Quantification of Cox4 in the prefrontal cortex in post stroke, vascular, and other ageing related dementias

Jack Eklid* supervised by Vincent Foster, Arthur Oakley and Prof Raj Kalaria

Introduction

- Stroke is a major risk factor for dementia, with a reported 24% incidence of post-stroke dementia within 3.8 years following stroke.
- Little is known about PSD pathology compared to cognitively healthy stroke survivors.
- Lesions in the prefrontal circuitry are linked to executive dysfunction observed in vascular related dementias.
- It was previously found that pyramidal cell volumes in Layers III and V (indicated by arrows) of the dorsolateral prefrontal cortex (dlPFC) of post stroke subjects to be reduced by 30-40% compared to controls and post stroke non-dementia cases¹.

Aims/Objectives

- To quantify Cytochrome c oxidase subunit IV (Cox4) in layers III and V pyramidal neurons of the dorsolateral prefrontal cortex (dlPFC) in post mortem demented brains.
- To investigate Cox4 relationship to pyramidal neuron volumes and executive function in post stroke dementias.

Method

- 60 cases (10 X Control, PSD, PSND, VaD, AD and Mix).
- Neuropsychometric measures were available for post-stroke cases through the CogFAST study.
- Serially cut 10µm wax-embedded paraffin tissue blocks were stained with anti-Cox4 antibody and visualised using diaminobenzidine (DAB). 10 images were taken per case at 10X magnification using a Zeiss Axioplan 2 microscope.
- Image analysis: Using Image Pro software, Cox4 was quantified by measuring Per Area (P/A) and Integrated Optical Density (IOD) from 10 images of layer III and 10 images of layer V for each case, see Image 1. Pyramidal neuronal counts were performed on 10 images and the mean was calculated for each case.

Results

Per Area (PA) analysis of Cox4 showed a significant decrease in Layer III in PSD (P=0.019), VaD (P=0.028) and Mix (P=0.034) when compared to controls. Layer V showed a significant decrease in PSND (P=0.019), PSD (P=0.004), VaD (P=0.049) and Mix (P=0.004) compared to controls. A trend between PSND and PSD patients was found in Layer V, with P/A of PSD comparably less than PSND (P=0.086). See Graph 1

IOD analysis showed a significant decrease in Layer III in PSD (P=0.034) and VaD (P=0.041), with a trend in Mix (P=0.070) compared to controls. Layer V showed a significant decrease in PSND (P=0.019), PSD (P=0.003), VaD (0.049) and Mix (P=0.004) compared to controls. See Graph 2

On examination of relationships between Layer V PA data from PSND and PSD patients with neuropsychometric measures, we found correlations with Memory-Learning (rhow=-0.553, P=0.017), attention (rhow=-0.566, P=0.014) and MMSE score from CAMCOG examination (rhow=-0.474, P=0.047). Layer V IOD data from PSND and PSD patients correlated with attention (rhow=-0.502, P=0.034). See Graphs 3 and 4

The number of Cox4 +ve pyramidal neurons in layer III significantly decreased in PSND (P=0.001), PSD (P=0.013), VaD (0.001) and Mix (P=0.001) compared to controls. Layer V showed a similar decrease, with PSND (P=0.002), PSD (P=0.002), VaD (P=0.031), Mix (P=0.002) and AD (P=0.019) all showing a significant decrease in pyramidal neurons compared to controls.

Discussion

- Cox4 was stained regardless of neuronal type. Layers III and V contain high numbers of pyramidal neurons and so we infer that the quantitative changes observed were attributed to pyramidal neurons.
- It is difficult to delineate from this study whether the quantitative changes in Cox4 are attributable to a decrease in expression in all pyramidal neurons or a decrease in number of Cox4 +ve pyramidal neurons.

Future work:
A further stain (e.g. Nissl) needs to be integrated into the study to obtain a ratio of cells expressing Cox4 to cells not expressing Cox4.

Conclusion

- We showed a decrease in COX4 expression in those suffering CVD based dementia when compared to controls, suggesting a link between vascular pathology and metabolic expression.
- Quantity of Layer V Cox4 positive neurons in post stroke cases may indicate executive dysfunction.

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References: ¹Foster V et al. Pyramidal neurons of the prefrontal cortex in post stroke dementia. Brain 2014; 137(Pt 9):2509-21

*Student number 120023426, BSc Physiological Sciences, j.eklid@ncl.ac.uk