Young research workers' prize finalists

A MYOSTATIN REGULATES CARDIOMYOCYTE GROWTH THROUGH MODULATION OF Akt SIGNALLING IN VITRO AND IN VIVO

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Myostatin is a highly conserved, potent negative regulator of skeletal muscle hypertrophy in many species from rodents to humans. It also appears necessary for adipogenesis and diabetes in some models, though its mechanisms of action are incompletely understood. Transcript profiling of hearts from a genetic model of cardiac hypertrophy revealed dramatic upregulation of myostatin, not previously recognised to play a role in the heart. Here we show that myostatin abrogates the physiological cardiomyocyte growth response to phenylephrine in vitro through inhibition of the serine-threonine kinase, Akt, a critical determinant of cell size in many species from drosophila to mammals. Evaluation of myostatin null mice, revealed that their cardiomyocytes and hearts overall were slightly smaller at baseline than littermate controls. More detailed echocardiographic analysis showed that myostatin null hearts had smaller systolic and diastolic chamber diameters and unexpected wall thickening predominantly affecting the interventricular septum. As expected, myostatin null hearts grew more exuberantly in response to chronic phenylephrine infusion, which corresponded with an increase in Akt activation in vivo after PE treatment. Biochemical analysis of skeletal muscle revealed a dramatic increase in baseline Akt phosphorylation and activation in myostatin deleted mice. Together these data demonstrate that myostatin is dynamically regulated in the heart and acts more broadly than previously appreciated to regulate growth of multiple types of striated muscle.

Conclusion: These data demonstrate that myostatin is dynamically regulated in the heart and inhibits Akt signalling in response to hypertrophic stimuli. These findings may have implications for ongoing efforts to inhibit myostatin to treat skeletal muscle diseases. The unanticipated finding that myostatin modulates Akt may contribute to its effects on skeletal muscle growth and diabetes.

B GENETIC ALTERATION OF MARROW DERIVED STROMAL CELLS WITH HIF-1α/VP16 ENHANCES COLLATERAL FORMING POTENTIAL IN VITRO AND IN VIVO

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Background: Marrow derived stromal cells (MSCs) release many arteriogenic cytokines and augment the collateral response to ischaemia when delivered locally. Furthermore, cell transduction with the hypoxia inducible factor-1α/VP16 construct (HIF-1α/VP16) increases expression of hypoxia dependant genes. Therefore the hypothesis that this study addresses is that expression of a constitutively active form of a hypoxia inducible factor-1α analogue (HIF-1α/VP16) could enhance the arteriogenic potential of marrow stromal cells.

Methods and Results: Human MSCs were either transduced with the Ad2/HIF-1α/VP16 vector, or exposed to normoxia or 1% O2. VEGF and bFGF levels increased significantly in the conditioned media (MSCCM) from HIF-1α/VP16 transduced MSCs compared with controls. When compared with MSCCM from MSCs under normoxia or hypoxia, MSCCM from transduced MSCs increased endothelial and smooth muscle cell proliferation and migration. Successful adenoviral transduction and significant augmentation of VEGF release was observed in MSC derived from both normal volunteers and from patients with ischaemic heart disease. In a murine model of hindlimb ischaemia (n=9 per group), adductor muscle injection of HIF-1α/VP16 transduced murine MSCs increased distal limb perfusion, improved limb function and attenuated calf muscle atrophy over controls. Adductor muscle collateral vessel area and VEGF expression was increased significantly compared to non-transduced cells.

Conclusion: Constitutive overexpression of HIF-1α/VP16 augments the in vitro and in vivo arteriogenic effects of MSCs and may represent a novel approach for therapeutic arteriogenesis.

C THE ROLE OF ANGIOGENIC GROWTH FACTORS IN INTIMAL HYPERPLASIA

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Introduction: Arterial thickening due to accumulation of vascular smooth muscle cells (VSMCs) and macrophages is an important early stage in atherosclerosis.

Aim: Examination of the effects of angiogenic factors upon the growth and cellular composition of arterial neointimal lesions in an animal model.

Method: Lesion formation was induced by the placement of a biologically inert collar around the carotid artery of rabbits maintained on either a normal or cholesterol enriched diet. Localised lesions containing predominantly VSMCs with and without macrophages resulted in the normal and hypercholesterolaemic diets, respectively. Localised gene delivery and the biological influence of the endothelium to contribute to the observed effects are advantages of this model.

Results: Low efficiency liposome mediated gene transfer of vascular endothelial growth factor-A (VEGF-A), reduced intimal thickening, macrophage infiltration, and endothelial vascular cell adhesion molecule (VCAM-1) expression in hypercholesterolaemic rabbits without significantly increasing angiogenesis. In contrast, high efficiency Ad VEGF-A transduction resulted in loss of the arterioprotective effect. Transfer of Ad-Placental Growth Factor-2 increased neointimal macrophage infiltration and thickening, endothelial VCAM-1 expression, and angiogenesis in hypercholesterolaemic rabbits. PR39 induces angiogenesis via inhibition of hypoxia inducible factor degradation and was also studied. Ad PR39 increased angiogenesis and increased intimal thickening. Both VEGF and fibroblast growth factor pathways were implicated in mediating the response because specific inhibition of these pathways abolished the effects of PR39. There was a striking correlation between the degree of intimal thickening and adventitial neovascularisation, but the results indicate that PR39 induced neointima formation in this model has both angiogenesis independent and dependent phases.

Conclusion: (1) There is a concentration dependent effect of VEGF in the regulation of neointima formation. (2) PlGF induces atherogenic changes in the arterial wall. (3) Studies with PR39 suggest that intimal thickening is initially angiogenesis independent, but has a later angiogenesis dependent phase. These results have implications for the therapeutic use of angiogenic cytokines in vascular disease.

D LEFT ATRIAL RADIOFREQUENCY ABLATION DURING MITRAL VALVE SURGERY FOR CONTINUOUS ATRIAL FIBRILLATION: RESULTS OF A PROSPECTIVE RANDOMISED CLINICAL TRIAL

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Background: Although left atrial (LA) radiofrequency ablation (RFA) is increasingly used for the treatment of atrial fibrillation (AF) during MV surgery, its efficacy has not been tested in the context of a randomised trial. We assessed the hypothesis that left atrial RFA increases the long term (>1 year) prevalence of sinus rhythm after MV surgery and improves functional status without increase in perioperative morbidity or mortality.

Methods: Ninety seven patients with continuous AF were randomly assigned to undergo MV surgery (n=48) or MV surgery plus RFA of the LA (n=49) using a monopolar radiofrequency probe. Stratified blocked randomisation secured that the two groups were comparable in terms of age, LA dimensions, and disease aetiology. Additional cardiac procedures were performed as required. Clinical examination, 12 lead ECG, echocardiograms, and a shuttle walk test were performed preoperatively, at hospital discharge, and six and 12 months postoperatively. The primary endpoint of the study was the presence of AF at any of the clinical follow up appointments. Secondary endpoints included functional status, atrial transport function, left atrial and left ventricular dimensions, and function.

Results: The two groups had similar preoperative clinical profile. There were no significant intergroup differences on perioperative mortality (6.1% in RFA group *v* 8.3% in controls p=0.71) and morbidity. No

complications related to RFA were noted. Twelve months postoperatively, SR was present in 44.4% of RFA patients and 4.5% of controls ($p<0.0001$). Adequate LA contractility was documented in 64% of those regaining SR. At 12 months, the RFA group had significantly better ejection fraction 59 (SD 7) *v* 54.2 (SD 7) % ($p=0.004$), left ventricular end systolic diameter (3.93 (SD 0.7) *v* 4.26 (SD 0.6) cm $p=0.03$) and exercise capacity (380 (SD 122) m *v* 317 (SD 120) m) ($p=0.02$) than the control group.

Conclusion: Left atrial RFA during MV surgery in patients with continuous AF significantly increases the rate of SR restoration one year postoperatively, improving cardiac function and patient exercise capacity. On the basis of its efficacy and safety, routine use of left atrial RFA during MV surgery in patients having continuous AF is justified.

E HIGH FREQUENCY RE-ENTRY CIRCUITS DRIVING AF: EVIDENCE FROM GLOBAL LEFT ATRIAL MAPPING IN HUMANS

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Background: Fast Fourier transformation (FFT) of electrograms in animal models and epicardial arrays in human atria suggest highest frequency activation arises in the left atrium (LA) driving AF. We studied FFT of global endocardial electrograms in permanent human left AF, hypothesising that areas driving AF are characterised by organised high frequency signals that remain in a constant location.

Methods: Patients with permanent AF underwent catheter ablation in the LA guided by non-contact mapping (NCM). Before ablation 19.5 s of AF was recorded and FFT applied to a grid of 64 virtual electrograms evenly distributed over the LA. The dominant frequency (DF) of each electrogram was defined as the largest peak in the resultant frequency spectrum. Thus DF maps of the entire LA were obtained and at areas revealed as potential drivers activation maps analysed to identify wavefront characteristics.

Results: Twenty five patients aged 51 (SD 9) years were studied. In four patients FFT demonstrated a discreet area in the LA where electrograms were highly organised, with a frequency gradient away from that area. NCM revealed stable re-entry circuits; two were clockwise around the os of the left superior pulmonary vein (LSPV), including the roof and base of LA appendage (LAA), one anticlockwise around the LAA, and one clockwise following a circuit including the LAA, LSPV os, roof, and posterior LA. The mean DF at this area was 7.6 (SD 0.7) Hz (132 (SD 12) ms). The shortest pathway length and velocity were 93 (SD 21) mm and 71 (SD 19) cm/s. In 14 patients, FFT revealed areas with discreet peaks DF 7.1 (SD 0.7) Hz (144 (SD 17) ms) but spectral analysis was much more irregular. NCM either did not reveal re-entry circuits or they were intermittent during the sampled period. In seven patients FFT revealed no areas of organisation in the LA. Compared to the other 18 patients they had lower amplitude electrograms (−0.6 (SD 0.4) *v* −1.6 (SD 0.5) mV $p<0.01$) and a longer duration of AF (77 (SD 33) *v* 35 (SD 24) months $p=0.04$). NCM either demonstrated no or short lived re-entry circuits.

Conclusion: Organised electrogram activity identified by FFT is due to stable re-entry circuits. Tissue surrounding the LAA and LSPV can support re-entry circuits that drive AF in some patients. Ablation of these drivers may be a successful strategy but would require energy delivery outside regions enclosed by most current strategies.

F COMBUSTION DERIVED NANOPARTICULATE IMPAIRS VASCULAR FUNCTION AND ENDOGENOUS FIBRINOLYSIS IN MAN: AN EXPLANATION FOR THE INCREASED CARDIOVASCULAR MORTALITY ASSOCIATED WITH AIR POLLUTION

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Background: Although the mechanisms are unknown, air pollution is associated with an increase in cardiovascular morbidity and mortality. We have previously demonstrated vascular dysfunction in cigarette smokers. As combustion products and particulate matter are common to both air pollution and cigarette smoke, we hypothesised that air pollution would cause detrimental vascular effects. We investigated the effects of (a) dilute diesel exhaust and (b) ambient particle inhalation on vascular and fibrinolytic function in man.

Methods: Studies were conducted in a double blind randomised crossover design with: (A) 15 healthy men exposed to dilute diesel exhaust (300 $\mu g/m^3$) or air for 1 hour; and (B) 12 male patients with stable ischemic heart disease and 12 age matched volunteers exposed to concentrated ambient particles (CAPs) or air for 2 hours. Six hours after all exposures bilateral forearm blood flow and plasma fibrinolytic markers were measured before and during unilateral intrabrachial bradykinin, acetylcholine, and nitroprusside infusions.

Results: There was no difference in resting blood flow following all exposures. (A) There was a dose dependent increase in blood flow with each vasodilator ($p<0.001$ for all) that was attenuated following exposure to diesel exhaust: bradykinin ($p=0.006$), acetylcholine ($p=0.07$), and sodium nitroprusside ($p<0.001$). Bradykinin caused a dose dependent increase in plasma tissue plasminogen activator (t-PA) concentration ($p<0.001$) that was suppressed following exposure to diesel exhaust ($p=0.04$; area under the curve decreased by 34%). (B) The mean CAPs exposure concentration was 190 (SD37.2) $\mu g/m^3$. Chemical analysis of ambient particulate identified low levels of carbon and a primary constituent of sodium chloride. Although there was a dose dependent increase in blood flow with each vasodilator and plasma t-PA release ($p<0.001$), this was unaffected by CAPs or air in either group.

Conclusion: Inhalation of combustion derived, but not inorganic particulate matter, markedly impairs the regulation of vascular tone and endogenous fibrinolysis in man. These findings provide a potential mechanism that links combustion derived air pollution to the pathogenesis of atherothrombosis and acute myocardial infarction.