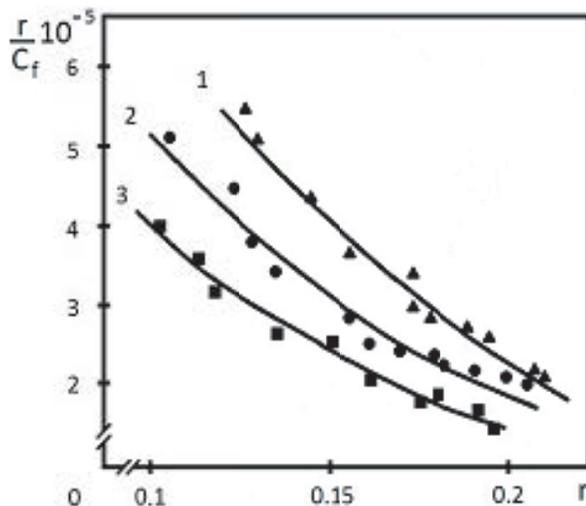


Figure 2. The binding isotherms of doxorubicin with non-irradiated tDNA at temperatures 1 – 290 K, 2 – 300 K, 3 – 310 K.

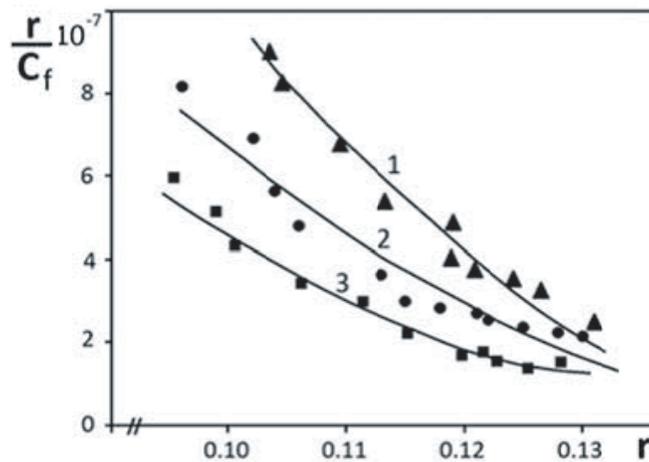


Figure 3. The binding isotherms of netropsin with calf thymus DNA at temperatures 1 — 290 K, 2 — 300 K, 3 — 310 K.

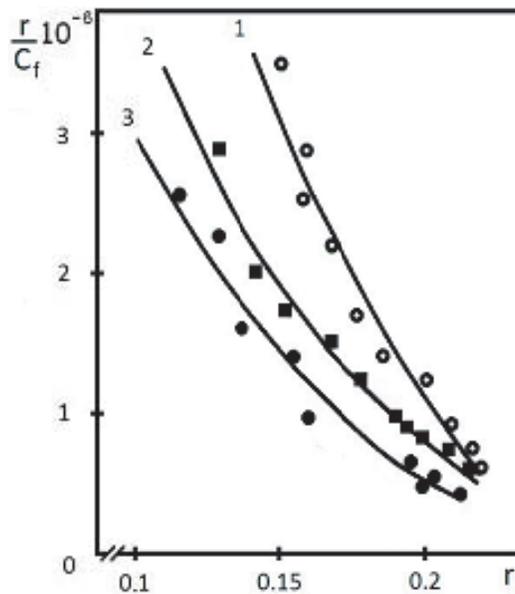


Figure 4. The binding isotherms of doxorubicin with irradiated tDNA at frequency of 64.5 GHz at temperatures 1 — 290 K; 2 — 300 K; 3 — 310 K.

shown in Table 1, when DNA is irradiated with millimeter waves the binding constant (K) increases: DX forms a stable complex with irradiated DNA. Binding constant to DX and NT for DNA irradiated with resonant for water structures frequencies of 64.5 GHz and 50.3 GHz is almost an order of magnitude more than that for the non-irradiated DNA. When DNA is irradiated at non-resonant frequencies (e.g., 48.3 GHz), K increases, but not as much. At the same time from Table 1 it follows that DX and NT with irradiated and non-irradiated tDNA form a more stable complex, and when tDNA is irradiated at 64.5 GHz and 50.3 GHz frequencies DX and NT form a much stronger complex ($K = 57.4 \cdot 10^{-5} \text{ M}^{-1}$ for that irradiated at 64.5 GHz frequency tDNA-DX complexes at 300 K, and $K = 10.1 \cdot 10^{-5} \text{ M}^{-1}$ for that irradiated at 48.3 GHz non-resonant frequency).

Table 1. Summary of the binding constant K and complexation parameter n of doxorubicin and netropsin with non-irradiated and irradiated DNA.

Type of complex T, K	$K \cdot 10^{-5}, M^{-1}$	n	
Healthy DNA-doxorubicin			
Non-irradiated	290	6.0 ± 0.1	4.0
	300	5.2 ± 0.1	4.0
	310	4.5 ± 0.1	4.0
Irradiated at 50.3 GHz	290	64.5 ± 0.2	4.0
	300	50.3 ± 0.2	4.1
	310	39.4 ± 0.2	4.0
Irradiated at 64.5 GHz	290	62.0 ± 0.2	4.0
	300	48.1 ± 0.2	4.1
	310	38.2 ± 0.2	4.0
Irradiated at 48.3 GHz	290	6.9 ± 0.1	3.9
	300	5.9 ± 0.1	4.0
	310	5.1 ± 0.1	4.1
Tumor DNA — doxorubicin			
Non-irradiated	290	8.7 ± 0.1	4.1
	300	7.4 ± 0.1	4.0
	310	6.3 ± 0.1	4.1
Irradiated at 50.3 GHz	290	74.9 ± 0.2	4.0
	300	58.3 ± 0.2	4.1
	310	46.1 ± 0.2	4.2
Irradiated at 64.5 GHz	290	75.0 ± 0.2	4.1
	300	57.4 ± 0.2	4.0
	310	44.9 ± 0.2	4.2
Irradiated at 48.3 GHz	290	12.1 ± 0.2	3.9
	300	10.1 ± 0.1	4.0
	310	8.5 ± 0.1	4.0
DNA — netropsin			
Non-irradiated	293	5.0 ± 0.2	6.0 ± 0.1
	303	3.0 ± 0.2	5.9 ± 0.2
	313	1.9 ± 0.2	6.0 ± 0.2
DNA — netropsin			
Irradiated at 50.3 GHz	293	36.1 ± 0.3	5.9 ± 0.2
	303	21.1 ± 0.2	6.0 ± 0.2
	313	12.8 ± 0.3	6.0 ± 0.2
Irradiated at 64.5 GHz	293	38.4 ± 0.2	6.0 ± 0.2
	303	22.3 ± 0.3	6.1 ± 0.2
	313	13.0 ± 0.2	6.1 ± 0.2
Irradiated at 48.3 GHz	293	6.9 ± 0.2	6.0 ± 0.3
	303	4.1 ± 0.2	6.2 ± 0.3
	313	2.4 ± 0.2	6.1 ± 0.2

4. CONCLUSION

The effect of non-ionizing and non-thermal electromagnetic radiation of the millimeter range on the degree of binding of anti-cancer drugs to irradiated DNA was experimentally investigated. The irradiation was carried out at resonance frequencies of 50.3 GHz and 64.5 GHz, which coincide with the frequencies of oscillations molecular structures of water (triads and hexagons). The calculations carried out on the basis of the experimental results showed that during irradiation at resonant frequencies there was a significant increase in the binding constant K (almost by an order of magnitude) to netropsin and doxorubicin. This means that the same anti-tumor effect can be achieved at much lower doses of medicines (considerable dose reduction). This is essential from the point of view of the application of gentle therapies for patients and the reduction of expenses associated with acquisition of expensive medicines. The obtained data suggest that the treatment by millimeter therapy in combination with anticancer drugs may be promising for clinical oncology in the treatment of malignant tumors.

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