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INHIBITING AUTOPHAGY IN HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELLS INCREASES BLOOD BRAIN BARRIER PERMEABILITY

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Vascular cognitive impairment (VCI) places a tremendous emotional and financial burden on patients, their families and society. While aging is the main risk factor, the precise mechanisms leading to VCI are unclear. Cerebral arterial perfusion defects and blood brain barrier (BBB) defects are two main factors that have been proposed to contribute to VCI. Functional hyperemia facilitates delivery of sufficient nutrients to and the removal of metabolic end products from highly active neurons. BBB integrity provides a barrier between the brain and the potentially toxic circulating milieu protecting neurons from potential damage. It is known that age-associated deterioration of arterial function and BBB integrity occur, but what is the mechanism? Autophagy is an intracellular degradation pathway that clears damaged proteins and organelles from the cytosol. We have shown that repressed age-associated endothelial cell (EC) autophagy contributes to age-associated cerebral arterial dysfunction. Further, adult mice with genetic repression of EC autophagy display increased BBB permeability assessed in vivo. We sought to substantiate the latter finding using a reductionist approach. Here we test the hypothesis that human brain microvascular ECs (HBMVECs) with repressed EC autophagy display increased EC permeability in vitro. HBMECs were seeded on a 96 well Electric Cell-substrate Impedance Sensing (ECIS) plate. ECIS detects changes in barrier permeability by measuring the resistance across cultured ECs in response to high and low frequency electrical current. The current across the HBMVECs was monitored for approximately 20 h until a stable monolayer of ECs formed, as determined by a stable cell index over several hours. Once evidence for a stable monolayer was present, HBMVECs were treated with vehicle (PBS), interleukin 1 beta (IL-1β), or 3-Methyladenine (3-MA). A vehicle was used as a negative control and IL-1β was used as a positive control. 3-MA is a class III phosphoinositide 3-kinase (PI3-K) inhibitor that disrupts autophagy initiation. Permeability was assessed from 22-40 h at 5 min intervals, in 16 wells of a 96-well plate per treatment, and 2 experiments were completed. Confirming our ability to detect differences, IL-1β, heightened permeability in HBMVECs from 20-40 h vs. results obtained from the vehicle control. Providing strong proof of concept for our hypothesis, heightened permeability was observed in HBMVECs treated with xx IL-1β from 24-40 h vs results obtained from the vehicle control. These results indicate that impaired EC autophagy may contribute to increased BBB permeability. To complement this pharmacological approach, ongoing studies are measuring permeability across: (i) HBMVECs with and without genetic disruption of EC autophagy; (ii) primary ECs from mice with EC-selective depletion of autophagy-related genes vs. wild-type animals; and (iii) primary ECs from adult vs. older mice. The overall goal of this research is to determine whether targeting EC autophagy might represent a therapeutic intervention strategy to treat vascular cognitive decline.