Mitochondrial dysfunction in Alzheimer's disease: an investigation into whether excessive damage or impaired clearance contributes to selective neuronal vulnerability in the hippocampus.

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ABSTRACT
Selective neuronal vulnerability (SNV) in the hippocampus is a hallmark feature of Alzheimer's disease (AD) pathology. For example, the Cornus-Ammonis (CA)-1 subfield is particularly vulnerable to AD pathology compared to CA3. Although the underlying mechanism of SNV in the AD hippocampus remains elusive, the development of techniques studying of the metabolic processes within cells has highlighted an important roles for both mitochondrial dysfunction and impaired cellular clearance mechanisms in the brain of patients with Alzheimer's disease. Through the use of immunolabelling techniques on post-mortem tissue of different regions of the hippocampus, this project aims to provide further data of the presence of abnormal metabolic activity or oxidative stress in healthy and diseased brain tissue, which may be useful in clarification of the role of mitochondrial dysfunction in SNV.

INTRODUCTION

Alzheimer's disease is one of the most common neurodegenerative conditions facing society, and is characterised by the presence of extracellular amyloid β (Aβ) plaques, intracellular neurofibrillary (tau) tangles, and brain atrophy. Although the majority of treatments developed in the attempt to treat AD have focussed on these hallmarks, the key to preventing the progression of neurodegeneration in patients may lie in greater understanding and targeting of the selective neuronal vulnerability exhibited in patients with the disease. The development of therapies able to restrict tau tangles and Aβ plaques to the entorhinal cortex and CA1 regions of the hippocampus can limit cognitive difficulties experienced by patients, and there is potential to provide neuroprotection to all cells of the CNS before any pathology can develop.

SELECTIVE NEURONAL VULNERABILITY

The concept that neurons in some regions of the brain may be more susceptible to the development of AD pathology was first highlighted in 1991 by Braak et al, in which progression of tau tangles from the transentorhinal region, through the limbic regions, and onto the neocortex in AD was organised into 'stages'. Cells are currently thought to succumb to pathology if intracellular and extracellular stressors exceed the capacity of neurons and glia to maintain homeostasis. Because of this, cells that are exposed to a greater stressor load, or have a lower threshold of capacity to combat these stresses, will be much more vulnerable to damage and death than unstressed neurons. The identification of several potential causes of SNV in AD, such as endoplasmic reticulum (ER) stress, impaired intracellular clearance, alterations to Ca^{2+} handling, and inflammation have highlighted the complexity of pathways involved in maintenance and restoration of homeostasis. As understanding of these mechanisms progress, mitochondrial dysfunction is increasingly well-posited to take a central role in SNV as both an initiator and exacerbator of cellular stress.

MITOCHONDRIAL DYSFUNCTION

Changes to mitochondrial morphology can be seen at the earliest stages of AD pathology in vulnerable regions of the CNS, with Zhang et al in 2016 using transmission electron microscopy and super-resolution immunofluorescence to show the presence of a 'mitochondria on a string' phenotype. These alterations in organelle structure are also accompanied by a deterioration in

organelle function, reflected by significant reduction in the rate of glucose metabolism seen with positron emission tomography (PET) scanning.
The mechanism in which abnormal mitochondrial activity can increase neuronal vulnerability to AD pathology are currently unclear, but multiple pathways are likely to be involved. For example, the reduced ability of mitochondria to act as intracellular stores of Ca^{2+} may lead to pathologically high cytosolic Ca^{2+} concentrations, leading to disruption of intracellular signalling, calcification of cellular structures, and activation of apoptotic or necrotic pathways.
The generation of reactive oxygen species (ROS) as a consequence of electron leakage from the respiratory chain complex is also likely to be involved in cellular distress. ROS have the capacity to inflict substantial oxidative damage on many intracellular components, which has detrimental effects on multiple cellular pathways and structures. Because of this, cells with an increased rate of synthesis of ROS are more likely to overcome the protective mechanisms responsible for the maintenance and restoration of homeostasis. Cells exhibiting selective neuronal vulnerability may also have a lower tolerance to the effects of mitochondrial dysfunction: neurons within vulnerable regions of the brain exhibit a much greater ratio of ROS:antioxidant generation, therefore are more likely to suffer oxidative damage from the same degree of mitochondrial dysfunction compared to neurons with a faster rate of antioxidant production.
However, mitochondrial dysfunction may not necessarily be the initiator of Alzheimer's disease pathology; abnormal Ca^{2+} handling and ROS production may occur in response to damage inflicted on mitochondria by other agents. As well as this, insulin resistance and vascular insufficiency may contribute to insufficient ATP generation and oxidative stres, potentially explaining the increased risk of AD in patients with cardiovascular disease and diabetes mellitus.
Factors independent to mitochondrial function can also contribute to abnormal metabolism. For example, a 1998 study by Mutsaers and Carroll has indicated that inhibited intracellular trafficking in demyelinated axons can cause focal accumulation of mitochondria and prevent their transport to sites of high ATP demand and increased intracellular Ca^{2+} concentration, and multiple experiments have highlighted defects in the mitophagy pathway responsible for clearance of damaged mitochondria.
This could suggest that mitochondrial dysfunction is not necessarily the sole cause of cellular dysfunction, but can greatly exacerbate the stress neurons are exposed to. If this occurs in cells with pre-existing vulnerabilities to the effects of oxidative stress, it may then tip the balance of homeostatic mechanisms to result in neuronal dysfunction.

PROJECT AIMS AND POTENTIAL LIMITATIONS

This project aims to compare markers mitochondrial function and clearance mechanisms in different regions of hippocampus in order to identify potential pathways contributing to the differences in susceptibility to oxidative damage and other Alzheimer's disease pathology. Medial temporal cortex and hippocampal CA1 and CA3 subfields of human post-mortem tissue of AD cases (n=8) and non-neurologic controls (n=5) will be immunolabelled for relevant markers of oxidative stress, mitochondrial function, and the mitochondrial clearance pathways for downstream qualitative and quantitative analyses. Time permitting, additional techniques, such as laser capture microdissection of neuronal and microglial populations from CA1 and CA3 subfields, will be used for proteomic analyses. Both of these techniques will hopefully provide further evidence to support the hypothesis of mitochondrial dysfunction and oxidative damage playing a major role in the mechanisms underlying SNV in AD, and may even highlight pathways with the potential to be exploited clinically as therapeutic targets or biomarkers of disease.
However, there are substantial potential limitations that may impede the ability to meet the objectives of this project. The COVID-19 pandemic has already significantly delayed the start of the laboratory work, and social distancing restrictions threaten my ability to learn and perform the techniques described above. As well as this, the small sample size of tissue used reduces the

reliability of any findings, however the use of human brain tissue samples, opposed to animal models less reflective of disease pathology, does somewhat compensate for this.

POTENTIAL THERAPIES AND FUTURE QUESTIONS

Therapies that act to reduce oxidative stress have already shown promise in treating AD, such as the finding in 1991 by Crapper McLachlan that dementia patients given intramuscular injections of the iron chelator desferrioxamine had reduced disease progression compared to controls, as well as the Rotterdam study highlighting a correlation between dietary intake of vitamins A, C, and E with reduced risk of Alzheimer's disease. This emphasises the importance of promoting a healthy diet and lifestyle in patients to prevent AD pathology from developing, as well as the potential for therapies such as selective delivery of antioxidants to support vulnerable neurons to prevent progression of AD. In addition to this, monoclonal antibodies could be used to downregulate neuronal expression of insulin receptors to adequately supply cells with glucose, intrabody therapies may potentially stimulate mitophagy pathways, and transcriptional upregulation of Ca^{2+} binding protein expression to prevent the accumulation of Ca^{2+} within the cytoplasm. However, much of the mitochondria's role in selective neuronal vulnerability remains to be understood before effective and safe therapies targeting metabolic dysfunction can be developed.