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Functional NIRS study of blood brain barrier disruption when induced by focused ultrasound and intra-arterial mannitol infusion

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ABSTRACT

Enhancing brain fluid movement across blood brain barrier (BBB) has been recognized as a potential treatment of neurodegenerative diseases. Moreover, BBB opening is of high interest also in brain drug delivery in the treatment of brain tumors/cancers. However, efficient therapies which are based on BBB opening are still limited because of insufficient understanding of mechanisms and safety issues. Currently, there are few promising methodologically diverse BBB opening approaches.

In this paper, we use functional near-infrared spectroscopy (fNIRS) for the first time for monitoring cerebral hemoglobin and water concentration changes during BBB opening in mouse brain by using two different techniques: intra-arterial mannitol infusion (IAM) and focused ultrasound (FUS). Both of these BBB opening techniques are already in clinical use but their hemo- and hydrodynamic implications have not been investigated from comparative aspect. Two fibre detectors were attached on both sides of the mouse brain and the source fibre was attached on middle of forehead. Further, by using a combination of three wavelengths 690nm, 830nm and 980nm, that have sufficient light penetration in the mouse brain, we can show average dynamics of hemoglobin and water in the whole brain, synchronized with BBB opening. To validate the level of BBB opening we used Evans blue dye and show its accumulation in the brain parenchyma tissue with the corresponding fNIRS responses.

Keywords: BBB disruption, brain, cerebral hemodynamics, cerebrospinal fluid, glymphatic, mannitol, water.

1. INTRODUCTION

The blood-brain barrier (BBB) regulates the central nervous system (CNS) homeostasis, and allows proper neuronal function, as well as prevents toxins, pathogens and inflammation to affect the CNS [1]. However, for treatment of brain disease such as brain tumors, BBB opening is needed, to enable chemotherapy drug to penetrate into the CNS. In addition, increasing brain fluid movement and glymphatic activity by modulating the BBB has been recognized as a potential treatment method for neurodegenerative diseases [2]. For instance, findings on Alzheimer Disease mouse models indicate that increasing of BBB permeability with focused ultrasound (FUS) can facilitate the removal of the protein accumulations and reverse memory loss [3]. Consequently, development of efficient and safe BBB opening techniques and monitoring, understanding BBB mechanism and the brain drainage system are of high interest [4-8].

BBB opening is already clinically exploited in brain drug delivery by using FUS [9] and mannitol infusion [10, 11]. In FUS based BBB opening, microbubbles are excited by ultra sound energy and interactions of the bubbles with the endothelial wall primarily creates BBB opening [9]. Generally seen, intra-arterial mannitol (IAM) based BBB opening is primarily based on osmotic shrinkage of endothelial cells which consequently opens tight junctions between the cells [6, 12].

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There is a large number of studies on FUS therapies. Biological effects of FUS can be invisible, visible or destructive, depending on the rate of acoustic energy deposition. In particular, transcranial FUS is non-invasive, making it suitable for wide range of therapies, where existing treatment options are lacking, limited or not suitable for all patients. Munoz et al. reviewed in 2018 recent developments in understanding of how FUS stimulates the brain, and concludes that effects mediated by BBB may stay open longer than FUS treatment, and because of this, future challenges include refining control of the duration of neural and behavioral effects [13]. There are also many preclinical and clinical studies showing that transiently disrupting the BBB with IAM during chemotherapy holds much promise as a therapeutic method. However, researchers still have limited understanding of the BBB opening mechanisms of both FUS and IAM and, in particular, how BBB disruption affects fluid exchange in the brain.

Only a limited number of studies are focusing on the hemodynamic effects of BBB opening. Yoon et al. used spectral range of 700 nm to 900 nm, to quantify hemodynamic changes in brain cortex when stimulating mouse brain by FUS. Optical fibers were positioned on the mouse scalp at source-detector distance of 4 mm. High frequency (8 MHz) FUS induced a pronounced increase in HbO and decrease in HbR, showing a pattern similar to the general hemodynamic response to neural activity [14]. Moreover, some interesting studies using fMRI Blood oxygenation level dependent (BOLD) during FUS BBBD show that BOLD is increasing when FUS is applied, for example by Chu et al. [15]. Milej et al. [16] quantified BBB permeability using dynamic contrast-enhanced NIRS utilising indocyanine green (ICG, 67 kDa) and 800CW carboxylate (IRDye, 1166 kDa), and generated permeability surface-area maps. This study demonstrates that BBB breakdown (accidentally) may contribute to secondary brain injury and lead to vascular edema accompanied with infiltration of white blood cells. Todd et al. show that neurovascular response is attenuated by FUS BBB opening, particularly BOLD and cerebral blood flow (CBF) changes are suppressed in response to forepaw pinching stimulation [17].

Ergin et al. documented several physiological responses due to IAM and ICG [18]. Mannitol injection brought significant changes in heart rate, blood pressure, and CBF, but the hemodynamic parameters return to normal after the injection. Myllylä et al. showed the cardiovascular effect of IAM in both human and mouse with comparable results: IAM causes strong immediate cardiovascular effects [19]. Recently, we presented a combined method using direct-current electroencephalography (DC-EEG) and fNIRS for monitoring BBBD in human [8]. IAM generated a prolonged increase in the ratio of oxyhemoglobin (HbO) and deoxyhemoglobin (HbR). In this paper, we further study the effects of BBB opening and show cerebral hemoglobin and water concentration changes during BBB opening in mouse and compare these responses between two BBB opening mechanisms.

2. METHODOLOGY

2.1 fNIRS monitoring

In our fNIRS setup, optical fibre having three light source inputs and one output tip was used as a source. This enables mixing of three narrow band wavelengths, each modulated at a specific frequency [20, 21]. Light sources are high-power light emitting diodes having wavelengths of 690 nm, 830 nm and 980 nm. Two fibre detectors were attached in ear canals and the source fibre was placed on middle of the head, see Figure 1. The diameter of the fibre tips are 2.5 mm. Distance between the source and the detectors in sensor placement is roughly 1 cm, enabling measurement of whole brain NIRS effect, rather than only surface of the brain cortex when using a shorter source-detector distance [14]. All fibres have the same physical dimensions.

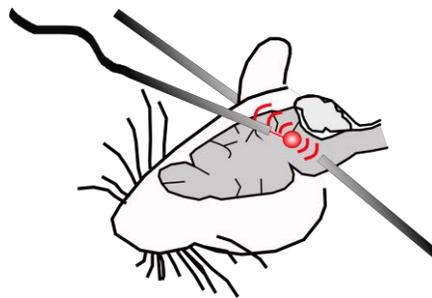


Figure 1. Placement of the fibre sensors. Two fibre detectors are attached in ear canals and the source fibre is placed on middle of the head.

Before starting the analysis, the protocol was translated into a code in Matlab 2019b for plotting purpose. It included setting time for injections of IAM, microbubbles, Evans blue and FUS. A 5th-order Chebyshev LPF at 5 Hz was applied prior further process. HbO, HbR, and water concentrations were calculated using the modified Beer-Lamber law (MBLL) [22] and analysis model described by Myllylä et al. [23].

2.2 BBB opening procedure in mouse

Mannitol

Laboratory Mice (CD-1 strain) were anesthetized with ketamine-xylazine-asepromazine. External carotid artery was exposed and thin (<200 μm) heparine filled polyethylene cannula was introduced into the vessel. 360 μl of pre-warmed 25% mannitol was infused into carotid artery at rate of 720 $\mu\text{l}/\text{min}$. 3 min post IAM 200 μl 2% (W/V in 0,9% saline) Evans blue was infused into tail vein to verify BBB opening after termination of experiment.

FUS

Mice are anesthetized ketamine-xylazine-acepromazine. Ring-shaped transducer was used, a FUS system RK-20 (Fus Instruments), that operates at 1.1-1.2 MHz frequency. Acoustic pressure at the focused area was up to 1 MPa. FUS transducer was modulated at 1.1 MHz frequency having focused length of from 1.5 to 4.5 mm and focused area of up to 5 mm (with 70% acoustic energy). The skin was removed above the skull and the ring transducer mounted on the skull with 1% agarose.

10 sec before sonication 200 μl of Sonovue microbubbles were injected into tail vein in 1:3 or 1:10 dilution in 0,9% saline were injected from mouse tail vein 10 seconds before ultrasonic emission. Ultrasonic modulation total exposure time was 1-2 min. As with IAM, Evans blue dye (2%, w/vol) was used to verify BBB opening.

Animal experiments were performed under the licence of National Animal Experiment Board of Finland (License number: Esavi/2362/04.10.07/2017 and Esavi/826/04.10.07/2018).

3. RESULTS

Figures 2 and 3 show typical hemodynamic and water responses for IAM and FUS induced BBB opening in mouse.

IAM BBBD

Timing

60 s: injection of mannitol
90 s: injection of mannitol ends
(240 s: Injection of Evans blue)

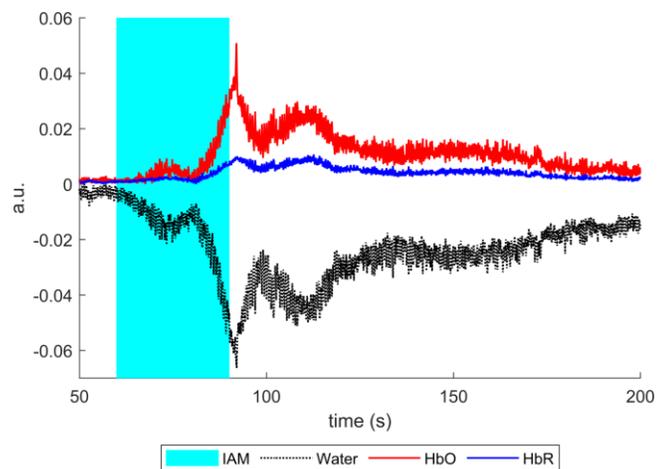
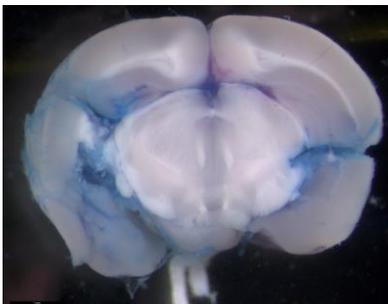


Figure 2. IAM based BBB opening in mouse brain. On the left, Evans blue extravasation associated with BBB opening is visible as blue hue in brain parenchyma. On the right, hemodynamic and water responses for IAM.

It is supposed that IAM causes osmotic shrinkage of endothelial cells and consequent opening of BBB. This seems to cause remarkable decrease in water concentration from the brain. This is accompanied with vasodilation and hyperemic response explaining increase in HbO and drop in HbR, showing strong anti-correlation between water and HbO signals.

FUS BBBD

Timing

60 s: injection of Evans blue
 150 s: injection of Sonovue bubbles
 160 s: FUS is set on
 270 s: FUS is set off

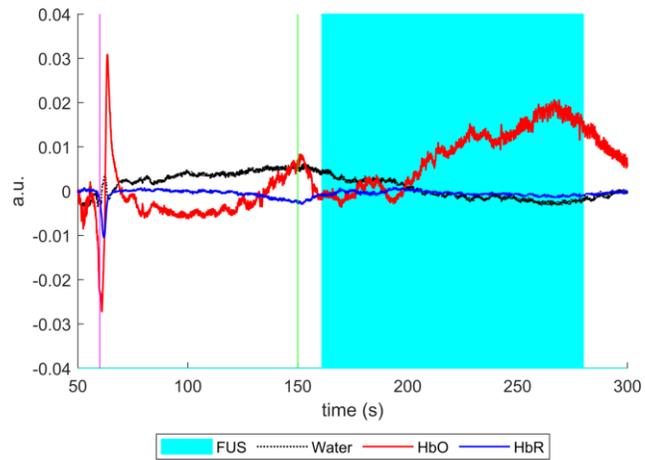
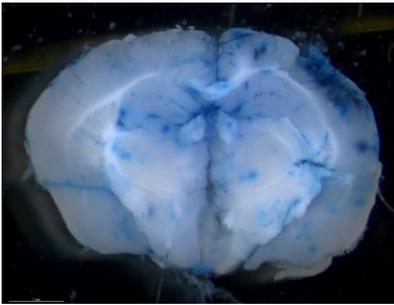


Figure 3. BBBD induced by FUS. On the left, Evans blue extravasation following BBB opening induced by FUS. On the right, hemodynamic and water responses for FUS. Note: high peak visible in fNIRS signal at 60 s is motion artefact caused by the Evans blue injection.

Earlier studies have shown that FUS can cause neuromodulation depending on the rate of acoustic energy deposition [14]. In Figure 3, shown HbO/HbR pattern is similar to the general hemodynamic response to neural activity thus indicating also an effect of neuromodulation. However, it still remains open, and requires further studies to evaluate if there are noticeable differences in HbO, HbR and water signals between FUS neuromodulation and FUS BBB opening.

Interestingly, when compared to IAM, in FUS BBB opening we could not detect noticeable change in HbR and water concentration which indicates that BBB opening induced by FUS has different mechanism than IAM based BBB opening.

4. CONCLUSION

To our knowledge, this was the first time when hemodynamic and water concentration changes have been measured during BBB opening and compared between FUS and IAM based techniques. Evidently, IAM based BBB opening causes noticeable immediate changes in both haemoglobin and water concentrations. On the contrary, FUS BBBD causes noticeable immediate changes only in HbO indicating differences between the BBB opening mechanisms. Also the water reaction was different in IAM and FUS methods. In both cases BBB opening was verified by using Evans blue. However, further studies are still required to be performed, in order to visualise and validate more the measured effects of BBB opening.

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