Sophie Truman BPS Vacation Studentship July/August 2021

During the summer of my final year studying BSc Pharmacology at King's College London, I had the opportunity to gain invaluable laboratory experience by joining Professor Susan Brain's group in the Department of Vascular Biology and Inflammation. The vacation studentship I was awarded by the British Pharmacological Society enabled me to complete an 8-week project that meaningfully contributed to the post-doctoral research project of Dr Fulye Argunhan, who is currently investigating the protective effect of CGRP in a murine heart failure model. Recent findings have shown that in murine models, αCGRP attenuates the cardiac hypertrophy and dysfunction associated with both hypertension and pressure-overload-induced heart failure. As αCGRP is a potent vasodilator, the question remains whether its cardioprotective effect is independent of its anti-hypertensive properties in vivo. To investigate this, Dr Argunhan identified a subpressor dose of CGRP of which to chronically administer to mice which had undergone left coronary artery ligation surgery and will subsequently develop heart failure. When I joined the lab in July, the first cohort of mice had just completed a four-week chronic dosing regimen of this subpressor CGRP dose and left ventricular tissue samples from these mice had been processed ready for histological examination. My project was therefore predominantly centred around sectioning, staining, imaging, and analysis of these cardiac samples to obtain valuable and much anticipated histological data on behalf of Dr Argunhan.

The first histological technique I employed during my time with the Brain group was microtomy. As noted by Dr Argunhan, I was quick to hone my skills on the microtome and completed the required 5μm sectioning of samples from the first cohort ahead of the project schedule. Following the processing of the second cohort's cardiac samples, I was again required to utilise the microtome but was this time faced with a number of challenges due to a fault with the equipment. This gave me an opportunity to develop my troubleshooting skills, such as how to prevent the splitting of sections often seen at higher temperatures or with blunt blades and how to ensure the microtome is cutting at a consistent desired thickness.

In terms of histological examination, we were interested in determining the collagen content and composition within the cardiac samples and therefore employed a number of histological staining protocols to achieve this, including Haematoxylin and Eosin and PicroSirius Red. Once complete, the next stage of the project was to capture a number of microscopy images at various magnifications. I particularly enjoyed the imaging of samples stained with PicroSirius Red which were taken under both brightfield and polarised light microscopy. At this point in my project, I was able to delve into the pathophysiology of heart failure and visualise the composition and extent of fibrosis induced in the mouse model through LAD ligation. What's more, Dr Argunhan gave me the opportunity to work independently and search through the literature to advise her on whether we can rely upon PicroSirius Red staining and polarised light microscopy to accurately determine collagen subtypes in infarcted tissue. After considering a vast amount of literature and taking my own observations into account, we came to a suitable conclusion for the data we had obtained. This was a valuable experience as I learnt to both critically appraise published literature and establish my own informed opinions, to then apply this knowledge to the data I was obtaining first-hand.

Upon completion of microscopy imaging, it was then necessary to conduct analysis of the images I had taken with ImageJ, an automated software analysis programme. Both Dr Argunhan and I were unsure how to proceed with ImageJ in order to examine the extent or lack of fibrosis within the processed murine cardiac samples. After consulting the literature and former colleagues of the Brain group, we concluded that provided we standardise the process across all images, there were many ways to obtain the data we required. Therefore, through trial and error I developed my own successful protocol for image analysis and was able to determine the % fibrosis in each cardiac sample ready for statistical analysis. I presented my method during a Brain group weekly lab meeting and was asked to document my work as a Standard Operating Procedure, making it accessible for Brain group members in the future.

The scope of my project then increased further than anticipated due to the good progress I was making with my assigned project tasks. Following the completion of ImageJ analysis, I utilised GraphPad Prism 9 to assess the statistical significance of the data I had collated over the past 8 weeks on behalf of Dr Argunhan. The graphs below were produced from analysis of both Haematoxylin and Eosin-stained slides and PicroSirius Red stained slides from the first two cohorts. Haematoxylin and Eosin staining allowed us to analyse both the mean number of nuclei and % area of nuclei present in the samples, whilst PicroSirius Red staining allowed us to analyse the % area fibrosis within the samples which due to the nature of the project was of most importance to us. The trend from the data so far suggests that this subpressor dose of αCGRP is not producing a statistically significant protective effect in the murine myocardial infarction-induced heart failure model. However, a third and final cohort receiving this chronic subpressor dose is due for completion in October 2021. It will be interesting to see whether this increase in n number influences the statistical significance of the overall data.

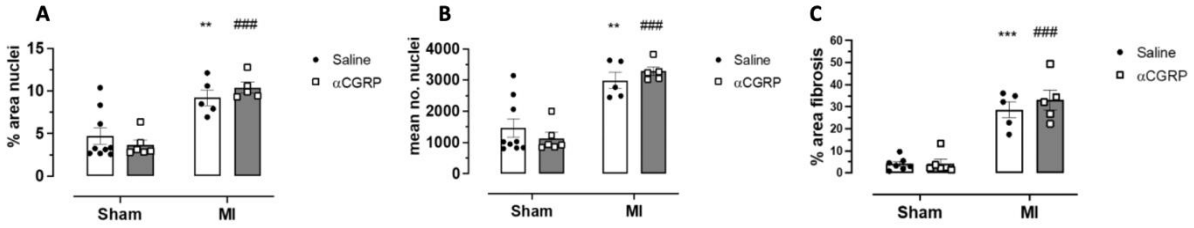

Figure 1: A and B A graph to show the % area of nuclei and the mean number of nuclei in Haematoxylin and Eosin stained left ventricular murine cardiac samples from both sham and myocardial infarction (MI) mice, who had received chronic 4-week administration of either saline or αCGRP. **C** A graph to show the % area fibrosis in PicroSirius Red stained left ventricular murine cardiac samples from both sham and myocardial infarction (MI) mice, following chronic 4-week administration of either saline or αCGRP.

Thanks to the financial support of the British Pharmacological Society, I gained the vital laboratory experience I needed to take the next steps towards my career progression and I thoroughly enjoyed completing my project under the supervision of Dr Argunhan and Professor Brain. In addition to developing many useful histological techniques, I also improved upon several transferable skills such as effectively communicating scientific data, the ability to work independently within a laboratory and to troubleshoot effectively.