Effect of low intensity EMW EHF on suspension density of rat blood erythrocytes

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Abstract. The effect of electromagnetic waves of extremely high frequencies on suspension density of rat blood erythrocytes has been studied. It was shown that the irradiation results in decreasing of suspension optic density, consequently of intact erythrocyte number and the value change of this parameter depends on suspension irradiation duration. It was also shown that suspension density change of erythrocytes in the cases of irradiated and non-irradiated samples differs from each other. In non-irradiated suspension, density increases with concentration enhancement, in the case of irradiation by 50.3 GHz frequency, vice versa, it decreases.

Keywords: Irradiation, Erythrocyte suspension, Optic density, Density, Number concentration.

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Introduction

Electromagnetic waves (EMW) permanently exist in surrounding medium and influence all processes occurring in living and nonliving nature. Numerous studies show that EMW of different diapason have a certain effect on all levels of organization of living compound (Zhao et al., 2007; Zare, et al., 2007; Khayyat and Abou-Zaid, 2009; Gerner et al., 2010; Zhang et al., 2013). Radiofrequency EMW have been studied for many years and their applicative value is tremendous. According to new subdivision of radiofrequency waves, frequencies of 30-300 GHz are considered as extremely high (EHF). The natural background of them is not much higher on The Earth, but connected to increasing of artificial sources of EMW EHF, intensity of irradiation in this diapason always increases (Ongel et al., 2009). Living organisms are continuously exposed to this effect (Hood, 2001; Kalantaryan et al., 2010). That is why recently the investigation of EMW EHF effect on living organisms has become an actual and important topic. Taking into consideration the aforementioned it is actual to study the effect of this physical factor on internal medium of multicellular organisms, particularly, on different physicochemical parameters of the blood. Different mechanisms of EMW EHF effect, the permeability of which is sufficiently low, are discussed and the mediation of EMW EHF effect on biological systems through water is considered to be the most accepted hypothesis (Loginov et al., 1999; Chunyan and Tribat, 2008; Nerkararyan et al., 2014). Water composes 90-95% of plasma that is why it is important to study the effect of external physical field created by EMW EHF on parameters of intact blood and corpuscles as well as plasma. Earlier it was shown that the irradiation of animal blood plasma resulted in increasing of surface tension of biological liquid, it was also shown that the irradiation of erythrocyte suspension invokes a decreasing of electrokinetic potential of rat blood erythrocytes (Mikaelyan, 2014; Nerkararyan et al., 2014). In this work dependency of suspension optic density of erythrocytes on irradiation duration by 50.3 GHz frequency (resonant for water) as well as suspension density on suspension concentration at fixed irradiation duration is presented.

Materials and methods

White outbred laboratory rats (*Rattus norvegicus*, “Vistar”) were used in experiments. Animals were kept in single-type conditions and fed by combined food. Animals were decapitated and blood was gathered in glass where previously 1-2 mL 5% solution of Na^{+}-citrate prepared by physiological solution was added. From each animal 3-3.5 mL blood was received. Animal blood was centrifuged during 10 min with 1,500 g acceleration (Electronic Centrifuge Capacity). Erythrocytes were separated from supernatant. Physiological solution was added to sediment of erythrocytes and suspensions with different optic densities were obtained. Dilution degree was sufficiently high: optic density values of suspension were in 0.1-0.3 interval and there was not a need to elute erythrocytes. Suspension with 0.7 optic density at $\lambda = 670$ nm wavelength was subjected to the irradiation, which was further diluted by physiological solution irradiated by the same frequency as stock suspension of erythrocytes. The non-irradiated suspension of erythrocytes was as a control. Suspension of rat blood erythrocytes was irradiated during 15 min, 30 min, 1 h, 1.5 h and 2 h with 50.3 GHz frequency. The source of EMW EHF was G4-141 generator with 37.5-53.5 GHz interval of frequencies and 64 μWt/cm² power flux density. Electromagnetic field was homogeneous and the irradiating object was far from waveguide by 180 mm. Optic density measurement was carried out by photoelectrocolorimeter (KPK-2) at 670 nm wavelength. After irradiation optic density of irradiated suspensions by above mentioned durations was determined. At wavelengths more than 600 nm suspension optic density consists of only light scattering, while at wavelengths of more than 600 nm the optic density is a sum of absorption and scattering. Evaluating light weakening at λ=670 nm the concentration of erythrocytes in suspension may be determined. Quantitatively light weakening taken place due to scattering is described by formula similar to Buger-Lambert-Berr principle (Potapenko et al., 2006):

$$I = I_0 e^{-knl} \text{ or } D = \frac{knl}{0.4343}$$

Where:

I_0 and I are intensities of falling and weakened light bands respectively,

D - sample optic density,

e - base of natural logarithm (2.73…).

k - weakening coefficient (0.025 cm^2).

N - concentration of erythrocytes ($1/cm^3$).

l - optic pathway length in sample (cm).

Potapenko et al. (2006) showed that on absorption spectrum of erythrocyte suspension it is clearly obvious that at wavelengths more than 600 nm absorption does not depend on wavelength, that is why concentration may be calculated unambiguously, accepting k = 0.025 cm².

Control and irradiated during 1 h suspensions were diluted by physiological solution until getting 0.1, 0.2, and 0.3 optic densities at $\lambda = 670$ nm. Mentioned values of optic densities were chosen to measure suspension density. Density of samples (control and irradiated) was measured by

Anton Paar DMA 4500 vibrating tube densitometer. The measurements were carried out in two temperatures: 293 K (≈ 20 °C) and 303 K (≈ 30 °C). The statistic treatment of obtained data was carried out.

Results

Densities of control and irradiated suspensions of rat blood erythrocytes have been determined. It was revealed that the irradiation results in decreasing of suspension optic density. The values of optic densities of control and irradiated suspensions are presented in Figure 1.

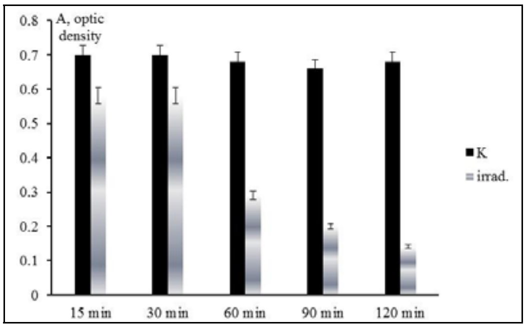

Figure 1. Values of optic densities of control and irradiated by 50.3 GHz frequency with different durations suspensions of rat blood erythrocytes.

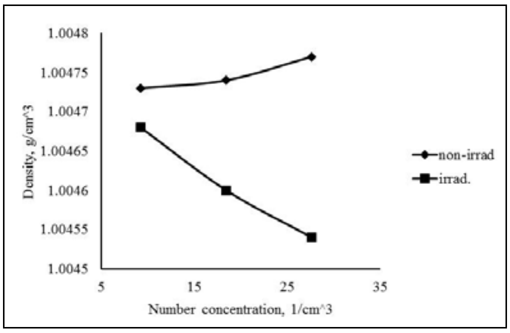

Figure 2. Dependence of suspension density of erythrocytes on number concentration at 293 K (≈ 20 °C).

Dependence of suspension density of erythrocytes on suspension concentration at 293 K (≈ 20 °C) is presented in Figure 2. As it is obvious from Figure 2, in control suspension the density of erythrocytes enhances with concentration increasing. In irradiated suspension, vice versa, with concentration decreasing the density enhances. The same regularity is observed at 303 K (≈ 30 °C), but in this case at the similar absorptions the density in suspension is lower which indicates an increasing of suspension volume of erythrocytes due to temperature enhancement (Figure 3).

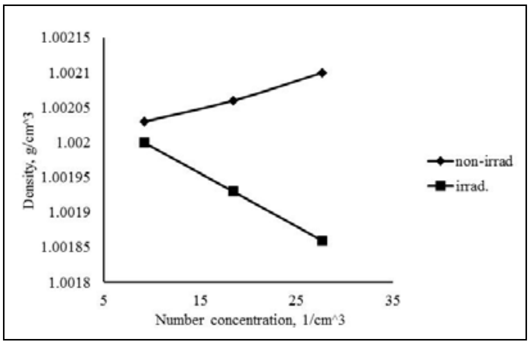

Figure 3. Dependence of suspension density of erythrocytes on number concentration at 303 K (≈ 30 °C).

Discussion

It is obvious from Figure 1 that suspension irradiation results in reliable decreasing of suspension optic density and the value of change depends on irradiation duration, this dependence has a proportional character: the longer is irradiation duration, the bigger is deviation from control values of studying parameter, which indicates that long irradiation results in more significant alterations. Taking into consideration that at $\lambda = 670$ nm light scattering in suspension of erythrocytes shows a portion of intact, undamaged erythrocytes (Potapenko et al., 2006), at all rest single-type conditions (density, concentration etc.) optic density decreasing indicates that sedimentation of erythrocytes occurs and the part of preserved intact erythrocytes is reduced. Based on the fact that optic density measurement was carried out at 670 nm, it may be assumed that EMW EHF is a factor accelerating sedimentation of erythrocytes. On the other hand, it may be connected to aggregation of erythrocytes. It is in coincidence of literature data (Loginov et al., 1999; Rybalko et al., 2002). Maltseva et al. (2011) showed that a development of pronounced hemolysis is associated with enhanced aggregation of erythrocytes. The irradiation of suspension of erythrocytes results in decreasing of surface charge density which in its turn contributes in approaching of erythrocytes and aggregation.

It is known that the irradiation results in disorder of water structure (Petrosyan et al., 2001), on the other hand, aggregation of erythrocytes occurs (Loginov et al., 1999; Rybalko et al., 2002). From aforementioned it is followed that in irradiated suspension after irradiation the part of erythrocytes is subjected to hemolysis or forms aggregates and the total suspension density decreases. In suspensions with big optic density, i.e. big concentration of erythrocytes the possibility of aggregation after irradiation is higher, consequently a decrease of density is bigger.

Earlier it was shown that suspension irradiation of erythrocytes results in decreasing of electrokinetic potential value, which in its turn influences on sedimentation rate of erythrocytes (Samoylov, 2007). Electrokinetic potential value is determined by surface charge value of cell membranes and makes a significant effect on intercellular interactions, counteracting attraction forces. The decrease of electrokinetic potential value results in increasing of attraction forces, which in its turn, may contribute aggregation of erythrocytes.

Therefore, the irradiation results in significant decreasing of suspension optic density which, apparently, is connected to aggregation and disorder of erythrocyte part. The measuring optic density indicates the amount of more stable erythrocytes to the irradiation. Moreover, if in control suspension with increasing of erythrocyte number concentration we obtain a respective increasing of density, in the irradiated suspension, vice versa, the density decreases.

Conclusion

Therefore, the irradiation results in significant decreasing of suspension optic density which, apparently, is connected to aggregation and disorder of erythrocyte part. The measuring optic density indicates the amount of more stable erythrocytes to the irradiation. Moreover, if in control suspension with increasing of erythrocyte number concentration we obtain a respective increasing of density, in the irradiated suspension, vice versa, the density decreases.

Conflict of interest statement

Authors declare that there is not any conflict of interest.

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