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Lay Summary

Brain microvascular endothelial cells (BMECs), the cells that line cerebral blood vessels and form the blood-brain barrier, play a vital role in supplying the brain with glucose by controlling blood flow and selectively taking up glucose. BMECs also form a tight barrier that prevents infiltration of toxic or inflammatory compounds into the brain. Changes in cholesterol levels can impact nutrient transporters, ion channels that regulate blood flow, as well as the tight junction proteins that form the barrier. Our previous research had shown that BMECs treated with U18666A, a chemical inhibitor of the NPC1 protein, increased barrier permeability but decreased total glucose transport across the barrier. Niemann-Pick Disease (NPC) alters intracellular cholesterol transport and its localization in the cells. In this project, we sought to test how NPC1 impacts BMEC-mediated glucose transport and supply to the brain.

Blood flow regulation is important for brain health as it supplies the brain with vital nutrients (glucose, oxygen, etc.) during activity. BMECS regulate blood flow through neurovascular coupling (NVC), a process in which highly active brain cells (such as neurons) release potassium that is then detected by brain endothelial cells, triggering an electrical signal that dilates upstream blood vessels and increases regional blood flow. To test whether there are changes in blood flow, we examined blood flow dynamics in healthy and NPC1-null mice. Our preliminary results indicate that NPC1-null mice had decreased baseline blood flow and a negligible NVC response as indicated minimal increases in blood flow following exposure to a potassium stimuli. We are currently following up on this work by patch clamping isolated BMECs to specifically test whether ion channel activity in these cells is altered.

We also further investigated glucose metabolism and transport in U18666A-treated cells in cell culture experiments. As we previously noted increased glucose uptake in U18666A-treated BMECS, we examined expression of glucose transporters and metabolic enzymes. Despite increases in glucose uptake, we did not observe any changes in glucose transport protein in these cells. However, we did observe increased RNA levels of rate-limiting glycolytic enzymes. Follow up experiments demonstrated that U18666A treatment diminishes mitochondrial function and metabolism, which may trigger increased glycolysis to compensate for decreased energy production. Interestingly, we also observed that co-treated U18666A-treated cells with 2-deoxyglucose, a glycolytic inhibitor, partially prevented loss of barrier function.

In our last aim, we further examined changes in barrier function in BMECs following NPC1 inhibition. Our results indicated significant protein depletion and altered morphology of the critical tight junction protein, Claudin-5, which is vital for controlling permeability of small molecules. To confirm that permeability changes were primarily due to claudin-5, we examined permeability of tracers across a range of sizes and found that it was primarily lower weight tracers that show increased permeability across the barrier. This effect appeared to be reversed by co-treating the cells with hydroxypropyl beta cyclodextrin (HPβCD). Finally, we attempted to validate our results with U18666A by using a CRISPR gene editing system to knockout the NPC1 protein in our cell line and generate genetically-deficient NPC1 knockout models. Our results with this model confirmed that NPC1 depletion diminishes barrier integrity, however we noted that the magnitude of change was not as large as what was observed in U18666A-treated cells. Our preliminary results also indicate that HPβCD can restore barrier function in the CRISPR NPC-KO lines as well.

Our work has several important implications for understanding and treating NPC. Changes in blood flow and nutrient delivery may contribute to neurodegeneration as neurons and other brain cells fail to receive the proper level of nutrients to maintain their activity. Our current results show decreased blood flow in NPC1-null mice and limited NVC, which may contribute to neurodegeneration in this disease. Furthermore, we observed metabolic dysfunction and increased glucose uptake in NPC1-inhibited BMECs, which suggests that these cells may consume more of the available

glucose for themselves, leaving less for recipient cells and potentially further promote neurodegeneration. Finally, our results show increased barrier permeability in NPC1 deficient cells, which can further contribute to neurodegeneration by increasing permeability to neurotoxic compounds. Restoration of barrier function with HPβCD is promising as it may indicate a potential method to minimize neurodegeneration due to infiltration of neurotoxic or inflammatory compounds. Together, these results show that targeting BMECs may be a potential strategy to prevent and/or treat NPC disease manifestations.

Publications during NNPDF funding period:

1. Moiz B, Sriram G, Clyne AM. Interpreting metabolic complexity via isotope-assisted metabolic flux analysis. Trends Biochem Sci. 2023 Jun;48(6):553-567. doi: 10.1016/j.tibs.2023.02.001. Epub 2023 Mar 1.

Pending publications relevant to NNPDF project:

1. **Moiz, B***. Weber, C*. Clyne, AM. "Brain Endothelial Cell Metabolism in Health and Disease"
2. **Moiz, B.*,** Alpizar, V. Li, A. Qin, A. Walls, M. Hart, S. Brandon, K. Pepper, T. Weber, C. Sangha, G. Davidson, C. Wassif, C. Porter, F. Clyne, AM. NPC1 inhibition and cholesterol depletion increase small molecule permeability in the blood-brain barrier by disrupting claudin-5.
3. **Moiz, B. *,**Li, A. Alpizar, V. Weber, C. Walls, M. , A. Qin, A. Walls, M. Hart, S. ,Sangha, G. Wassif, C. Porter, F. Clyne, AM. NPC1 inhibition in brain microvascular endothelial-like cells diminishes glucose transport into the brain and alters metabolism of recipient cells.