Study of the Relationship between the Recording Parameters of the Laser Doppler Flowmetry and Fluorescence Spectroscopy During Occlusion Test

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***Abstract*—This paper presents evaluation results of the parameter changes of microcirculatory-tissue systems of the human body during occlusion functional tests. It describes the methodology for conducting experiments as well as results of calculations of the relationship between the recording parameters of the laser Doppler flowmetry and fluorescence spectroscopyon healthy volunteers. The article analyses the data, which has practical significance for physiology and medicine.**

Keywords—non-invasive diagnostics; ***laser Doppler flowmetry***; ***fluorescence spectroscopy***; ***microcirculatory-tissue system***; ***occlusion test***; ***redox ratio***; ***metabolism***.

I. INTRODUCTION

Microcirculatory-tissue systems (MTS) of the human body have an important role in oxygen tissues supply. Therefore, their study and development of new noninvasive functional diagnostics methods is still relevant in current clinical practice.

Currently, various technologies are applied for the MTS study. But optical non-invasive technologies such as laser Doppler flowmetry (LDF) and fluorescence spectroscopy (FS) are particularly widely adopted. The main advantage of these technologies is that they allow to non-invasively evaluate the processes occurring in biological tissues. LDF method is based on an optical noninvasive sensing of tissue by laser light and analyzing of scattered and reflected emission from the moving red blood cells in the tissues [1]. Recorded parameter is an index of the microcirculation, which depends on the concentration of red cells in the probed volume and their movement speed. FS method is based on excitation of the fluorescence of endogenous and exogenous biotissue fluorophores and recording emission in the visible region of the spectrum [2-3]. This method is highly sensitive and allows for non-invasive tissue oxygen metabolism diagnosis. The most perspective and appropriate use of the FS method is in such areas as oncology, cosmetology, surgery and transplantation [4-9]. An integrated approach to the MTS performed daily from 11:00 till 13:00 am to avoid any influence of circadian rhythms on the blood circulation. study is the most informative. It includes the simultaneous use of several diagnostic methods.

There are various functional tests to study the possible reactions of MTS to the external action, among which an occlusive test (OT) is released. The essence of this test is cross clamping arteries on one of the limbs of the subject for a few minutes. In response to the occlusion blood flow changes occur in the area distal to the clamping after the liberation limb. This test allows to evaluate the blood flow amount in the absence of arterial inflow and to assess the reserve possibilities of the microcirculatory bed by the increase in blood flow during reactive postocclusive hyperemia. It is believed that the metabolic processes may depend on perfusion [10-11]. In this context, the aim of this work is to find the relationship between metabolic processes and biological tissue perfusion in the area of human skin containing nutritive blood flow during OT.

II. EXPERIMENTAL STUDIES

Experimental studies were carried out by LDF and FS methods using the multifunctional laser noninvasive diagnostic system (MLNDS) LAKK-M (modification 3). This system realizes the LDF method at a wavelength of 1064 nm and spectrophotometric channel at two-wavelengths. The peculiarity of this MLNDS is the possibility of simultaneous registration of perfusion and fluorescence spectra.

Measurements were carried out on 10 apparently healthy volunteers of approximately the same age in physical and mental rest. Volunteers were pre-adapted to the room temperature of 24-25°C. Experimental studies were carried out on the midline of the dorsal surface of the lower third of the right forearm at a point located 20 mm above the styloid process, which is almost devoid of arteriolar-venular anastomoses (AVAs) and dominated by nutritional blood flow (skin area without AVAs). All measurements were performed daily from 11:00 till 13:00 am to avoid any influence of circadian rhythms on the blood circulation. Optical fiber was installed perpendicular to the studding surface without applying any pressure. The arrangement of the optical fiber is shows in Fig.1.

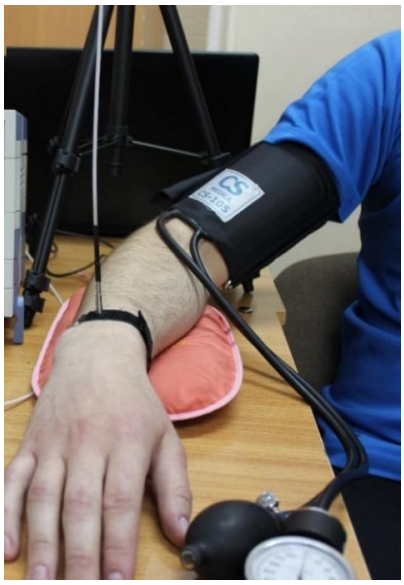


Fig. 1. Optical fiber location on the apparently healthy volunteers forearm.

The study involved registration of changes in blood flow and the biological tissue coenzymes fluorescence during 4 of the successive stages. The first stage included registration of a base test of LDF-gram for an 1 minute-period. Shoulder occlusion test with the pressure in the cuff 220 mm. Hg. Art. held during 3 minutes during the second stage. The third and fourth stages of the study included recording a base test for 6 minutes, while the response of the microcirculation in the provocative test and a gradual recovery of parameters by the end of the study were investigated. In carrying out experimental studies LDF-gram was presented as 20 fragments by 30 seconds each with the simultaneous recording of each fragment and registration of two fluorescence spectra upon excitation of UV (365 nm) and blue (450 nm) light. Research scheme is shown in Fig. 2.

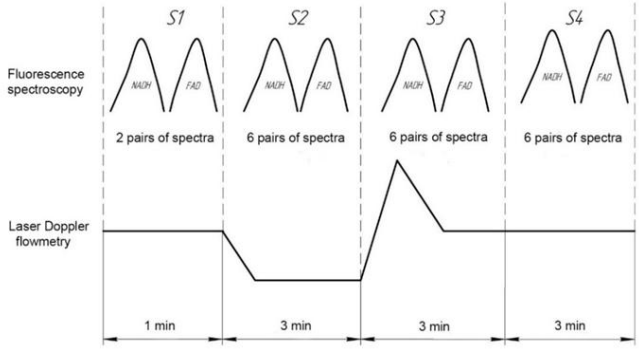


Fig. 2. Scheme of experimental studies.

All the experiments were carried out in a sitting position with the right hand on the table at heart level. The duration of each person study was about 10 minutes.

During the experiments, registration of the following indicators was performed: blood microcirculation (I_m) index and the NADH and FAD fluorescence amplitude. In addition, a common metabolic rate (redox ratio – *RR*) was calculated by two ways:

$$RR_1 = \frac{I_{NADH}}{I_{FAD}} \tag{1}$$

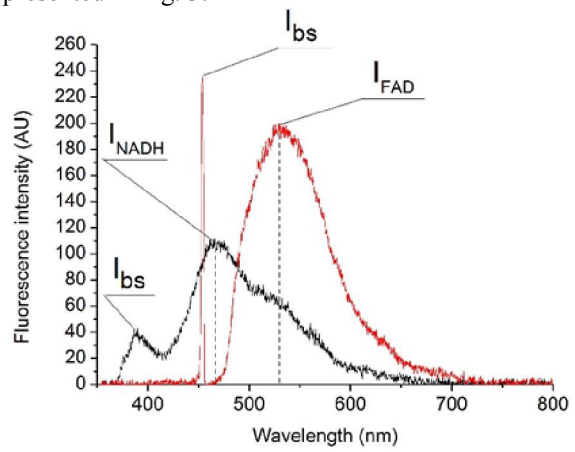
$$RR_2 = \frac{k_f(NADH)}{k_f(FAD)} \tag{2}$$

where, I_{NADH}, I_{FAD} is the amplitudes of fluorescence of NADH and FAD. and $k_f(NADH)$, $k_f(FAD)$ – fluorescent contrast coefficients, that calculated by the formula:

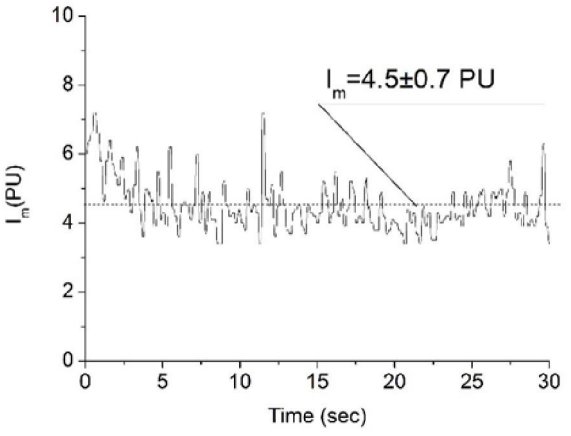
$$k_f = 1 + \frac{I_f - I_{bs}}{I_f + I_{bs}} \tag{3}$$

I_{bs} – the maximum intensity of the backscattered laser radiation.

Typical view of co-registered LDF-grams and fluorescence spectra for the first fragment during the experimental studies presented in Fig. 3.



a)



b)

Fig. 3. Typical view of co-registered fluorescence spectra (a) and LDF-gram (b) for the 1st fragment during the experiment.

III. RESULTS

Based on the experimental data resulting for each volunteer Pearson correlation coefficients between the co-registered LDF and FS parameters during OT were calculated. The resulting data are presented in Table 1.

The obtained data show that inverse Pearson correlation coefficients between the I_m and the amplitude of the NADH fluorescence intensity for the 8 out of 10 volunteers were in the range of 40-88%, between the I_m and FAD fluorescence intensity were in the range of 68-96%, respectively. It is also should be noted that was revealed fairly large scatter of coefficients of correlation between perfusion and redox ratios.

It should also be noted that the correlation coefficients between the coenzymes fluorescence intensity and perfusion for some volunteers (for example, №1), were positive, in contrast to other results. Currently, the cause of these phenomena is unknown. Further research to study these differences is supposed.

TABLE I. THE CORRELATION COEFFICIENTS BETWEEN THE CO-REGISTERED LDF AND FS PARAMETERS DURING OT

Parameters	**Pearson correlation coefficients**									
	#1	***#2***	***#3***	***#4***	***#5***	***#6***	***#7***	***#8***	***#9***	***#10***
I_{NADH}, a.u.	0,154	-0,763	-0,888	-0,402	-0,918	-0,661	-0,759	-0,092	-0,876	-0,976
I_{bsNADH}, a.u.	0,451	-0,881	-0,835	-0,713	0,068	-0,801	-0,330	0,525	-0,735	-0,953
I_{FAD}, a.u.	0,289	-0,885	-0,957	-0,705	-0,921	-0,853	-0,676	0,203	-0,805	-0,982
I_{bsFAD}, a.u.	0,279	-0,796	-0,933	-0,806	0,012	-0,879	-0,865	0,094	-0,773	-0,990
RR_1, a.u.	-0,154	0,413	0,164	0,040	0,135	-0,186	0,440	-0,371	-0,091	0,308
RR_2,a.u.	-0,661	0,707	-0,369	0,511	0,564	0,391	-0,431	-0,601	0,506	0,850

IV. CONCLUSION

As the result of experimental studies during the occlusion test, a high correlation between perfusion and metabolic parameters recording by fluorescence spectroscopy was revealed. The results of this study assume the need of further studies for the methodological base improvement with the simultaneous use of laser Doppler flowmetry and fluorescence spectroscopy methods in clinical practice.

REFERENCES

[1] A.V. Dunaev, V.V. Sidorov, N.A. Stewart, S.G. Sokolovski, E.U. Rafailov, "Laser reflectance oximetry and Doppler flowmetry in assessment of complex physiological parameters of cutaneous blood microcirculation", Proc. SPIE. 8572, 857205, 2013.

[2] J.R. Lakowicz, Principles of fluorescence spectroscopy, New York: Kluwer Academic Publishers; 2006.

[3] M.A. Mycek, B.W. Pogue, Handbook of biomedical fluorescence, Taylor & Francis; 2003.

[4] V.V. Tuchin, Handbook of optical biomedical diagnostics, 2002.

[5] O. Castro-e-Silva, A.K. Sankarankutty, R.B. Correa, J. Ferreira, J.D. Vollet-Filho, C. Kurachi, et al., "Autofluorescence spectroscopy in liver transplantation: preliminary results from a pilot clinical study", Transplant Proc, vol. 40, pp. 722–5, 2008.

[6] E.Y. Ershova, L.N. Karimova, S.S. Kharnas, S.G. Kuzmin, V.B. Loschenov, "Photodynamic therapy of acne vulgaris", Proc. SPIE. 4949, pp. 62–7, 2003.

[7] S.A. Dadvani, S.S. Kharnas, K.E. Chilingaridi, V.B. Loshchenov, S.P. Vetshev, "Laser auto- fluorescent spectroscopy – a new method of express-diagnostics in surgery", Surgery, vol. 10, pp. 75–9, 1999.

[8] D.C. DeVeld, M.J.H Witjes, H.J. Sterenborg, J.L. Roodenburg, "The status of *in vivo* autofluorescence spectroscopy and imaging for oral oncology". Oral Oncology. vol. 41, pp. 117–131, 2005.

[9] N. Akbar, S. Sokolovski, A. Dunaev, J.J.F. Belch, E. Rafailov, F. Khan, "In vivo noninvasive measurement of skin autofluorescence biomarkers relate to cardiovascular disease in mice", J Microsc. vol. 255, pp. 42–8, 2014.

[10] A.I. Krupatkin, V.V. Sidorov, V.V. Dremin, A.V. Dunaev, I.N. Novikova, S. Zhu, G. Nabi, K.S. Litvinova, A.P. Baklanova, R.M. Bakshaliev, S.A. Ravcheev, "Evaluating adaptation options of microcirculatory-tissue systems based on the physiological link of nutritive blood flow and redox ratio" Proc. SPIE. 9448, 944803, 2014.

[11] A.V. Dunaev, V.V. Dremin, E.A. Zherebtsov, I.E. Rafailov, K.S. Litvinova, S.G. Palmer, N.A. Stewart, S.G. Sokolovski, E.U. Rafailov, "Individual variability analysis of fluorescence parameters measured in skin with different levels of nutritive blood flow", Medical Engineering & Physics, vol. 37, pp. 574-583, 2015.