Longitudinal assessment of blood-brain barrier disruption in a photothrombotic rodent stroke model using histology and permeability MRI

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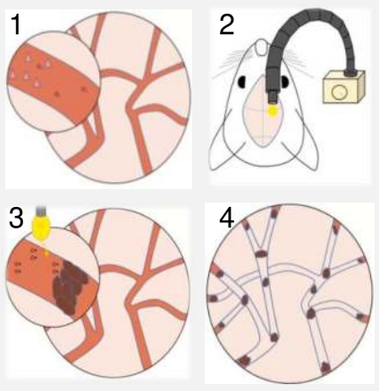
Introduction

- In ischemic stroke, blood clots obstruct the arteries supplying blood to the brain, resulting in brain tissue death.
    - Significant morbidity and mortality in both adult and pediatric populations[1,2].
- Disruption of the blood-brain barrier (BBB), which maintains optimal environment for neuronal functioning by regulating movement of solutes into the brain[3,4], contributes to further injury after ischemic stroke in adults.
    - Can lead to inflammation and influx of toxic substances.
- In the pediatric stroke, knowledge of the extent of BBB disruption is currently lacking, thus hindering administration of effective treatment strategies and increasing the likelihood of poor outcome.
    - Stroke research in children is sparse due to logistical and ethical limitations.
- Animal models of stroke provide an effective alternative for studying aspects of stroke injury otherwise not feasible in human subjects (e.g. longitudinal studies on BBB integrity and histology).
- Previous research have characterized the extent of BBB disruption after stroke in adult and neonatal rats showing distinctive differences which may have major implications for treating neonatal stroke[5].
- However, the extent of BBB permeability after stroke in juvenile rats is unknown.

Objective & Hypothesis

- The objective of this study is to assess, using both in-vivo and ex-vivo methods, the evolution of BBB permeability in a photothrombotic stroke model in a juvenile rat (see Figure 1).
- We hypothesized that the induced ischemic stroke causes BBB disruption in juvenile rats and that the degree of disruption will vary over time.

Figure 1. Stroke Model: Photothrombosis[6]



1. Oxygen flows in vessels as O_2.
2. After Rose Bengal dye injection, cold light is illuminated upon the target area, creating oxygen intermediates.
3. Oxygen intermediates induce endothelial cell membrane peroxidation, leading to platelet adhesion and aggregation.
4. This leads to the formation of thrombi which determine local cerebral flow interruption.

Methods

- Male Sprague-Dawley rats (5 weeks old, 145±15 g) from Charles River Laboratories (Charles River Canada facilities)

Procedure

- Rats were anesthetized with isoflurane (5% induction, 2% maintenance at 1L/min) .
- Incision is then made in scalp (no craniotomy).
- Cold light is illuminated upon the target area at an appropriate wavelength (in the green spectrum).
- Illumination time: 20 min
- Light intensity: 150 W, 100%

Histology and MRI

- Stroke outcome was confirmed by Triphenyl tetrazolium chloride (TTC) and hematoxylin and eosin (H&E) staining as well as in-vivo T2-weighted (T2W) and Diffusion-weighted Imaging (DWI) (see Figure 2).
- BBB permeability was quantitatively assessed ex-vivo by measuring Evan's Blue leakage longitudinally on days 0, 1, 2, and 7 (n=3 for every time point).
    - 4% Evan's Blue was injected into the tail vein and allowed to circulate for 2 hours before sacrifice.
    - Evan's Blue concentration was measured with spectrophotometer, and normalized against the weight of the tissue sample to generate a value of Evan's Blue (µg)/ brain tissue (mg).
    - Evan's Blue leakage was then expressed as mean infarct to control hemispheric ratio.
    - Dynamic contrast-enhanced (DCE) MRI was performed on a subset of rats to measure BBB permeability in-vivo.

Statistical Analysis

- Student t-test was performed to compare the amount of Evan's blue in the stroke hemisphere with the non-stroke hemisphere.
- Standard correlation test was conducted between DCE results and Evan's Blue leakage results.

Results

Confirmation of stroke using MRI and histology

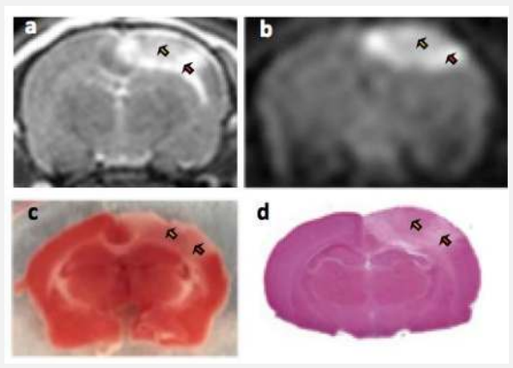
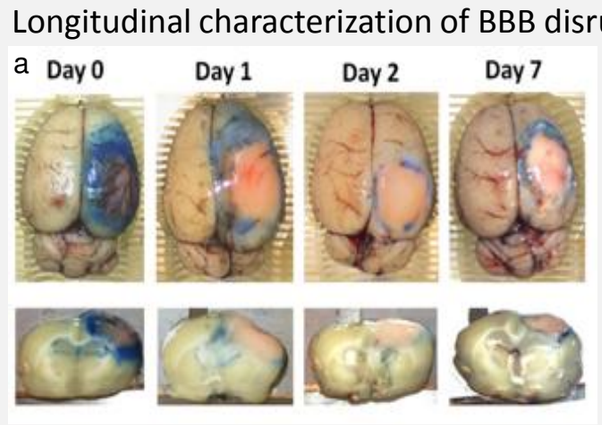


Figure 2. a, Coronal T2W image of a representative slice; **b,** Coronal DWI of a representative slice; **c,** Coronal TTC stain of a representative slice; **d,** Coronal H&E stain of a representative slice. All images are representations of day 0 post-stroke. Red arrow indicates a representative area of lesion (brighter area on all images) and yellow arrow indicates a representative area of penumbra (darker area similar to healthy tissue on all images).

Longitudinal characterization of BBB disruption using Evan's Blue leakage



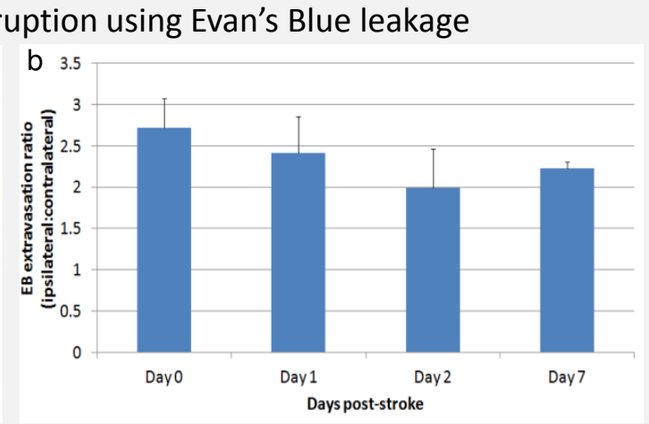
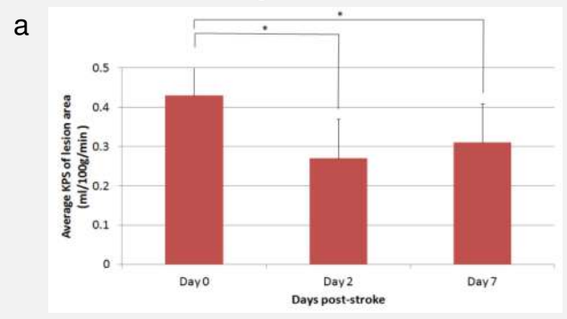


Figure 3. a, Representative whole brains with their corresponding coronal slices, showing the degree of EB extravasation on days 0, 1, 2, and 7. **b,** Graph representing EB extravasation based on the aforementioned days (n=3 per day).

Longitudinal characterization of BBB disruption using DCE imaging & comparison with Evan's Blue leakage



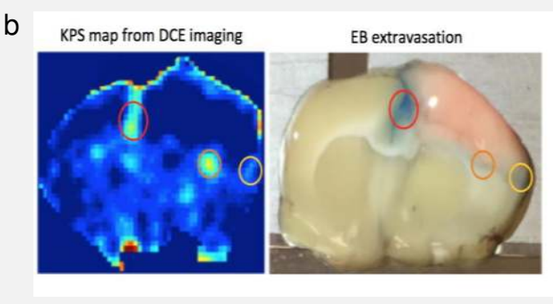


Figure 4. a, Graph representing Gadolinium extravasation (n=3). **b,** Visual comparison between KPS map from DCE imaging of a representative slice of a rat brain and corresponding EB-injected coronal slice.

- DCE results are correlated with EB leakage results (R^2= 0.588, p<0.05).

Discussion

- Our photothrombotic method yielded a highly reproducible stroke that was confirmed with histology and MRI.
- BBB leakage was shown to be higher in the stroke hemisphere compared to the contralateral hemisphere in stroke rats.
    - Highest permeability on day 0
    - Persisting BBB disruption up to day 7
- The model helps us to elucidate differences between ages and test therapeutic effects of neuroprotective drugs stabilizing the BBB at different time points after childhood stroke.
- Our results show that in-vivo MRI and ex-vivo staining are correlated.
    - Allow us to investigate BBB disruption in childhood stroke using DCE imaging.

References

1. Mathers, C. D., Boerma, T. & Ma Fat, D. *Br. Med. Bull.* **92,** 7–32 (2009); 2. Tsze, D. S. & Valente, J. H. *Emerg. Med. Int.* **2011,** 1–10 (2011); 3. Krueger, M. *et al. J. Cereb. Blood Flow Metab.* **37,** 2539–2554 (2017); 4. Daneman, R. & Prat, A. *Cold Spring Harb. Perspect. Biol.* **7,** a020412 (2015); 5. Fernandez-Lopez, D. *et al. J. Neurosci.* **32,** 9588–9600 (2012); 6. Labat-gest, V. & Tomasi, S. *J. Vis. Exp.* (2013).