A GENE-BASED RESTORATION OF AKT ACTIVITY IN ENDOTHELIAL PROGENITOR CELLS FROM HUMAN SUBJECTS AT HIGH CARDIOVASCULAR RISK RESCUES VASCULAR REPARATIVE CAPACITY

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Introduction Late-outgrowth endothelial progenitor cells (LEPC) are putative mediators of endogenous vascular repair, and represent an attractive tool for future cell-based cardiovascular repair strategies. However, progenitor function may be impaired in high cardiovascular risk populations, such as those of South Asian (SA) ethnicity, in whom cell based therapies are likely to offer most benefit.
Methods Detailed analysis of in vitro LEPC function (abundance, proliferation, migration, angiogenesis and senescence) was performed in 12 SA men and 12 matched White European (WE) controls. Molecular abnormalities within the Akt/eNOS signalling axis were analysed with PCR and western blotting. LEPC were transfused into immunodeficient mice subsequent to femoral artery injury to assess in vivo reparative function. In vitro and in vivo studies were repeated after lentiviral gene delivery of constitutively active Akt1 (E17K) or control (EGFP) to SA LEPC; augmented Akt activity was confirmed using a Glycogen Synthase Kinase phosphorylation assay. Data are expressed as mean [SEM] and compared with t tests as appropriate; statistical significance is defined as $p<0.05$ (denoted by *).
Results The two groups were matched for age and cardiovascular risk factors, although the SA group was comparatively insulin resistant (HOMA-IR 1.2 [0.2] vs 0.5 [0.1] au*). SA LEPC exhibited impaired colony formation (0.06 [0.02] vs 0.19 [0.03] colonies/ml blood*), migration to vascular endothelial growth factor (5 [0.7] vs 10 [1.7] cells/microscopic field*) and in vitro angiogenesis (1.9 [0.6] vs 3.8 [0.5] tubular structures/microscopic field*), associated with markedly decreased abundance of the phosphorylated forms of the pro-angiogenic molecules S473-Akt (0.14 [0.05] vs 0.81 [0.2] au*) and S1177-eNOS (0.05 [0.02] vs 0.15 [0.01] au*). Transfusion of WE LEPC into immunodeficient mice after wire-induced femoral artery luminal injury augmented re-endothelialisation; however, neither SA LEPC, nor vehicle, augmented re-endothelialisation (WE: 54.2 [6.4], SA: 36.9 [3.4], vehicle: 31.1 [2.4] % re-endothelialised; WE vs SA*; SA vs vehicle $p=0.2$). Lentiviral gene delivery of a E17K, but not EGFP control, to SA LEPC was associated with augmented Akt1 activity and rescue of in vivo re-endothelialisation capacity (E17K: 55.2 [4.4] vs EGFP 24.1 [1.3] % re-endothelialised; E17K vs EGFP*; E17K vs WE non-transduced cells $p=0.9$).
Conclusions These data provide proof of principle for human LEPC based vascular repair therapy, and demonstrate a mechanism by which to rescue marked progenitor dysfunction in a group at high risk of cardiovascular events, whom are likely to benefit from cardiovascular repair therapies.

B ASSESSMENT OF VALVULAR CALCIFICATION AND INFLAMMATION BY POSITRON EMISSION TOMOGRAPHY

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Background The pathophysiology of aortic stenosis is incompletely understood and the relative contributions of valvular calcification and inflammation to disease progression are unknown.
Methods Patients with aortic sclerosis and mild, moderate and severe stenosis were prospectively compared to age and sex-matched control subjects. Aortic valve severity was determined by echocardiography. Calcification and inflammation in the aortic valve were assessed by sodium 18-fluoride (18F-NaF) and 18-fluorodeoxyglucose (18F-FDG) uptake using positron emission tomography. Histological analysis was performed on the valves of five patients who subsequently underwent aortic valve replacement.
Results 121 subjects (20 controls; 20 aortic sclerosis; 25 mild, 33 moderate and 23 severe aortic stenosis) were administered both 18F-NaF and 18F-FDG. Quantification of tracer uptake within the valve demonstrated excellent inter-observer repeatability with no fixed or proportional biases and limits of agreement of ±0.21 (18F-NaF) and ±0.13 (18F-FDG) for maximum tissue-to-background ratios. Activity of both tracers was higher in patients with aortic stenosis than control subjects (18F-NaF: 2.87±0.82 vs 1.55±0.17; 18F-FDG: 1.58±0.21 vs 1.30±0.13; both $p<0.001$). 18F-NaF uptake displayed a progressive rise with valve severity ($r^2=0.540$, $p<0.001$) and colocalised to osteocalcin staining on histology. Uptake was observed both in the presence and absence of underlying calcium on CT with the latter predominating. 18F-FDG displayed a more modest increase in activity with valve severity ($r^2=0.218$; $p<0.001$) and mapped to areas of macrophage accumulation. Among patients with aortic stenosis, 91% had increased 18F-NaF (>1.97) and 35% increased 18F-FDG (>1.63) uptake. A weak correlation between the activities of these tracers was observed ($r^2=0.174$, $p<0.001$) and while 18F-NaF activity was higher in the aortic valve than aortic atheroma (2.68±0.84 vs 2.07±0.30; $p<0.001$) the reverse was true for 18F-FDG (1.56±0.21 vs 1.80±0.25; $p<0.001$).
Conclusions Positron emission tomography is a novel, feasible and repeatable approach to the evaluation of valvular calcification and inflammation in patients with aortic stenosis. Calcification appears to be the predominant process that is particular to the valve and disproportionate to the degree of inflammation, indicating it to be a more attractive target for therapeutic intervention.

C ENDOTHELIAL SPECIFIC NOX2 OVER-EXPRESSION INCREASES SUSCEPTIBILITY TO ANGIOTENSIN II INDUCED AORTIC DISSECTION

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Aortic dissection is a detrimental disease with high mortality. However, the mechanisms regulating the susceptibility to aortic dissection remain unknown. We hypothesise that endothelial oxidative stress due to the activation of the reactive oxygen species (ROS)-generating Nox2 enzyme play an important role in the development of aortic dissection. To investigate this, we generated transgenic mice (C57BL/6J background) with endothelial specific over-expression of Nox2 (Nox2-Tg) under the control of a tie-2 promoter. Expression of the human Nox2 transgene was confirmed by qRT-PCR to be found only in endothelial cells (EC) isolated from transgenic mice, and not in WT EC or vascular smooth muscle cells (VSMC) and macrophages isolated from either genotype. Wild-type (WT) littermates and Nox2-Tg male mice (6 months, n=11) were treated with vehicle or AngII (1 mg/kg/day) via subcutaneous minipump for 28 days. There was no significant difference in the pressor responses to AngII between WT and Nox2-Tg mice (WT 121±7 mm Hg vs Nox2-Tg 122±6 mm Hg). However, 5/11 Nox2-Tg mice developed aortic dissections compared to 0/11 WT mice after AngII infusion ($p<0.001$). Immunohistochemistry revealed significant increases in endothelial VCAM-1 expression, MMP activity and CD45+ inflammatory cell recruitment in the aortas of Nox2-Tg mice after 5 days of AngII infusion. Inflammatory cell recruitment was further confirmed by FACS analysis of cells derived