superoxide release in atrial samples of patients with post-operative AF but had no effect in patients with permanent AF. Similarly, atorvastatin did not induce a mevalonate-reversible changes in the atrial BH4 concentration and NOS uncoupling in neither group.

Conclusions Together, these findings indicate that upregulation of NOX2-NADPH oxidases is an early but transient event in the natural history of AF, as mitochondrial oxidases and uncoupled NOS account for the statin-resistant increase in atrial superoxide production in permanent AF. Variation in atrial sources of reactive oxygen species with the duration and substrate of AF may explain the reported variability in the effectiveness of statins in the prevention and management of AF.

143 TISSUE FACTOR PATHWAY INHIBITOR REGULATES ANGIOGENESIS INDEPENDENTLY OF TISSUE FACTOR VIA INHIBITION OF VASCULAR ENDOTHELIAL GROWTH FACTOR SIGNALLING

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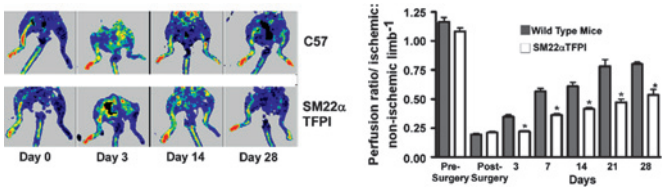
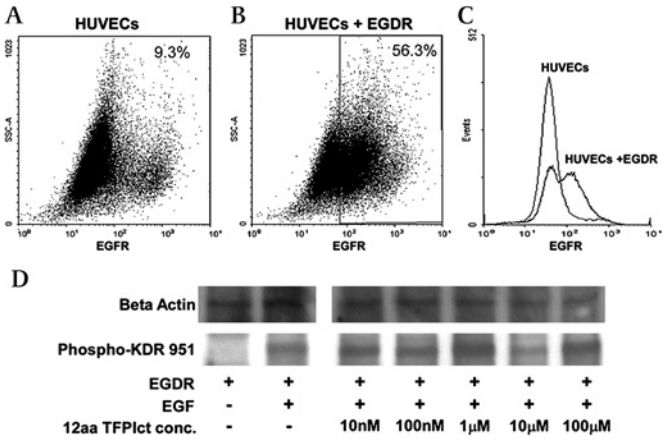
Introduction The biological systems of coagulation and angiogenesis show considerable interdependence. Proteases and inhibitors within the tissue factor (TF) pathway of coagulation have emerged as potential regulators of angiogenesis. Tissue factor pathway inhibitor (TFPI), as the primary physiological inhibitor of tissue factor (TF)-mediated coagulation, is ideally situated to modulate the pro-angiogenic effects of TF. However, TFPI may also have effects on angiogenesis independent of its anti-TF ability.

Methods We determined the effects of altered TFPI expression on the regulation of angiogenesis in vivo using genetically-modified murine models of vascular overexpression (SM22áTFPI strain) and endothelial-specific deletion of the TF-binding domain of TPFI (Tie2TFPI). We then defined the mechanism of these effects in vitro using Human Umbilical Vein Endothelial Cells (HUVECs) over-expressing TFPI or via exogenous addition of TFPI-derived peptides in assays of angiogenesis.

Results Vascular-directed over-expression of TFPI (SM22áTFPI strain) inhibited angiogenesis in vivo (Abstract 143 figure 1). SM22áTFPI showed significantly impaired recovery from ischaemia in the hindlimb ischaemia model after 3 days ($p<0.05$, $n=5$ per group), which persisted throughout the experiment. Survival (until 1-cm tumour dimension) of SM22áTFPI mice vs wild-type control (median survival 14 cf. 10 days) following s.c. B16 melanoma injection ($n=7$ per group, $\chi^2=4.325$, $^*p<0.05$). Endothelial-specific deletion of the TF-binding domain of TFPI failed to reveal a pro-angiogenic phenotype. This led us to suspect that the anti-angiogenic action of TFPI may be independent of TF. Systemic delivery of the murine TFPI carboxyl-terminus (mTFPIct) replicated the effects of endogenous overexpression. In vitro, overexpression of TFPI inhibited endothelial cell tube formation on Matrigel and migration using an injury migration model. Human TFPIct (hTFPIct) inhibited tube formation and migration through inhibition of Vascular Endothelial Growth Factor Receptor-2 (VEGFR2) tyrosine-951 phosphorylation, a key event in migration. hTFPIct did not inhibit VEGF121-induced migration, which lacks the heparin-binding domain of VEGF165. Utilising the chimeric receptor, EGDR, which contains the extracellular domain of epidermal growth factor (EGF) and the intracellular domain of VEGFR2/KDR, a direct effect of TFPIct on the intracellular domain of VEGFR2 was excluded (Abstract 143 figure 2) TFPIct did not block phosphorylation of EGDR when stimulated with EGF.



Abstract 143 Figure 1



Abstract 143 Figure 2

Conclusion Angiogeneis is a key biological system in health and disease; enabling cells in a hypoxic environment to stimulate new blood vessel growth. These data demonstrate, both in vivo and in vitro, an inhibitory role for TFPI in angiogenesis that is TF-independent. In addition to it classical role as a TF-antagonist, TFPI, via TFPIct, interferes with the interaction of VEGF165 with the extracellular domain of VEGFR2, thereby limiting angiogenesis.

144 A DRUGGABLE INHIBITOR OF CARDIAC HYPERTROPHY IDENTIFIED THROUGH AN INNOVATIVE CHEMICAL LIBRARY SCREEN

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Cardiac hypertrophy is a prerequisite for the development of heart failure. It currently affects almost one million people in the UK. Few effective anti-hypertrophic agents with druggable properties have been identified. Recently, our group showed that plasma membrane calcium ATPase isoform 4 (PMCA4) knockout mice showed a reduced response to hypertrophic stress prompting us to hypothesise that a novel PMCA4 specific inhibitor would modify the development of cardiac hypertrophy. A library of 1280 medically optimised compounds was screened using a novel in vitro assay which measures the Ca2+ dependent ATPase activity of PMCA4. The compound AP2 was identified, which inhibited PMCA4 activity with high affinity (IC_{50}=300 nM) but not other PMCAs (PMCA1, PMCA2 and PMCA3) or related ATPases which are expressed in the heart including the sarcoplasmic reticulum calcium ATPase and Na/K ATPase. In isolated neonatal rat cardiomyocytes (NRCM), AP2 showed dose dependent inhibition of phenylephrine-induced hypertrophy, indicated by an 85% reduction in cell surface area as well as in BNP activity. In vivo studies showed that AP2 (5 mg/kg body weight/day IP) significantly reduced pressure-overload induced hypertrophy following 2 weeks transverse aortic constriction (TAC) (heart weight/tibia length (mg/mm): sham, 5.5±0.3, vehicle treated TAC mice, 8.7 ±0.2, AP2 treated TAC mice, 7.0 ±0.5, $n=10$ in each group, $p<0.01$). AP2 treated TAC