O_2^{-} production, MAPK and NFkB activation, and monocyte adhesion (all $p < 0.05$; one-way AVOVA). These C242T effects were further confirmed using $p22^{phox}$ shRNA engineered HeLa cells and coronary microvascular endothelial cells isolated from Nox2 knockout mice. Clinical significance was investigated using saphenous vein segments from non-CHD subjects after phlebectomies. Informed consents were obtained from the patients and the project was approved by the local NHS and the university ethical committees according to the UK regulation. TT (C242T) allele was common (prevalence of ~20%) and compared to CC, veins bearing TT allele had significantly lower levels of Nox2 expression and O_2^{-} generation in response to high glucose challenge (all $p < 0.05$).

In summary, our study for the first time provides mechanistic insight into the protective effect of the $p22^{phox}$ C242T SNP against the inflammatory oxidative stress-related cardiovascular diseases. $p22^{phox}$ C242T SNP causes $p22^{phox}$ structural changes that alter its interaction with the catalytic subunit Nox2 and inhibits endothelial oxidative response to TNFalpha or high glucose stimulation.

168 TARGETING THE HIPPO SIGNALLING PATHWAY TO ENHANCE THE THERAPEUTIC POTENTIAL OF IPS-DERIVED CARDIOMYOCYTES

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Introduction Cell based therapy using stem cell derived cardiomyocytes, has emerged as a potential therapeutic approach for cardiac diseases such as myocardial infarction and heart failure. Adult skin fibroblasts can be reprogrammed into induced pluripotent stem cells (iPSC) which could be an ideal source of iPS-derived cardiomyocytes (iPS-CM). Challenges facing cell therapy include the high number of viable cells needed to survive in pathological conditions. The Hippo signalling pathway has been described as a key pathway involved in regulating cardiomyocyte proliferation and survival in both embryonic and adult hearts. The purpose of this study is to test whether modification of the Hippo pathway will enhance the efficiency of iPS-CM generation and will increase iPS-CM survival and viability in pathological conditions.

Methods Skin fibroblasts were reprogrammed to iPS cells and then differentiated to cardiomyocytes. The Hippo signalling pathway was modified by genetic ablation of MST1, a major upstream regulator of the Hippo pathway, or by overexpressing YAP, the main downstream effector of the pathway. Cell proliferation was analysed using EdU incorporation assay and staining for cytokinesis markers Ki67 and phospho-histone H3. Cell death and viability were analysed using caspase 3/7 and MTT activity and trypan blue staining in both normal and hypoxic conditions.

Results Analysis of cell proliferation shows that genetic ablation of Mst1 leads to significantly increased proliferation (12 ± 1.5% $P < 0.001$), survival and viability (20 ± 4.3% $P < 0.001$) of iPSC in both normal and hypoxic conditions compared to controls. In addition overexpression of YAP, which is normally inhibited by upstream Hippo pathway components, and overexpression of mutated constitutively active form of YAP (S127A) increases cell proliferation in iPS-CM compared to control iPS-CM as shown with EdU assay (+20.8 ± 1.6% $P < 0.01$) and Ki67 staining (4.9 ± 0.9% $P < 0.001$). Overexpression of YAP leads to up regulation of genes associated with inhibition of apoptosis and promotion of cell proliferation.

Conclusion Targeting the Hippo pathway in iPS cells and iPS-CM significantly increases proliferation and survival in both normal and hypoxic conditions. Therefore, modulation of the Hippo pathway could become a new strategy to enhance the therapeutic potential of iPS-CM.

169 SOLUBLE GUANYLATE CYCLASE ACTIVATORS AS COMBINATION ANTI-PLATELET THERAPY WITH P2Y12 INHIBITORS AND PDE INHIBITORS: *IN VIVO* AND *EX VIVO* STUDIES

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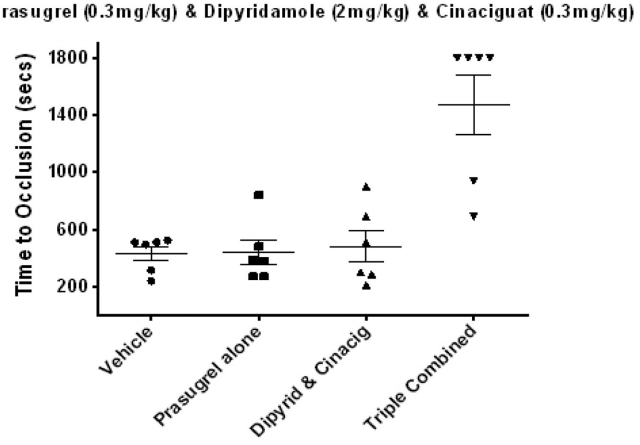
Methods Mice were pre-treated with vehicle, prasugrel (0.3 mg/kg), cinaciguat (0.3 mg/kg) + dipyridamole (2.0 mg/kg), or prasugrel + cinaciguat + dipyridamole (triple) and anesthetized. To measure in vivo thrombus formation, the carotid artery was then exposed and thrombosis induced by placement of a piece of filter paper saturated with 10% ferric chloride in contact with the adventitial surface of vessel for 3 minutes. Carotid artery blood flow was monitored by a Doppler probe with the time to form an occlusive thrombus being taken as the time required for blood to stop flowing completely for >1 minutes. Time to occlusion from different treatment groups was compared. To measure ex vivo platelet function, blood was taken from the vena cava of treated mice and aggregation of platelets in whole blood in response to arachidonic acid (AA) 1 mM, PAR-4 amide 30 μM, and collagen 10 μg/ml was then determined using flow cytometry.

Results In mice treated with vehicle, prasugrel and cinaciguat + dipyridamole complete vessel occlusion occurred within 8 minutes. Conversely, triple combination of prasugrel + cinaciguat + dipyridamole blocked thrombus formation (time to occlusion > 24 minutes). In ex vivo platelet function tests, we observed reduced platelet aggregation in mice treated with the triple combination compared to other treatments. Results as mean ± SEM. AA; vehicle 83 ± 9%, prasugrel 67 ± 7%, cinaciguat + dipyridamole 62 ± 9%, triple 27 ± 27%: PAR-



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4; vehicle 52 ± 11%, prasugrel 27 ± 14%, cinaciguat + dipyridamole 26 ± 16%, triple 11 ± 6%: collagen; vehicle 40 ± 16%, prasugrel 42 ± 10%, cinaciguat + dipyridamole 44 ± 19%, triple 18 ± 11%.

Conclusion Our animal studies suggest that combinations of low doses of cinaciguat, prasugrel and dipyridamole could provide a focused and powerful anti-platelet effect. This could be an effective therapeutic antithrombotic approach with potentially lesser effects at other sGC/PDE sites, particularly the vascular smooth muscle, reducing the incidence of headache and hypotension.

170 PLAKOGLOBIN DEFICIENCY PREDISPOSES TO LEFT ATRIAL ELECTRICAL REMODELING FOLLOWING CHRONIC EXPOSURE TO ANABOLIC STEROIDS

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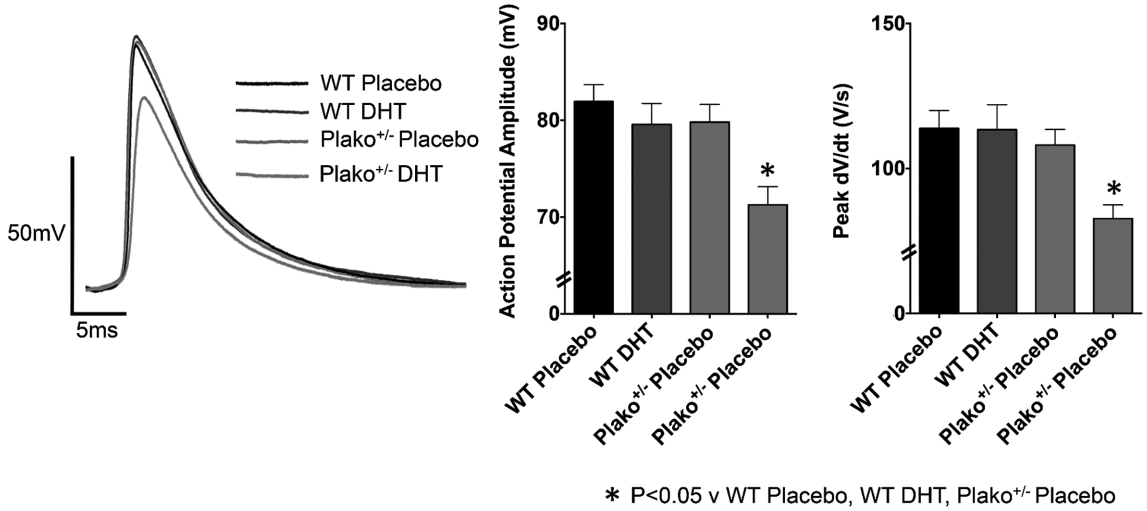
Background The recreational abuse of anabolic steroids is an emerging global health concern and may disrupt cardiac electrophysiology. Every fifth man joining a gym in the UK uses anabolic steroids for performance enhancement. Based on molecular signaling analyses and considering the volume-loading effects of testosterone, individuals with vulnerable desmosomal (cell-cell contact) proteins may be at increased risk of anabolic steroid induced cardiac electrical remodeling. We therefore studied the impact of chronic anabolic steroid exposure on left atrial (LA) electrophysiology in wildtype (WT) and plakoglobin (Plako; or gamma-catenin, a key desmosomal protein) deficient mice using dihydro-testosterone (DHT-a stable derivative of testosterone).

Methods Young adult WT and Plako$^{+/-}$ male mice, bred on 129/sv background were fitted with subcutaneous osmotic mini-pumps containing either DHT (62.5mg/ml) in ethanol, or ethanol alone (Placebo), for 6 weeks. Following treatment, we examined transmembrane action potentials, and generated high spatial resolution activation maps in the Di-4-Anepps loaded LA using the Hamamatsu ORCA flash 4. Significance was taken as $P < 0.05$ v Placebo, one way ANOVA with Bonferroni post hoc analysis. Experiments and analysis were performed blinded to genotype and treatment.

Results DHT minipump exposure lead to supraphysiological DHT plasma levels and increased the ventricular mass:tibial length ratio of both genotypes (WT Placebo 79 ± 2 Plako$^{+/-}$ Placebo 78 ± 2 WT DHT 86 ± 3 Plako$^{+/-}$ DHT 92 ± 3 mg/cm, $P < 0.05$, n = 56 mice total). DHT treatment increased Plako$^{+/-}$ LA mass:tibial length ratio (WT Placebo 2.4 ± 0.1 Plako$^{+/-}$ Placebo 2.3 ± 0.1 WT DHT 2.6 ± 0.2 Plako$^{+/-}$ DHT 3.2 ± 0.2mg/cm, $P < 0.05$, n = 56 mice total) and LA cardiomyocyte fiber size (WT Placebo 0.16 ± 0.01 Plako$^{+/-}$ Placebo 0.14 ± 0.006 WT DHT 0.16 ± 0.004, Plako$^{+/-}$ DHT 0.24 ± 0.04 mm^2, $P < 0.05$ n = 20 LA total).

DHT reduced action potential amplitude in Plako$^{+/-}$ LA, but not WT LA (WT Placebo 82 ± 2 Plako+/- Placebo 80 ± 2 WT DHT 80 ± 2 Plako+/- DHT 71 ± 2mV, $P < 0.05$, n = 65 cells total, n = 22 LA) and peak dV/dt (WT Placebo 114 ± 6 Plako+/- Placebo 108 ± 5 WT DHT 113 ± 9, Plako$^{+/-}$ DHT 82 ± 5 V/s, $P < 0.05$, (see Figure 1). Conduction velocity was significantly attenuated in Plako$^{+/-}$ LA following DHT exposure (WT Placebo 40 ± 0.3 Plako$^{+/-}$ Placebo 40 ± 0.9 WT DHT 37 ± 1.3 Plako$^{+/-}$ DHT 36 ± 1.4 cm/s, $P < 0.05$, n = 29 LA total). Chronic DHT treatment did not alter the action potential duration in either genotype.

Conclusion Reduced cardiac plakoglobin expression increased susceptibility to steroid-induced left atrial hypertrophy, reduced action potential amplitude and lead to significant conduction slowing. These differences are likely to provide an enhanced substrate for atrial arrhythmogenesis. Our results suggest a potentially important interaction between reduced mechanical cell-cell contacts and anabolic steroid use.



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