AUSTRALIAN SOCIETY FOR BIOPHYSICS

Abstract: 108O

How Do Steroids Impact Batten's Disease?

Trang D. Le[a], Alvaro Garcia[b] Evelyne Deplazes [c], Ronald J. Clarke[b], Charles Cranfield[a]

[a] School of Life Sciences, University of Technology Sydney, Sydney, Australia.
[b] School of Chemistry, University of Sydney, Sydney, Australia.
[c] School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Australia.

Cell death can occur if cellular materials are not properly recycled [1, 2], an action linked to the lysosome of cells [1]. Battenin, encoded by ceroid-lipofuscinosis neuronal 3 (CLN3) gene, is an integral membrane protein located within the lysosomal membrane, but its function is still unknown [3]. Mutation in the CLN3 gene would lead to lack of functional battenin, leading to Batten's disease (BD) [4] which is a fatal inherited disorder where cell death occurs due to build-up of lipofuscins (cell waste material) in cells [2]. Schultz et al 2018[5] demonstrated that some steroids, particularly carbenoxolone (CBX), enoxolone (EXO) and 7-ketocholesterol (7-keto) can correct several types of structural defects in the membranes of Batten's disease mice and alleviate their symptoms [5]. Our research investigates possible mechanisms for this improvement by observing which membrane properties would differ in presence of those steroids.

By using Tethered Bilayer Lipid Membranes (tBLMs) to measure membrane conductance, we can observe changes in the permeability of DOPC membrane to cations in the presence of aforementioned steroids. However, the data did not suggest any significant effects on permeability when the steroids were added directly onto formed tBLMs. It's an unusual result for substances that demonstrated the ability to correct membrane's structural properties. Hence, we made tethered membrane with steroids embedded into them and identified changes in membrane's conductance that way. We then identified if the presence of these steroids could alter the *apparent* dissociation constant (K_d) of the membrane for Ca^{2+} by contrasting the conductance decreases caused by this divalent cation against a fixed concentration of Na^+ using a method reported previously [6]. Changes in the K_d were hypothesised to be as a result of membrane dipole potential (Ψd) changes. To test this, we looked for indications of dipole potential changes through fluorescent experiments involving the voltage-sensitive probe RH421. As a way to show steroids' capability in altering membrane's structure, we also investigated the effect of the steroids on the strength of intermolecular forces within the membrane using differential scanning calorimetry (DSC).

[1] Cotman, S.L. & Staropoli, J.F. 2012, 'The juvenile Batten disease protein, CLN3, and its role in regulating anterograde and retrograde post-Golgi trafficking', Clinical Lipidology, vol. 7, no. 1, pp. 79–91.
[2] Double, K.L., Dedov, V.N., Fedorow, H., Kettle, E., Halliday, G.M., Garner, B. & Brunk, U.T. 2008, 'The comparative biology of neuromelanin and lipofuscin in the human brain', Cellular and Molecular Life Sciences, vol. 65, no. 11, pp. 1669–82.
[3] Bennett, M.J. & Rakheja, D. 2013, 'The neuronal ceroid-lipofuscinoses', Developmental Disabilities Research Reviews, vol. 17, no. 3, pp. 254–9.
[4] Shematorova, E.K., Shpakovski, D.G., Chernysheva, A.D. et al. Molecular mechanisms of the juvenile form of Batten disease: important role of MAPK signaling pathways (ERK1/ERK2, JNK and p38) in pathogenesis of the malady. Biol Direct 13, 19 (2018).
[5] Schultz, M.L., Tecedor, L., Lysenko, E., Ramachandran, S., Stein, C.S. & Davidson, B.L. 2018, 'Modulating membrane fluidity corrects Batten disease phenotypes in vitro and in vivo', Neurobiology of disease, vol. 115, pp. 182–93.
[6] Deplazes, E., Tafalla, B. D., Murphy, C., White, J., Cranfield, C. G., & Garcia, A. (2021). Calcium ion binding at the lipid–water interface alters the ion permeability of phospholipid bilayers. Langmuir, 37(48), 14026-14033.