BMS Laboratory Visit Award Report

Elucidation of the impact of VEGF-Axxxb isoforms on retinal endothelial cell permeability in hyperglycaemia.

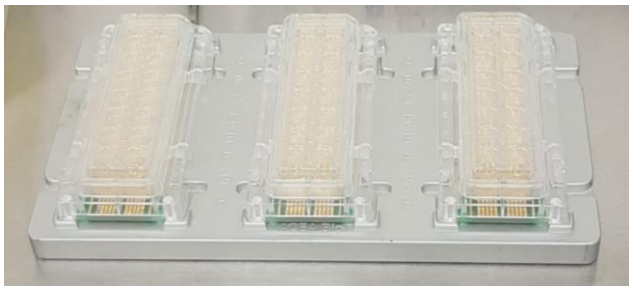
I would firstly like to begin this report by thanking the British Microcirculation Society for their invaluable support in making my trip to the University of Ulm possible. This was a very productive time, I was able to generate a lot of data over a short space of time to answer important scientific questions in my thesis and that will be written up into a manuscript soon.

Diabetic retinopathy (DR) is the leading cause of blindness in the working population, currently treated by highly invasive and expensive therapies. A major systemic symptom of diabetes is increased permeability of blood vessels which is apparent across the body including in the retina as presented in last year's BMS meeting.

Cell culture – where I cultured the iBREC line

Microvascular damage results from ischaemia-driven pro-angiogenic vascular endothelial growth factor (VEGF-A) production. Proximally spliced VEGF (VEGF-A_{165}a) is upregulated in the ischemic diabetic retina and is implicated as the principal driver of DR pathogenesis. VEGF-A can be alternatively spliced to produce anti-angiogenic VEGF-A_{xxx}b family of isoforms. Serine rich protein kinase-1 (SRPK1) regulates VEGF-A splicing and inhibition of this kinase has been shown to reduce choroidal neovascularization in mice by decreasing pro-angiogenic and increasing anti-angiogenic VEGF-A isoforms. We have previously published VEGF-A_{xxx}b blocks the increase in permeability induced in diabetic conditions in retinal pigmented epithelial cells of the outer blood retinal barrier and across the whole retina. However, it is relatively unknown as to what the effects are of VEGF-A_{165}b directly on inner blood retinal barrier made up of retinal endothelial cells of the retinal vasculature.

I arrived in Germany on 1st October and was immediately greeted by Dr. Deißler who gave me a tour of her lab and helped me move into the accommodation I would call home for the following six weeks. We began immediately the following day, she taught me how to culture her unique immortalised bovine retinal endothelial cell line and I was trained to use the xCELLigence system. This system requires cells to be grown to a monolayer on a set of gold microelectrodes. Cell medium serves as an electrical conductive buffer that allows electron flow when imposed to an electrical potential. The electron flow can be impeded by cells, the magnitude of which is dependent on the size, shape and adherence of cells. Thus, this system can be utilised to assess changes in monolayer permeability in response to hyperglycaemia and also elucidate how VEGF-A isoform expression can effect permeability. My research group



xCELLigence electronic microtiter plates (E-Plates®)

do not have this equipment, so these experiments would have not been possible without this laboratory visit.

Hyperglycaemia induced a reduction in cellular impedance thus increase in monolayer permeability after five days of treatment. This effect appeared to be due to changes in Claudin-5 and Claudin-1 expression. Inhibiting SRPK1 did not have any effect on monolayer permeability, however this could be due to the fact that the primary retinal sources of VEGF-A are retinal pigment epithelial cells and Müller cells. Recombinant VEGF-A_{165}a treatment induced an increase in monolayer permeability, whilst VEGF-A_{165}b had no effect. Interestingly, co-treating the cells with both isoforms protected against the increase in permeability produced by hyperglycaemia. These findings will be built upon in our laboratory in Nottingham.


Ulmer Münster – the tallest church in the world!

This experience developed me both professionally and personally, my collaborative skills have improved alongside my ability to work independently. I thoroughly enjoyed working with Heidrun, every morning we would begin our day with riveting scientific discussion followed by chat about the looming Brexit! I feel like I made a friend as well as an exciting collaborator. Ulm is a beautiful old town with the tallest church in the world and was a pleasure to visit. The data generated from my trip has allowed me to further understand the changes to the retinal vasculature in response to hyperglycaemia and how to potentially treat it. This strengthens my thesis and should hopefully lead to a publication after follow-up experiments. Without the generous funding from the British Microcirculation Society, this would not have been possible, so I am very grateful to the committee for their support!