Title: Identifying matrix metalloproteinases as a potential therapeutic target to protect the endothelial glycocalyx in diabetic cardiomyopathy

Presenting Author: Stanley Buffonge[1]

Co-author Names: Dr Yan Qiu[1], Dr Rebecca Foster[1], Prof Simon Satchell[1]

[1]Bristol Renal, University of Bristol, UK

Aim: The endothelial glycocalyx is a brush-like layer of membrane bound proteoglycans and glycoproteins lining the luminal side of vascular endothelial cells. It is therefore a vital regulator of vascular permeability. We have recently shown that damage to the coronary microvascular endothelial glycocalyx (CMVEGlx) contributes to the pathology of diabetic cardiomyopathy. Restoration of the glycocalyx improved diastolic function and reduced cardiac oedema. Matrix metalloproteinases (MMPs), specifically -2 and -9 are known to cause the shedding of the glycocalyx by cleaving core proteins such as syndecan 4 (SDC4). With this knowledge, we aimed to investigate whether inhibition of MMP-2 and -9 can protect the CMVEGlx in diabetes.

Methods:

In vitro: To investigate if inhibition of MMPs helps protect the glycocalyx in vitro, coronary microvascular endothelial cells were treated with 10ng/ml of TNF- α for 6 hours and also treated with the MMP-2 and -9 specific inhibitor SB-3CT. Quantitative PCR arrays, immunofluorescence, permeability assays, and SDC4 ELISAs were used for investigation.

In vivo: To induce a mouse model of diabetic cardiomyopathy, type 1 diabetes was induced in male FVB mice by injection of streptozotocin (STZ). Nine weeks post-STZ injections mice developed diastolic dysfunction and were culled after 12 weeks. To visualise and quantify the glycocalyx, lectin staining was conducted, and a peak-peak fluorescent intensity measurement was taken between the membrane and the lectin to give an indication of glycocalyx depth. MMP-2 and -9 activity assays were done on the hearts and urine.

Results: In vitro: TNF-α induced SDC4 mRNA upregulation by 12-fold (***$p<0.001$) and MMP9 upregulation by 2.3-fold (**$p<0.01$). Inhibition of MMP-2 and -9 significantly reduced SDC4 mRNA upregulation (**$p<0.01$). A significant increase of SDC4 in the conditioned media was found after TNF-α treatment with a mean concentration of 65 pg/ml compared to the control of 18 pg/ml (****$p<0.001$). This was significantly reduced by treatment with the SB-3CT inhibitor (**$p<0.01$). Cell surface SDC4 fluorescent intensity was also significantly reduced by TNF-a (****$p<0.001$) and restored with MMP inhibition (*$p<0.05$). Inhibition of MMPs also significantly improved endothelial permeability, which was increased as a result of TNF-a.

In the mouse model of diabetic cardiomyopathy, a significant reduction in glycocalyx depth was observed from lectin staining (*$p<0.05$). This was accompanied by a significant increase in MMP 9 in both the heart (**$p<0.01$), and the urine (*$p<0.05$) of the diabetic group. Glycocalyx depth was found to be positively correlated to diastolic function (*$p<0.05$) whilst MMP activity was found to be negatively correlated to diastolic function of the heart (*$p<0.05$).

Conclusion: Both the in vitro and vivo work prove that diabetes causes shedding of the coronary microvascular endothelial glycocalyx. The results show that MMPs are a potential therapeutic target to protect the glycocalyx and improve diastolic function in diabetic cardiomyopathy.