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Lysosomal dysfunction induced cytosolic vacuolation and increased intracellular amyloid-beta 42 (Aβ42) in human brain endothelial cells (HBEC-5i)

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Abstract

Lysosome is a primary degradative organelle and is crucial in cellular homeostasis. A reduction in its function due to ageing has been associated with the development of Alzheimer's disease (AD), a common neurodegenerative disorder characterised by the deposition of neurotoxic amyloid plaque in the brain and cerebral vessel walls. The breakdown of the blood-brain barrier (BBB) plays a vital role in the pathogenesis of AD. However, the impact of lysosomal dysfunction on brain endothelial cells, the key component of the BBB, in the disease progression is yet to be fully understood. In this study, human brain endothelial cells (HBEC-5i) were exposed to a lysosomotropic compound, chloroquine (CQ) for 24 h. Cell viability was assessed with the 3-(4,5-dimethylthiazol-2-yl)− 2,5-diphenyltetrazolium bromide (MTT) assay to determine the inhibitory concentration (IC) at IC10 (17.5 μM), IC25 (70.5 μM), and IC50 (125 μM). The morphological changes observed include vacuoles arrested in the cytosols and cell shrinkage that were more prominent at IC25 and IC50. Lysosomal dysfunction was evaluated by measuring the lysosomal-associated membrane protein-1 (LAMP-1) and microtubule-associated protein light chain 3-II (LC3-II) using the capillary-based immunoassay. LC3-II was significantly increased at IC25 and IC50 (p < 0.05 and p < 0.001, respectively). The concentration of intracellular and extracellular Aβ42 was quantitated using the enzyme-linked immunosorbent assay, which demonstrated a significant increase (p < 0.05) in intracellular Aβ42 at IC25. This study showed that perturbation of lysosomal function impairs autophagy that leads to intracellular increment of Aβ, indicating the important roles of lysosomes in endothelial cells homeostasis and disease progression. © 2023 The Authors

Author Keywords

Alzheimer's disease; Amyloid angiopathy; Autophagic vacuoles; Lysosome inhibitor; Neurodegenerative diseases; Neurovascular dysfunction

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