IHS Fellowship report

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The link between migraine aura and cerebrovascular endothelial dysfunction

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Overview

Migraineurs can have vascular dysfunction and are at significantly higher risk for ischaemic or haemorrhagic stroke, cerebral white matter disease and cardiovascular events. Migraine aura is a vascular risk biomarker but mechanisms underlying this association remain unknown. Cortical spreading depolarisation (CSD) can alter characteristics of cerebrovascular endothelium.

This study aimed to determine vascular endothelial features that are altered by migraine aura to understand the underlying mechanism. We hypothesised that the massive potassium increase during CSD traverses biological barriers to reach microvascular endothelial cells and induce a phenotype change resulting in blood-brain barrier disruption and other yet unknown alterations, such as expression changes of adhesion molecules or endothelial anti-thrombotic and fibrinolytic factors, predisposing to cerebral ischaemic events.

We used non-invasive induction of cortical spreading depolarisation to prevent tissue injury and injury-related effects on neuronal cells and vasculature using optogenetics stimulation through intact skull of channelrhodopsin-2-positive mice, expressing light-sensitive ion channels in cortical neurons. After recovery we quantified adhesion molecule expression and pro/anti-thrombotic factors in the piriform cortex.

Our second experiment focused on CSD-induced transcriptome changes specifically in endothelial cells that were harvested from Tie2 mice with green fluorescent protein labelled endothelial cells, using RNA sequencing after FACS sorting.

Summary of research

In Tie-2 mice, multiple CSDs were induced through thinned skull and detected non-invasively using optical intrinsic signals from white light illumination. Ipsi- and contralateral hemispheres were harvested separately and homogenised for FACS sorting of endothelial cells. Endothelial cell samples were grouped based on experimental condition: i) CSD induction hemispheres; ii) hemispheres contralateral to CSD induction sides as internal controls; iii) both hemispheres from sham experiments as negative controls. The samples were sent to our collaborators at Leiden University in the Netherlands where RNA was isolated for RNA sequencing. Initial analysis of the first dataset looks promising, as we could identify the majority of genes known to be expressed specifically in brain endothelial cells. For some of these genes, samples collected from CSD hemispheres and control groups differed in expression levels. Additional data sets are being included to be able to state whether this trend will be significant.

In addition to my IHS project, I got the chance to contribute to several other projects in the lab, on synaptically triggerd SD in migraine mutant mice, on SD as an innate antiseizure mechanism, and on the relation between SD and seizures.

Outcome of research

Initially, we aimed to determine whether non-invasive optogenetically-triggered CSD was able to induce an endothelial phenotype change and to identify CSD-associated gene expression changes and thereby molecular mechanisms responsible for disruption of the blood-brain barrier.

Since RNA sequencing and analysis of the results is still ongoing, it is hard to say whether our aims have been met at this point. Initial results do show CSD-associated changes in gene expression levels compared to control groups, but additional analysis will have to point out which genes are up- or downregulated and in which molecular pathways this will play a role.

Conclusion

The opportunity to work in this world-leading migraine research group was a great experience in which I got to learn new techniques, broaden my scientific knowledge, and strengthen my enthusiasm for translational research. This will all help my in finishing my PhD in the Netherlands and continue my career after that. The international experience will enhance my curriculum which will be very valuable for my future career.

I would recommend future IHS Fellowship applicants make sure they are well prepared before starting the Fellowship abroad. For a smooth start, it definitely helps to familiarise yourself with the procedures you will be using. Also, it helps to ask what training you will have to follow or tests you will have to pass before you are allowed to start your experiments. It will save a lot of time if you can start those before or right after your arrival in the lab.