Figure 1. Changes in the absorption spectra of doxorubicin binding with irradiated at a frequency of 64.5 GHz with DNA of sarcoma-45 tumor in buffer solution at temperature of 300K. During titration doxorubicin concentration remains constant, equal to C_0=4.8·10^{-5} M (spectrum 1). DNA concentration varies from zero (1) to 10^{-4} M/P (6)

NON-IONIZING MILLIMETER ELECTROMAGNETIC WAVES INCREASE THERMODINAMIC PARAMETERS OF THE BINDING OF ANTICANCER DRUG DOXORUBICIN WITH DNA

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INTRODUCTION

Studies in recent decades have shown that low intensity non-ionizing millimeter electromagnetic waves (MMEWs) without causing a significant increase in temperature in the tissues and cells, promote activation of a number of physical and chemical processes taking place in biological systems.Therefore MMEWs currently are widely used in several fields, including biology and clinical medicine [1,2]. In particular, in recent years, due to the combined use of MMEWs with anticancer drugs, it became possible with experimental animals in chemotherapy significantly to reduce toxic side effects of anticancer drugs without reducing their antitumor activity[3,4]. The anthracycline-doxorubicin (DX) is widely used in chemotherapy due to its efficacy in fight against a wide range of cancers such as carcinomas, sarcomas and hematological cancers [5]. Although the molecular mechanisms of DX action has not yet been completely revealed, it can be considered proven that DX molecules, penetrating the cell, **bind to DNA.** This binding takes place mainly by intercalation and groove binding mechanism. At high DX concentrations, exceeding the concentration of base-pairs of DNA it is also possible formation of an external aggregate complex with DNA [6]. The aim of this work is to identify the features of interaction of antitumor drug DX with DNA isolated from sarcoma-45 tumor pre-irradiated by resonant frequencies of oscillations of molecular water structures.

EXPERIMENT

In our experiments DNA samples were used, which were isolated from liver of healthy white rats (hDNA) and from tumor of affected with sarcoma-45 animals (tDNA). As a source of MMEWs radiation the generators of coherent Extremely High Frequency oscillations G4-141 and G4-142 (Russian made) were used, operation in a range of 38.5-78.8 GHz frequencies. The incident power density at the location of object was about 20 μW/cm^2. 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) and at a frequency of 48.3 GHz, which does not coincide with resonant frequencies. In [7] was shown that irradiation *in vitro* with resonant 50.3 GHz and 64.5 GHz frequencies for 90 minutes the greatest change in the DNA melting parameters was observed. Therefore, in further experiments hDNA and tDNA solutions were irradiated for 90 min.

RESULTS and DISCUSSION

Table 1 shows the values of the DNA melting parameters during irradiation with resonant and non-resonant coherent millimeter waves. As seen from the Table, when irradiated with resonant frequencies similar regularities of changes of the melting parameters are observed, however T_m greatest change occurs during irradiation with frequency of 64.5 GHz, which coincides with the resonant frequency of oscillations of water triad structures.

It is known that the resonant frequencies of DNA absorption are within 2÷9 GHz range. Therefore, summarizing the literature and our experimental data, it can be assumed that DNA thermostability growth by irradiating with 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 [8].

It is known that in the process of malignation (neoplastic transformation) the content of 5-methylcytosine significantly increases in DNA extracted from solid tumors [8]. Relatively recently it has been shown [9] 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 [3,4], we can assume that the interaction DX with tDNA can to some extent be changed and be selective if tDNA was prior irradiated with resonant frequencies of vibrations of the water molecular structures.

The experimental data shows 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 no longer are 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 unirradiated and irradiated complexes of hDNA- DX and tDNA-DX for three different temperatures.

At *in vitro* irradiation of DNA solutions certain structural changes occur in DNA molecules (due to the partial dehydration of DNA caused by irradiation), which are stronger in tumor DNA, owing to which the irradiated DNA molecules form more stable complexes with an anticancer drug Doxorubicin (DX). When DNA is irradiated with millimeter waves the binding constant (K) increases: **Doxorubicin forms a stable complex with the irradiated DNA**. For a DNA irradiated with resonant for water molecular structures frequencies of 64.5 GHz and 50.3 GHz the coefficient of binding K to DX is almost **an order of magnitude more than for the non-irradiated DNA.** With irradiated and non- irradiated tumor DNA anticancer drug forms more stable complexes, and when tumor DNA is irradiated with 64.5 GHz and 50.3 GHz frequencies DX forms much stronger complexes (for irradiated at 64.5 GHz frequency tumor DNA-DX complexes at 300K K = **57.4**·10^{-5} M^{-1}, and K = **7.4**·10^{-5} M^{-1} for non-irradiated). An increase in the thermodynamic binding parameter K in *in vitro* complexation of DX with irradiated DNA **indicates the prospects of development of the millimeter therapy complex with anticancer drug for clinical oncology in the treatment of malignant tumors. 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.**

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High-frequency generator to irradiate laboratory animals. In the right corner of the enlarged image of the antenna of generator

CONCLUSION

Summarizing the experimental data can be said that at *in vitro* irradiation of DNA solutions certain structural changes occur in DNA molecules (due to the partial dehydration of DNA caused by irradiation), which are stronger in tDNA, owing to which the irradiated DNA molecules form a more stable complex with DX. Increase in the thermodynamic binding parameters (K, ΔH) in *in vitro* complexation of anticancer drug DX with irradiated DNA indicates to the **prospects of development of the millimeter therapy complex with anticancer drug for clinical oncology in the treatment of malignant tumors.**

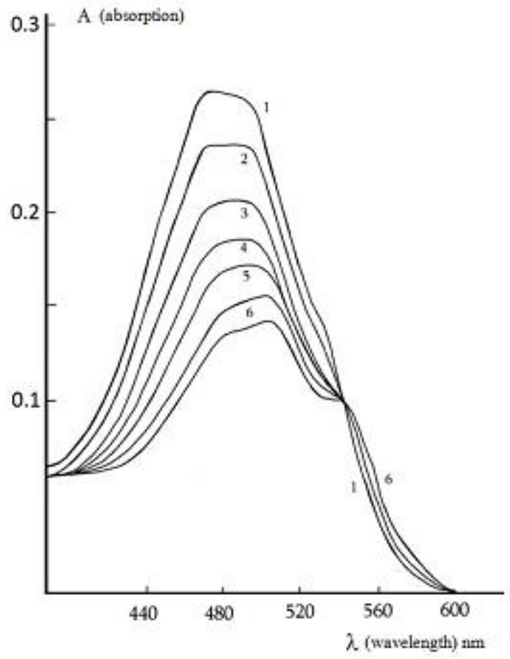

Figure 1. Changes in the absorption spectra of doxorubicin binding with irradiated at a frequency of 64.5 GHz with DNA of sarcoma-45 tumor in buffer solution at temperature of 300K. During titration doxorubicin concentration remains constant, equal to C_0=4.8·10^{-5} M (spectrum 1). DNA concentration varies from zero (1) to 10^{-4} M/P (6)

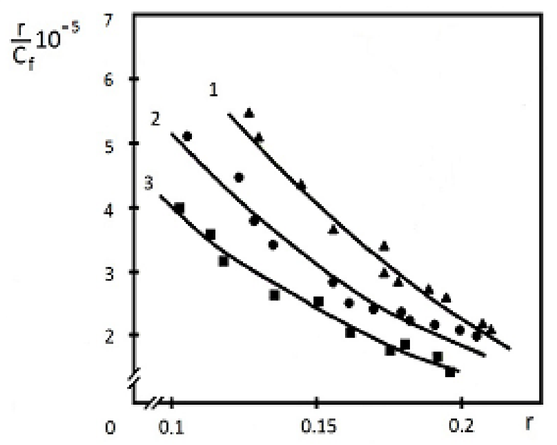

Figure 2. The binding isotherms of doxorubicin with non-irradiated tDNA at temperatures: 1-290K, 2-300K, 3-310K

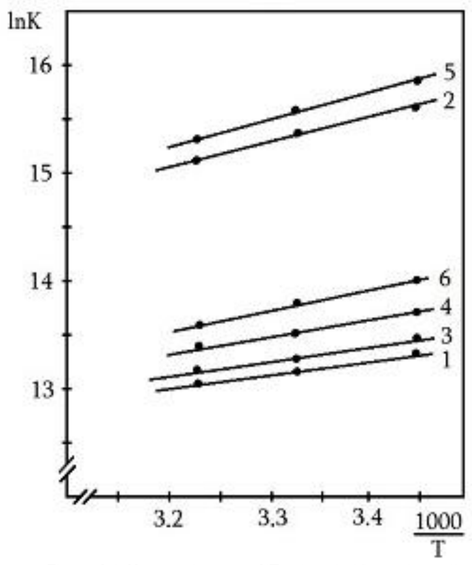

Figure 4. lnK dependence on 1/T calculated from absorption isotherms for non-irradiated hDNA-DX (1) and tDNA-DX (4); irradiated with resonant 64.5 GHz frequency hDNA-DX (2) and tDNA-DX (5); irradiated with non-resonant 48.3 GHz frequency hDNA-DX (3) and tDNA-DX (6).

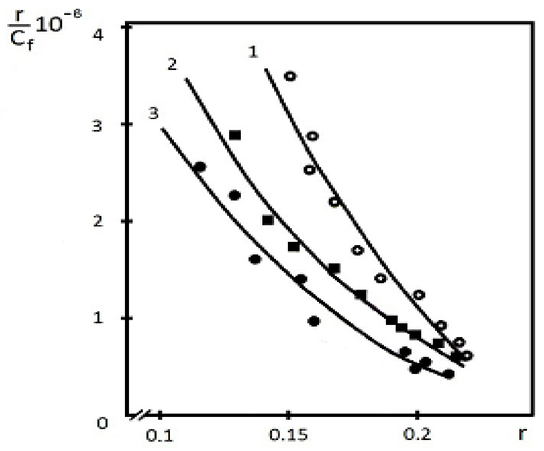

Figure 3. The binding isotherms of doxorubicin with irradiated tDNA at frequency of 64.5 GHz at temperatures: 1-290K; 2-300K; 3-310K.

Table 1. The values of the parameters thermal denaturation of DNA exposed to the millimeter waves for 90 minutes

Frequency of radiation, GHz	healthy DNA		Tumor DNA	
	T_m, °C	, °C	T_m, °C	, °C
0	83.0 ± 0.1	5.7 ± 0.1	82.0 ± 0.1	6.6 ± 0.1
64.5	84.1 ± 0.1	5.6 ± 0.1	83.5 ± 0.1	6.2 ± 0.1
50.3	83.8 ± 0.1	5.6 ± 0.1	83.2 ± 0.1	6.3 ± 0.1
48.3	83.2 ± 0.1	5.7 ± 0.1	82.3 ± 0.1	6.5 ± 0.1

Table 2. Summary of thermodynamic data for the binding of doxorubicin with non-irradiated and irradiated DNA

Type of complex	T, K	K·10^{-5}, M^{-1}	- ΔG, kcal/mol	- ΔH , kcal/mol	ΔS, cal/mol K	n
healthy DNA-doxorubicin						
Non-irradiated	290	6.0± 0.1	7.7 ±0.1	2.6	17.6	4.0
	300	5.2± 0.1	7.9 ±0.1			4.0
	310	4.5± 0.1	8.1 ± 0.1			4.0
Irradiated with 50.3GHz	290	64.5 0.2	9.1 0.1	4.3	16.5	4.0
	300	50.3 ±0.2	9.3± 0.1			4.1
	310	39.4 0.2	9.4± 0.1			4.0
Irradiated with 64.5GHz	290	62.0± 0.2	9.1 ± 0.1	4.4	16.1	4.0
	300	48.1 ±0.2	9.2 ±0.1			4.1
	310	38.2± 0.2	9.4 ±0.1			4.0
Irradiated with 48.3GHz	290	6.9 ±0.1	7.8 ± 0.1	2.7	17.6	3.9
	300	5.9 ± 0.1	8.0± 0.1			4.0
	310	5.1 ± 0.1	8.1± 0.1			4.1
tumorDNA-doxorubicin						
Non-irradiated	290	8.7 ±0.1	7.0 ± 0.1	2.9	17.3	4.1
	300	7.4 ±0.1	8.1 ±0.1			4.0
	310	6.3± 0.1	8.3 ±0.1			4.1
Irradiated with 50.3GHz	290	74.9± 0.2	9.2± 0.1	4.4	16.6	4.0
	300	58.3 ± 0.2	9.4 ±0.1			4.1
	310	46.1 ± 0.2	9.5 ± 0.1			4.2
Irradiated with 64.5GHz	290	75.0 ± 0.2	9.2 ± 0.1	4.6	15.8	4.1
	300	57.4 ±0.2	9,3 ±0.1			4.0
	310	44.9 ± 0.2	9.5± 0.1			4.2
Irradiated with 48.3GHz	290	12.1 ±0.2	8.1 ±0.1	3.1	17.2	3.9
	300	10.1 ±0.1	8.3 ±0.1			4.0
	310	8.5 ±0.1	8.5 ±0.1			4.0

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