

Original Article

The effects of lysosomal dysfunction on cytoplasmic vacuolation and amyloid-beta 40 (Aβ40) level in human brain endothelial cells (HBEC-5i)

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Abstract



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Lysosomes are an important intracellular organelle that regulates cellular degradation. Dysfunctional lysosomes disrupt this process, leading to the accumulation of toxic proteins that are meant to be degraded inside the cell, leading to cellular stress and potential toxicity. One of the proteins is beta-amyloid which is associated with conditions like cerebral amyloid angiopathy (CAA) and Alzheimer's disease (AD). To identify its effects on the vascular compartment, the current study explored lysosomal dysfunction's impact on cytosolic vacuole formation and amyloid beta 40 (Aβ40) levels in human brain endothelial cells (HBEC-5i). Cells treated with the lysosomotropic compound chloroquine (70.5 μM) exhibited morphological changes, including prominent cytosolic vacuole formation. The vacuole density was recorded at 11.86 ± 1.907 vacuoles per cell ($p < 0.05$), and its diameter was significantly increased (3.76 ± 0.182 μm, $p < 0.05$) compared to the negative control group. However, the average cell size remained unchanged despite the vacuole formation in CQ-treated cells. ELISA tests on lysate and supernatant revealed no significant differences between treatment and control groups in intracellular and extracellular Aβ40 levels. This suggested that while lysosomal dysfunction induced cytosolic vacuole changes, it did not significantly alter Aβ40 levels. Further research is needed to elucidate the pathways involved in Aβ40.

Keywords: Alzheimer's disease, Autophagy, Chloroquine, Lysosomotropic agent, Vacuoles

1. Introduction

Alzheimer's disease (AD) is one of the most prevalent forms of dementia, with nearly 10 million new cases diagnosed annually [1]. Its symptoms include a deterioration in physical, cognitive, and behavioural functioning that interferes with day-to-day activities [2,3]. A proposed pathogenic mechanism for Alzheimer's disease (AD) is the presence of Aβ pathology, in which the Aβ peptide is produced, aggregated, and deposited in the brain tissue and the walls of blood vessels in the brain [3,4]. The Aβ peptide plaque is generated from amyloid precursor protein (APP), a transmembrane protein in human brain endothelial cells (HBEC) [5], which later will be engaged in either one of two pathways, amyloidogenic and non-amyloidogenic. In the non-amyloidogenic pathway, the enzymes involved in the cleavage process are α-secretase and γ-secretase to produce the C83 fragment and extracellular APP alpha (sAPPα). Meanwhile, in the amyloidogenic pathway, the

β- and γ-secretase cleavage will generate sAPPβ, APP intracellular C-terminal dominant (AICD) and Aβ peptide monomers, with the most important Aβ are Aβ40 (80-90%) and Aβ42 (5-10%) [6,7].

Aβ40 is a soluble isomer with 40 amino acid residues and is abundantly found in the cerebral spinal fluid [8]. It plays a significant role in forming Aβ deposits despite its low amyloidogenicity [3,8]. Aβ40 served as a proxy of total Aβ production by preferably binding to the proto-fibrillar Aβ42, hence inhibiting Aβ42 from being aggregated [9]. Aside from that and in the relative level of the Aβ42/40 ratio, it plays a vital role in Aβ42/40 ratio, a well-known biochemical feature in AD diagnosis [3]. Previously, Aβ42 was found to be increased with lysosomal dysfunction in HBEC-5i, but the status of Aβ40 level has not yet been identified [2]. In addition, a previous study had identified that a marked reduction of CSF Aβ42/40 has consistently been found in patients with different stages of AD [3,10].

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There also have been studies that linked lysosomal dysfunction with the accumulation of A β -peptides. Lysosomes are membrane-bound organelles whose main function is to degrade waste materials from outside the cells and the worn-out components of the cells. One of the major pathways in degrading intracellular macromolecules is the autophagy-lysosomal pathway (ALP). This cellular process involves the fusion of autophagosome-filled macromolecules with lysosomes to form autolysosomes and, later, the degradation process [11].

However, in the event of lysosomal dysfunction, the fusion with autophagosome is impaired, accumulating intracellular autophagic vacuoles through cytosolic vacuolation. Cytosolic vacuolation is a formation of giant membrane-bound subcellular structures in the cytoplasm, which has been observed in cells following bacterial or viral infection or exposure to low molecular weight drugs or chemicals [2,12,13]. Aside from that, lysosomal dysfunction also caused the accumulation of intracellular macromolecule waste, inclusive of toxic proteins, such as A β [14,15]. This accumulation will lead to anatomical disruption and functional breakdown of the endothelial cells of the brain (BEC) and an increase in blood-brain barrier (BBB) permeability. These two conditions are the clinical characteristics of cerebral amyloid angiopathy (CAA) and AD [3,4].

The previous investigation conducted in our laboratory demonstrated that the use of Chloroquine, a lysosomotropic drug, resulted in a decrease in lysosomal function. This impairment of the autophagy process led to a rise in the intracellular level of A β -42 in the HBEC-5i in vitro model [2]. However, there is less evidence on the effects of the lysosomal dysfunction on the cytosolic vacuolation formation and the intracellular and extracellular A β -40 level in the same model, despite its significant role in forming A β deposits and importance in the A β 42/40 ratio. Therefore, this study investigates whether chloroquine-induced lysosomal dysfunction in the human brain endothelial cell line (HBEC-5i) could affect the cell line's cytosolic vacuolation formation and extracellular and intracellular A β 40 level, providing insights into its role in Alzheimer's disease pathology.

2. Materials and methods

2.1. Cell Culture Procedure

Human brain endothelial cells (HBEC-5i) (Addexbio Technologies, USA) were cultured in a complete medium of Dulbecco Modified Eagle's Medium/Ham's F-12 (Sigma, USA) with 1% Penicillin-Streptomycin mixed solution (Nacalai Tesque, Japan) and 10% Fetal Bovine Serum (FBS) (Capricorn Scientific, South America). The flasks were pre-coated with 1% gelatin (ATCC, USA) and were incubated in 5% CO₂ incubator at 37°C. The cells were from passages 6-8 with about 80-90% confluency. Upon reaching the confluence, adherent cells were detached and counted using the Trypan blue method and a hemacytometer.

2.2. Cell Treatment Procedure

A total of 3.0×10^5 cells were seeded in each T75 flask. The flasks were divided into the negative control (NC) (no treatment) and 70.5 μ M Chloroquine diphosphate salt (CQ) (ThermoFisher Scientific, USA). The concentration was established from the IC₂₅ value that induced lysoso-

mal dysfunction in HBEC-5i from previous study [2]. All the flasks were incubated for 24 hours in a 37°C incubator with 5% CO₂. The study was performed in three (3) biological replicates (n=3).

2.3. Cell Vacuolation Microscopic Examination

After 24 hours of incubation, the general morphology of all HBEC-5i cells and the presence of cell vacuolation in each group were observed under an inverted light microscope (Olympus, Japan). Thirty-six images were captured by Cell[^]A (Olympus) imaging systems software (x 100 magnification) for each cell group (NC and CQ) and then analysed by using the ImageJ software (USA). In this analysis, data collected were vacuolation density (number of vacuoles per cell), vacuole average diameter (μ m), and cell surface area (μ m²) of untreated and vacuolated-treated cells. All data then were analysed statistically. The cells in both flasks were prepared for subsequent sample preparation.

2.4. Cell Lysate Preparation

After microscopic examination, the media supernatant from each group flask was collected and kept at -20°C for extracellular A β 40 level analysis. For intracellular A β 40 level, the cells were rinsed with cold PBS, lysed with cold RIPA lysis buffer (VWR Chemicals, USA), with protease inhibitor (Sigma, USA) and incubated on ice for 15 minutes. The cells were scraped and transferred into a microcentrifuge tube, vortexed for 1 minute (3 times) and centrifuged at 13,500 rpm, 4°C for 15 minutes. The proteins containing supernatant (cell lysate) were collected, and the total protein quantity was determined by BCA protein assay.

2.5. BCA Protein Assay

Pierce[™] BCA Protein Assay Kit (ThermoScientific, USA) was used to assess total protein concentration. BCA working reagents were prepared, and standard dilutions (BSA) with the 25-2000 μ g/mL range were diluted before the assay. Briefly, 200 μ L of working reagent were pipetted into a 96-well microplate, and 10 μ L of each standard solution or supernatant or lysate (from each cell group) were added into each well. Both solutions were mixed well with a plate shaker for 30 seconds and incubated at 37°C for 30 minutes. After cooling to room temperature, the absorbance of the solution was measured at 562 nm on a plate reader. The calibration graph of the standard solution (absorbance vs concentration) was prepared, and the concentration of each solution of media or lysate (from each cell group) was identified from the graph. The assay was performed in triplicate, n=3, and the media and lysate from each cell group (NC and CQ) were homogenised and kept on ice throughout the process.

2.6. Measurement of A β 40 concentration HBEC-5i using ELISA

A total of 100 μ L of each standard, blank and samples (cell lysate and supernatant) were added into the ELISA kit (Elabscience, USA) provided microplate wells, sealed, and incubated for 90 minutes at 37°C. After the incubation, the solution was decanted and 100 μ L of biotinylated A β detection working solution was immediately added into each well and the plate was incubated for 1 hour at 37°C. Then, 100 μ L of the HRP conjugate working so-

lution was added and incubated for 30 minutes at 37°C. Following this incubation, the samples were washed with the washing buffer. Finally, 90 µL of substrate reagent was added and incubated for 15 minutes at 37°C under dark before being added with stop solution to each well and read using a microplate reader at 450 nm.

2.7. Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) Version 27. All data were expressed as mean $\pm$ standard error of the means (SEM). Descriptive and independent t-tests were employed to compare the morphology changes and cytosolic vacuolation. Meanwhile, independent t-tests were used to compare the intracellular and extracellular A β 40 levels of NC and CQ-treated cell groups. A $p < 0.05$ will be considered statistically significant.

3. Results

3.1. Morphological and cytosolic vacuolation in HBEC-5i

3.1.1. Morphological Changes of HBEC-5i and Cytosolic Vacuolation

The observed morphological changes indicated that the HBEC-5i cell morphology in the NC cell group resembled a cobblestone pattern (Fig. 1(a)). In Fig. 1(b), the CQ-treated HBEC-5i cells exhibited morphological alterations characterised by a greater degree of cell shrinkage compared to the negative control cell group. In addition, the cell's cytoplasm exhibited prominent vacuoles.

3.1.2. Cytosolic Vacuole Analysis

Figure 2(a) demonstrated a notable difference in the average density of cytosolic vacuolation per cell between the CQ-treated group (11.86 ± 1.907) and the NC group, which had no cytosolic vacuolation. The statistical analysis revealed a t-value of -6.221 and a p-value of 0.025 ($p < 0.05$). Figure 2(b) demonstrated a statistically significant difference ($p < 0.05$) in the diameter of the cytosolic vacuole in the afflicted cells of the CQ-treated group ($3.76 \pm 0.182 \mu\text{m}$) compared to the NC group, which did not exhibit cytosolic vacuolation; $t(2) = 20.696$, $p = 0.02$. Meanwhile, Figure 2(c) showed that the average surface size of vacuolated impacted HBECs (1238.80 ± 34.678

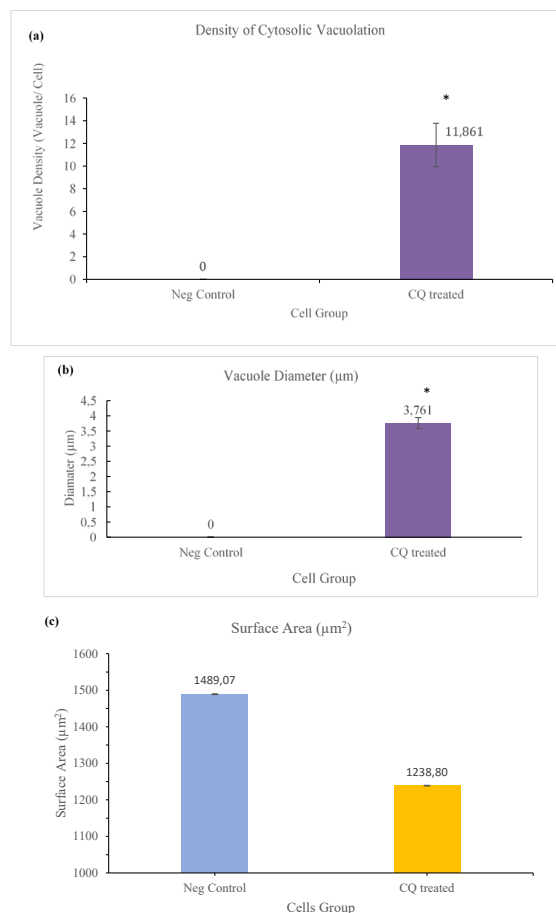


Fig. 2. Prominent increases in cytosolic vacuolation density and diameter were observed in the HBEC-5i CQ-treated group compared to the NC group. The graphs show the analysis of cytosolic vacuolation using ImageJ image processing. (a) The density of cytosolic vacuolation per cell in the CQ-treated group was significantly higher (11.86 ± 1.907) than in the NC group ($*p\text{-value} < 0.05$); (b) The average vacuole diameter in cytosolic vacuolation in the CQ-treated group was larger ($3.76 \pm 0.182 \mu\text{m}$) than in the NC group ($*p\text{-value} < 0.05$); (c) The surface area size (μm^2) of HBEC-5i in NC group was higher compared to CQ-treated cell groups, however, it was not significant.

μm^2) was not statistically different from the cells in the NC group ($1489.07 \pm 122.199 \mu\text{m}^2$); $t(4) = 1.970$, $p = 0.120$.

3.2. CQ increased A β 40 concentration in HBEC-5i

3.2.1. Intracellular A β 40 Level

Fig. 3 showed the result of intracellular A β 40 level on HBEC-5i cells lysate in both the negative control and CQ-treated groups. The graph showed a decreased level of A β 40 in the CQ-treated cells group as compared to the negative control cell groups. However, there is no significant mean difference in intracellular A β 40 level in the CQ-treated cell ($17.36 \pm 0.196 \text{ pg/mL}$) as compared to the negative control HBECs ($17.76 \pm 0.154 \text{ pg/mL}$); $t(4) = 1.603$, $p = 0.184$.

3.2.2. Extracellular A β 40 Level

Meanwhile, Fig. 4 showed extracellular A β 40 level of HBEC-5i supernatant after CQ treatment as compared to the negative controls. This result showed that the A β 40 level in CQ-treated cells was higher ($16.02 \pm 0.030 \text{ pg/mL}$) than the A β 40 level in negative control cells ($15.45 \pm$

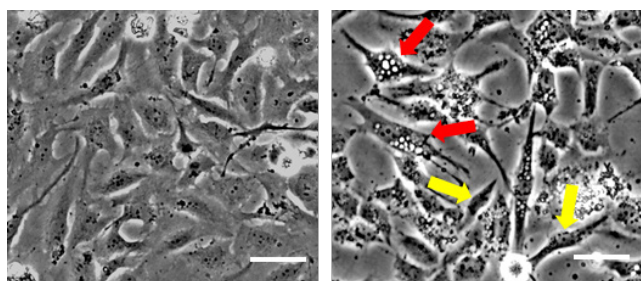


Fig. 1. Morphological alterations and the formation of vacuoles were observed in HBEC-5i cells after 24 hrs of treatment. (a) HBEC-5i in the control group (NC), with no treatment, showing no vacuole formation; (b) HBEC-5i treated with CQ at $70.5 \mu\text{M}$, where vacuole formation was observed along with distinct cell shrinkage. Microscopic observations were performed at 100x magnification. The yellow arrow showed minor shrinkage of the HBEC-5i cells, and red arrow showed prominent vacuole of the cell's cytoplasm. Scale bar measurement: 1 cm = 50 μm .

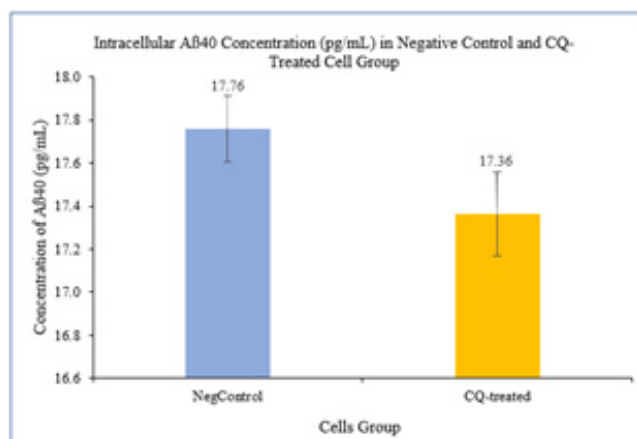


Fig. 3. Intracellular Amyloid beta 40 (Aβ40) level in HBEC-5i cell lysate of negative control and CQ-treated group (70.5μM) for three biological replicates (n=3). Low level of intracellular Aβ40 in CQ-treated cells group (17.36 ± 0.196 pg/mL) as compared to the NC cell groups (17.76 ± 0.154 pg/mL), however, it was not significant.

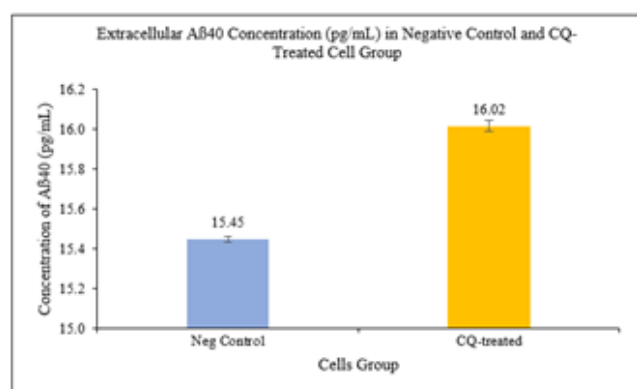


Fig. 4. Extracellular Amyloid beta 40 (Aβ40) level in HBEC-5i's supernatant of negative control and CQ-treated group (70.5μM). High level of extracellular Aβ40 level (16.02 ± 0.030 pg/mL) in HBEC-5i's compared to NC group (15.45 ± 0.015 pg/mL), however, it was not significant.

0.015 pg/mL).

4. Discussion

Lysosomes are cellular organelles that play a vital role in cellular homeostasis as they are involved in various cellular degradation pathways, including autolysosome formation for intracellular macromolecule degradation [16,17]. However, when the lysosomal function is compromised, the autophagosome and lysosomal fusion are impaired, leading to the failure of the cellular degradation process. This situation then will cause the accumulation of intracellular degradation products, including toxic proteins such as Aβ [3]. The deposition of Aβ protein will cause failure and dysfunction of endothelial cells of the brain and blood-brain barrier (BBB), which is one of the clinical characteristics of cerebral amyloid angiopathy (CAA) and Alzheimer's disease (AD) [3,4].

In this study, the lysosomal dysfunction in the HBEC-5i cell line was induced by Chloroquine (CQ) (70.5 μM). This concentration was established from previous study that was found to induce cellular changes associated with lysosomal dysfunction, such as LC3-II accumulation and an increase in Aβ42 levels in HBEC-5i cells while maintaining cell viability [2]. After 24 hours of incubation, mi-

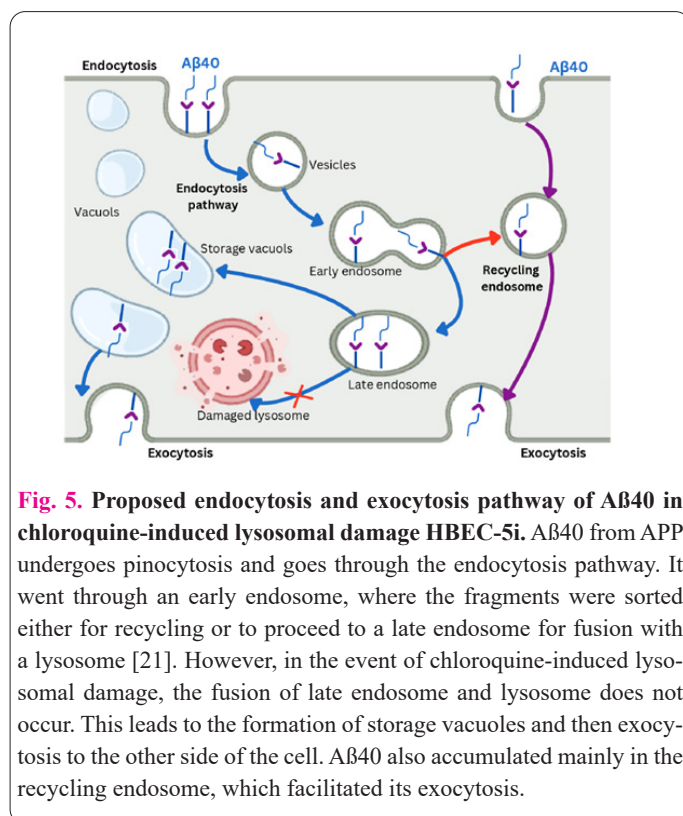
croscopic examination revealed elongation of cells treated with CQ and prominent cytosolic vacuole formation compared to NC cells. These observations aligned with existing studies that showed induction of cytosolic vacuolation in cellular systems after CQ treatment [2,18,19]. The study suggested that vacuole formation in HBEC-5i was an indicator of altered acidification and inhibition of lysosomal enzymes by CQ compound. Aside from that, the increased number of cytosolic vacuolation indicated a significant disturbance of the autophagosome-lysosome fusion. Thus, our findings suggest a potential role for BBB dysfunction as an early contributor to AD pathology with lysosome disruption on endothelial cells.

The analysis of cytosolic vacuolation by using ImageJ program showed that the lysosomal dysfunction model had induced a significant increase in the vacuolation density per cell. However, there is only a slight decrease in the surface area size despite no significant difference compared to NC groups. This suggests that at the concentration of 70.5 μM, CQ could change the cell's cellular function but not the size of surface area or cell viability [2].

This study also found that CQ-treated cells exhibited decreased intracellular Aβ40 levels but increased extracellular Aβ40 compared to controls, despite their no significant mean difference as compared to the NC cells group. This led to the proposal that Aβ40 oligomers underwent transcytosis by being transported out of cells [20,21]. Studies on endocytosis mechanisms support this idea, indicating different endocytotic pathways for Aβ40 and Aβ42 [21,23]. Aβ42 accumulates more in lysosomes, which could lead to its higher degradation and/or aggregation at lower lysosomal pH. Meanwhile, Aβ40 exhibits differential trafficking, showing significant accumulation in recycling endosomes. This accumulation may facilitate its transcytosis across the blood-brain barrier (BBB), which could explain its low levels in the endothelium [21].

This suggestion is aligned with a study on endocytosis mechanisms of Aβ in polarised human cerebral microvascular endothelial (hCMEC/D3) cell monolayers. In the study, they proved that following endocytosis, the Aβ protein was sorted by endo-lysosomal system but different endocytotic pathways. Aβ40 were transported into the cell through early and late endosomes, then robustly accumulated in recycling endosomes, which facilitate its exocytosis by the endothelial cells. In comparison, Aβ42 was accumulated more in late endosomes and subsequent lysosomes, which made it more susceptible to lysosomal degradation compared to Aβ40 (Fig. 5) [21]. In addition to that, the exocytosis rate of intracellular Aβ40 was found to be 3-fold higher than Aβ42 [22]. Other research on CQ's effects on endothelial growth factor receptors suggested that CQ selectively influences biological pathways, impacting Aβ40 levels differently [23].

While lysosomal dysfunction induced morphological changes and cytosolic vacuolation, it did not significantly alter Aβ40 levels. This suggests an efficient transcytosis pathway for Aβ40 transport across cells during lysosomal dysfunction. Further investigations could explore the routing of tagged Aβ40 and Aβ42 within the cytosolic vacuole to deepen the understanding of other BBB components such as astrocytes and pericytes. Since this in-vitro study was found to induce cytosolic vacuoles in endothelial cells, developing new lysosomal-targeting drugs that can selectively improve the degradation of organelles without



exacerbating vacuolation or inducing toxicity could provide a new avenue for AD treatment. The ability to modulate lysosomal activity might reduce the accumulation of any toxic components in the cells and preserve the BBB normal physiology.

5. Conclusion

Lysosomal dysfunction induced in HBEC-5i resulted in prominent morphological changes impacting general morphology, vacuolation density and vacuole diameter. The observed changes in cytosolic vacuolation indicated a disturbance in cellular systems. However, there was no significant difference in intracellular and extracellular Aβ40 levels, suggesting that Aβ40 has gone through different pathways of degradation in the cells after CQ treatment. Even though Aβ40 showed less toxicity than Aβ42, its importance in the Aβ42/40 ratio was undeniable. Comprehensive studies should be performed to further understand the behaviors of both Aβ isoforms within cells under CQ-induced lysosomal dysfunction in a co-culture system that closely recapitulates the blood-brain barrier, thereby elucidating the interaction between lysosomal dysfunction in the BBB compartment and its implications in AD.

Abbreviation

Aβ: Amyloid beta; AD: Alzheimer disease; CAA cerebral amyloid angiopathy; CQ: Chloroquine; HBEC: Human brain endothelial cells; NC: Negative control

Conflict of Interests

The authors declare that there are no conflicts of interest in this work.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

No human or animals were used in the present research.

Informed Consent

The authors declare that no patients were used in this study.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Nor Azma Hannah Mazlan: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft. Nurul Farhana Jufri: Funding, Conceptualization, Methodology, Validation, Writing - review & editing, Supervision. Mohd Hamzah Mohd Nasir: Conceptualization, Methodology, Validation, Supervision, Writing - review and editing. Farah Wahida Ibrahim, Asmah Hamid: Writing - review. Iffah Nadiah Laili: Investigation.

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References

1. WHO, "Global action plan on the public health response to dementia, 2017 – 2025.," WHO.
2. Laili IN, Nasir MHN, Jufri NF, Ibrahim FW, Hamid A (2023) Lysosomal dysfunction induced cytosolic vacuolation and increased intracellular amyloid-beta 42 (Aβ42) in human brain endothelial cells (HBEC-5i). *Biomed Pharmacother* 161:114501. doi: 10.1016/j.biopha.2023.114501.
3. Hampel H, Hardy J, Blennow K (2021) The Amyloid-β pathway in Alzheimer's disease. *Mol Psychiatry* 26: 5481–5503. doi: 10.1038/s41380-021-01249-0.
4. Qi X, Ma J (2017) The role of amyloid beta clearance in cerebral amyloid angiopathy: more potential therapeutic target. *Transl Neurodegener* 6: 22. doi: 10.1186/s40035-017-0091-7.
5. d'Uscio LV, He T, Santhanam AV, Katusic ZS (2018) Endothelium-specific amyloid precursor protein deficiency causes endothelial dysfunction in cerebral arteries. *J. Cereb. Blood Flow Metab* 38:1715–1726. doi: 10.1177/0271678X17735418.
6. Qiu T, Liu Q, Chen Y, Zhao Y, Li Y (2015) Aβ 42 and Aβ 40: similarities and differences. *J Pept Sci* 21: 522–529. doi: 10.1002/psc.2789.
7. Murphy MP, LeVine H (2010) Alzheimer's disease and the amyloid-β peptide. *J. Alzheimer's Dis* 19: 311–323. doi: 10.3233/JAD-2010-1221.
8. Wilczyńska K, Waszkiewicz N (2020) Diagnostic utility of selected serum dementia biomarkers: amyloid β-40, amyloid β-42, tau protein, and YKL-40: A review. *J Clin Med* 3452. doi: 10.3390/jcm9113452.
9. Chang Y, Chen Y (2014) The coexistence of an equal amount of Alzheimer's amyloid-β 40 and 42 forms structurally stable and toxic oligomers through a distinct pathway. *FEBS J* 281: 2674–2687. doi: 10.1111/febs.12813.
10. Fandos N, Pérez-Grijalba V, Pesini P, Olmos S, Bossa M, Villemagne VL, Doecke J, Fowler C, Masters CL, Sarasa M, & AIBL Research Group (2017) Plasma amyloid β 42/40 ratios as biomarkers for amyloid β cerebral deposition in cognitively normal individuals. *Alzheimers Dement (Amst)* 8: 179–187. doi: 10.1016/j.dadm.2017.07.004.

11. Yim WWY, Mizushima N (2020) Lysosome biology in autophagy. *Cell Discov* 6. doi: 10.1038/s41421-020-0141-7.
12. Shubin AV, Demidyuk IV, Komissarov AA, Rafieva LM, Kostrov SV (2016) Cytoplasmic vacuolization in cell death and survival. *Oncotarget* 7: 55863–55889. doi: 10.18632/oncotarget.10150.
13. Sharma S, Ghufra SM, Ghose S, Biswas S (2021) Cytoplasmic vacuolation with endoplasmic reticulum stress directs sorafenib induced non-apoptotic cell death in hepatic stellate cells. *Sci Rep* 11: 3089. doi: 10.1038/s41598-021-82381-3.
14. Zhang W, Xu C, Sun J, Shen HM, Wang J, Yang C (2022) Impairment of the autophagy–lysosomal pathway in Alzheimer’s diseases: Pathogenic mechanisms and therapeutic potential. *Acta Pharm Sin* 12: 1019–1040. doi: 10.1016/j.apsb.2022.01.008.
15. Whyte LS, Lau AA, Hemsley KM, Hopwood JJ, Sargeant TJ (2017) Endo-lysosomal and autophagic dysfunction: a driving factor in Alzheimer’s disease? *J Neurochem* 140: 703–717. doi: 10.1111/jnc.13935.
16. Malik BR, Maddison DC, Smith GA, Peters OM (2019) Autophagic and endo-lysosomal dysfunction in neurodegenerative disease. *Mol Brain* 12(100). doi: 10.1186/s13041-019-0504-x.
17. Cooper GM. Lysosome. *The Cell: A Molecular Approach*. 2nd edition., 2nd Edition. Sunderland (MA): Sinauer Associates, 2000.
18. Hampel H, O’Byrne SE, Molinuevo JL, Zetterberg H, Masters CL, Lista S, Kiddle SJ, Batrla R, Blennow K (2018) Blood-based biomarkers for Alzheimer disease: mapping the road to the clinic. *Nat Rev Neurol* 14: 639–652. doi: 10.1038/s41582-018-0079-7.
19. Korolenko TA, Johnston TP, Vetvicka V (2020) Lysosomotropic features and autophagy modulators among medical drugs: evaluation of their role in pathologies. *Molecules* 25(5052) doi: 10.3390/molecules25215052.
20. Serra ND, Sundaram MV (2021) Transcytosis in the development and morphogenesis of epithelial tissue. *EMBO J* 40. doi: 10.15252/embj.2020106163.
21. Wang Z, Sharda N, Omtri RS, Li L, Kandimalla KK (2023) Amyloid-beta peptides 40 and 42 employ distinct molecular pathways for cell entry and intracellular transit at the blood–brain barrier endothelium. *Mol Pharmacol* 104 : 203–213. doi: 10.1124/molpharm.123.000670.
22. Swaminathan SK, Ahlschwede KM, Sarma V, Curran GL, Omtri RS, Decklever T, Lowe VJ, Poduslo JF, Kandimalla KK (2018) Insulin differentially affects the distribution kinetics of amyloid beta 40 and 42 in plasma and brain. *J Cereb Blood Flow Metab* 38: 904–918. doi: 10.1177/0271678X17709709.
23. Wesén E, Jeffries GDM, Matson Dzebo M, Esbjörner EK (2017) Endocytic uptake of monomeric amyloid- β peptides is clathrin- and dynamin-independent and results in selective accumulation of A β (1–42) compared to A β (1–40). *Sci Rep* 7. doi: 10.1038/s41598-017-02227-9.