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Fluorescence-Based Method for Assessment of Blood–Brain Barrier Disruption*

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Abstract — **We report on a fluorescence-based optical method for assessment of blood-brain barrier in humans. The technique is based on monitoring of fluorescence light excited in the dye circulating in the brain. Measurements were carried out in healthy volunteers and in patients with disruption of the blood-brain barrier with the use of time-resolved method during inflow and washout of indocyanine green after its intravenous injection. We show large differences in the fluorescence signals - in healthy subjects a fast washout of the dye can be observed whereas in patients the washout is significantly prolonged. We conclude that the monitoring of the fluorescence signals during injection of exogenous optical contrast agent can be used for the assessment of the condition of blood-brain barrier at the bedside. The technique may be of benefit for diagnosis of the patients suffering from damage of the blood-brain barrier and in monitoring of therapies used in such patients.**

I. Introduction

Optical methods of brain perfusion assessment based on monitoring of exogenous contrast agent are extensively tested in several clinical applications. Indocyanine green (ICG) is used in these studies as contrast agent revealing high absorption and emission in near infrared wavelengths range [1-4] and relative low toxicity [3, 5]. It was reported that optical monitoring of inflow of the dye allows to estimate cerebral blood flow [6, 7] and cerebral blood volume [8]. Recently, time-resolved optical spectroscopy was successfully applied in estimation of changes in absorption of the tissue with depth discrimination [9-12]. This technique allowed to evaluate inflow and washout of the ICG separately in extra- and intracerebral tissue compartments [13, 14]. Thus, methodology of cerebral blood flow was significantly improved [15]. It was also reported that time-resolved fluorescence light detection can be utilized in assessment of inflow and washout of ICG [16-20].

Blood-brain barrier is an important feature of cerebral circulation. In many clinical studies the barrier breakdown under diverse pathological conditions was demonstrated [21, 22]. Recently, it was suggested that analysis of the washout of ICG can be used for assessment of blood-brain barrier. Feasibility of in-vivo monitoring of blood-brain barrier disruption was presented in a mouse model [23]. Furthermore, in experiments carried out on rabbits it was shown that the blood-brain barrier disruption can be initiated after an injection of Mannitol and that this effect can be assessed by an analysis of ICG accumulation in the brain [24].

Aim of this study is to validate usefulness of an optical minimally invasive method in assessment of blood-brain barrier disruption. The proposed technique is based on optical monitoring of the inflow of the ICG and estimation of the clearance rate of the dye. This dye washout efficiency is related to the permeability of blood-brain barrier and an effect of retention of the dye in the brain tissue. Thus, it may be used for assessment of blood-brain barrier disruption.

II. Method

A. Instrumentation

A time-resolved optical setup was utilized in this study [16]. The instrument consists of picoseconds semiconductor leaser heads (PicoQuant, Germany), photomultipliers (Hamamatsu Photonics, Japan) and time-correlated single photon counting electronics (Becker&Hickl, Germany). The laser pulses at wavelength of 760 nm are delivered to the tissue with the use of multimode optical fibers. The light reemitted from the tissue is transmitted to the detectors using fiber bundles. Long-pass filters are used to block the excitation light and allow for detection of fluorescence photons excited on the ICG. The excitation wavelength was selected considering absorption spectra of the ICG [1], selectivity of the filters used and sensitivity spectra of the photomultiplier tubes.

The source fibers and detection bundles were positioned on the head using a flexible optode holder in such a way that the source-detector separation was fixed at 3cm. The optode construction allowed to measure in 4 source-detector pairs forming a square on each hemisphere. The middles of the squares were located in C4 and C4 points (according to 10-20 system used in EEG recordings).

B. Data analysis

Eight time-correlated single photon counting cards allow to acquire simultaneously distributions of times of arrival (DTAs) of fluorescence photons for 8 source-detector pairs. Furthermore, after background subtraction, statistical moments of the DTAs were calculated in Matlab environment. Total number of photons N_{tot} (zeroth statistical moment of the DTA), mean time of flight $<t>$ (first statistical moment of the DTA) and variance V of the distribution (second order centralized statistical moment)

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were analyzed [25]. In order to quantify the rate of ICG washout an algorithm based on analysis of maximal changes of the moments (N_{tot}, $<t>$ and V) caused by the inflow of the dye was applied. Furthermore, relative changes in the signals of moments after period of 3 min from the maxima were calculated. These relative changes were considered as measures of ICG washout rate.

Measurements

The measurements were carried out in supine position after fixing optodes on the head of the subjects/patients. A dose of 5 mg of ICG was dissolved in 3 ml acqua pro injectione and injected rapidly into forearm vein. The measurements were carried out in 3 healthy subjects and 3 patients with the blood-brain barrier disruption which was diagnosed by biochemical analysis of the cerebrospinal fluid. The study protocol was approved by the Ethics Committee of the Medical University of Warsaw.

III. Results

Examples of time courses of the statistical moments of the DTAs recorded in a healthy volunteer and a patient with disrupted blood-brain barrier were presented in Fig.1. It can be clearly noted in all considered moments of DTAs that their values return to the initial levels much faster in the healthy subject than in the patient.

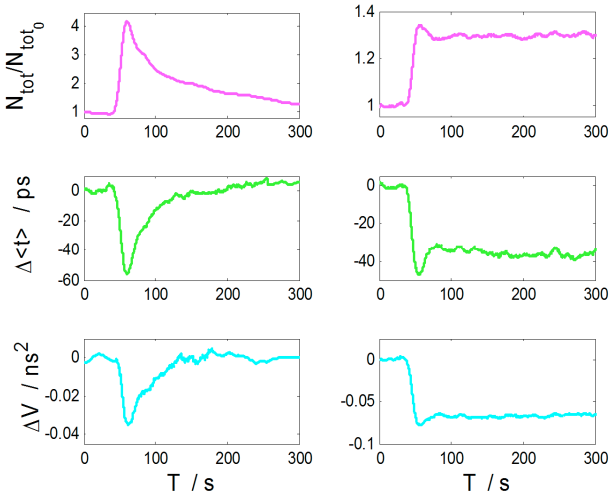

Figure 1. Time courses of moments of DTAs (total number of photons N_{tot}, mean time of flight $<t>$ and variance V) recorded in a healhy volunteer (left column) and patient with blood-brain barrier disruption (right column).

TABLE I. ICG WASHOUT RATES

Moments of DTAs	*Healthy subjects*	*Patients with BBB disruption*
Total number of photons N_{tot}	70.2±7.7 %	9.4±7.8 %
Mean time of arrival <t>	73.4±19.3 %	10.9±7.4 %
Variance of DTA	57.0±13.5 %	7.9±5.3 %

Results of a preliminary statistical analysis of the ICG washout rate in the healthy volunteers and the patients with blood-brain barrier disruption are shown in Table.1. The mean values were presented together with the standard deviations derived for all subjects and all source-detector pairs used in the measurement. It can be noted that all moments return to the initial levels much faster in the healthy volunteers than in the patients with blood-brain barrier disruption.

IV. Conclusions

Obtained results suggest that the ICG washout rates calculated from the time courses of statistical moments of DTAs can be used in the assessment of retention of ICG in the brain tissue caused by the blood-brain barrier disruption. Differences in the washout rate estimated from the signals of different moments are rather small which suggests that even a technically simple measurement of total number of photons with the use of continuous wave fluorescence detection may be used in the assessment of the blood-brain barrier.

We conclude that the monitoring of fluorescence signals during injection of the exogenous optical contrast agent can be used for the assessment of the condition of blood-brain barrier at the bedside. The technique may be of benefit for diagnosis of the patients suffering from the damage of the blood-brain barrier and in the monitoring of therapies used in such patients.

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