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Instant Effects of Radiofrequency Electromagnetic Wave on Hemoglobin in Single Living Intact Red Blood Cell

Cheng Can YAO[1], Xiao Kun LI[1], Yao Xiong HUANG[2]*

[1]College of Pharmacy, Jinan University, Guangzhou 510630
[2]Institute of Biomedical Engineering, Jinan University, Guangzhou 510630

Abstract: The absorption spectrum of the hemoglobin (Hb) in single living intact red blood cell (RBC), exposed in 900 MHz radiofrequency electromagnetic wave (RF-EMW), was non-invasive, *in situ*, real-time measured by employing a highly sensitive fast multi-channel micro-spectrophotometer system. Both the absorption intensity and site of intracellular Hb were altered after RBCs were exposed in 900 MHz RF-EMW with power density at 5 mW/cm^2. It was indicated that not only the concentration of Hb in living RBCs was decreased, but the molecular structure of Hb was changed by the RF-EMW action.

Keywords: Radiofrequency electromagnetic wave, hemoglobin, absorption spectrum, structure and function.

With the development and widespread applications of mobile communication in daily life, the effects of exposure of radiofrequency electromagnetic wave (RF-EMW) on health has become a hot and concerning topic[1,2]. RF-EMW not only alters the shape, structure and function of cell membrane, but also influences the structure and function of intracellular substance, such as the structural change of intracellular protein and the rupture of DNA chain[3-5]. Red blood cells (RBCs) is the important experimental object in biochemistry and cell biology because they are cells with the simplest structure in body. The main substance in RBCs is the hemoglobin (Hb) solution[6]. In this study, the instant effects of 900 MHz RF-EMW on the structure and function of Hb in single living human RBC was investigated. It can supply some references on the study of other chemical or biological effects of RF-EMW exposure.

Experimental

Vein blood from health adult men was placed in heparinized tubes, and the young RBCs were separated by density gradient centrifugation at room temperature. The washed RBCs were incubated in isosmotic phosphate buffered saline (PBS), consisting of 150 mmol/L NaCl, 5 mmol/L Na phosphate, and 5 mmol/L glucose (pH 7.4), at 37℃ for at least 1 h, then kept cold, and used on the same day. All preparations of RBCs

* E-mail: tyxhuang@jnu.edu.cn

suspension were carried out at room temperature and all measurements were carried out at 37℃.

RBCs suspension was assessed by a phase contrast microscopy on glass slide, put into a special mini-cell culture pool. The antenna of RF-EMW generator was aimed at the glass slide. The power density of RF-EMW was set at 5 mW/cm^2 by adjusting the antenna. The absorption spectra of intracellular Hb of single living intact RBC were measured by means of a multi-channel microspectrophotometer[7]. In the measurements, the absorption spectrum from 350 nm to 800 nm of Hb in RBC was obtained, and two absorption peaks at wavelength around 540 nm (β peak) and 575 nm (α peak), were observed in the spectrum. The intensity of the two peaks are not only correlated with the content of intracellular Hb, but also with the physical and chemical properties of Hb, such as solubility, stability, oxidation state and so on; while the positions of the two peaks reflect the molecular structure of Hb with different oxygen carrying capacity[7]. It is suitable for monitoring the instant change of the structure and function of the intracellular molecules in single living intact RBC in response to RF-EMW exposure.

The exposure protocol for 900 MHz RF-EMW was shown in **Figure 1**. The RBCs suspension was exposed in 900 MHz RF-EMW at 5 mW/cm^2 for 30 minutes, 30 minutes' ×3, respectively. The absorption spectra were measured at four points: before exposure (O point), at the beginning of the exposure (A point), at the end of exposure for 30 min (B point) and at the end of exposure for 30 min ×3 (C point). Only one RBC was measured at every checkpoint, and 50-60 RBCs were observed for one group.

Due to the individual differences among RBCs, the absorbances of Hb in every RBC were not same. For obtaining a comparable results, the relative change rate of the absorbance (ΔA), at t point (t=A, B, or C) (A_t) to the absorbance at O point (A_0) of Hb in the same RBC was applied. The relative change rate of the absorbance was expressed as $\Delta A(\%)=100\times(A_t-A_0)/A_0$.

Results and Discussion

In exposured group, the intensity of absorption of β peak and α peak at A point raised by 1.63% and 1.03%, respectively, compared to that at O point **(Figure 2)**, while the absorbances of β peak and α peak at B point decreased by 6.04% and 8.55%, respectively. The difference peak intensities between at B point and A point was significant ($p<0.05$). The absorbances of β peak and α peak at C point decreased by 12.73% and 17.47% respectively, compared to that at O point. The ΔA value at C point and at A point was even more significant ($p<0.01$). In control group, there is no significant difference of the ΔA among all points ($p>0.05$). After RF-EMW exposure the components of the membrane and cytoskeleton can be recombined, the permeation of cell membrane increased, and the structure of RBCs membrane can be changed[8]. In the result, the Hb and ions released from RBCs, and the absorbance of Hb decreased.

Figure 1 Experimental protocol of 900 MHz RF-EMW exposure

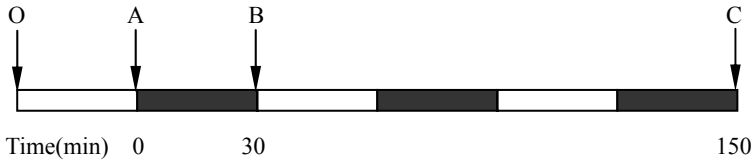


Figure 2 Effect of 900 MHz RF-EMW on the absorbance of Hb in single living intact RBC

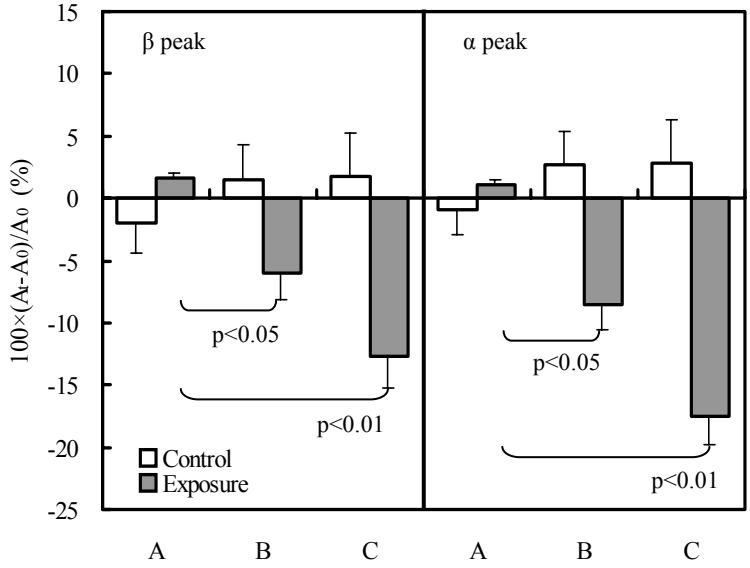
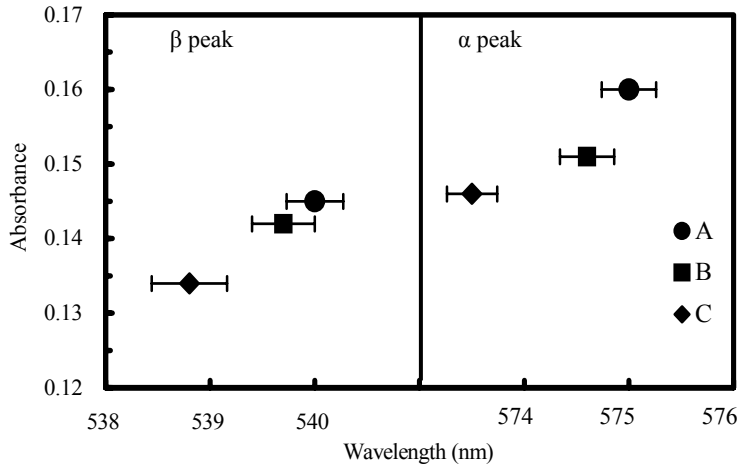


Figure 3 Effect of 900 MHz RF-EMW on the absorption peak of Hb of single living intact RBC



The absorbing unit in Hb is heme, and it has two absorption peaks at 575 nm and 540 nm in visible region. The absorption peaks would shift, when the structure or number of conjugated double bond in heme was altered. So, the change of Hb's structure can be reflected by the position of absorption peaks to a certain extent[9]. The wavelengths of two absorption peaks at B point (after RF-EMW exposed for 30 min) shifted to short wavelength about 0.5 nm compared to A point (before exposure). The difference was not significant ($p>0.05$) **(Figure 3)**. The wavelengths of two absorption peak at C point (after exposed by RF-EMW for 30 min three times) were shifted to short wavelength by 1.0-1.5 nm compared to A point, the difference was significant ($p<0.05$). It indicated that the molecular structure of Hb in RBCs was changed. The shape of active combination sites of ligand such as Fe^{2+} in heme might be altered by RF-EMW, and caused changing the molecular vibration frequency. So the shifts of the absorption peaks of Hb could reflect the change of the structure of heme. The theoretical calculation has proved that the structure and the absorption spectrum of protoporphyrin-metal complexes could be altered, after it absorbed the energy of 2450 MHz microwave[10]. In addition, the membrane proteins, especially the integral proteins, are influenced by RF-EMW, which may alter the combination of membrane proteins and intracellular Hb. That might also be one of the reasons for the shift of the absorption peaks.

In summary, 900 MHz RF-EMW could lead to the change of both the concentration and the molecular structure of Hb in single living intact RBC, and influence its physiological functions such as the oxygen carrying capacity of RBCs.

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