Background

• Aging and cerebral small vessel disease (SVD) have deleterious effects on the white matter of the brain including an increase in pro-inflammatory microglia and blood-brain barrier dysfunction 1,2.

• Blood brain barrier dysfunction can involve the extravasation of serum proteins such as fibrinogen, thrombin or albumin into the brain parenchyma 3,4.

• The uptake of fibrinogen into the white matter is associated with an increase in microglia, however the effects on microglia activity are not fully understood 5.

• Extracellular vesicles (EVs) are small lipid-membrane bound vesicles that have emerged as key regulators of cell-cell signaling.

• The role of EVs in aging, neuroinflammation and pro-inflammatory signaling in the context of aging and blood-brain barrier dysfunction requires further investigation.

Objectives

1. Investigate fibrinogen mediated activation of BV2 cells

2. Investigate the ability of extracellular vesicles released from fibrinogen-exposed cells to propagate pro-inflammatory signaling through transcriptional priming of the NLRP3 inflammasome

3. Determine if extracellular vesicles are carriers of fibrinogen

4. Determine if repeated exposure to fibrinogen or extracellular vesicles can prolong up-regulation of pro-inflammatory signaling

Methods

1. Fibrinogen Treatment (0.5-4 mg/ml) for 3-12 hours

2. EV Isolation via ultracentrifugation

3. mRNA Isolation, RT-qPCR

4. Counting of EVs using Apogee A50 MicroPlus nanoflow cytometer

5. Fluorescent labeling of EV cargo using nanoflow cytometer

6. EV Treatment (4k events/cell) for 3-6 hours

7. RNA & Cell lysate collection for RT-qPCR and Western blotting

Results

Fibrinogen exposure activates pro-inflammatory microglia signaling in a dose-dependent manner

• IL-1β expression following single exposure to EVs (4k/cell) released from fibrinogen treated cells results in sustained up-regulation of pro-inflammatory microglia.

• IL-1β mRNA expression measured by RT-qPCR of cells after exposure to a range of EV doses (2k-12k/cell) * indicates values that are statistically different from control treatment determined using one-way ANOVA (p-value <0.05)

Extracellular vesicles released from fibrinogen-exposed cells propagate pro-inflammatory signaling to naive cells in a dose-dependent manner

• Extracellular vesicles released from fibrinogen-exposed cells carry fibrinogen as cargo

Discussion

• Fibrinogen induced robust microglia activation at concentrations 20-fold lower than circulating-plasma levels, suggesting that leakage through the blood brain barrier is sufficient for the induction of pro-inflammatory microglia.

• EV-mediated signaling may represent a novel mechanism enhancing fibrinogen-induced microglial activity.

• Future work investigating EV-mediated propagation in vivo will improve our understanding of blood-brain barrier dysfunction and microglia activation in the context of aging and SVD.

References

1. Li et al., Higher blood-brain barrier permeability is associated with higher white matter hyperintensities in older adults. J Neuroinflammation. 2021;18(1):330


3. McAleese et al., Extravascular fibrinogen in the white matter of Alzheimer’s Disease and normal aged brains: implications for fibrinogen as a biomarker for Alzheimer’s disease.

4. Raj et al., Increased white matter inflammation in aging- and Alzheimer’s disease brain.


Acknowledgements

We would like to thank our funding sources CIHR, NSERC, and CCNA. We would like to thank Dr. Lynn Wang for technical assistance.
IL-1β mRNA levels (r.u.)

Fibrinogen Concentration (mg/ml)

IL-1β