Summary Report

Atlantic Indigenous Mentorship Network Kausattumi Grants Program, 2022-2023

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Quantification of neurovasculature changes in a post-hemorrhagic stroke animal model

Description of Research

Despite the high-mortality rate, treatment of hemorrhagic stroke has not seen as notable advances as ischemic stroke. Therapies targeting downstream molecular cascades are desirable, but the complexity of hemorrhagic stroke pathophysiology poses a challenge. Previous studies have shown that the decreased arterial pressure due to blood loss causes subsequent systemic vasoconstriction as a means of compensation - however, beyond this generalized statement, the detailed changes in brain vasculature have not been visualized and investigated. The objective of this study is to quantitatively analyze neurovascular network parameters, such as volume and connectivity, in pre and post-stroke animal models using high-resolution imaging and a novel perfusion casting agent. This fundamental understanding will aid in the development of pivotal preventative and treatment strategies.

Micro-Computed Tomography (micro-CT) is one of the few imaging techniques that can visualize even the smallest vessels without destroying surrounding tissues (preventing further analysis). Soft tissues, such as cerebral blood vessels, are not easily detected using micro-CT under normal conditions - however, when coated with a radiopaque compound, such as VascupaintTM, the vessels harden to a density that is detectable. The imaging power of this technique can be advanced even further when coupled with a fluorescent imager (ART Optix FLUOR) and a fluorescent dye (Evan's Blue, EB). Due to its albumin-binding nature, EB extravasates at the site of a hemorrhage, allowing the exact site of the stroke to be identified.

My role involves performing the surgical operation to infuse the required agents into the animal. Last year, optimized Standard of Operating Procedures (SOP) were developed and sampling commenced with n=12 pre-stroke and n=12 post-stroke (equal stratification among sexes). The samples have since undergone microCT and fluorescence imaging. Preliminary findings align with our hypothesis that post-stroke brains will exhibit less intact vasculature. For the 2024 calendar year, my focus will be to conduct extensive analysis using specialized software to identify patterns and relationships pertaining to an array of factors (ie. age, sex, weight, stroke site, etc). Insightful interpretation of the data will require thorough literature review and engagement with mentors. Deliverables will then be produced, with an aim to complete the project by the end of year.

All experimental procedures and animal breeding were carried out in compliance with the guidelines and recommendations, set forth and approved by the Animal Care ethics committee (protocol # 20-30-ND). We use the spontaneously-hypertensive stroke-prone rat (SHRsp) which develops hemorrhagic stroke by 11-13 weeks of age. We sampled the animals at 10 weeks (pre-stroke) and at signs of stroke development (post-stroke). Upon anesthetic and heparin administration, the femoral artery is isolated and infused with EB dye (30mg/kg) for 25 minutes, after which the thoracic chest is opened for flushing of blood and fixation via intracardially perfusion. VascupaintTM is introduced at a constant flow rate of 0.6ml/min, with the sublingual and ocular vasculature turning yellow within a minute as indicators of successful perfusion. Micro-CT images are acquired at 9μm resolution and reconstructed using SkyScan 1176 software to create over 2500 coronal slices of each brain. The standardized region of the scan in which the primary analysis is performed is centered around the middle cerebral artery (MCA), as this is the most common location for hemorrhagic stroke. Infarct-specific analysis will investigate stroke sites outside this region. A comprehensive 3D analysis will be computed to produce relevant values such as Euler number, vessel thickness, and percent vessel volume.

Research Outcomes

I have made significant progress over the past year and am on track to complete my MSc studies in August 2024, as initially anticipated, equating to nine semesters of part-time study. My required course work is complete, and all core data has been collected.

Over the past year I:

- Concluded the pre and post-stroke rat sampling required for my thesis (n=24 total). Each experiment takes approximately 3 hours to complete, with subsequent analysis to follow. An additional complicating factor is the time-sensitive and unpredictable nature of the sampling - as the strokes occur spontaneously at any time. I made every effort to sample stroking rats within a time span of mere hours to minimize suffering
- Worked alongside colleagues in the University of Alberta to advance my knowledge of micro-CT imaging techniques and began in-depth analysis of collected samples using specialized software delivered a seminar to faculty/staff and graduate students within the School of Pharmacy
- Conducted extensive review of literature and wrote a paper titled Theranostics: Pharmacokinetics, Pharmacodynamics and Imaging

- Facilitated my annual committee meeting
- Commenced immunohistochemistry and confocal microscopy experiments
- Participated in Memorial University's School of Pharmacy Research Day poster competition
- Assisted in lecture delivery in the area of pharmacokinetics for Doctor of Pharmacy students (independently delivering three lectures)
- Completed related work as a teaching assistant within the School

The above was completed all while maintaining strong ties to my community and advocating for Indigenous health equity.

Knowledge Sharing

- *Quantification of Neurovasculature in a Post-Hemorrhagic Stroke Animal Model* poster presentation delivered at MUN School of Pharmacy Research Day in Nov 2022
- *Hemorrhagic Stroke: Behind the Scenes* (public 60-minute seminar for faculty & graduate students within MUN School of Pharmacy in Jan 2023)

Completed Project Deliverables

- *Theranostics: Pharmacokinetics, Pharmacodynamics & Imaging* (major review paper, pending publication in Journal of Pharmaceutical Sciences)
- *Quantification of Neurovasculature in a Post-Hemorrhagic Stroke Animal Model* abstract published by in Journal of Pharmaceutical Sciences, as part of Canadian Society of Pharmaceutical Sciences (CSPS) Annual Symposium summary document

Forthcoming Project Deliverables

- *Quantification of Neurovasculature in a Post-Hemorrhagic Stroke Animal Model* (thesis - currently being drafted, submission due Aug 2024)

Activities Outside of Your Research

- Asked by provincial health authority (NL Health Services) to present at Pharmacy Grand Rounds on '*Indigenous Health: Considerations and Complexities'*
- Graduated with my Doctor of Pharmacy (PharmD) in Spring 2023. I am now better equipped to translate my basic sciences observations to clinical practice. With this being said, I have been making an active effort during my knowledge translation activities to incorporate clinical knowledge with the findings from my experiments in animal-models. This has helped convey the relevance and context of my research within each of the broader population, Atlantic Canadians and Indigenous individuals.

Future Research Plans

Throughout the upcoming year, I will be conducting subsequent analysis to provide further insight into the samples collected. This includes extensive analysis of microCT data using specialized software (Bruker SkyScan1176), as well as statistical analysis using SigmaPlot. The analysis will serve to detect patterns/trends pertaining to factors translatable to human populations, including age, weight, sex and infarct site. This data will then be graphed and/or tabulated for use in future knowledge translation activities.

Immunofluorescence techniques used to detect relevant markers of inflammation contributing to hemorrhage pathophysiology are also ongoing. Currently one-half of samples have undergone fluorescence imaging, with the latter half anticipated to be scanned in Fall 2023. ART Optix FLUOR imaging helps to quantitatively define association between extravasation of EB and destruction of surrounding vasculature. Immunohistochemistry (IHC) to detect endoglin (marker of angiogenesis) has also been underway since Feb 2023. The samples for IHC have all been collected, sliced and imaged. Analysis of this data is still outstanding but is anticipated to commence late Fall 2023 once other time-sensitive actionable items (such as pending publications and forthcoming presentations) have been completed.

Throughout Winter/Spring 2024, I will be heavily involved in training a new graduate student with the Daneshtalab lab, who will be taking over the research pertaining to SHRsp rats once I complete my Masters program. Their project will build upon the work I have done so far. I will be teaching them the surgical and analytical techniques I have used, such that they can continue the same in rats with blood pressure medications introduced (to identify if there are differences in stroke outcomes, as compared to my unmedicated rats). While the timeline for this work will not fit into my thesis, I will play a large role in initiating this project.

Aside from this, routine activities such as animal care, literature review and paper preparation are being conducted. I will also be presenting my research at a national conference (Vascular 2023 in Montreal Oct 25-29th) and participating in the learning sessions to deepen my knowledge, as well as my connections with other investigators in my field. My annual committee meeting is scheduled for Feb 2024, and I am also set to deliver another seminar presentation to MUN School of Pharmacy faculty/graduate students in Winter 2024.