Visualization & Quantification of Cerebrovascular Network Changes in a Hemorrhage Stroke-Prone Animal Model

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Introduction & Intention

- Hemorrhagic stroke is associated with high mortality, morbidity and disability - with a 1-year mortality rate of up to 58%[1].
- Visualization and quantification of the changes in cerebrovascular microcirculation, that occur as part of hemorrhagic stroke is needed as foundation to support development of novel treatment agents.
- Micro-Computed Tomography (micro-CT), coupled with a radiopaque contrast agent, can produce 3D reconstructions of the vascular network at a resolution sufficient to resolve small blood vessels, while leaving the tissue intact for subsequent analysis.
- The imaging power of this technique is advanced even further when coupled with a fluorescent imager (GE/ART Optix) and dye, allowing the exact site of stroke to be identified.

Methods

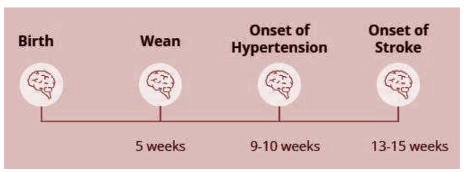


Figure 1: SHRsp timeline

- Uncontrolled hypertension is the leading controllable factor contributing to hemorrhagic stroke in humans[2], thus use of a spontaneously hypertensive stroke-prone (SHRsp) animal model is translatable.
- Evan's Blue (EB) Dye (30mg/kg) was infused through the femoral artery prior to fixation with 4% PFA
- Vascupaint™ (VP) *Medilumine*, a bismuth vanadate latex casting agent, was then infused via the aortic arch at 1.0mL/min until first sign of yellow coloring observed in key indicator regions (Figure 2), followed by 0.5mL/min until 6ml depleted, to opacify vasculature

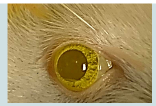
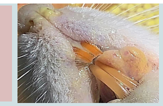


Figure 2: Key Indicators of VP Perfusion to Brain. Conjunctival/ episcleral vessels (left) & exterior nares/sublingual vasculature (right)



- 9 µm resolution produced image projections were reconstructed using the bundled SkyScan 1176 software (*Bruker*) NRecon program to create >3000 coronal slices
- Region of interest (ROI) of 500 slices - surrounding the middle cerebral artery (MCA), as it is the most common location for stroke - was analyzed using CTAn.
- For brains with a non-MCA stroke site (n=5), stroke-specific ROI were also analyzed. Corresponding anatomical sites in age/sex matched pre-stroke brains were used as controls.

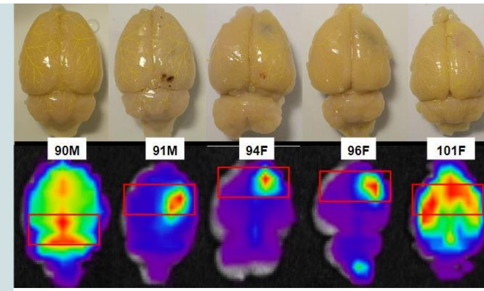


Figure 4: Macroscopic & Fluorescent Dorsal Images of Non-MCA Infarct Sites

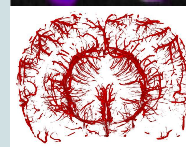
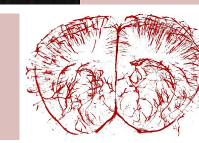


Figure 5: Coronal Slices Central Vascular Dysfunction
87M pre-stroke (left)
100M post-stroke(right)



Results

Table 1: Micro-CT Data	Pre-Stroke (n=12)	Post-Stroke (n=12)	Pre-Stroke (n=5)	Post-Stroke (n=5)	Interpretation
	MCA ROI		Infarct ROI		
Percent Vessel Volume	2.937 ± 0.822	0.691 ± 0.192	9.941 ± 3.575	8.174 ± 0.214	% of the total volume that is vessel - ↓ in post-stroke due to vasoconstriction and less intact vessels
	p<0.001		*p = 0.36*		
Fractal Dimension	1.888 ± 0.117	1.392 ± 0.054	1.296 ± 0.217	1.604 ± 0.082	Indicator of surface complexity - how the object's surface fills space - ↓ in post-stroke because less intact vessels
	p<0.001		*p = 0.032*		
Euler Number	933.6 ± 232.0	695.4 ± 331.9	466 ± 153.7	451 ± 104.9	Indicator of redundant connectivity - the number of connections holding object together - ↓ in post-stroke due to less intact vasculature
	p = 0.26		*p = 0.88*		
Vessel Thickness (µm)	4.846 ± 0.806	3.468 ± 0.524	9.503 ± 1.831	4.120 ± 1.176	- ↓ in post-stroke due to vasoconstriction
	p = 0.01		*p = 001*		

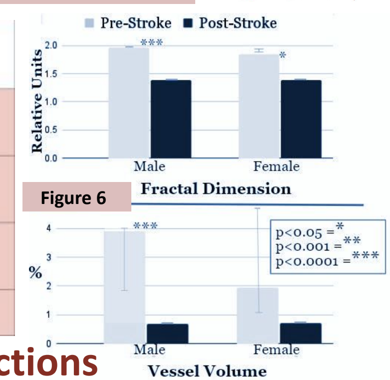


Figure 6

n=24 total: n=12 pre-stroke & post-stroke (n=6 male & female in each). Post-stroke brains exhibited EB extravasation and decreased VP perfusion, as evidenced by fluorescence & micro-CT respectively

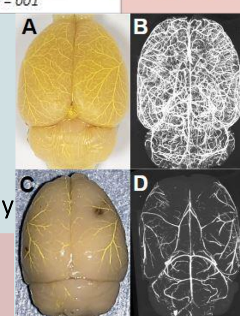


Figure 3: Rat 61F pre-stroke macroscopic (A), micro-CT (B); **Rat 93F post-stroke** macroscopic(C), micro-CT (D)

Future Directions

- *In-vivo* imaging using the established method
- Immunohistochemistry - markers of angiogenesis
- Introduction of antihypertensive drugs

1. Smajlović et al. 2006. Basic Med Sci. (3):17–22.
2. Wajngarten et al. 2019. Eur Cardiol. v.14(2)

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