Parallel development and characterisation of a anti-oxidant stent coating and an *in vitro* biological model for qualitative assessment.

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Restenosis is a major cause of coronary artery stent failure and is linked to vascular endothelium damage with resultant oxidative and inflammatory stress. Drug eluting stents (DES) have failed to eliminate this risk; particularly for diabetic patients. Here, we have assessed the potential therapeutic effects of a novel antioxidant stent coating using (i) a common chemical assay, DPPH (2,2-diphenyl-1-picrylhydrazyl) and (ii) superoxide scavenging ability using NBT (nitroblue tetrazolium) reduction and have identified high antioxidant potential that is not dependent on drug incorporation within the coating.

To assess the biological effect of this novel antioxidant coating, we have initially used human umbilical vein endothelial cells (HUVECs) treated with pro-inflammatory cytokines to mimic the inflammation stress encountered post-stent placement. Pro-inflammatory signalling was assessed by measuring phospho-P65 (pP65) expression using quantitative western blotting and oxidative stress assessed by measuring reactive oxygen species (ROS) generation using DCFDA. In addition, we have specifically examined expression and activation (via oxidation) of an enzyme called Ca2+/Calmodulin-dependent protein kinase II-delta (CaMKIIδ) that is known to be a central component of vascular pathology during acute and chronic inflammation and oxidative stress .

HUVECs were stimulated with pro-inflammatory cytokines, Tissue Necrotic Factor alpha (TNFα) and Interleukin 1-beta (IL-1β). Stimulation with IL-1β (10 ng/ml) for 1h resulted in the highest overall level of P65 phosphorylation (7.80 ± 1.41 (fold increase ± S.E.M. in pP65 expression in IL-1β -stimulated cells c.f. unstimulated controls, n=4, $p<0.05$). Conversely, stimulation with TNFα (10 ng/ml) for 6h led to the highest overall increase in ROS (5.02 ± 0.54, fold-stimulation ± S.E.M. in ROS in TNFα -stimulated cells c.f. unstimulated controls, n=3, $p<0.05$).

We have shown that CaMKIIδ is highly expressed in HUVECs and that stimulation with IL-1β (10 ng/ml) for 3h significantly induced CaMKII oxidation (1.56 ± 0.06 (fold-increase ± S.E.M. in oxCaMKII in IL-1β stimulated cells c.f. unstimulated controls, n=4, $p<0.05$). Preventing the oxidation-induced activation of CaMKII may present a therapeutic mechanism by which antioxidant stents can improve endothelial recovery and future work will examine the reduction or reversal of this effect in the presence of our novel stent coating.

In conclusion, we have developed a novel anti-oxidant stent coating and established an in vitro system to test biological activity. This approach will now be applied to more physiologically relevant cell types before examining the novel coating's efficacy.