Experimental study of the influence of blood flow on the fluorescence signal of biological tissue

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In recent years, fluorescence spectroscopy (FS) has been successfully used in studies of biological objects at the micro level, which allows the monitoring of temporal and spatial dynamics of molecular processes. This method is based on analyzing the characteristics of induced endogenous fluorescence in probed biological tissues with low-intensity optical radiation at certain wavelengths. However, in general, one of the main problems with this method so far, is the correct interpretation of the data. It is known that changes in blood flow can affect the fluorescence spectra: it may result from signal attenuation due to the absorption capacity of the blood and the change in the concentration of fluorescent coenzyme occurring during concomitant changes in metabolism. In this study, we tried to experimentally study the effects of blood flow on the NADH and FAD fluorescence signals in biological tissues.

Experimental studies were conducted on the palmar surface of the skin of the middle and ring fingers of the right hand of healthy volunteers. To evaluate the intensity of blood flow, perfusion was recorded by laser Doppler flowmetry (LDF) with a "LAKK-02" laser analyzer (sensing wavelength – 1064 nm). The fluorescence spectrum of biological tissues was detected by means of non-invasive diagnostic FS channel complex "LAKK-M" (SPE "LAZMA", Moscow) for 2 excitation wavelengths of endogenous fluorophores NADH and FAD – 365 and 450 nm, respectively. The main idea of the research was the implementation of provocative actions (functional tests) on blood flow through changes in extremity temperature (heat and cold pressor test) and application of the brachial arterial occlusion cuff with pressure of 200-220 mm Hg (occlusion test). Special tooling designed and printed on a 3D-printer, allows the placement of the measuring LDF and FS channel fibre along the arm, as well as the ability to secure additional 2-channel temperature sensors specially developed for the temperature measuring instrument. One study recorded the fluorescence of either NADH or FAD. The study consisted of 7 stages, during which the fluorescence spectra and the perfusion of biological tissues are registered at the same time: background recording when placing your hands in the air – 2 min; in warm water (42 °C) – 4 min (heat test); in cold water (15-20 °C) – 10 min (cold pressor test); occlusion of the brachial artery in cold water – 3 min (in conjunction with occlusive cold pressor test); relaxation step in cold water – 10 min; heating of hot water (42 °C) – 11 min (final thermal break). The duration of the experiment was a full 40

minutes, during which about 70-90 fluorescence spectra of biological tissues were recorded. A total of 37 experiments were performed on 10 volunteers.

With the help of non-invasive methods LDF and FS, we have shown the relationship between blood flow (perfusion) and recorded fluorescence signals. All volunteers displayed a high inverse correlation (~0.4-0.8) between registered back reflected radiation and the intensity of fluorescence from biological tissue perfusion and temperature for both fluorophores investigated. Thus, we can assume that factors affecting the absorption of the blood change the fluorescence spectra of biological tissue.

The results obtained must be considered in fluorescence spectroscopy research to determine the concentrations of various fluorophores in biological tissue and the development of new diagnostic criteria and normalizing factors. Also, the results of these experiments indicate a need for further studies, since this generally leads to improved methodology and instrumentation technology for use in medical fluorescence spectroscopy.

Apoptotic changes visualization in cisplatin-treated leukemic cells using second-harmonic generation imaging

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Apoptosis, or programmed cell death type I, is a process in which sequence of events leads to degradation of cell content, cell shrinkage, membrane changes and fragmentation of nucleus. In the execution phase of apoptosis, breaking down of cell cytoskeleton causes the cell membrane to bulge outward - phenomenon known as membrane blebbing. The end result is formation of apoptotic bodies, membrane-bound vesicles containing organelles, sometimes with nuclear fragments. Induction of apoptosis is considered as best approach in anticancer therapy with cytotoxic drugs [1]. Therefore, detecting morphological features of tumor cells under the influence of cytotoxic agents is of great scientific importance. One of the obstacles is to visualize and analyze morphological changes in living suspension cells (e.g. leukemic cells) using conventional light and/or fluorescence microscopy. Therefore, transmission electron microscopy is often used. Although this gives deep insight into subcellular morphology and changes, methodology employed is often time-consuming, expensive and involves usage of multiple toxic substances.

Given all of the above, we analyzed apoptotic changes in commercial human acute promyelocytic leukemic (HL-60) cell line treated with well known apoptosis- inducing antitumor agent cisplatin [2], using second-harmonic generation (SHG) imaging, using