Reactive oxygen species, DNA damage and Solar-Earth communications

E E Tekutskaya* and I S Ryabova¹

¹Kuban State University of the Ministry of Science and Education of the Russian Federation, 149 Stavropol’skaya, Krasnodar, the Russian Federation

*etekytska@gmail.com

Abstract. Reactive oxygen species play a significant role in regulating the main functions of the cell both in the normal conditions and when exposing the cell to exogenous and endogenous factors under electromagnetic and radiation emission at various levels of solar activity. The research studied the impact of low-intensity alternating magnetic field; the intensity level is comparable to the geomagnetic field, in the frequency range from 3 to 60 Hz and γ-radiation on the generation of the most stable ROS which are hydrogen peroxide, in vitro. A degree of oxidative DNA damage in the blood serum of health donors, after being exposed to the alternating magnetic field, was investigated by determining the content of 8-hydroxy-2-deoxyguanosine (8-OHdG) in the blood serum and DNA single breaks in the lymphocytes. An increase of the content of 8-OHdG in DNA by a factor of 1.5 – 2 in comparison with non-emitted samples, and non-linear change in the level of lipid peroxide in the blood serum with maxima at MF frequencies of 8 and 50 Hz. In lymphocytes are observed. When exposed to gamma radiation, the number of single-strand breaks (SSB) of DNA increased with increasing exposure time from 44.3 ± 0.4% (irradiation for 30 minutes) to 68.3 ± 0.8% (irradiation for 90 minutes) compared to the control samples. It is concluded that the obtained effects can be associated with the generation of reactive oxygen species in the aquatic environment with variations in solar activity and affect the adaptive capabilities of the human body as a whole.

1. Introduction

According to modern ideas, reactive oxygen species play a significant role in regulating the main functions of cell both in the normal conditions and when exposing the cell to exogenous and endogenous factors. Depending on the force of pathogenic factor affecting the cell ROS can induct adaptation processes or apoptosis. It is known that living organisms are very sensitive to ROS content in the environment, which are both damaging and signal agents. The level change of these compounds in the atmosphere determines the correlation, discovered by Chizhevsky, between solar activity (SA) and the biological activity of living organisms on Earth [1]. ROS are able to exert a direct destructive effect on the cellular structures, as well as initiate free radical oxidation of lipids, proteins, and nucleic acids. ROS realize their physiological and pathological effects in close interaction with other regulatory factors of the cell, modulating their activity [2-4].

Nucleic acid-based biopolymers cause a particular interest when studying the magnetic field (MF) effect on living systems at frequencies close to the geomagnetic field. Known manifestations of stress-reaction in cells are biologically important molecules damages and, above all, DNA. Violation or poor
repair processes and the resulting mutations can have catastrophic consequences [2]. One of the crucial oxidative damages biomarker of nucleic acids mediated with ROS generation is the formation of 8-hydroxy-2-deoxyguanosine (8-OHdG) in DNA. Content levels of 8-OHdG and its analogues 8-hydroxiguanosine and 8-hydroxiguanin are associated with many degenerative diseases. Thus, the relationship between ROS and the use of 8-OHdG as a marker of oxidative stress has been studied in many diseases, including prostate cancer, cystic fibrosis, atopic dermatitis, and rheumatoid arthritis.

It was demonstrated in the articles [3-5] exposure to aqueous DNA solutions and human whole blood in vitro by an electromagnetic field of low frequency (LF) can lead to the formation of ROS. It can contribute to the damage of the primary structure of DNA, the accumulation of oxidized nitrogenous bases (8-OHdG) and subsequently lead to the formation of single-strand breaks (SSB) in the DNA of human blood cells [3].

The objective of the research was to study the exposure of low-intensity alternating MF, the intensity level of which is comparable to the geomagnetic field, in the frequency range from 3 to 60 Hz and γ-radiation on the generation of the most stable ROS – hydrogen peroxide; and to assess the amount of oxidative damage to 8-OHdG and the appearance of SSB in DNA of human lymphocytes.

2. Materials and methods

Samples of peripheral blood collected from healthy donors (20 people), men, non-smokers, aged from 21 to 23 years were objects of the research. Blood sampling was performed in 2.5 ml plastic tubes with EDTA added as an anticoagulant at a final concentration of 2.0 mg / ml.

Processing of blood samples in vitro by MP was carried out in sterile plastic containers with an irradiated layer thickness of 2 mm. In the course of the experiments, we used a device developed by us for the automated study of biological fluids in an alternating MF, described in article [6]. The processing time was 15 minutes. The intensity of the magnetic component of the field reached $24 \pm 4 \text{ A/m}$, the frequency was changed in the range from 3 to 50 Hz with a step of 1 Hz. The MF strength was measured with the "Ekofizika-110A" device with a digital measuring transducer for measuring alternating electric and magnetic fields P3-80-EH500.

The degree of oxidative DNA damage was assessed by the concentration levels of 8-OHdG in the blood serum of donors before and after exposure to variable MF using enzyme-linked immunosorbent assay. A ready-made DNA Damage ELISA Kit containing monoclonal antibodies to 8-OHdG was used. After adding the stop solutions, the optical density of the samples was measured at a wavelength of 450 nm using a Thermo Fisher Scientific Multiskan microplate reader (Finland).

The determination of the total level of hydrogen peroxide in aqueous solutions of DNA isolated from the blood of donors by the method described in [3] was carried out using a spectrophotometric method using a ready-made kit PerOxImmundiagnostic AG (Germany).

The number of single-strand breaks (SSB) of DNA in donor blood lymphocytes was estimated by the ratio of the fluorescence values of the control and experimental samples. After alkaline treatment of the lysates and the addition of ethidium bromide, the fluorescence intensity of the obtained samples was measured in a quartz cuvette on a Hitachi F-2700 fluorescence spectrophotometer at an excitation wavelength of 540 nm and $\lambda_{\text{abs}} 610 \pm 5 \text{ nm}$ at a right angle to the direction of the exciting light. The results were presented as a percentage ratio of the number of alkaline-labile DNA sites containing SSB to the total amount of DNA before and after treatment of lymphocytes with variable MF [4]. When studying the effect of gamma radiation on the content of SSB of DNA in lymphocytes, a radioactive preparation 137Cs with an activity of 0.104 MBq was used.

The laboratory diagnostic examination was carried out in accordance with the mandatory observance of the ethical standards set out in the 1975 Declaration of Helsinki with amendments of 1983. Statistical processing of the obtained data was carried out by the methods of variation statistics using the Student's t-test (significant difference at $p <0.05$).
3. Results and discussion

The concentration of oxidative nucleobases damage 8-OHdG in human blood serum DNA was determined after alternating MF of 550 ± 30 A/m exposing to them in the frequency range from 3 to 50 Hz for 30 minutes in vitro. There was an increase in the concentration of 8-OHdG in DNA by a factor of 1.5 - 2 in comparison with non-irradiated samples, as well as a nonlinear change in the level of lipid peroxide in blood serum with maxima at MF frequencies of 8 and 50 Hz. The results are shown in Table 1.

Table 1. Concentration of 8-OHdG in DNA and lipid peroxide in the blood of healthy donors serum after exposure with alternating MF in vitro (n=20, p<0.05).

| Frequency of MF, Hz | Concentration of 8-OHdG, ng/ml | Lipid peroxide level, mmol/l |
|-------------------|-------------------------------|-----------------------------|
| non-exposed       | 7.7±0.5                       | 9.1±0.1                     |
| 3                 | 14.5±0.4                      | 12.0±0.2                    |
| 8                 | 11.4±0.8                      | 19.0±0.1                    |
| 30                | 14.1±0.7                      | 15.1±0.1                    |
| 50                | 12.1±0.5                      | 20.1±0.2                    |

With an increase in the MF frequency, the concentration of 8-OHdG in the donor blood serum DNA changed in a complex manner. Thus, the exposure of samples with an alternating MF with frequencies up to 40 Hz did not lead to a noticeable increase in the amount of 8-OHdG in DNA. With a further increase of the MF frequency, the accumulation of 8-OHdG in DNA was observed, and for a frequency of 50 Hz it was 1.5 times higher than in the control. The observed change in the amount of 8-OHdG in DNA cannot be caused by depurinization of the modified base, since this modification leads not to a weakening, but to an increase in the stability of the glycosidic bond. It is known that guanine in DNA has the lowest redox potential among natural bases and is more susceptible to oxidation. As an electron donor, it is able to donate its electron to acceptors, forming a guanine radical cation, which then migrates along the DNA chain along guanines in a hopping manner until it is oxidized to form 8-oxoG, as shown in [7]. Under the action of MFs with frequencies of 3, 30, and 50 Hz on DNA, further oxidation of 8-oxoG to such products as oxazolone and guanidino hydantoin can occur.

The isolated suspension of donor peripheral blood lymphocytes was exposed with 137Cs gamma radiation with an activity of 0.104 MBq for 30, 60 and 90 minutes. In lymphocytes when exposed to 137Cs gamma radiation, an increase in the amount of DNA SSB was observed with an increase in the exposure time from 44.3 ± 0.4% (irradiation for 30 minutes) to 68.3 ± 0.8% (irradiation for 90 minutes) compared with control samples (without irradiation). Such types of DNA damage, leading to the formation of oxidation products of nitrogenous bases 8-OHdG and SSB, are based on a universal mechanism associated with the generation of ROS in aqueous solutions under the action of alternating magnetic fields and ionizing radiation, including those changing the geomagnetic field. This process can significantly impact the integrity of human immunocompetent cells, in particular, lymphocytes and, in general, on the immune response under the action of exogenous factors, including changes in SA.

When living organisms are exposed to energetically weak physical factors, the energy of which is insufficient for the decomposition of water molecules into radicals, water is likely to be the primary target, and ROS is the primary mediators. Despite the fact that the energy of the LF MF is too low for any significant direct damage to DNA, this energy is sufficient to trigger the initial stage of the ROS formation mechanism - the transition of dissolved oxygen from the triplet to the singlet state. Singlet oxygen is reduced to a superoxide anion radical, the protonated form of which dismutates to form the longest-lived ROS, hydrogen peroxide. The start of the water oxidation process leads to a cyclic autocatalytic process of the formation-decomposition of hydrogen peroxide. The cyclic reaction
mechanism probably determines the quasiperiodicity of the $H_2O_2$ formation reaction in aqueous systems. The rate of these reactions in natural conditions correlates with SA. According to Bruskov [8], a diurnal rhythm of the ROS synthesis reaction and, in particular, $H_2O_2$, is observed in water and water bodies.

The researches summarized in the articles [9-10] have shown that LF MF, especially at a frequency of 50 Hz, reduces the production of melatonin and, thus, causes disturbances in the circadian rhythm with possible consequences for the immune system and, according to the authors, with different cancer morbidity. The authors of [10] found that exposure to 0.1 mT, 50 Hz MF can increase the expression of the clock genes BMAL1, PER2, PER3, CRY1, and CRY2, which confirms our hypothesis that the LF MF, in terms of the level of intensity, is comparable to the geomagnetic field, and can control circadian physiological processes by modulating gene expression through the formation of ROS.

One of exogenous factor such as change of UV-radiation intensity can cause DNA damage. UV-A range (320–400 nm) (95%) and UV-B range (5%) cause damaging affects due to absorption of radiation in the UV-C range (<280 nm) and in the greater part of the UV-B range (280–320 nm) by the stratospheric ozone layer. With direct excitation of the DNA molecule by UV radiation, nitrogenous bases are affected in the head. The generation of cyclobutanopyrimidines and thymine dimers, adducts and breaks (single- and double-stranded), which can be sources of errors in DNA replication, occurs. In this case, it can lead to cytotoxic and mutagenic effects. As noted in [12], the SA is used as the main mugen factor of the maximum of UV radiation from the Sun and solar cosmic rays. At the SA minimum, at the minimum ultraviolet level, galactic processes come to the fore. Variations of galactic cosmic rays turn out to be a significant biotropic factor. With prolonged X-ray flares and low minimum intensity of UV radiation from the Sun during the protracted years in 2008-2009, the ozone layer of the Earth's atmosphere decreases. At the same time, the intensity of cosmic cosmic rays and their mutagenic role for the biosphere are increased [12-13].

ROS are involved in the initial stages of cell signaling (redox signaling) under stress, hypoxia, and other pathological conditions. The nature of the cellular response in this case depends on the duration and intensity of exposure to the above factors. With moderate exposure, a nonspecific response is formed, which generally increases the adaptation of the human body to new conditions of variations in electromagnetic and radiation radiation at different levels of SA.

4. Conclusion

The research of the degree of oxidative damage to DNA as a molecular predictor of exogenous disorders showed that after treatment of blood serum and lymphocytes isolated from the peripheral blood of healthy donors with variable MF, in terms of the intensity level comparable to the geomagnetic field, and gamma radiation, a significant change in the $8-OHdG$ level is observed in blood serum and SSB of DNA of lymphocytes. Such types of DNA damage, leading to the formation of oxidation products of nitrogenous bases and DNA SSB, are based on a universal mechanism associated with the generation of ROS in aqueous media under the action of an alternating magnetic field and ionizing radiation, which change the geomagnetic field. This process has a significant effect on the integrity of human immunocompetent cells and, in general, on the immune response under the action of exogenous factors in conditions of variations in electromagnetic and radiation radiation at different levels of SA.

5. References

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