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Assessment of the proliferation rate of mesenchymal stem cells of adipose tissue *in vitro* after nanosecond microwave pulses exposure

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Abstract. Data are presented on the effect of nanosecond repetitively pulsed microwave radiation (RPMs) on the proliferation rate of mesenchymal stem cells from the femur of laboratory rats *Wistar*. The impact was carried out with the following parameters: carrier frequency of 9.4 GHz, pulse repetition rate of 22, 25 Hz, 50–100 pulses, peak power flux density (pPFD) of 140 W/cm^2, absorbed energy value in 50 pulses at a depth of 1 cm of 699×10^{-6} J/cm^3. The effect of the exposure was assessed by the change in the number of cells in the culture 24 and 72 hours after a single irradiation with RPMs with different modes of exposure. Depending on the pulse repetition rate and number of pulses of RPM an increase in the rate of cell division was observed. The most pronounced stimulating acceleration of cell division is provided by RPMs with a frequency of 25 Hz and a minimum number of pulses (50 pulses), and maximum proliferation is recorded after 72 hours.

Keywords: stem cells, adipose tissue, rate of division, proliferation, nanosecond microwave pulses, intensity.

1. Introduction

Significant experimental and clinical material on the effective use of mesenchymal stem cells (MSCs) has been accumulated to date. MSCs have a high differentiation potential and a certain degree of plasticity; they retain their properties even after freezing, which ensures their repeated use [1]. In addition to MSCs isolated from red bone marrow and umbilical cord blood, a special place is occupied by adipose-derived mesenchymal stem cells (AMSCs). This is because AMSCs can be easily and abundantly in amount extracted from tissue and processed *ex vivo*. It was previously shown that different modes of exposure to nanosecond microwave pulses [2] could effectively accelerate the proliferation of mesenchymal stem cells isolated from the red bone marrow of laboratory animals. One of the practically important tasks of cell therapy is the rapid production of a significant number of cells reproduced from stem cells, and this can be successfully implemented using nanosecond pulse technology. The purpose of this study was to evaluate the effect of nanosecond microwave pulses on the rate of proliferation of mesenchymal stem cells from the adipose tissue of laboratory rats *in vitro*.

2. Materials and methods

An experimental study was carried out on 15 cultures of MSCs isolated from preadipocytes of the abdominal adipose tissue of *Wistar* laboratory rats [3]. All procedures with animals were performed in accordance with international rules (RF GOST R-53434-2009, Principles of Good Laboratory Practice, 2010) [4]. Electric signals from the probes were recorded with a Rigol MSO8204 high-speed digital oscilloscope.

MSCs were isolated from 15 mature female Wistar rats of the same age, weighing 250–300 g. Cells in culture flasks were viewed using an Optika XDS-2SFL microscope (Italy) at 20x magnification. The viability of MSCs after cultivation was 91.5±2%. A single irradiation of experimental cultures was carried out on the 14th day of cultivation when the cells reached 80% coverage of the culture surface. Cell cultures were divided into groups: control group – cell cultures that were not subjected to any influences and were located in a CO_2 incubator; experimental (4

groups) – cell cultures that were subjected to a single exposure to 50 and 100 nanosecond microwave pulses with a power flux density of 140 W/cm^2 and pulse repetition rates of 22 or 25 Hz, respectively. Statistical processing of the research results: when processing the obtained data, the average value of the obtained values (X), the error of the mean (m), the median (Me), the upper and lower quartiles ($Q1$, $Q3$) were calculated, comparison criteria were used, including the Friedman and Kruskal-Wallis tests. The Shapiro-Wilk test was used to assess the normality of distribution. When accepting the hypotheses about the difference, the level of statistical significance was less than 5% ($p < 0.05$).

3. Results

To generate microwave radiation, a compact device based on a magnetron MI-459 (product was serially produced by Pluton JSC, Russia) was developed. The measuring path consists of a 31 dB directional coupler, a 26 dB attenuator and a semiconductor detector. The detected signal is fed to the oscilloscope. The power of microwave pulses emitted from the horn antenna is determined by the amplitude of the detected signal on the oscilloscope. The carrier frequency of the generator was 9.4 GHz, the peak output power was at least 20 kW, and the pulse duration at half power level was 100 ns. During irradiation, the cell culture was placed at a distance of 20 cm from the generator antenna horn, in the zone of the generated nanosecond RPM wave. During radiofrequency electromagnetic exposure, heating of tissues and, accordingly, an increase in temperature in the irradiated object/tissue is possible. The measured heating value is used to calculate the specific absorbed rate (ΔU, SAR), but due to the impossibility of measuring heating in relation to nanosecond pulses, it is impossible to correctly estimate the SAR. Therefore, it is more appropriate to use the value of absorbed energy, which can be calculated based on the measured pPFD. To estimate the absorption of an electromagnetic wave in a layer of biological tissue of thickness L, simplified formulas (1–3) can be used [5]:

$$\alpha = (\pi\varepsilon'')/(\lambda_0 \sqrt{(\varepsilon')}), \quad (1)$$

$$\varepsilon'' = \sigma/(2\pi f \varepsilon_0), \quad (2)$$

$$L = -20 \cdot \lg(e^{\alpha l}), \quad (3)$$

where α – is the absorption constant, ε' and ε'' – are the real and imaginary parts of the dielectric constant, σ – is the conductivity of the medium, ε_0 – is the electrical constant, λ_0 and f – are the wavelength in free space and frequency, l – is the depth (cm). Having made some assumptions, we obtain an expression for the absorbed dose that takes into account the dielectric properties of the cell solution (4):

$$\Delta U = NtS_0 (1-1/L), \quad (4)$$

where ΔU – is absorbed dose (J/cm^3), N – is the number of pulses, t – is the pulse duration, S_0 – is the power flux density of the incident wave (pPFD, W/cm^2), L – is the attenuation (in times) of power at a depth of centimeter. However, it should be taken into account that in the direction of wave propagation, attenuation occurs according to an exponential law. Substituting into these expressions the characteristics of biological tissues presented in [6-7], for bone marrow cells $\varepsilon' = 28$ and $\sigma = 10$ S/m, one can obtain attenuation values close in value to the results of computer modeling. In accordance with the RPM 140 W/cm^2 used in the work, the calculated value of the absorbed energy of a nanosecond RPM in 50 pulses at a depth of 1 cm was 699×10^{-6} J/cm^3.

Fixed pulse repetition rates of 22 and 25 Hz and the number of nanosecond pulses (50 and 100 pulses) were selected based on the results of a previous study as the most effective in stimulating the proliferation of mesenchymal stem cells [2]. The peak power flux densities used, which did not

cause measurable heating of the cellular environment, were determined and recorded using standard methods based on antenna measurements and calorimetric calibrations [8].

The experimental results showed (Fig. 1) that MSC cultures before the start of the study contained an average of 5×10^6 cells. In cultures irradiated with 100 microwave pulses with a repetition frequency of 22 Hz stimulation of MSC growth by 30% was observed after 24 hours, and by 40% – after 72 hours. Exposure to a smaller number of pulses (50) did not initiate a significant effect after 24 hours, but provided an increase in the number of cells relative to the control by 27% after 72 hours. After exposure to 100 nanosecond microwave pulses with a repetition rate of 25 Hz, there was no statistically significant effect after 24 hours, and the number of MSCs increased by 21% after 72 hours. Exposure to a pulse repetition rate of 25 Hz with a smaller number of pulses (50) provided stimulation of proliferation by 27% after 24 hours and 45% after 72 hours.

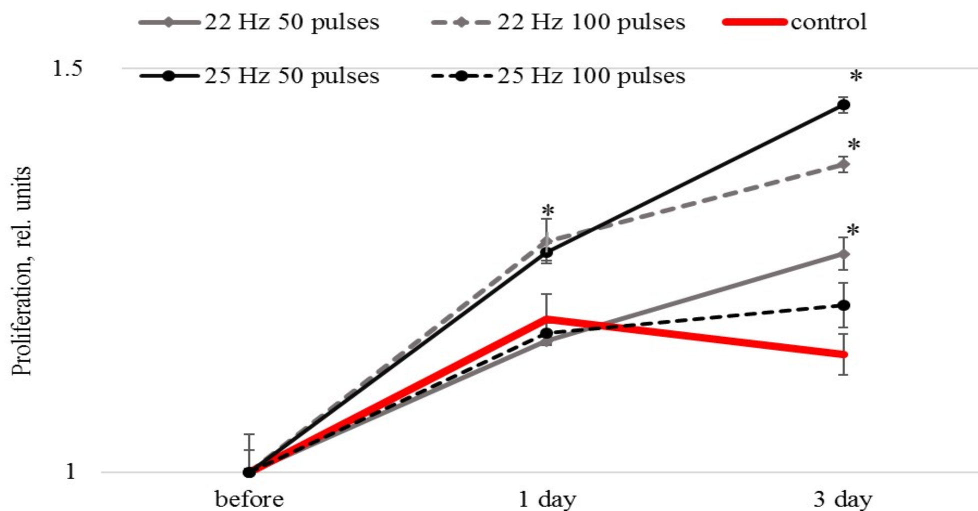

Fig. 1. Change in the rate of proliferation of mesenchymal stem cells in adipose tissue of laboratory rats after exposure to nanosecond RPMs with pPFD of 140 W/cm^2 and different pulse repetition rates and number of pulses. Note: proliferation indicators are presented in relative units based on the number of cells before irradiation. * – differences are statistically significant in relation to the indicators of the control cells.

4. Conclusions

The results obtained suggested the possibility of the existence of a universal mechanism for the response of MSCs from adipose tissue and MSCs from red bone marrow to exposure to nanosecond microwave pulses. The mechanism of action of nanosecond microwave pulses on MSCs is apparently associated with the activation of molecular signals of the microenvironment (MSC niche) [2, 9], the influence on Ca^{2+}-dependent processes inside and on the surface of cells [10, 11], as well as the induction of energy to overcome activation barriers target molecules, in particular protein globules [12]. In order to develop effective technologies for controlling stem cell proliferation using nanosecond microwave pulses, it is necessary to find optimal combinations of modes: peak power flux density, repetition rate and number of pulses. At the same time, studying the general patterns and biophysical and physiological mechanisms of MSC response to exposure of nanosecond microwave pulses with different parameters will make it possible to stimulate the proliferation of stem cells in vitro for biomedical purposes.

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