THE EFFECT OF NON-COHERENT IMPULSE RADIATION ON FUNCTIONAL STATUS OF MONONUCLEAR CELLS IN EXPERIMENT

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The aim of the investigation was to study the effect of non-coherent impulse radiation on functional status of mononuclear cells in experiment.

Materials and Methods. *In vivo* experiments were carried out on white outbred male rats exposed to non-coherent impulse radiation with the following set-up parameters: impulse time — 10 μs, amperage — 1 kA, electrode voltage — 10 kV, pulse energy — 5 J, frequency — 1 Hz. We used two exposure modes on animals: three times within a minute, and three times within 2 min.

We studied the state of oxygen-dependent neutrophil metabolism using spontaneous and induced NBT-test (NBT — nitro blue tetrazolium), assessed the activity of phagocytosis by latex particles phagocytosis, and determined spectrophotometrically the concentration of nucleic acids in lymphocytes of peripheral blood.

Results. One-minute exposure caused no significant changes of functional status of mononuclear cells. Two-minute exposure resulted in NADPH-oxidase activation in neutrophil plasma membrane. Cell phagocytic rate was found to increase when the animals were exposed to non-coherent impulse radiation. Phagocytic index and phagocytic number increased of 21.84 and 45.28% respectively. There was revealed the increase of DNA concentration in lymphocytes of peripheral blood in rats.

Key words: mononuclear cells; phagocytosis; neutrophil NADPH-oxidase; DNA; RNA; non-coherent plasma radiation.

The immune system cells are considered not only as the factors of adaptive and nonspecific immunity, but also as the universal indicators of homeostasis [1]. That is why the assessment of functional activity of mononuclear cells under various physicochemical effects on the body is of the utmost interest.

Recently, plasma technologies have been frequently used in practical and experimental biology and medicine [2, 3]. There are known bactericidal and cytotoxic effects of gas-discharge plasma [4]. Non-coherent impulse radiation of UV emission is found to be the main operative factor of spark discharge plasma [2].

The generation of pulse discharges is known to result in the formation of radical products [5, 6] enable to have an effect on oxidation-reduction processes and modification of macromolecules and different cell structures [7–9]. On the other hand, free radicals are the products of normal cell metabolism [7]. Physiological effects of radicals are cytotoxic action when radicals are the part of phagocytes, cell proliferation regulation, B-lymphocyte differentiation, T-lymphocyte activation, and transcription induction of particular genes [10, 11].

The functional status of mononuclear cells after non-coherent plasma radiation exposure has not been assessed so far.

The aim of the investigation was to study the effect of non-coherent impulse radiation of spark discharge plasma on functional status of mononuclear cells in experiment.

Materials and Methods. We carried out the experiments on laboratory animals — white outbred male rats weighting 200–250 g. The animals were kept in standard conditions of an animal facility.

When carrying out the investigations, ethical principles were kept inviolate according to European Convention for the protection of vertebrata used for experimental and other scientific purposes (the Convention was passed in Strasburg, 18.03.1986, and adopted in Strasburg, 15.06.2006).

As an operative factor we used non-coherent impulse radiation of spark discharge plasma generated by the device developed by All-Russian Scientific Research Institute of Experimental Physics (Russian Federal Nuclear Centre, Sarov, Nizhny Novgorod region, Russia) [12]. The device had the following set-up parameters: impulse time — 10 μs, amperage — 1 kA, electrode voltage — 10 kV, 1 pulse energy — 5 J, frequency — 1 Hz. An anterior abdominal wall of the animals was exposed to radiation. The target of the research was lymphocytes isolated from rat peripheral blood, and blood neutrophils and monocytes. Peripheral blood cells of intact animals served as the control.

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To assess the effect of non-coherent impulse radiation on functional activity of mononuclear blood cells, the animals were divided into the following groups: intact animals (control group), the animals with three-time exposure within 1 min, and the animals with three-time exposure within 2 min. The animals were exposed once a day for three days. The exposure modes and frequency were had been determined in the previous experiments [13].

The animals were administered 0.1 ml of heparin intraperitoneally, 10 min later they were anesthetized by ether, and decapitated. The blood was kept in heparinized test-tubes to prevent blood coagulation.

The lymphocytes were isolated in ficoll-urografin gradient (ρ=1,076 g/cm). We used trypan blue test to determine cell viability, which was at least 95% throughout the experiment.

The neutrophil metabolic activity was estimated using spontaneous and zymozan-induced tests with nitro blue tetrazolium (NBT-test). In the course of the reaction, colorless NBT is absorbed by neutrophils, and recovered in response to their dehydrogenase system into dark-violet diformazan granules visible and determined under microscope.

The phagocytic activity was judged by the absorption of latex particles of 0.8 μm in size. The microscopic study enabled to calculate the percentage of the number of active cells (phagocytic index) and the average number of latex particles absorbed by a phagocyte (phagocytic number).

Nucleic acid concentration was determined by alkaline and acid hydrolyses by removing lipid acid-soluble fractions with the following spectrophotometry [14].

The findings were statistically processes using software package Excel and Statistica 6.0 according to the biomedical statistical recommendations [15].

The findings were represented as M±G, where M — arithmetic mean, G — root-mean-square deviation. We determined the reliability of differences according to Kruskal–Wallis test. Two samplings were considered to belong to different parent entities if $p<0.05$.

Results and Discussion. Phagocytosis is the center link of nonspecific protection of the body. The cell phagocytic activity increase is the key factor indicating cell activation [16]. The analysis of the non-coherent impulse radiation effect on blood cell phagocytic activity of the test animals (Table 1) showed that there were no statistically significant changes of phagocytic activity under three-time exposure within 1 min.

Two-minute exposure promotes phagocytosis activation, and it is reflected by the increase of phagocytic index of 21.84% (p=0.019) and phagocytic number – of 45.28% (p=0.017) compared to the control group. Thus, the effect of non-coherent impulse radiation results in phagocytosis activity induction.

Phagocyte activation is characterized by reactive oxygen species formation and accompanied by respiratory burst [1]. NBT-test reflects oxygen-dependent metabolism of neutrophils and free radical production [17]. The study of neutrophil metabolic activity indices after one-minute non-coherent impulse radiation revealed no significant changes (Table 2). Two-minute exposure was found to activate oxygen-dependent metabolism of neutrophils. The number of positively reacting cells of spontaneous NBT-test increased of 15.33% (p=0.032). The increase of spontaneous activation index indicating the activity of neutrophil enzyme systems was 28.40% (p=0.032). We revealed the statistically significant increase of metabolic activity coefficient of 8.79% (p=0.034) indicating the neutrophil stimulation degree. The decrease of reserve neutrophils characterizing potential ability to activation was 16.39% (p=0.034).

The intensity of reactive oxygen species (ROS) generation by neutrophils is known to be determined mainly by the NADPH-oxidase activity that depends on the

Table 1

The effect of non-coherent impulse radiation on blood phagocytic activity in animals

Exposure time	Phagocytic index, %	Phagocytic number
Control	81.5±6.8	5.30±0.75
1 min	78.0±10.2	4.70±0.58
2 min	99.3±1.2* (p=0.019)	7.7±0.6* (p=0.017)

* — statistically significant difference of values with the control group.

Table 2

The effect of non-coherent impulse radiation on neutrophil oxidative metabolism

	Exposure time		
	Control	1 min	2 min
NBT-test, spontaneous, %	68.50±2.38	68.50±2.69	79.00±2.65* (p=0.032)
NBT-test, induced, %	83.75±2.99	79.00±1.00* (p=0.037)	80.67±1.15
SNAI	0.81±0.05	0.80±0.04	1.04±0.04* (p=0.032)
ANAI	1.15±0.11	1.00±0.03	1.10±0.06
NMAC	1.82±0.05	1.87±0.03	1.98±0.04* (p=0.034)
NRI	1.22±0.07	1.16±0.04	1.02±0.04* (p=0.034)

Note: SNAI and ANAI — spontaneous and activated neutrophil activity indices; NMAC — neutrophil metabolic activity coefficient; NRI — neutrophil reserve index. * — statistically significant difference of values with the control group.

type, concentration and time of stimulation [18]. In addition, there is the concept of two-stage neutrophil activation known as cell priming. Cytokines, chemotoxic factors, metabolic regulators, lipid peroxidation products serve as priming agents [19–21]. Neutrophil transition into activation may be due to the cell interaction with ROS formed by non-coherent impulse radiation [6]. H_2O_2 in weak concentration is known to induce neutrophil chemotaxis, a short-term increase of intracellular calcium, and increase respiratory burst [22].

Thus, the observed reactive oxygen species generation increase and the reduction of cell reserve potential indicate that non-coherent impulse radiation induces phagocytosis and promotes the activation of neutrophil oxygen-dependent metabolism.

The interest in the biological effect of non-coherent impulse radiation has started growing recently [23]. Photobiological effects are known to realize through the cascade of oxidative reactions [24]. The activation of free radical processes in the body has an impact on the state of cell genetic apparatus. Medium-intensity oxidative stress caused by ROS performs a regulatory function and promotes proliferation [7, 11, 25, 26], while high ROS concentrations can have a damaging effect on nucleic acids, and induce cell death [7, 8, 25, 27]. The controversial effect of ROS on cells, and nucleic acids in particular, was the background for the assessment of the DNA and RNA number in peripheral blood lymphocytes in animals.

The study of the non-coherent impulse radiation effect on the concentration of nucleic acids in lymphocytes of animals under three-time exposure within 1 min did not show statistically significant changes of DNA and RNA levels (Table 3). Three-time exposure within 2 min was found to statistically significantly increase DNA concentration ($p=0.027$) compared to the control group, as well as there was the tendency for RNA downregulation.

The observed increase of DNA number in lymphocytes, on the one hand, can be explained by regulatory function of oxygen radicals (superoxide anion radical and H_2O_2) on proliferative process activation [11, 24]. On the other hand, DNA concentration increase can be due to the formation of covalent links between DNA and proteins, as well as between the neighboring pyrimidine and purine bases that can reduce DNA molecule resistance to destruction [27, 28]. However, according to the data reported in literature [6, 27], the intensity level of non-coherent impulse radiation we use (pulse energy — 5 J) is still insufficient for both a direct destructive effect on DNA molecules, and through ROS formation in the course of plasma chemical reactions.

Thus, the study of non-coherent impulse radiation effect on cell genetic apparatus requires further investigations by high-technology methods of molecular biology.

Conclusion. The study of non-coherent impulse radiation effect of low-temperature plasma of spark discharge on functional activity of mononuclear cells in the experiment showed that three-time exposure radiation within 2 min promotes the activation of phagocytic reaction and oxygen-dependent metabolism of neutrophils, DNA concentration increase in peripheral blood lymphocytes of animals.

Table 3

The concentration of nucleic acids in lymphocytes of the animals under non-coherent impulse radiation

Exposure time	DNA, μg/ml	RNA, μg/ml
Control	13.45±4.05	6.52±4.21
1 min	12.36±3.32	7.18±4.09
2 min	28.88±16.42* (p=0.027)	3.50±1.39

* — statistically significant difference of values with the control group.

References

1. Medzhitov R., Dzhanevey Ch. Vrozhdennyy immunitet [Congenital immunity]. *Kaz Med Z — Kazan Medical Journal* 2004; 85(3): 161–167.

2. Ivanova I.P., Trofimova S.V., Piskarev I.M., et al. Vliyanie aktivnykh form kisloroda nizkotemperaturnoy gazorazryadnoy plazmy na rezistentnost' membran kletok [The effect of reactive oxygen species of low-temperature gas-discharge plasma on cell membrane resistance]. *Vestnik Nizhegorodskogo gosudarstvennogo universiteta im. N.I. Lobachevskogo — Herald of Nizhny Novgorod State University named after N.I. Lobachevsky* 2011; 2(2): 190–195.

3. Vasilets V.N., Gutsol A., Shekhter A.B., Fridman A. Plazmennaya meditsina [Plasma medicine]. *Khimiya vysokikh energiy — High-Energy Chemistry* 2009; 43(3): 276–280.

4. Ivanova I.P., Zaslavskaya M.I. Biotsidnyy effekt nekogerentnogo impul'snogo izlucheniya iskrovogo razryada v eksperimente in vitro i in vivo [Biocydic effect of the spark discharge non-coherent impulse radiation in experiments in vitro and in vivo]. *Sovrem Tehnol Med — Modern Technologies in Medicine* 2009; 1: 28–31.

5. Kanazawa S., Kawano H., Watanabe S., Furuki T., Akamine S., Ichiki R., Ohkubo T., Kocik M., Mizeraczyk J. Observation of OH radicals produced by pulsed discharges on the surface of a liquid. *Plasma Sources Sci Technol* 2011; 20(3): 383–391.

6. Ivanova I.P., Trofimova S.V., Karpel Vel Leitner N., Aristova N.A., Arkhipova E.V., Burkhina O.E., Sysoeva V.A., Piskaryov I.M. Analiz aktivnykh produktov izlucheniya plazmy iskrovogo razryada, opredelyayushchikh biologicheskie effekty v kletkakh [The analysis of active products of spark discharge plasma radiation determining biological effects in tissues]. *Sovrem Tehnol Med — Modern Technologies in Medicine* 2012; 2: 20–30.

7. Dontsov V.I., Krut'ko V.N., Mrikaev B.M., Ukhanov S.V. Aktivnye formy kisloroda kak sistema: znachenie v fiziologii, patologii i estestvennom starenii [Reactive oxygen species as a system: significance in physiology, pathology and natural ageing]. *Trudy ISA RAN — Proceedings of System Analysis Institute of Russian Academy of Sciences* 2006; 19: 50–69.

8. Artyukhov V.G., Nakvasina M.A., Popova L.I., et al. Strukturno-funktsional'nye modifikatsii limfotsitarnykh kletok cheloveka v usloviyakh vozdeystviya aktivnykh form kisloroda [Structural functional modifications of human lymphocytic cells when exposed to reactive oxygen species]. *Vestnik VGU Ser. Khimiya, biologiya, farmakologiya — VSU Review, Series Chemistry, Biology, Pharmacology* 2005; 2: 110–115.

9. Ivanova I.P., Trofimova S.V., Piskaryov I.M., Burkhina O.E., Sysoeva V.A., Karpel Vel Leitner N. Issledovanie mekhanizmov biotsidnogo deystviya izlucheniya plazmy iskrovogo razryada [The study of biocidal mechanisms of spark discharge plasma radiation]. *Sovrem Tehnol Med — Modern Technologies in Medicine* 2012; 3: 12–18.

10. Droge W. Free radicals in the physiological control of cell function. *Physiological Reviews* 2002; 82: 47–95.

11. Gamaley I.A., Klyubin I.V. Roles of reactive oxygen species: signaling and regulation of cellular functions. *Int Rev Cytol* 1999; 188: 203–215.

12. Spirov G.M., Luk'yanov N.B., Shlepkin S.I., Volkov A.A., Moiseenko A.N., Markevtsev I.M., Ivanova I.P., Zaslavskaya M.I. *Ustroystvo dlya vozdeystviya na bioob"ekt* [Bio-object exposure device]. Patent RF №2358773. 2009.

13. Ivanova I.P., Prodanets N.N., Burenina Yu.V. Effekt vliyaniya vysokoenergeticheskogo impul'snogo izlucheniya na metabolizm zhivotnykh v norme i s limfosarkomoy Plissa [The effect of high-energy impulse radiation on metabolism in healthy animals and in animals with Pliss lymphosarcoma]. *Byulleten' Sibirskoy meditsiny — Bulletin of Siberian Medicine* 2005; 2: 88–90.

14. *Biokhimiya i molekulyarnaya biologiya* [Biochemistry and molecular biology]. Pod red. Titovoy N.M., Zamay T.N., Borovkovoy G.I. [Titova N.M., Zamay T.N., Borovkova G.I. (editors)]. Krasnoyarsk: IPK SFU; 2008; 103 p.

15. Glants S. *Mediko-biologicheskaya statistika* [Biomedical statistics]. Moscow: Praktika; 1999; 459 p.

16. Pinegin B.V., Krasnova M.I. Makrofagi: svoystva i funktsii [Macrophages: characteristics and functions]. *Immunologiya — Immunology* 2009; 4: 241–249.

17. Gerasimov I.G., Ignatov D.Yu. Osobennosti aktivatsii neytrofilov in vitro [The characteristics of neutrophil activation in vitro]. *Tsitologiya — Cytology* 2004; 46(2): 155–158.

18. Mayanskiy A.N. NADFN-oksidaza neytrofilov: aktivatsiya i regulyatsiya [Neutrophil NADPH-oxidase: activation and regulation]. *Tsitokiny i vospalenie — Cytokines and Inflammation* 2007; 6(3): 3–13.

19. Sheppard F.R., Kelher M.R., Moore E.E., et al. Structural organization of the neutrophil NADPH oxidase: phosphorylation and translocation during priming and activation. *Journal of Leukocyte Biology* 2005; 78: 1025–1042.

20. Alvarez-Maqueda M.E., Bekay R., Monteseirin J., Alba G., Chacon P., Vega A., Santa M.C., Tejedo J.R., Martin-Nieto J., Bedoya F.J., Pintado E., Sobrino F. Homocysteine enhances superoxide anion release and NADPH oxidase assembly by human neutrophils. Effects on MAPK activation and neutrophil migration. *Atherosclerosis* 2004; 172: 229–238.

21. M'Rabet L., Coffer P., Zwartkruis F., et al. Activation of the small GTPase rap1 in human neutrophils. *Blood* 1998; 92: 2133–2140.

22. Kovalenko E.I., Semenkova G.N., Cherenkevich S.N. Vliyanie peroksida vodoroda na sposobnost' neytrofilov generirovat' aktivnye formy kisloroda i khlora i sekretirovat' mieloperoksidazu in vitro [The effect of hydrogen peroxide on neutrophil ability to generate reactive oxygen and chloride species and secrete myeloperoxidase in vitro]. *Tsitologiya — Cytology* 2007; 49(10): 839–847.

23. Beebe S.J. and SchoenbachK. H. Nanosecond pulsed electric fields: a new stimulus to activate intracellular signaling. *Journal of Biomedicine and Biotechnology* 2005; 4: 297–300.

24. Karu T.Y. Kletochnye mekhanizmy nizkointensivnoy lazernoy terapii [Cell mechanisms of low-intensity laser therapy]. *Uspekhi sovremennoy biologii — Advance of Modern Biology* 2001; 121(1): 110–120.

25. Men'shchikova E.B., Lankin V.Z., Zenkov N.K., Bondar' I.A., Krugovykh N.F., Trufakin V.A. *Okislitel'nyy stress*. *Prooksidanty i antioksidanty* [Oxidative stress. Pro-oxidants and antioxidants]. Moscow: Slovo; 2006; 556 p.

26. Hattoria H., Subramaniana K. Small-molecule screen identifies reactive oxygen species as key regulators of neutrophil chemotaxis. *PNAS* 2010; 107(8): 546–551.

27. Artyukhov V.G., Trubitsina M.S., Nakvasina M.A., Solov'eva E.V. Fragmentatsiya DNK limfotsitov cheloveka v dinamike razvitiya apoptoza, indutsirovannogo vozdeystviem UF-izlucheniya i aktivnykh form kisloroda [Fragmentation of human lymphocyte DNA in dynamics of apoptosis induced by UV radiation and reactive oxygen species]. *Tsitologiya — Cytology* 2011; 53(1): 61–67.

28. Finkel T., Holbrook N. Oxidants, oxidative stress and the biology of ageing. *Nature* 2000; 408: 239–247.