Two-photon microscopy imaging of blood brain barrier leakage in fetal alcohol disorder mice

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Abstract: We demonstrated blood brain barrier leakage associated with fetal alcohol spectrum disorder using two-photon microscopy. Evaluation was enhanced by concurrent imaging with different conjugated dyes and second harmonic generation imaging of collagen.

OCIS codes: 110.4234 Multispectral and hyperspectral imaging, 300.6410 Spectroscopy, multiphoton

1. Introduction

The fetal alcohol spectrum disorder (FASD) is a broad term describing fetal developmental deficits that are directly as a result from maternal alcohol consumption during pregnancy [1]. Patients with FASD often suffer from adverse co-morbidities such as epilepsy, and cognitive and behavioural abnormalities [2,3]. The underlying mechanisms for high-prevalent co-morbid epilepsy remain elusive, however recent findings suggest that FASD-mediated hyper-excitability is associated with abnormal gap junction protein expression in astrocytes [4]. Astrocytes are established to play an essential role in regulating blood-brain barrier function, raising the speculation that an abnormal BBB function might be one of the contributing factors to the disease progression [5–7]. The BBB is an important multicellular anatomical structure that consists of endothelial cell lining connected by tight-junction protein complexes, astrocytes and pericytes, each cell type interconnected by gap junctions. It seals around cerebral vasculature and functions as a selective interface which tightly regulates molecular trafficking between brain vessels and the extracellular fluid in the central nervous system (CNS), hence maintaining a precise interior milieu [6]. Severe BBB damage has been previously described in several brain disorders such as epilepsy, stroke and Alzheimers Disease [8–10]. Thus, BBB disruption may significantly impair brain functions. Currently, there is a lack of direct evidence illustrating BBB leakage in FASD. The conventional method of using albumin-binding 68 kDa Evans blue to evaluate vascular permeability is insensitive to subtle leakages in the BBB. Therefore, we utilized two dextran-conjugated dyes with different sizes (10 kDa, 70 kDa) and separate emission and excitation spectra (FITC, Texas Red) to sensitively quantify BBB integrity both spatially and temporally in an FASD mouse model. To capture leakage dynamics, we used a fast resonant laser scanning mirror and spectral separation of the emitted fluorescent light for simultaneous dual-dye imaging.

2. Methods

2.1. FASD Mouse Model

C57 Bl6 mice were used in the following experiments in accordance with guidelines of the animal welfare committee of the UHN. Breeding cages were set up and 10% Ethanol was administered for 7 days to females following a vaginal plug indicating mating had occurred. Regular water was used for the rest of the 21 day gestation period. Male offspring at age of 7-8 weeks were used for following FASD experiments.

2.2. In vivo Preparation and imaging

A craniotomy was performed under isoflurane to expose the parietal lobe of the cortex and an optical window was created for in vivo imaging using 1.2% agarose, a 8 mm diameter glass cover slip. A mixture of dyes, 10 kDa FITC

conjugated dextran and 70 kDa Texas Red conjugated dextran, were injected intravenously through the tail vein. Two-photon fluorescence microscopy was performed *in vivo* through the cranial window of control mice and FASD mice, using a Nikon A1R MP^{+}.

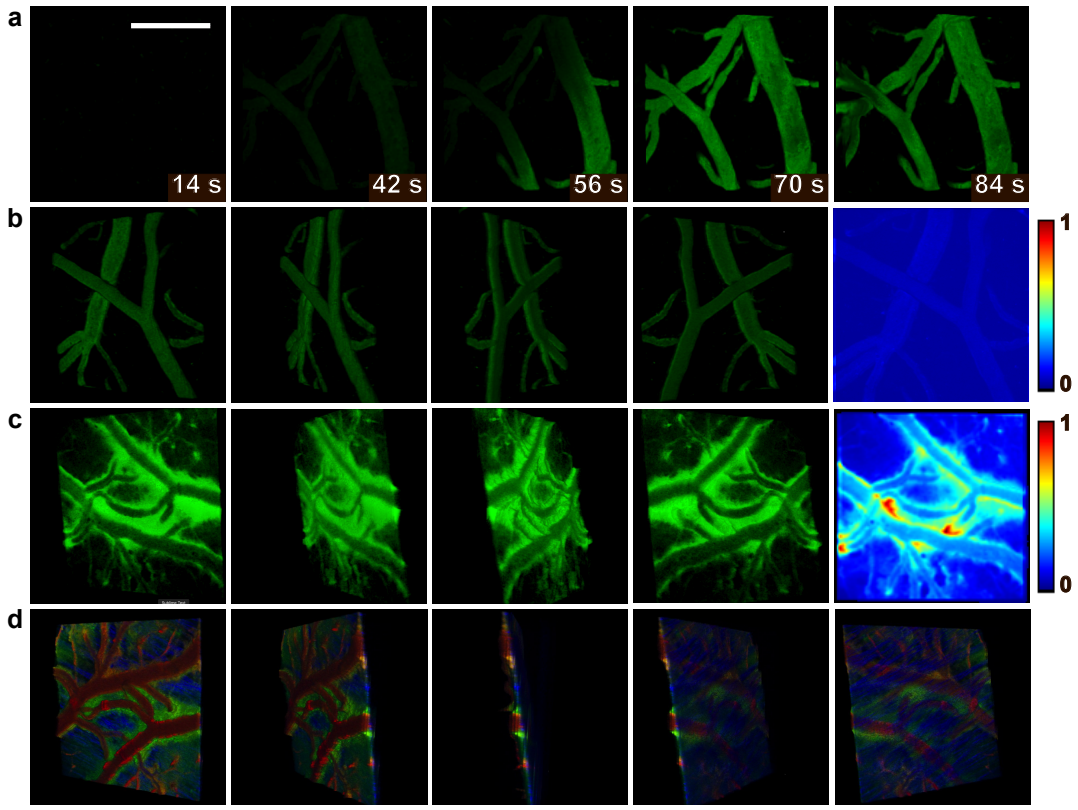

Fig. 1. (a) Confocal imaging showing fluorescent dye in blood vessels following bolus injection. Two-photon microscopy (750nm excitation) of 10 kDa dextran conjugated FITC dye in age-matched (b) control and (c) FASD mouse cortical vasculature (several 3D views, one time point) and corresponding temporal leakage maps (right panel, arbitrary units). (d) 3D rendition of mouse brain using two-photon microscopy showing leakage of 10 kDa dextran conjugated FITC dye (green channel) with vessels labelled with the larger 70 kDa dextran conjugated Texas Red (red channel), and SHG imaging of collagen (blue channel) outlining the intact dura on the surface of the brain (900nm excitation). (Scale bar = 200 μm)

3. Results and Discussion

The sensitive two-dye injection method allowed for the characterization of an accurate in-vivo BBB leakage profile of the FASD model (see Fig. 1). Vessel structures were outlined by segmentation using the larger Texas Red dextran dye and the extravascular signal intensity was then calculated. Rapid dye uptake in vessels is shown in Figure 1(a). FASD mice showed a significant higher extravascular fluorescence signal intensity of the smaller FITC-dextran dye (see Fig. 1 (b) *vs.* (c)). This phenomenon was also observed with the Texas Red dextran dye in the FASD mice, however the leakages was significantly reduced. In contrast, age-matched controls showed no visible extravascular fluorescence signal, which indicated an intact BBB. The location of the brain surface was localized using second harmonic generation (SHG) imaging of collegen in the dura matter (see Fig. 1(d)). The temporal profile of the increased leakage rate for an FASD mouse over an age-match control is shown in Figure 2. Our results provide direct evidence of the FASD-associated BBB disruption. Such disruption is susceptible for the entry of small blood-borne metabolites such as thrombin and hemoglobin-derived iron, which are proven to be highly toxic to brain functions [11, 12]. Therefore, a

prolonged exposure to small blood-borne neurovascular toxins might be a potential explanation of the co-morbid neurological disorders exhibited in FASD patients. Our results also revealed a small degree of larger Texas Red dextran dye leakages in the FASD animals, suggestive of a greater BBB damage in various vascular regions.

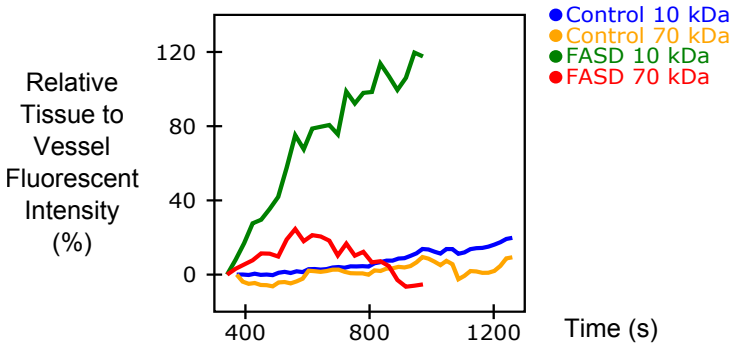

Fig. 2. Changes in fluoresent intensity in tissue relative to vessels for FASD mouse and control.

4. Conclusion

This work has revealed that a compromise in the BBB is associated with FASD. Concurrent two-photon imaging of different dextran conjugated dyes and SHG imaging of the dura enabled more complete leakage characterization. The FASD-mediated BBB disruption could permit the entry of blood-borne toxic molecules leading to neurological dysfunction. Our results implicate BBB permeability as a potential target for postnatal treatment of FASD.

Acknowledgements

We would like to thank Dr. Philippe Monnier for useful contributions. Research supported by NeuroDevNet and CIHR.

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