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Application of Repetitively Pulsed X-Ray Radiation in Experimental Oncology

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***Abstract*—Development of new technologies in the field of radiation required new approaches and strategies for their application. Power radiation when one continued pulsed divided to serial pulses with different specific repetition rate could provide more complicated and expressed reaction of the biological objects. We used different normal and tumor cell lines in vivo and in vitro to compare efficacy of different pulse repetition rate of X-ray radiation when the total absorbed dose didn't exceed 1 Gy. We observed strong dependent of tumor cell reaction to repetition rate. Using this parameter we can stimulate or inhibit tumor growth up to 90% compare to control group. Irradiation of tumor-bearing mice inhibited growth of primary tumor up to 60% with the total absorbed dose 0.4 Gy. Moreover same experimental conditions allowed to reduce number of metastasis in mouse lung at 70%. That resulted in longer survival of experimental animals compare to control group. Thus we can conclude that pulsed radiation with nanosecond pulse duration has a potential for application in oncology.**

Keywords—pulse radiation, low doses, tumor mice, metastasis.

I. Introduction

The use of low-temperature atmospheric plasma in biology and medicine is becoming more popular. It is used in various applications, such as disinfection, wound healing, an antibacterial agent and a means of controlling the physiological state of cells [1–4].

Last years there has been a search for new effective technologies for antitumor therapy, generally low-traumatic and non-invasive. In this case, preference is given to methods of medical ablation of the tumor using ultrasound (HIFU) [1] and radio frequency [2], including microwave radiation [3]. Within the framework of this approach, methods of inhibiting tumor growth with subsequent death of tumor cells by means of nanosecond pulse-periodic microwave and X-ray are actively being developed.

An important feature of the biological action of such pulses is the absence of a violation of the thermal balance of the irradiated object, since with a significant energy in the pulse and its short duration (several tens of nanoseconds), the average power of the exposure is quite low. Moreover, the high efficiency of nanosecond pulses is primarily associated with physical (modulation) exposure parameters. Essentially important is the temporary organization of the pulse duration, pulse repetition rate, number of pulses per session, as well as the number of exposure sessions and the time between sessions.

It is necessary to take into account the patterns established by Schoenbach et al. [4], which takes into account the reorientation time of charges in the membrane of eukaryotic cells, which can vary between 0.5 ns and 100 ns. The choice of pulse repetition frequencies is determined mainly by the results of W.R. Adey [5]. He showed that the pulse repetition frequencies in a range between 6–20 Hz are most effective for biological objects. It has been showed that pulse regime of non-ionizing radiation with special pulse repetition rate less than 40 Hz produced different reactions of biological objects [6–8]. In our previously studies we have observed inhibition of tumor cell proliferation when low-dose nanosecond pulsed X-ray radiation applied with the pulse repetition rate 10–13 Hz [9]. We also observed inhibition of tumor growth in vivo with pulse repetition frequency 10 Hz. But there are no data if other frequencies could increase antitumor efficacy.

II. Experimental Setup

A. Irradiation

Irradiation of the animals was performed using a generator of pulsed periodic X-ray ("Sinus-150", Institute of High-Current Electronic SB RAS, Tomsk, Russia). A high-voltage pulse had a half-height duration of 4 ns. The calculated photon energy spectrum had a maximum at 90 keV, and most of the quantum flux was in the 60–200 keV range. Pulse amplitude stability of the diode voltage in the operating mode was constantly controlled by a capacitance sensor. The absorbed doses were verified using thermoluminescent dosimeters LiF. Direct-reading dosimeters ("Arrow-Tech", USA) in the plastic

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casing were also used for continuous dose control. Pulse repetition rate was 10, 13 and 16 pulses per second.

B. Analysis of Tumor Growth and Metastasis

Female C57Bl6 mice (6–8 weeks old) were subcutaneously injected with 2×106 LLC cells. Irradiation was started when the tumor volume reached 430±25 mm^3. The regime for mice treatment presented as 5 days local irradiation at 1.8 mGy/min. Time of mice irradiation was limited by the absorbed dose equaled to 0.02 Gy. Total absorbed dose for 5 days was 0.1 Gy. Control mice were sham-exposed (generator at the off-mode) in identical conditions. On 19th day the weight and size of tumor was measured. For measurement of spontaneous metastases, lungs were fixed with Bowen fixative and amounts of colonies were calculated in each mouse under a dissecting microscope. All animal studies were carried out in accordance with the regulations and with permission of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

Statistical analysis. The Mann-Whitney U test for non-parametric trials was used for the statistical analysis of the results and *p* values less than 0.05 were regarded as significant.

III. Results

A. Tumor Growth Inhibition

Pulsed X-ray irradiation of tumor-bearing mice led to inhibition of LLC tumors. Delay of tumor growth measured on 19th day reached 30–40 % (Fig. 1). Highest inhibition level was when 10 pulses per second applied. Other pulse repetition frequencies didn't exceed 30% compare to control group.

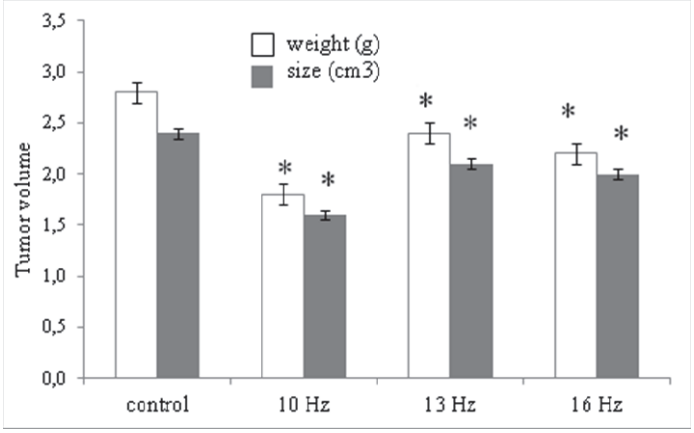

Fig. 1. Volume of solid tumor of C57Bl6 mice after exposure to pulsed X-ray. Error bars indicate the standard error of the mean (SEM) for $n = 3$ independent experiments; a sign * indicates statistical significance ($p \leq 0.05$).

B. Metastasis Inhibition

5 day irradiation local irradiation with pulsed X-ray significantly with daily dose 0.02 Gy decreased the number of metastatic colonies (Fig. 2) when 10 pulses per second rate applied. Average number of colonies in control group was 72.1±3.21. Irradiation of mice with pulse repetition rate 10 pulses per second inhibited colony formation by 62% (35.6±4.1). Irradiation with the pulse repetition rate 13 and 16 pulses per second resulted in inhibition of lung colonies at 23 and 12%, respectively. These results demonstrate that pulse ionizing radiation of tumor-bearing mice when the total absorbed dose lower 1 Gy strongly inhibits tumor growth and metastasis in depend on pulse repetition rate.

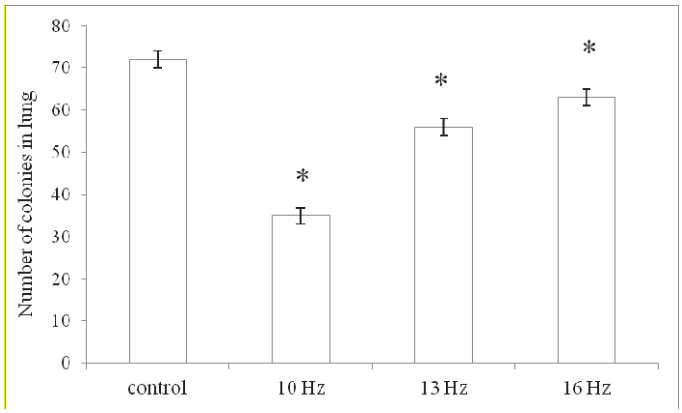

Fig. 2. Number of metastatic colonies in the lung of C57Bl6 mice after exposure to pulsed X-ray. Error bars indicate the standard error of the mean (SEM) for $n = 3$ independent experiments; a sign * indicates statistical significance ($p \leq 0.05$).

IV. Discussion

Power radiation when one continued pulsed divided to serial pulses with different specific repetition rate could provide more complicated and expressed reaction of the biological objects. Cells and tissues with their biological rhythms and intercellular processes which can be measured in a period of a few nanoseconds are depended on time period of acting factor. In this case, tumor cells presents a perspective object for investigation of repetitively pulsed X-ray radiation with nanosecond pulse duration.

Traditional (non-pulsed) X-ray irradiation shows antitumor efficacy against Lewis lung only when absorbed dose is 40 Gy and divided for 2 or 5 fraction [10]. Another data showed inhibition of tumor growth with doses lower than 0.2 Gy but only when irradiation was applied before tumor inoculation [11–12]. That means that X-ray low-dose radiation stimulates immune system, but doesn't possess direct influence on tumor. Application of pulsed regime allow to decrease total dose 200-times and save the antitumor efficacy of ionizing radiation.

V. Conclusion

That results evidence real opportunity for application low-dose repetitively-pulsed X-ray in clinical treatment of oncological patients. Decreasing of total absorbed dose with saving antitumor and antimetastatic effects is a main aim of the radiation oncology.

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