Speckle-contrast imaging of pathological tissue microhemodynamics at optical clearing

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ABSTRACT

The study of blood microcirculation is one of the most important problems of the medicine. This is caused by the fact that many diseases, such as cardio-vascular diseases, atherosclerosis, diabetes, chronic venous insufficiency, oncology diseases, cause functional and morphological changes of microcirculation of blood flow. The results of experimental study of changes of blood microcirculation of pancreas in rats with diabetes measured by using Laser Speckle Contrast Imaging (LSCI) at application of optical clearing agents are presented. Laser speckle contrast techniques are based on the spatial and temporal statistics of the speckle pattern, calculating of contrast of time-averaged dynamic speckles in dependence on the exposure time at the registration of the speckle-modulated images. In research, 28 Wistar rats weighing 300-500 g were used. Alloxan induced animal model of diabetes was explored. The influence of solution of glycerol, PEG-300 was investigated. Application of 70%-aqueous glycerol solution demonstrates 50%-decrease of blood flow velocity in the group of diabetic animals, to 10^{th} min blood flow velocity was completely restored. Blood flow in the control group almost stopped, to 10 min has not recovered. Application of solution of PEG-300 demonstrates 25%-decrease of blood flow in the group of diabetic animals. Blood flow in the control group show 65%-decrease of blood flow. The results obtained at the study of blood microcirculation disorders of pancreas in diabetes show that diseases development in animals causes changes in the microcirculatory system and application of optical clearing agents demonstrates changes in vascular permeability in conditions of development of pathologies.

Keywords: laser speckles, speckle-contrast, microhemodynamics, optical clearing agent, diabetes, pancreas, pathological tissue, blood microcirculation.

1. INTRODUCTION

Many diseases, such as cardio-vascular diseases, atherosclerosis, diabetes, chronic venous insufficiency, cause functional and morphological changes of blood flow in single vessels and blood microcirculation within the microvasculature. In the case of diabetes, diabetic angiopathy (generalized defeat of blood vessels) arises. There are two types of diabetic angiopathy: microangiopathy arising as result of destruction of small vessels - capillaries, arterioles, and venules, and macroangiopathy - ischemic brain disease, peripheral vascular occlusion - arising as result of destruction of large vessels[1]. Diabetes is characterized by the elevation of blood glucose level, for the reason that the peptide hormone insulin is produced insufficiently in the beta cells of the pancreas (type I insulin-dependent), or the body cells not responding properly to the insulin produced (Type II, non insulin-dependent or "adult onset diabetes")[2,3]. According to the data of World Health Organization, the incidence of diabetes in the world is about 347 million people. Diabetes is ranked as the third in the world after cardiovascular and oncological diseases. Hemodynamic changes in diabetes contribute to the emergence of hypoxemia in various organs, leading to retinopathy, neuropathy, nephropathy, coronary heart disease, and

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peripheral arterial disease. Thus, all these changes in blood flow and microcirculation within the microvasculature are primarily associated with endothelial dysfunction. The multiple functions of vascular endothelium include regulation of vessel integrity, vascular growth and remodeling, tissue growth and metabolism, immune responses, cell adhesion, angiogenesis, hemostasis and vascular permeability. Endothelial dysfunction is characterized by the following features: reduced endothelium-mediated vasorelaxation, hemodynamic deregulation, impaired fibrinolytic ability, enhanced turnover, overproduction of growth factors, increased expression of adhesion molecules and inflammatory genes, excessive generation of reactive oxygen species, increased oxidative stress[4].

Basic information about the etiology and pathogenesis of diabetes mellitus has become known thanks to experiments conducted on animals. A model of alloxan diabetes that occurs when animals are administered alloxan has become a widespread. This substance selectively damages the β-cells of the pancreatic islets, and therefore insulin deficiency of varying severity develops. As a result, animals develop hyperglycemia and diabetic syndrome, similar to insulin-dependent type I diabetes mellitus[5,6]. In these studies, we chose this particular model of experimental diabetes mellitus type I due to its ease of use and functionality.

Currently, the most effective diagnostic methods for determining the physiological parameters of blood flow and microcirculation are the methods based on dynamic light scattering[5]. One of the prospective methods for the assessment of blood flow is the Laser Speckle Contrast Imaging (LSCI)[7-12]. LSCI is a noninvasive, contactless method that allows for imaging of blood flow in real-time without scanning by the laser beam. LSCI is based on calculation of the local contrast of time-averaged dynamic speckles in dependence on the exposure time at the registration of the speckle-modulated images[7].

In this study, we present results of blood microcirculation measurements within the microvasculature of the pancreas in rats with diabetes and under influence of optical clearing agents by using Laser Speckle Contrast Imaging (LSCI). The influence of aqueous solution of 70% glycerol and of PEG 300 was investigated.

2. MATERIAL AND METHODS

2.1 Laser Speckle contrast imaging

The local estimation of the contrast *K* for the fixed exposure time done within the areas with given number of speckles makes it possible to visualize tissue regions with essentially different velocity of the scatterers[7,8]:

$$K_k = \sigma_{Ik} \big/ \bar{I}_k \,, \tag{1}$$

where k is the number of frames in a sequence of speckle-modulated images, $\bar{I}_k$ and σ_{Ik} are averaged over the analyzed frame scattered light intensity and the *rms* (root-mean-square) value of the fluctuation component of the pixel's brightness, respectively:

$$\bar{I}_k = (1/MN)\sum_{m=1}^{M}\sum_{n=1}^{N} I_k(m,n), \tag{2}$$

$$\sigma_{Ik} = \sqrt{(1/MN)\sum_{m=1}^{M}\sum_{n=1}^{N}\left\{I_k(m,n) - \bar{I}_k\right\}^2}\,, \tag{3}$$

where M and N are the number of pixels in rows and columns of the analyzed area of the frame, respectively; $I_k(m,n)$ is the brightness of the (m,n)-pixel of the k-frame.

To perform the measurements and calculate the contrast, we developed a program in the LabVIEW 8.5 environment (National Instruments, USA. The monitoring of blood microcirculation was carried out using a specially designed experimental setup (Fig.1).

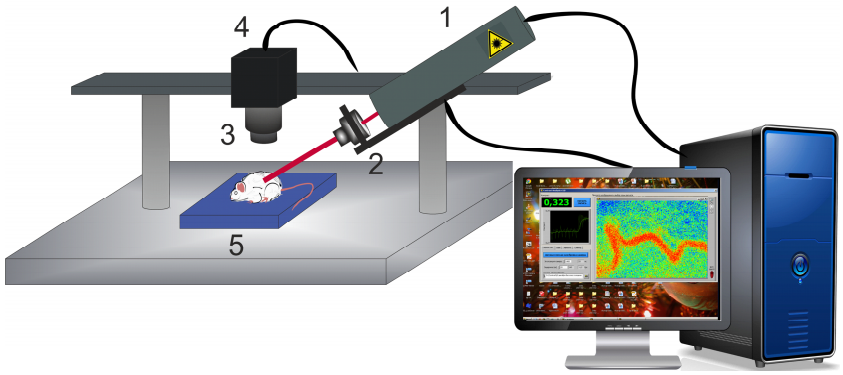


Figure 1. 1-He-Ne laser (632.8 nm); 2-objective (LOMO 20×); 3 - microscope tube lens with objective (LOMO 10×); 4-CMOS-camera Basler A602f (656 × 491 pxls, pxl size 9.9 × 9.9 μm, 8 bits/pxl); 5- rat under study.

The problem of quantitative velocity measurements is associated with understanding the interconnection between the contrast of speckles K and the velocity of scattering centers (or velocity distribution)[12-14].

Calibration was conducted out in the following way: the blood was passed through a channel of 3 mm in diameter at a given velocity of 1.5 mm/s by using a dispenser of drugs (MLW Lineomat, Germany). Recording and processing of speckle images were made by using software designed in the LabVIEW 8.5 environment (National Instruments, USA).

Under the assumption of purely ordered flow, the speckle contrast K can be defined as follows[14]:

$$K = \frac{\sigma_s}{\langle I \rangle} = \left[\frac{\tau_c}{2T} \left\{ \sqrt{2\pi} erf\left(\frac{\sqrt{2}T}{\tau_c} \right) - \frac{\tau_c}{T} \left(1 - \exp\left(-\left(\frac{2T}{\tau_c} \right)^2 \right) \right) \right\} \right]^{1/2}, \quad (4)$$

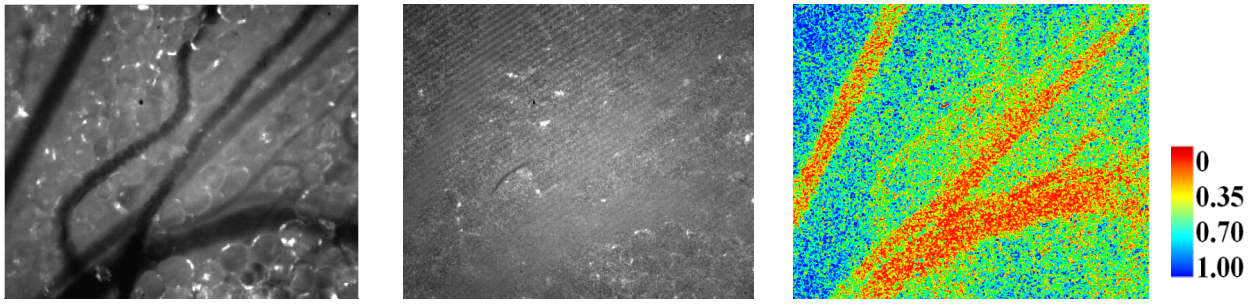
where T is the exposure time of the camera, τ_c is time of correlation. Again, it is worth noting that the above equation is in actuality a cumulative distribution function of a Gaussian probability distribution function, which is characteristic to directed flows.

The simplest approach leads to a characteristic velocity defined as follows[7,14]:

$$\upsilon_c = \lambda / 2\pi k \tau_c, \quad (5)$$

where λ is the light source wavelength, k is the normalization factor which depends on the parameters of a Gaussian curve from Eq. (4), and the scattering properties of biological tissue or phantom. Calibration allowed us to determine the value of this coefficient as ~ 0.14. In this regard, we can introduce the concept of "reduced" velocity using Eqs. (4) and (5) to process phantom experimental data for contrast K at the particular exposure time of the camera T. "Reduced" velocity can be associated with the velocity of blood flow determined from the speckle contrast K measurements for the further assessment of blood circulation in *in vivo* studies.

Figure 2 shows the images of a typical blood vessels (a), obtained by a digital microscopy, the spatial distribution of the measured speckle local contrast (b) in coherent light, and its normalized distribution (c). The diameter of the investigated vessels varied in the range from 100 μm to 250 μm.



a) b) c)

Figure 2. The image of the vessels obtained by a digital microscopy (a), speckle image in coherent light (632.8 nm) (B&W) (b) and calculated distribution of laser speckle contrast (1-0) (colored).

2.2 Animal models

In research, 28 Wistar rats weighing 300-500 g were used. Animals were divided into two groups: control (14 rats) and diabetic (14 rats). For modeling of diabetes in animals, we used alloxan-induced diabetes model. Experimental diabetes in rats was induced by a single subcutaneous injection of alloxan with a dose of 220 mg/kg body weight of the animal. Alloxan disturbed pancreas functioning leading to development of diabetes in rats. Status of diabetes was confirmed by the increase of glucose level in the blood that was tested by using a commercial glucometer Accu-Chek Active (Roche Diagnostics, Germany). Average values of the glucose in the blood before the introduction of alloxan, and the day of the experiment (after 30 days) were 120 ± 16, 403 ± 105 mg/dl, respectively. We can assume that this is an initial stage of diabetes of Type I[5,6]. Under general anesthesia solution Zoletil 0.2 mL, laparoscopy was performed. Then the changes of blood flow were evaluated.

All procedures with animals were performed in strict accordance with "Rules for Conducting Qualitative Clinical Trials in the Russian Federation" (approved by the Ministry of Health of the Russian Federation and enacted on January 1, 1999), appendix 3 to Order No. 755 of the Ministry of Health of the USSR of 10.08.77, the provisions of WMA Declaration of Helsinki (2000) and the recommendations contained in the European Community Directives (No. 86 / 609EC). Approval for this study was obtained from the Ethics Committee of Saratov State Medical University of the Ministry of Health of the Russian Federation according to Protocol No. 8, dated May 17, 2016

In addition, vascular permeability studies were performed by applying optical clearing agents, such as 70%-aqueous glycerol solution and PEG-300, the analysis of its effects on blood flow in pancreatic vessels. The solutions were applied to the tissue site topically using a pipette in a volume of 0.5 ml.

3. RESULTS AND DISCUSSION

In Fig. 3, the normalized curves of changes of pancreatic blood flow of control and diabetic groups of laboratory animals under the influence of 70%-aqueous glycerol solution and in Fig. 3 under the influence of solution of PEG-300.

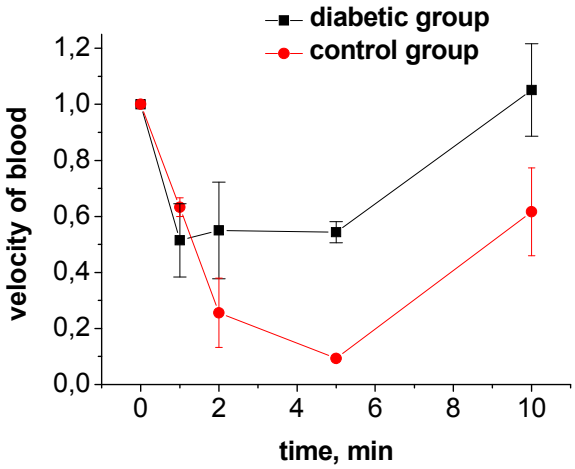


Figure 3. Blood flow in the pancreas of rats in both groups exposed to 70%-aqueous glycerol solution

In Fig. 4, the normalized curves of changes of pancreatic blood flow of control and diabetic groups of laboratory animals under the influence of solution of PEG-300.

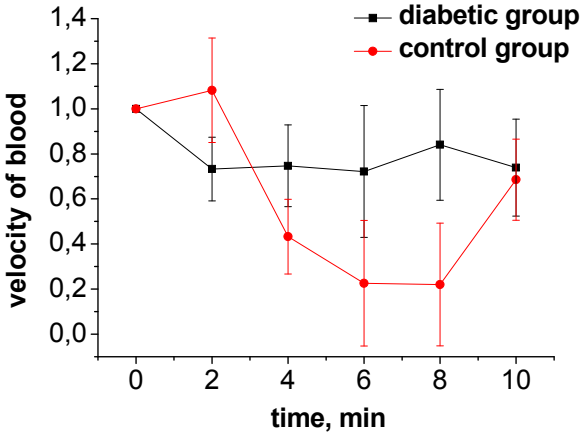


Figure 4. Blood flow in the pancreas of rats in both groups exposed to solution of PEG-300.

Application of 70%-aqueous glycerol solution demonstrates 50%-decrease of blood flow velocity in the group of diabetic animals, to 10th min blood flow velocity was completely restored. Blood flow in the control group almost stopped, to 10 min has not recovered.

Application of solution of PEG-300 demonstrates 25%-decrease of blood flow in the group of diabetic animals. Blood flow in the control group show 65%-decrease of blood flow. The difference in the effects of solutions on the blood flow in the diabetic and control groups could be caused by decreased vascular endothelial permeability at diabetes even in early stages of disease. Thus, application of optical agents could be used not only for getting of better speckle images of blood flow distribution in the living organ at surgical procedures, but also for monitoring of some complications related to changes of vascular endothelial permeability like at diabetes. The assessment of the effect of the agent on blood flow by LSCI opens very urgent perspectives to combine blood circulation measurements with tissue optical clearing in the course of transplantation. It is important to note that practically all OCAs are cryogenic agents used for keeping living organs before transplantation[15].

4. CONCLUSION

Laser speckle contrast imaging may be useful for monitoring of microhemodynamics of pancreas and other organs and study of vascular endothelial permeability at diabetes. The ability of LSCI to measure blood flow velocity in a real time is prospective feature to be used in transplantation technologies and in emergency surgery to assess the state of internal organs and microhemodynamic pathologies. Also, one of the prospective applications of LSCI technique is a noninvasive monitoring of cerebral blood flow in the brain of small animals without craniotomy under conditions of immersion of optical clearing of the scalp and cranial tissues by means of hyperosmotic agents.

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