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Thermostability of the Mitoxantrone - DNA Complexes Irradiated by Non-Thermal Millimeter Electromagnetic Waves

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Abstract. Thermostability of mitoxantrone complexes with DNA of sarcoma 45 and healthy rat liver earlier irradiated by non-thermal (50 μW/cm^2) resonant (64.5 GHz and 50.3 GHz) and non-resonant (48.3 GHz) of water hexagonal structure frequencies millimeter electromagnetic waves (MEW) oscillations has been studied. It was shown that at irradiation of solutions by resonant with water hexagonalstructurefrequencies, a dehydration of nucleotides of irradiated DNA and Na+ ions existing in the solution occurs. At relatively low concentrations of mitoxantrone it results in decreasing of thermostability of complexes with DNA, moreover this change is more pronounced at complex-formation with DNA from sarcoma 45 tumor. It was shown that the thermostability of the radiated by non-resonant frequents DNA-MTX complexes does not change.

Keywords: mitoxantrone, absorption, DNA, millimeter electromagnetic waves.

Introduction

Biochemical studies have shown that antitumor drug mitoxantrone (MTX) like anthracyclines penetrating into the cell changes the chromatin structure, by binding to nucleic acids [1,2].However, compared with the anthracycline, it has a relatively reduced toxicity [3,4]. It was shown that MTX intercalates to the double-stranded (dS-) nucleic acids [2,3,5]. At present, all the higher proof is a concept asserting that the effect of non-thermal millimeter electromagnetic waves (MEW) on biological systems due to the effect of waves on the water [6-8]. Recently it has been shown [6-10] that MEW waves of certain frequencies have antitumor activity. Half an hour a day irradiation of rats with sarcoma 37by 53.5GHz MEW tumor growth is reduced by 33,5%, and the degree of DNA methylation of the tumor is reduced by about 2.5 times [10]. Therefore, the activity of anticancer drug can be increased if we combine an effect of the drug and radiation [4.11].We have investigated the thermal stability of the complexes MTX with DNA of the liver normal rats and sarcoma 45, irradiated by non-thermal millimeter (MEW) resonant (64.5 and 50.3GHz) and non-resonant (48.3GHz) frequencies of water hexagonal structures.

Methods

In the experiments, healthy white rats weighing 100-120g and transplanted sarcoma 45 rats have been used. Liver and tumortissues have been extracted from the 15th day of the treated animals. DNA was isolated by *Marmur* modified method [8.10]. The absorption spectra and DNA melting curves are obtained by *Unicam* SP 8-100 (*England*) and Cary-219 (USA) spectrophotometers. Studies carried out in an aqueous solution containing 0.1 M NaCl, 0.01 M Tris and $5\cdot10^{-4}$M EDTA (pH 7.4). Melting was carried out by continuous heating of DNA solutions with the rate of 0.25^{o}C/min. The measurements results were taken as an average of 6-8

experiments. The accuracy of the determining temperature was ± 0,05ºC, and covered optical units for absorbance was 10^{-4}.

Irradiation of the solutions was carried out in a special glass containers covered by transparent for radiation fine vinyl chloride cover. The thickness of the irradiated samples did not exceed 1 mm. The generators GI-142 and GI-141 (*Russia*) were used for irradiation. The interval of oscillation frequency for the GI-141 is 37.5 - 53.57GHz (flux density at the location of the sample was60 μW/cm^2) for GI-142 - 53.3 - 78.33GHz (flux density – 50 μW/cm^2). Solutions of DNA isolated from the liver of normal rats (hDNA) and tumor rat sarcoma 45 (tDNA) were irradiated by the resonant of the hexagonal structure of water frequencies50.3 and 64.5 GHz and non-resonant frequency of 48.3 GHz [6].

MTX was from Sigma NaCl, Tris were from Serva. The concentration of MTX determined by spectrophotometry (extinction coefficient ε_{659} =25.900 $M^{-1}sm^{-1}$ [2]). All reagents were used without further purification. MTX molecules can associated in solution. In [2,5] it was shown that at the concentrations of MTX Coless than $3 \cdot 10^{-6}$ M, process of association within the experimental error can be ignored.

Results and discussion

Aqueous solutions of DNA prepared for spectrophotometric measurements, were irradiated 30, 40, 60, 90 and 120 minutes respectively. Experiments have shown that irradiation of the DNA solution pH did not change within experimental error. The melting curves of hDNA and tDNA irradiated and non-irradiated by resonant (64.5 and 50.3 GHz) and the non-resonant (48.3 GHz) frequencies of water hexagonal structures were determined. From the melting curves the parameters of the melting temperature (T_m.) and the melting range (ΔT) were determined. Melting curves are obtained both immediately after irradiation and after 10 and 24 hours of storage of the irradiated samples. Experiments have shown that the values, T_m and ΔT do not depend on the storage time of the irradiated DNA.

Table 1 shows the melting parameters of hDNA and tDNA irradiated by resonance frequency of 64.5 GHz, depending on the duration of exposure. As follows from Table 1, T_m of hDNA and tDNA increases when the duration increases up to 90 minutes. Since the greatest change of the melting (1ºCfor hDNA and 1,5ºC for tDNA) parameters occurs at 90 minutes irradiation, further investigations ions have been carried out after 90 minutes of exposure. Table 2 shows the parameters of the melting hDNA and tDNA irradiated at 90 minutes by resonant (50.3 GHz) and the non-resonant (48.3 GHz) frequencies ofMEW. As shown in Table 2, both resonance frequencies show similar patterns of change in the parameters of melting, however, changes in T_m. and ΔT more pronounced by irradiation with a frequency of 64.5 GHz, which coincides with the resonant frequency of hexagonal ring molecular structure of water [6]. The observed stronger changes of the tDNA melting parameter may be due to structural differences compared tDNA to hDNA[12,13], whereby the hydration of tDNA hyper methylated in certain areas may be very different from the rest of the hydration of DNA [14].

Table 1. The temperature and the melting range of DNA isolated from healthy rats and liver tumor sarcoma 45 irradiated by 64.5 GHz MEW.

Duration of irradiation min.	hDNA		tDNA	
	$T_{m.}$, ^0C	ΔT, ^0C	$T_{m.}$, ^0C	ΔT, ^0C
0	83,0±0,1	5,7±0,1	82,0±0,2	6,6±0,2
30	83,1±0,2	5,8±0,1	82,1±0,2	6,6±0,2
40	83,6±0,1	5,7±0,1	82,3±0,1	6,5±0,2
60	83,9±0,2	5,6±0,2	82,9±0,1	6,3±0,1
90	84,1±0,2	5,6±0,2	83,5±0,2	6,2±0,2
120	84,0±0,2	5,6±0,2	83,5±0,2	6,2±0,2

Table 2. The temperature and the melting range of DNA isolated from the liver of normal rats and tumor sarcoma 45, irradiated by MEM at 90min.

Frequency irradiation (GHz)	hDNA		tDNA	
	$T_{m.}$, ^0C	ΔT, ^0C	$T_{m.}$, ^0C	ΔT, ^0C
0	83,0±0,1	5,7±0,2	82,0±0,2	6,6±0,2
64,5	84,1±0,2	5,6±0,2	83,5±0,2	6,2±0,2
50,3	83,8±0,1	5,6±0,2	83,2±0,2	6,3±0,2
48,3	83,3±0,2	5,7±0,1	82,3±0,2	6,5±0,1

We assume that increasing the thermostability of DNA by irradiation of MEW of resonant frequencies may be due to the bound structure of DNA with water [8,14,15]. Based on literature data on the effect of thermal coherent MEW on the structure of water [6-8, 14, 15] and comparing them with our experimental data, we can assume that as a result of radiation with resonant frequencies (for vibration of water structures) dehydration takes place of nucleotide pairs and Na^+ ions, being in close proximity with regard to the DNA molecule, so that they efficiently stabilize the double helix and even do that stronger in the case of millimeter waves of resonant frequencies (Table. 2). As can be seen from Table 2, the errors of changes of melting parameters of the irradiated by non-resonant frequency of 48.3 GHz samples are within the experimental error. Therefore, summarizing up the effect in vitro influence of the resonant MEW it can be conformed for the thermal stability of hDNA and tDNA occurs during irradiation of 90 minutes by frequency to 64.5 GHz. Therefore, seceding experiments on investigation of thermal stability of complexes hDNA and tDNA with anticancer compound MTX have been performed

after irradiation of the samples by frequency 64.5 GHz in 90 min. Table 3 shows the decadences of the parameters of the melting of non-irradiated and irradiated DNA-MTX complexes on the concentrations of MTX. It is known that MTX is an intercalating drug [2, 3, 5, 16], which increases the thermostability of DNA [3, 5, 17]. From Table 3 is follow that at relatively low concentrations of MTX, when one MTX molecule accounts for about 100 or more base pairs of DNA, T_m remains constant within the experimental error, and for irradiated DNA even decreases (0.8 ^{0}C for oDNA with MTX complexes). Further concentration increase of MTX, as might be expected, or leads to increase T_m for both non-irradiated and irradiated complexes. From Table 3 is also follow that at relatively low concentrations of MTX ($C_0/C_p < 0.01$), T_m complexes are more strongly modified by complexing with irradiated DNA, which more expressed for tDNA. Decrease of DNA stability can apparently be due to the external binding of MTX with DNA double helix [3,5,18]. The irradiation causes dehydration of nucleotides [8,14,15], thereby increasing the possibility of MTX molecules bind into the surface of the DNA, which leads to destabilization of the local DNA [19]. It is known that when a malignancy is increased the content of 5-methylcytosine of DNA in solid tumors as compared with DNA the number of healthy animals is increased [8, 12, 13]. In [20] it was shown that methylate cytosine support the MTX complexation with DNA. Therefore, we can assume that at binding MTX with tumor DNA as compared with DNA extracted from organs of healthy animals, a binding selectivity is observed that increases by preliminary irradiation of DNA molecules with MEW, especially with resonant frequencies for vibrations of molecular water structures.

Due to the decrease in stability of tDNA, when they are involved in mitotic cycle, they become more "susceptible to degradation", and this is likely increases the activity of anticancer drugs at their combined *in vivo* use with radiation [11].

Table 3. Values of the melting parameters unexposed and exposed (with a frequency of 64.5 GHz) hDNA and tDNA in combination with mitoxantrone.

The relativ concentration MTX (C_0/C_p)	Non-irradiated				Irradiated (90 min.)			
	hDNA		tDNA		hDNA		tDNA	
	T_m, ^{0}C	ΔT, ^{0}C	T_m, ^{0}C	ΔT, ^{0}C	T_m, ^{0}C	ΔT, ^{0}C	T_m, ^{0}C	ΔT, ^{0}C
0	83,0±0,1	5,7±0,2	82,0±0,1	6,6±0,2	84,1±0,2	5,6±0,2	83,5±0,2	6,2±0,2
0,002	82,9±0,2	6,2±0,2	81,9±0,1	7,0±0,2	83,9±0,2	6,3±0,2	82,9±0,2	6,9±0,2
0,005	82,9±0,2	7,3±0,2	81,7±0,2	7,5±0,2	83,7±0,1	7,2±0,2	82,7±0,2	7,8±0,2
0,008	83,1±0,1	7,8±0,2	82,0±0,1	8,2±0,2	83,9±0,1	8,3±0,2	83,2±0,2	8,1±0,2
0,02	84,2±0,2	8,3±0,2	83,5±0,2	8,9±0,2	84,8±0,2	8,8±0,2	83,6±0,2	8,9±0,2
0,04	85,1±0,1	8,4±0,2	84,3±0,2	8,7±0,2	86,6±0,1	8,8±0,2	84,3±0,2	9,0±0,2

Note. Melting range ΔT is defined as the temperature difference at the points where the optical density of the DNA solution varies from 17 to 83%: C_0-MTX concentration and C_p – DNA concentration viewed at nucleotide.

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