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EFFECT ON TUMORAL CELLS OF LOW INTENSITY ELECTROMAGNETIC WAVES

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Abstract—Violations of the process of methylation reveal itself at the early stages of malignant transformation of cells, and the content of 5-methylcytosine can serve as a diagnostic test for tumor formation and treatment of disease. The carried out studies revealed the correlation of antitumor activity of MM-therapy (the use of coherent millimeter electromagnetic waves of low intensity for therapy) with inhibition of methylation of tumor DNA *in vivo*. It is revealed in our experiments that an half-hour exposure of MM-radiation results in the tumor growth inhibition by 33.5% and a sharp suppression of the level of DNA-methylation 2.5 times. The results obtained in this experiment indicate the prospects of working out the MM-therapy for clinical oncology in the treatment of malignant neoplasms.

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1. INTRODUCTION

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, including cancer of different organs, cardiovascular diseases, diabetic angioneuroropathies, peptic ulcers, leucopenia, pain relief, skin disorders, infantile cerebral palsy, bronchial asthma, wound healing, etc. [1–3]. According to literature, the millimeter-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 [4–6]. The literature has shown that millimeter 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 neurons in the supraoptic nucleus of the hypothalamus in rats [7, 8]. 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). This 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 proposed here is non-ionizing and hence is completely deprived of any harmful side effects. In [9–12], the influence of millimeter electromagnetic waves (MMWs) on various tumors has been investigated. The present study was undertaken to investigate whether low-intensity (nonthermal) MMWs at 42.2 GHz can act on tumor of mice in vivo without cytostatic agents.

In 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 millimeter-radio waves, in the absence of cytostatics, is useful, since the MM-therapy used in complex antineoplastic treatment promotes the reduction of toxic antitumor effect of chemo- and radiotherapy and increasing of its antitumor effect. In most of the literature data the tumor inhibition by means of MM-therapy, in the absence of cytostatic agents, was shown in experiments *in vitro* [13, 14].

The process of DNA-methylation is closely related with the

appearance of tumors. Imbalance of DNA-methylation is observed in all, without exception, studied neoplasias. The violation of methylation process manifests itself at the early stages of malignant transformation of cells; content of 5-methylcytosine (5MC), 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 [15, 16].

Hypermethylation of tumor-DNA, the mechanism of which in many tumors is not clear, destabilizes the secondary structure of DNA as well, what may cause the selective sensitivity of malignant cells toward the influence of millimeter-waves in the absence of chemo- and radiotherapy and to allow receiving of expressed antitumor effect.

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 mice were housed in an air-conditioned room with a controlled 12-h light-dark cycle and free access to standard chow and tap water. To reveal the features of influence of MM-therapy with different modes of an irradiation on DNA of tumor-bearing animals at the absence of cytostatic drugs, a study of effects of millimeter waves on mice which are injected a sarcoma 37 by a known method [17] has been carried out. The course of influence of millimeter-waves started 3 days before transplantation in order to raise activity of animals' immune system [18]. On the fourth day, the animals were injected by sarcoma-37, and daily exposure was continued during 15 days. Several groups of mice with four to five animals in each group were used in each experiment. The animals were randomly distributed over the groups. In each experiment there were a group of animals which were not treated or exposed (cage-control) and a group of control animals which were sham-exposed (sham-control). Animals of other groups were exposed to EHF EMR with different durations of exposure — 15 minutes and 0.5 hour. The experiments were independently repeated three times.

As a source of millimeter wave radiation the generator of coherent EHF-oscillations G4-141 was used, operating in range of frequencies of $38.5 \div 53.5$ GHz. A whole-body exposure of mice to EHF EMR 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 mice were exposed from the top in plastic containers with a size of 80 mm × 80 mm × 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.

The output power of the generator was measured with the help of a M5-49 thermistor head and a M3-10A wattmeter (Istok, Fryazino, Russia). The frequency of output signal was controlled by a CH2-25 wavemeter (Istok, Fryazino, Russia). To calculate the specific absorption rate (SAR), we used dielectric parameters of the skin $\varepsilon' = 14$, $\varepsilon'' = 18$ and skin density $\rho = 1.15\,\mathrm{g/cm^3}$ [19].

The specific absorption rate on the surface of skin of the animals back was calculated by the formula [20]

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

where $\sigma = \varepsilon_0\varepsilon''\omega = 42.3\,\mathrm{S/m}$ is the electric conductivity of skin at the frequency of 42.2 GHz; $\varepsilon_0 = 8.85 \cdot 10^{-12}\,\mathrm{F/m}$ is the vacuum dielectric constant; ω is the circular frequency; $\xi = 377\,\Omega$ 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.

On the 16th day of experiment all animals were decapitated under an 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 DNA-methylation, DNA was extracted, after the slaughtering of the animals, from tumor cells by phenol-chloroform method in the presence of 1.5%-SDS [21]. Hydrolysis to the nitrogen bases were carried out in the sealed glass ampoules in 85%-formic acid at 176°C for one hour (0.1 mL of acid per 1 mg of DNA). The separation of nitrogen bases: guanine (G), cytosine (C), 5-methylcytosine (5MC), 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.

3. RESULTS AND DISCUSSION

An increase in the level of DNA-methylation in the tumor without treatment was observed in our experiments (Table 1), which in many cases is confirmed by literature data, since there is significant interaction between chromatin modification and DNA-methylation and accessibility of DNA in it for the corresponding methylases and their activation [22, 23]. It is also assumed that single-stranded DNA formed during replication and repair may be subject for *de novo* methylation by DNA-methyltransferase, which often occurs in tumors [24]. 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), in the case of 15 minutes and half-hour exposure is clearly visible.

As can be seen from Table 1, the pronounced effect of MM-therapy appears in the group with a half-hour continuous irradiation. A sharp suppression of the level of DNA-methylation of sarcoma-37 is observed, which can be explained as follows: low intensity MM-waves, acting on the growth and proliferation of cells, on the enzyme activity, on the

Table 1. The nucleotide composition of tumor DNA in the control and under the influence of MM-waves.

Experimental conditions Source of DNA	Content of bases in DNA, mol. %					
	G	C	5MC±ζ	A	T	G+C+5-MC
Animals with tumors Tumor (C-37) without treatment	21.9	17.8	4.7 ± 0.01	28.0	28.0	44.4
C-37+MMimpact exposure for 15 min	21.8	17.4	5.7 ± 0.01	28.0	28.0	44.9
C-37+MMimpact exposure for 30 min	21.9	18.1	2.2 ± 0.01	28.9	28.9	42.2

Note: In each group — 10 animals. Number of definitions — 9. These changes were reliable ($p < 0.05$) compared with control.

Table 2. MM-therapy influence on growth C-37.

Time of influence of MM-therapy	Antitumor activity					
	Number of animals		Tumour weight in grammes		$T\%$	P
	Control group	Investigated group	Control group	Investigated group		
30 Minutes	8	8	1.49 ± 0.12	0.99 ± 0.1	33.5	$= 0.05$
15 Minutes	8	8	1.49 ± 0.12	1.47 ± 0.13	0	-

genetic apparatus of cells, not accelerating tumor growth, exert an inhibitory influence on the development of the grafted sarcoma and increase the lifetime of experimental animals [25]. A similar effect was detected in our experiments. It is established that the duration of the procedure, the 30 min-MM 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-therapy on the organism of animals in both groups (15 and 30) min is insignificant $Kp = -1.2 - 1.5$.

Correlation of tumor growth delay with the level of DNA-methylation is obvious. After 15 sessions of MM-therapy without cytostatic drugs, in animals of the third group (exposure 30 min) an inhibition of tumor growth by 33.5% was observed compared with a control group and a sharp suppression of DNA-methylation level 2.5 times. 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 action 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, which provides genomic instability and can cause apoptosis of tumor cells, leading to inhibition of tumor growth [24, 26].

In case of MM-therapy with 15 min-duration of action 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 seen, the weight of tumors was identical with that in control group.

4. CONCLUSION

These studies revealed a correlation between antitumor activity of MM-therapy and inhibition of methylation level of tumor DNA ***in vivo***, because the therapeutic effect of coherent MM-waves was estimated

by inhibition of tumor growth and changes in the level of methylation. General toxic influence of MM-waves on the experimental animals with sarcoma-37 without cytostatics was insignificant and has an inhibitory effect on the development of the grafted sarcoma. Consequently, the results obtained in this experiment, in our opinion, can be seen from the point of view of possibilities MM-therapy in relation to the mechanisms of anticancer drug resistance and may be an important criterion for assessing the molecular influence. The antitumor effect of MM-waves, obtained without cytostatics, shows promising development of MM-therapy for clinical oncology in the treatment of malignant neoplasms.

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