Summary:

Leber's Hereditary Optic Neuropathy (LHON) is a retinal ganglionic neurodegenerative disorder caused by a maternally inherited mitochondrial DNA point mutation. The dysfunction of complex I of the respiratory chain, affected by the mutation, results in an increased production of reactive oxygen species and a decrease in ATP synthesis. This situation can lead to an imbalance in the production and demand of energy required for cell survival. In such a state the cell may die by apoptosis, which in LHON disorder is most prominently apparent as optic nerve degeneration. Cybrid cells are the most frequently used model for this disease as the optic nerve of a LHON patient is not accessible until the death of the patient and by which time the degeneration is to its full extent.

The aim of this study was the establishment of a cell-mediated LHON-model for evaluation of antioxidants and neuroprotective drugs and to discover the involvement of the mitochondrial permeability transition pore (mtPTP) in development of the disorder. Using cybrid cells harbouring the mitochondrial 11778 LHON point mutation, the efficacy of the promising neuroprotective tetracycline derivative minocycline was investigated. The drug increased the cell viability of LHON cybrids in the MTT assay after addition of the apoptotic stimulus, thapsigargin (TG). In contrast, this pattern of protection was not evident in the parental control cell line (NT2). A comparable protection to minocycline was observed by treatment with cyclosporine A (CsA), a well known blocker of the mtPTP. Furthermore, a general caspase inhibitor (z-VAD-FMK) did not prevent the thapsigargin-induced cell death in either of the cell lines. Whereas, staining of the nuclei with DAPI showed nuclear condensation and apoptotic morphology of the cell. In support of this, immunocytochemistry revealed the release of the apoptosis-inducing factor (AIF) from the mitochondria and translocation to the nucleus, suggesting a mtPTP-induced caspase independent apoptosis. Ratiometric Ca^{2+} imaging reveals that acetylcholine/TG triggered elevation of the cytosolic calcium concentration is alleviated by both minocycline and CsA, also suggesting an involvement of permeability transition. In addition the mitochondrial membrane potential ($\Delta\Psi_m$) of LHON cybrid cells, as evaluated by the uptake of TMRM, was significantly conserved with both minocycline and CsA treatments. Western blots showed a decrease in the ratio of active-caspase-3 to procaspase-3 proteins in the minocycline and CsA treated groups of cybrid cells after TG-induced cell death, which suggests the regulation of downstream apoptotic events. This is in contradiction to the obtained results in the MTT assay using z-VAD-FMK, which indicates that the TG-triggered cell death is a non-caspase-dependent process. The release of pro-apoptotic factors (e.g. AIF) from the mitochondria is the proposed mechanism of cell death. But parallel pathways , causing activation of caspases, are also possibly active. Blockade of the mtPTP opening by CsA and minocycline prevents death of the LHON cybrids. Additionally, minocycline dose dependently decreased the oxidative stress in LHON cells, supporting the good anti-oxidant property of this tetracycline.

In conclusion, the opening of the mtPTP is involved in the onset of LHON and minocycline provides beneficial effects, beside its antioxidant property, through inhibition of mtPTP. Further investigation into the effect of minocycline, possibly in an *in vivo* model of the disease, such as a LHON ND4 mutant mouse, would be necessary to prove its protective mechanisms on the retinal ganglionic cell layer and the optic nerve.