Investigating the impact of hyperglycaemia on human coronary microvascular endothelial cells with a focus on interleukin-36

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Introduction: Myocardial infarction (MI) is associated with poor perfusion within coronary microvessels. Our intravital imaging of the mouse beating heart showed this was due to significant thromboinflammatory cell recruitment which occluded the coronary capillaries.[1] This could be inhibited, and perfusion improved, by targeting the receptor for IL-36, a recent addition to the IL-1 cytokine superfamily. Interestingly, thromboinflammation was increased in aged hearts, where we demonstrated increased endothelial expression of IL-36R.[2] Myocardial perfusion post-MI is worse in patients with type 2 diabetes (T2D) and is associated with poorer outcomes. However, it is not known whether hyperglycaemia can also modulate coronary endothelial IL-36R. Therefore, this study investigated the impact of hyperglycaemia ± IL-36 on coronary endothelial IL-36R expression, as well as changes in VCAM-1 expression, reactive oxygen species (ROS) generation and ROS mediated oxidative damage.

Methods: Confluent human coronary microvascular endothelial cells (HCMECs) were treated with normal (5mM) or high (30mM) glucose growth media for 16 hours. Some HCMECs were also treated with 0, 3, 30 and 300ng/mL of IL-36γ. IL-36R and VCAM-1 expression was assessed using an Alexa 647 anti-IL-1Rrp2 antibody and an APC anti-CD106 antibody respectively. ROS generation and oxidative damage was determined using the dihydroethidium (DHE) dye and a FITC anti-DNA/RNA damage antibody respectively. To compare the role of IL-36 in the context of more established pro-inflammatory cytokines, some cells were stimulated with IL-1β and TNF-α. To identify coronary specific responses, experiments were repeated using human pulmonary microvascular endothelial cells (HPMECs). Cells were then analysed immunohistochemically and flow cytometrically.

Results: Neither normal or high glucose alone consistently increased ROS generation, oxidative DNA/RNA damage or IL-36R and VCAM-1 expression in HCMECs or HPMECs. However, when coupled with the higher doses of IL-36γ, IL-1β or TNFα, significantly increased ($p<0.05$) oxidative DNA/RNA damage, IL-36R and VCAM-1 expression was observed. Interestingly, dual cytokine and hyperglycaemia stimulated HCMECs seemed to be significantly ($p<0.05$) more susceptible to changes in oxidative stress, IL-36R and VCAM-1 expression than HPMECs.

Conclusion: We have previously demonstrated that the IL-36 / IL-36R pathway is critical in mediating perturbations in aged coronary microvessels post-MI. Here we show that IL-36 was also as capable of eliciting oxidative stress and inflammatory changes in HCMECs exposed to hyperglycaemia in a similar manner to IL-1β and TNF-α. These effects were not specific to coronary ECs, although (surprisingly) IL-36R expression was higher in dual stimulated ECs of the heart compared to pulmonary ECs, suggesting IL-36 may have a stronger functional role in the heart. Identifying novel mechanisms by which hyperglycaemia detrimentally affects cells of the coronary microcirculation is essential if targeted therapies that will be effective in MI patients with T2D are to be developed.

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