Characterising endothelial CaMKII oxidation in a cellular model of inflammation to investigate the therapeutic potential of a novel anti-oxidant stent coating

Daniel Longhorn, Christopher McCormick, Susan Currie

UNIVERSITY OF STRATHCLYDE

Drug eluting stent use is widespread but restenosis due to endothelial dysfunction remains a challenge. Ca2+/Calmodulin-dependent protein kinase II-delta (CaMKIIδ) is implicated as a key modulator of cardiovascular pathology and hyper-activation of CaMKIIδ promotes endothelial inflammation and oxidative stress [1]. This study has characterised different endothelial cell responses for the purpose of testing a novel antioxidant stent coating. Interleukin 1-beta (IL-1β) or tissue necrosis factor-alpha (TNFα) have been used to mimic pro-inflammatory signalling and CaMKII activation observed following endothelial stress. Human umbilical vein and coronary artery endothelial cells (HUVECs and HCAECs) were treated with IL-1β or TNFα (both 10ng/ml) up to 6 h. Quantitative immunoblotting was used to assess pro-inflammatory signalling, phospho-P65 (pP65; Cell Signalling) and CaMKII activation, phospho-CaMKII (pCaMKII; Thermo Fisher) and oxidised-CaMKII (oxCaMKII; GeneTex) expression. Increased pP65 expression was observed following 30 min stimulation with either IL-1β or TNFα in HUVECs[2]. Similarly, significant increases in pP65 expression were observed in HCAECs following stimulation with both cytokines (IL-1β: 1.93±0.29 and TNFα: 1.54±0.15 mean fold-change c.f. control ±S.E.M; n=4, $p<0.05$). This indicates both cell types produce similar pro-inflammatory responses following cytokine stimulation. In contrast, CaMKII activation in response to cytokine stimulation was different across cell types. Significant activation (via oxidation) of CaMKII was observed in HUVECs only after IL-1β stimulation[2]. In HCAECs however, activation of CaMKII was observed only after TNFα stimulation, via both oxidation (TNFα (6h): 1.50±0.12, mean fold-change c.f. control ±S.E.M; n=4, $p<0.05$), and phosphorylation (TNFα (30min): 1.22±0.05, mean fold-change c.f. control ±S.E.M; n=4, $p<0.05$).This work highlights the differences in responses of HUVECs and HCAECs to stimulation by inflammatory cytokines. Results demonstrate the importance of considering the endothelial model used for research into therapeutic intervention.

References

[1] McCluskey, et al. (2019).Vascular Pharmacology,118-119 106560 doi: 10.1016/j.vph.2019.04.002[2] Longhorn, et al. (2020). Heart, 106(Suppl 1) A2-A3; doi: 10.1136/heartjnl-2020-SCF.5.

Keywords: endothelial cell response, endothelial stress, pro-inflammatory response, cytokine stimulation

Longhorn, D., McCormick, C., & Currie, S. (2021). *Characterising endothelial CaMKII oxidation in a cellular model of inflammation to investigate the therapeutic potential of a novel anti-oxidant stent coating*. Abstract from 36th Annual Meeting of the International Society for Heart Research European Section International Society of Heart Research