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Effect of Nanosecond Repetitive Pulsed Microwave Exposure on Proliferation of Bone Marrow Cells

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***Abstract*—The purpose was to study the proliferative activity of bone marrow mononuclear cells (BMNCs) of rats after irradiated by nanosecond repetitive pulsed microwave (RPM). It was found that the irradiated by nanosecond microwave pulses can affect the BMNCs proliferation in vitro. It is important that both stimulation and inhibition of proliferation were observed after exposure. The effect depended on the pulse repetition frequency. The amount of BMNCs increased after exposure to pulse repetition frequency of 13 Hz up to 30% in comparison with a control cells and up to 51% in comparison with a false-irradiated cells. In contrast, there was inhibition up to 40% of BMNCs after exposure to a frequency of 8 Hz, in comparison with a control group.**

Key words—microwave radiation, nanosecond pulses, proliferative activity, bone marrow mononuclear cells, cell cultures.

I. Introduction

Currently, cell therapy is actively used at correction of various pathological conditions [1, 2]. This approach supposes use of various stem cells [3]. Recently, close attention is paid to research focused on assessment of proliferative potential and regenerative features of stem cells. This is due to relevance of their application in various areas of medicine, especially cardiology, combustiology, and cosmetology.

The most important population of adult stem cells are mesenchymal stem cells (MSCs). Despite the fact that recently, many alternative sources of MSCs have been discovered in the world, bone marrow (BM) remains the most preferred source for obtaining MSCs, since the mononuclear cells of the human BM, in addition to MSCs, contain hematopoietic stem cells. Both these populations of stem cells are widely used in cell therapy and regenerative medicine [4, 5]. It is convincingly shown that the physiological renewal and regeneration of tissues in the whole life of animals and humans occurs due to stem cells [6–8], including bone marrow cells.

To date, to regulate cellular activity and cell proliferation one uses the import specialized nutrient media. Despite their effectiveness, these methods are quite expensive and require long-term use to achieve the required number of stem cells. The principal ability to stimulate cell growth and to increase the speed of stem cell proliferation is provided by application of various physical factors, in particular, EHF radiation [9], laser radiation [9], pulsed electric and magnetic fields [11]. Both inhibitory and stimulating effects of various impacts on stem cell proliferation in cell cultures have been identified.

Currently, the biological effect of irradiated by RPM is being actively studied. It is shown that irradiated by nanosecond microwave pulses with effectively influences on the functional state of a number of cells and tissues [12–14]. In addition, it was revealed that irradiated by nanosecond microwave pulses with certain parameters have a stimulating effect on the processes of regeneration of damaged tissues, in particular, healing of full-layer skin wounds is accelerated [15] and stomach ulceration in mice is eliminated [16]. Therefore, it is not excluded that the impact of irradiated by nanosecond microwave pulses can have a stimulating effect on proliferation of stem cells, in particular, bone marrow cells.

In view of the foregoing, the purpose of this work was to evaluate proliferative activity of bone marrow cells after irradiated by nanosecond microwave pulses.

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II. Methods

A. Isolation and cultivation of the fraction of rat bone marrow cells

To obtain the culture of bone marrow cells we used generally accepted standard method [17]. All procedures with animals were performed in accordance with international rules and norms [18]. Mononuclear cells isolated from rat bone marrow were cultivated in an atmosphere of 5% carbon dioxide at temperature of 37°C and humidity of 100%. In the process of cultivation the colonies of the adhered marrow mononuclear cells were formed at the bottom of the vial. Cultural medium was changed every 3 days for the elimination of non-adherent cells. As a result, up to 60–70% of the monolayer was formed on the 6^{th} or 7^{th} day, and the cell monolayer (95–100%) was finally formed on the 12^{th}–14^{th} day of cultivation. After the cultivation was completed the nutrient medium has been empted. The adhesive cells of the monolayer were removed from the surface of the culture vials and incubation was performed for 7–10 min at 37°C in the presence of 5 ml of 0.25% trypsin solution ("PanEco", Russia). Received cell suspension was washed with clean nutrient medium, after that cell viability was evaluated and culture cellularity was calculated. The cells were viewed using an Optika XDS-2SFL microscope (Italy) at a 20-fold increase.

Eight cultures of mononuclear cells of rat bone marrow forming monolayer on 12^{th}–14^{th} days (3–4 passage) were obtained to carry out experiments with the impact of nanosecond PPMR. Viability of bone marrow cells after cultivation was 91.5±2%. Further, 8 cultures were divided into the three groups: 1) the control one – 2 cell cultures that were not exposed to influence and were placed into a CO2 incubator; 2) the false-irradiated control one – 2 cell cultures that were placed one time near the source of microwave radiation for 5–8 minutes without switching on the generator; 3) the experiment one 1 and the experiment one 2–4 cell cultures that werre subjected to single exposure of nanosecond microwaves with frequencies of 8 and 13 Hz. Before starting the experiment each culture contained $4\times10^5\pm63\times10^3$ bone marrow cells.

B. Irradiation of cell culture by RPMs

The laboratory pulse generator on the basis of the MI-505 magnetron ("Tantal", Russia) was used as the source of RPM. Irradiation of bone marrow cell culture in the culture vials were carried out in the far zone of the antenna horn with a cross section of 40 mm ×90 mm (at distance of 20 cm) connected to the waveguide of the generator. This provided impact with the peak power flow density (pPFD) of 1500 W/ cm^2 (what corresponds to the average PFD value of 1.2 mW/cm^2). The nanosecond microwave pulses intensity was measured using standard method based on antenna measurements and calorimetric calibrations [19]. The cells were irradiated once time with 4000 RPMs (carrier frequency of the generator was 10 GHz, output peak power was 180 kW, and pulse duration at half level power was 100 ns) with pulse repetition frequencies of 8 and 13 Hz. Exposition duration was 8 and 5 minutes, respectively. Selection of modes of exposure was based on the results of previous experiments for stimulation of tissue regeneration. False-radiated cell groups were placed near the radiation source similar to those actually irradiated, but without switching on the generator. Control groups of cells were not subjected to impact.

The obtained data were subjected to statistical processing with the help of the "Statsoft STATISTICA for Windows 8.0" application program package that calculated the average arithmetic value of the quantity of cell ± error of the average value. Significance of differences between indices of irradiated and false-irradiated cell cultures were determined using the nonparametric Mann-Whitney U-criterion.

III. Results and Discussion

When evaluating the effectiveness of proliferation the cellular passage plays an important role. On the early passages MSCs have better colony forming ability in comparison with the later passages [20]. In this work we used bone marrow cell cultures of the 3^{rd} and 4^{th} passages. The performed study showed that to end of the experiment the number of cells in the culture of the control group has increased by 117% in comparison with the first day and was $9\times10^5\pm112\times10^3$ (Fig. 1). This confirmed the high proliferative potential of the obtained cultures of bone marrow cells.

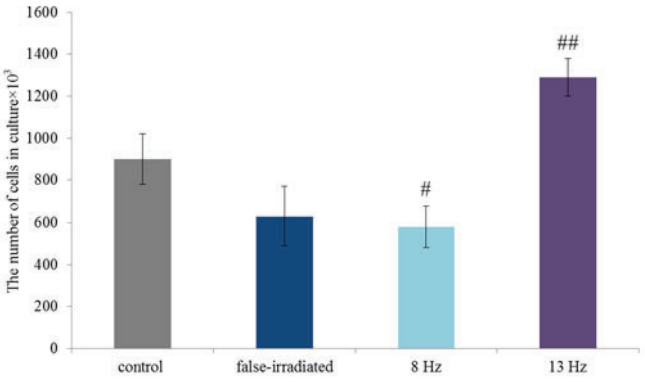

Fig. 1. Proliferative activity of rat bone marrow cells *in vitro* after irradiation by RPMs with pPFD of 1500 W/cm^2.

Note: * – differences are statistically significant in relation to indices of control cultures of bone marrow cells; ** - differences are statistically significant in relation to the indices of control and false-radiated cultures of bone marrow cells ($p \le 0.05$)

Analysis of the state of false-radiated cells showed that their proliferative activity decreased by 30% in relation to the control cells (Fig. 1). In this case, within the false-radiated group this index increased by 35% in relation to the first day of the experiment (Figure). Apparently, decrease of the proliferative potential in the false-radiated group during the experiment is associated with changes in the conditions of cultivation and ambient temperature during "false irradiation".

Assessment of the state of irradiated cell cultures showed that mononuclear cells of rat bone marrow are sensitive to action of RPMs. Proliferative activity of irradiated cells was changed in dependence on the frequency of pulse repetition. In particular, irradiation of cells with pulse repetition frequency of 8 Hz in 2 days after exposure was accompanied by 40% inhibition of cell proliferation in relation to the control group (Figure). This effect was statistically insignificant in relation to

the false radiated cells. Exposure with frequency of 13 Hz, on the contrary, increased the number of cells in the irradiated culture by 30% in relation to the control group, and by 51% in relation to the false-irradiated culture (Figure). This indicates on the possibility of effective stimulation of proliferation of bone marrow cell *in vitro* by exposure to Assessment of the state of irradiated cell cultures showed that mononuclear cells of rat bone marrow are sensitive to action of RPMs with a pPFD of 1500 W / cm^2 with pulse repetition frequency of 13 Hz.

Previously, the possibility of minor EHF stimulation (about 25%) with radiation of proliferation of "weakened" stem cells in cell cultures has been shown [9], while "normal" multipotent mesenchymal stromal cells did not respond to irradiation. After 5-minute exposure to continuous low-intensity laser radiation of the red and green spectra morphology and viability of MSCs was not changed, the proliferative activity was not stimulated [10]. In addition, it is known that low-intensity laser radiation with wavelength of 410 and 420 nm significantly suppresses fibroblast proliferation *in vitro* at daily illumination with EP 5–10 J/cm^2 [10]. The effect of stimulation of proliferation was shown at action of continuous laser radiation of 635 nm with power flux density of 32.6 mW / cm^2 and duration up to 90 seconds [21]. In the first three days after impact proliferation increased by more than 2 times, that the authors associate with activation of Ca^{2+} channels. The latter circumstance is very interesting from the point of view that according to the Eidy model [22] effects of modulated electromagnetic radiation results largely from Ca^{2+}- dependent processes. Currently there are no certain hypotheses in relation to the mechanisms of stimulation or inhibition of proliferation of stem cells. Within this framework, it is known that the stimulation of proliferation of rat bone marrow cells is mediated via MAPK/ERK signaling pathways [23, 24], or caused by proteins and molecules, associated with cell cycle arrest, such as Rb, cyclin E, CDK1, and CDC25B [23, 25] radiation in small doses [24]. Basing on these presentations we can assume both stimulating and inhibiting effects of non-ionizing radiation.

Thus, the experiments showed that irradiation of bone marrow cells with RPMs have mainly an effect on the proliferative activity of cells. At the same time, the impact with different pulse repetition frequencies initiates the opposite effects of changes in proliferation (stimulation and inhibition) that coincides with the results obtained earlier. The similar stimulating effect of RPMs on wound healing processes [15] and regeneration of neurogenic ulceration of the gastric mucosa [16] was shown in the previous studies. Apparently, reaction of stimulation of proliferation has common biological character for different cells. Since pulsed microwave radiations are not genotoxic [26], it actualizes the study of action of this factor on cell cultures. In addition, nanosecond repetitive pulsed microwave has more biotropic parameters in comparison with laser, and EHF radiations, electric and magnetic signals that will allow in the future to identify such mode of influence that will provide the most effective stimulation of proliferation of stem cells for rapid production of the necessary number of them required for the specific need of regenerative medicine.

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REFERENCES

[1] V. V. Novochadov, “The control of the cell settlement and scaffold remodeling in cartilage tissue engineering: a review,” Vestnik *Volgogradskogo gosudarstvennogo universiteta, Seriya 11: Estestvennye nauki*, no.1, pp. 19–28, 2013.

[2] E. R. Pavlovich, “Regenerative medicine: pluses and minuses of application of cell technologies in medical and biological researches,” *Sovremennye naukoemkie tekhnologii*, no. 2, pp. 103, 2013.

[3] K. S. Elbekyan, A. B. Khodzhayan, A. B. Muravyova, ”Features of disturbance of a micro-macroelement spectrum of blood serum at an experimental diabetes,” Fundamental'nye issledovaniya, no. 10, pp. 411–413, 2011.

[4] N. I. Kalinina, V. Y. Sysoeva, K. A. Rubina, E. V. Parfenova, V. A. Tkachuk, “Mesenchymal Stem Cells in Tissue Growth and Repair,” *Acta Naturae*, vol. 3, no. 4, pp. 32–39, 2011.

[5] N. I. Kalinina, V. Y. Sysoeva, K. A. Rubina, E. V. Parfenova, V. A. Tkachuk, “Mesenchymal Stem Cells in Tissue Growth and Repair,” *Acta Naturae*, vol. 3, no. 4, pp. 32–39, 2011.

[6] E. A. Lipunova, M. Y. Skorkina, *Sistema krasnoj krovi: sravnitel'naya fiziologiya: monografi*. Belgorod, Russia: BelGU, 2004. (in Russian)

[7] K. N. Yarygin, “The role of resident and circulating stem cells in physiological and reparative regeneration,” *Journal of Pathophysiology and Experimental Therapy*, no. 1, pp. 2–8, 2008.

[8] A. P. Yastrebov, D. Y. Grebnev, I. Y. Maklakova, *Stvolovye kletki, ih svojstva, istochniki polucheniya i rol' v regenerativnoj medicine: monografiya*. Ekaterinburg, Russia: UGMU, 2016. (in Russian)

[9] R. K. Chajlahyan, V. I. Yusupov, Y. V. Gerasimov, P. A. Sobolev, A. H. Tambiev, N. N. Vorob'yova, A. P. Sviridov, V. N. Bagratashvili, “Vliyanie gidrodinamicheskih processov i nizkointensivnogo izlucheniya s dlinami voln 0,63 mkm i 7,1 mm na proliferativnuyu aktivnost' stvolovyh kletok stromy kostnogo mozga in vitro”, *Biomedicina*, no. 2, pp. 24–29, 2011.

[10] S. V. Moskvin, D. Y. Klyuchnikov, E. V. Antipov, A. I. Gorina, O. N. Kiseleva, “The influence of continuous low-intensity laser radiation at the red (635 nm) and green (525 nm) wavelengths on the human mesenchymal stem cells in vitro: a review of the literature and original investigations,” *Voprosy kurortologii, fizioterapii i lechebnoj fizicheskoj kul'tury*, no. 2 pp. 32-42, 2016. (in Russian)

[11] M. V. Ulitko, S. Y. Medvedeva, V. V. Malakhov, “The influence of low-frequency pulsed electric and magnetic signals or their combination on the normal and modified fibroblasts (an experimental study),” *Voprosy kurortologii, fizioterapii i lechebnoj fizicheskoj kul'tury*, no. 3, pp.46–52, 2016.

[12] A. V. Kereya, L. P. Zharkova, V. V. Ivanov, I. R. Knyazeva, O. P. Kutenkov, Yu. N. Semjonova, V. V. Rostov, M. A. Bolshakov, “The epididymal adipose tissue of mice after nanosecond pulse-periodic microwave irradiation”, Radiation biology. Radioecology, vol. 54, no. 6, pp. 606–612, 2014.

[13] I. R. Knyazeva, V. V. Ivanov, L. P. Zharkova, O. P. Kutenkov, V. V. Rostov, M. A. Bolshakov, ”The effect of the repetitive pulsed microwaves on functional activity of isolated mitochondria of mice liver,” *Vestnik Tomskogo gosudarstvennogo universiteta. Biologiya*, no 4 p. 113–123, 2011. (in Russian)

[14] M. S. Korovin, M. A. Bolshakov, V. J. Gridneva, A. A. Yelchaninov, A. I. Klimov, V.V. Rostov, “Assessment of some metabolic parameters of white rats’ blood after exposure to repetitive x-ray or microwave pulses,” *Experimental and clinical gastroenterology*, no. 3, pp. 70–74, 2005.

[15] I. R. Knyazeva, M. A. Medvedev, L. P Zharkova., A. A. Gostyukhina, O. P. Kutenkov, V. V. Rostov, M. A. Bolshakov, “The influence of nanosecond microwave pulses on the regeneration process,” *Bulletin of Siberian Medicine*, no. 6, pp. 109–113, 2011.

[16] N. V. Mamonova, L. P. Jarkova, I. R. Knyazeva, O. P. Kutenkov, V. V. Rostov, M. A. Bolshakov, "Caused by ethanol gastric mucosa damage in mice before and after pulse-repetitive microwave exposure," *Experimental and clinical gastroenterology*, no. 8, pp. 46–49, 2011.

[17] K. V. Zaitsev, S. A. Mezheritskyi, N. P. Stepanenko, *et. al.*, "Immunophenotypical characteristics of cellular composition in breast milk," *Cell and Tissue Biology*, vol. 58, no. 7, pp. 543–547, 2016

[18] RF GOST R-53434-2009 Principles of good laboratory practice. Moscow, Russia: Standardinform, 2010.

[19] A. I. Klimov, A. A. Eltchaninov, E. Y. Konobeeva, "Measurements of Parameters of X-Band High-Power Microwave Pulses», *Russian Physics Journal*, vol. 49, no. 11, pp. 431–434, 2006.

[20] E. Y. Osipova, V. A. Nikitina, T. A. Astrelina, *et. al.*, "Human bone marrow mesenchimal stem cells growth rate dynamics, immunophenotype and genetic stability on early and late passages at ex vivo culturing," *Oncohematology*, vol. 1, pp. 44–50, 2009.

[21] M. Giannelli, F. Chellini, C. Sassoli, F. Francini, A. Pini, R. Squecco, D. Nosi, D. Bani, S. Zecchi-Orlandini, L. Formigli, "Photoactivation of bone marrow mesenchymal stromal cells with diode laser: effects and mechanisms of action," *Journal of Cellular Physiology*, vol. 228 no.1, pp. 172–181, 2013.

[22] W. R. Adey, "Biological effects of electromagnetic fields," *Journal of Cellular Biochemistry*,vol 51, no. 4, pp. 410–416, 1993.

[23] M. V. Pustovalova, A. K. Grekhova, A. N. Osipov, "Mesenchymal Stem Cells: Effects of Low-Dose Ionizing Radiation Exposure," *Radiation biology. Radioecology*, vol. 58, no. 4, pp.352–362, 2018.

[24] X. Liang, Y.H. So, J. Cui, K. Ma, X. Xu, Y. Zhao, L. Cai, W. Li, "The low-dose ionizing radiation stimulates cell proliferation via activation of the MAPK/ERK pathway in rat cultured mesenchymal stem cells," *Journal of Radiation research*, vol. 52, no. 3, pp. 380–386, 2011.

[25] W. Y. Guo, G. J. Wang, L. Cai, P. Wang, Q. Chen, Y. Tan, "Acceleration of diabetic wound healing by low-dose radiation is associated with peripheral mobilization of bone marrow stem cells,"*Radiation Research*, vol. 174, no. 4, pp. 467–479, 2010.

[26] A. B. Gapeev, E. N. Mikhailik, N. K. Chemeris, A. V. Rubanik, "Anti-inflammatory effects of high-peak-power pulsed microwaves as dependent on irradiation parameters," *Biophysics*, vol. 52, no. 5,pp. 947–952, 2007.