NO-MEDIATED NEUROINFLAMMATORY PATHWAYS AS TREATMENT TARGETS IN NEURODEGENERATION

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Numerous neurodegenerative diseases associated with protein misfolding (e.g. Alzheimer's and Parkinson's disease) exhibit enhanced oxidative and nitrergic stress conditions following initiation of neuroinflammatory pathways. The underlying activation of microglia within the central nervous system is responsible for release of pro-inflammatory molecules associated with nitric oxide (NO) production, a potent contribution to cytotoxic redox signaling. NO-mediated post-translational protein modifications impact upon protein functions and can exacerbate pathological processes. In addition, non-enzymatic and irreversible glycation signaling has been implicated as a pathway that promotes protein misfolding via the generation of advanced glycation end-products (AGE). Following activation of specific receptors recognizing AGEs (RAGE) further oxidative stress and cytokines production induces an upregulation of inflammatory mediators. However, the direct interactions between both, NO-mediated neuroinflammation and RAGE signaling remain poorly understood.
We investigated the therapeutic potential of suppressing NO signaling during early prion disease progression. To study the impacts of NO on the pathology, prion-diseased mice were injected daily with a NO synthase (NOS) inhibitor during disease onset. Neurophysiology and disease marker properties during early pathology were analysed.
Strong neuroinflammation characterized by enhanced nitrergic and oxidative stress was associated with a decline in hippocampal neuronal function in diseased mice during 6 to 10 weeks post inoculation (w.p.i.) with scrapie prion protein. Daily i.p. administration of the NOS inhibitor L-NAME between 6 and 9 w.p.i. prevented the functional degeneration of hippocampal neurons. We further found that this intervention reduced 3-nitrotyrosination of triose-phosphate isomerase (TPI), an enzyme involved in the formation of disease-associated glycation and AGE formation. Furthermore, L-NAME application reduced the degree of TPI-nitrotyrosination and the expression of RAGE. This work concludes that NO mediated post-translational modifications of TPI may enhance glycation signaling which contributes to further cytotoxicity and accumulation of misfolded prion proteins and thus illustrates an interaction between glycation and NO signaling.

6) SFRR-E Oral Presentation – Abstracts in Sequence of Presentation

SFRR-E Early Career Research Symposium

[YIA] CHANGES IN NADPH OXIDASE ACTIVITY IN E-CIGARETTE VAPOR CONDENSATE EXPOSED CULTURED CELLS AND THE ROLE OF ACROLEIN

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Even though electronic cigarettes (Ecig) are marketed as a healthier substitute for tobacco cigarettes, evidence is arising, that Ecig vapor could cause adverse health effects. It is assumed that Ecig liquid components that are degraded thermally are the ones responsible for the observed effects on human health, with toxic aldehydes being the most prominent group. The aim of this study is to evaluate the mechanistic effects of Ecig vapor toxicity on NADPH oxidase activation in immune and vascular cells, as well as to test if acrolein, a byproduct of Ecig liquid heating, is the main malefactor. My group previously showed that Ecig vapor exposure causes oxidative stress, inflammation, apoptosis, endothelial dysfunction and high blood pressure in a mouse model, through activation of NADPH oxidase [Kuntic et al. Eur. Heart J. 2020]. To understand the mechanism of NAPDH oxidase activation better, we have now exposed cultured endothelial cells (EA.hy 925) and macrophages (RAW 264.7) to condensed Ecig vapor (EcigCon). We observed that incubation of EA.hy 925 and RAW 264.7 cells with EcigCon leads to concentration-dependent cell death. In both EA.hy 925 and RAW 264.7 cells, EcigCon incubation promoted the transfer of the cytosolic NADPH oxidase subunits (p47phox, p67phox and Rac1) to the plasma membrane, hence showing the activation of the enzyme complex. Recent studies have shown that among toxic aldehydes found in Ecig vapor, acrolein plays a prominent role. Thus, we incubated both EA.hy 925 and RAW 264.7 cells with increasing concentrations of acrolein. It was again observed that p47phox, p67phox and Rac1 translocate to the plasma membrane. These data show that acrolein could be a significant part of Ecig vapor induced oxidative stress and cell death. More understanding is still needed to disclose the full mechanism of the negative effects of consumption of Ecig and the possible long-term toxicity.

[YIA] PROTEASOME ACTIVATION IN C. ELEGANS CAUSES MILD MITOCHONDRIAL DEFECTS; IS THIS THE LINK TO LIFESPAN EXTENSION?

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Protein homeostasis is extensively regulated by a variety of proteostatic mechanisms that are considered the guardians of the proteome and ensure precise synthesis, maintenance, function and elimination of proteins. These mechanisms tend to fail with ageing, thus contributing to loss of proteostasis, one of the ageing hallmarks. Our group and others have previously shown that activation of the proteasome, that is responsible for approximately 80% of protein degradation, can prolong the lifespan of C. elegans and D. melanogaster. However, how exactly proteasome activation facilitates lifespan extension, remains unknown. In this study, we have attempted to shed light onto this open question by focusing on the underlying molecular effects of proteasome activation. A proteomic analysis of C. elegans with an activated proteasome revealed differentially regulated glycolysis, a metabolic shift compatible with mitochondrial deficiency. Evaluation of various mitochondrial parameters showed that mitochondria were depolarized and fragmented in nematodes with an activated proteasome compared to control. Although further investigation is needed, our data may suggest that a mild mitochondrial defect accounts for the lifespan extension found in nematodes with an activated proteasome. Our future work will focus on determining how proteasome activation causes mitochondrial defects and if indeed the latter are responsible for the observed lifespan extension.

[YIA] EVIDENCE OF FERROPTOSIS INVOLVEMENT IN RETT SYNDROME PATHOGENESIS

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Rett syndrome (RTT) is a rare neurodevelopmental disorder caused in 90% of the cases by mutation in the X-linked gene encoding for MeCP2, an important epigenetic regulator. RTT patients show compromised metabolic processes including redox imbalance, dysfunctional mitochondrial bioenergetics and altered lipid metabolism. Since several molecular aspects involved in the pathophysiological mechanisms of RTT could suggest a possible role of ferroptosis, an iron-dependent cell death characterized by excessive lipid peroxidation, the aim of our study was to evaluate RTT susceptibility to this type of cell death using primary fibroblasts obtained from RTT patients. As a first step, we observed an increase of cell death rate in RTT compared to controls after treatment with several concentrations of two ferroptosis inducers: erastin (GPX4 inhibitor) or RSL3 (inhibitor of the cystine/glutamate antiporter). At the same time, the co-treatment with ferrostatin-1, a well know inhibitor of ferroptosis, reduced the levels of cell death. In addition, we found changes in GPx and GR activity after 3h treatment with 10 μM erastin or 5 nM RSL3, while Western blot analysis also showed an alteration in GPX4 protein levels and in formation of 4HNE protein adducts, after the treatment with the same doses of erastin and RSL3 for 3 and 6h. Finally, both the mitochondrial ROS production and lipid peroxidation levels were higher in RTT after the induction of ferroptosis with the two molecules, while ferrostatin-1 co-treatment significantly prevented these processes. In conclusion, our results indicate an increased vulnerability of RTT cells to ferroptosis that could contribute to the clinical features of RTT phenotypes, also