Differences in the response of male and female mouse coronary microcirculation to myocardial IR injury with inhibition of IL-36 pathway being vasculoprotective in both sexes

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Introduction: We have previously shown that the coronary microcirculation undergoes a number of thromboinflammatory and perfusion perturbations in the mouse beating heart in experimental models of myocardial infarction (MI).[1] Although several anti-inflammatory therapies have been successful in pre-clinical models of MI, they have failed in the clinical setting. This translational failure may be linked to a lack of an early benefit at the level of the coronary microcirculation. Sex differences in the onset and outcomes of MI are well established. However, little is known about how sex impacts the coronary microcirculation during health or MI. This study used intravital and laser speckle contrast imaging (LSCI) to compare the response of the coronary microcirculation, and overall ventricular perfusion, to myocardial ischaemia-reperfusion injury (IRI) in male and female mice. The vasculoprotective efficacy of an IL-36 receptor antagonist (IL-36Ra) was also investigated in both sexes.[2]

Methods: Myocardial IRI was induced in anaesthetised (100mg/kg-ketamine hydrochloride; 10mg/kg-medetomidine hydrochloride) male and female mice, receiving either vehicle or recombinant IL-36Ra (15ug/mouse;i.a.) during ischaemia and 60-minutes post-reperfusion. The beating heart coronary microcirculation was then imaged intravitally for neutrophils, platelets, and functional capillary density (FCD). LSCI was used to determine overall left ventricular (LV) perfusion. Infarct size was measured using dual TTC/Evans Blue staining. IL-36α/β / IL-36R / VCAM-1 expression and oxidative stress were investigated either immunohistochemically and/or using flow cytometry.

Results: A significantly ($p<0.01$) greater burden of thrombotic disease was noted in injured male coronary microvessels, whilst a significantly ($p<0.05$) greater neutrophil presence was identified in injured female coronary microvessels. FCD was reduced in both male ($p<0.05$) and female ($p<0.001$) hearts when compared to sham hearts, but with no significant differences between sexes. Unlike in female hearts, overall LV perfusion failed to reach pre-ischaemic baseline values in male hearts post-reperfusion and therefore remained significantly ($p<0.001$) lower than in female injured hearts. Expression of IL-36α/β cytokine and its receptor, but not VCAM-1, was significantly higher in healthy ($p<0.0001$) and injured ($p<0.0001$) female hearts compared to male hearts. Oxidative damage was significantly ($p<0.05$) higher on injured male cardiomyocytes, but a similar degree of oxidative damage was noted on male and female coronary endothelial cells. Infarct size was significantly ($p<0.05$) larger in injured female hearts. Despite these differences, IL-36Ra therapy resulted in similar beneficial effects (i.e., ↓ neutrophils, ↓ VCAM-1, ↓ oxidative damage, ↑ LV perfusion, ↑ FCD, ↓ infarct size) in both male and female injured mice.

Conclusion: A greater inflammatory cell presence, potentially linked to higher IL-36/IL-36R expression, was associated with larger infarcts in injured female hearts. These specific differences may explain why women are at a higher risk of mortality than age-matched men following an acute MI. Despite sex-related differences in the response of the coronary microcirculation to injury, we show that targeting the IL-36/IL-36R pathway utilises similar vasculoprotective mechanisms to improve infarct size in both sexes. This novel study also emphasises the importance of intervening rapidly to prevent thromboinflammatory events taking place immediately post-reperfusion.

1. Kavanagh *et al., Cardiovasc Res*. 2019; 115(13):1918-1932.
2. El-Awaisi *et al., JCI Insight.* 2022; 7(5):e155236.