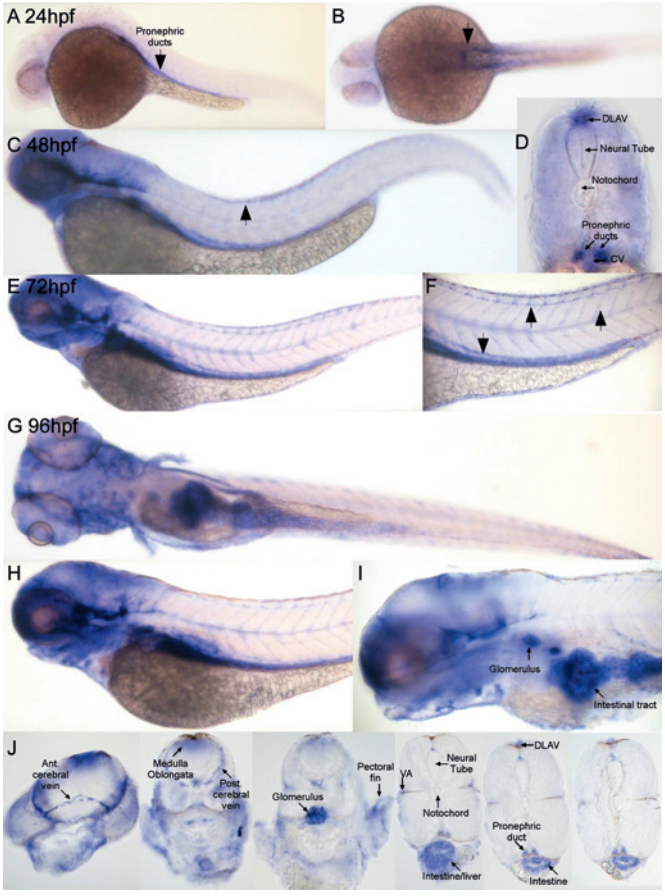
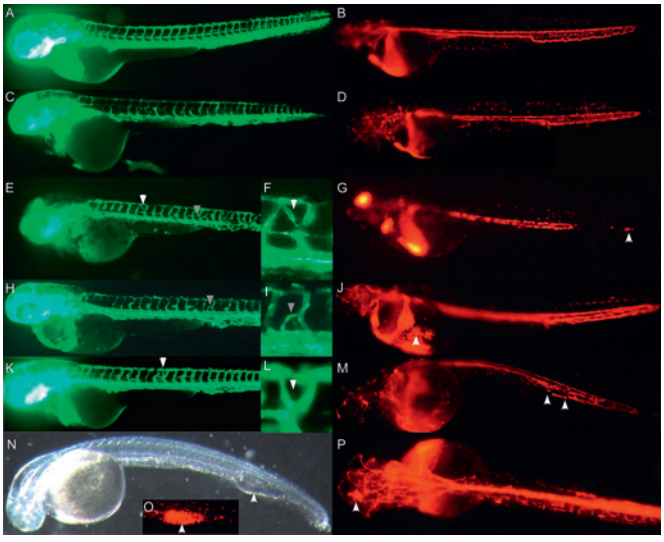


Abstract 141 Figure 1

developing kidney, gut, and vasculature. Morpholino (MO)-based knockdown of TFPI resulted in coagulopathy and disordered vascular development (Abstract 141 figure 2. left column: green endothelial Fli1GFP, right column: red erythrocytes. AB: uninjected controls. CD: MO controls. E–P: TFPI knockdown). Abnormally targetted (ie, vessels sprouting from normal site but growing in abnormal direction; grey arrows) and extra vessels (ie, superfluous vessels not seen in controls; white arrows in Abstract 141 figure 2EFHIK&L) were seen by 48hpf. TFPI MO induced coagulopathy (a spontaneous clot or bleed; white arrows Abstract 141 figure 2GJMP) in 25.2±2.3% ($p<0.01$ cf. uninjected controls) at 3 ng and 23.8±5.8% ($p<0.01$) at 9 ng. Control embryos did not demonstrate significant signs of coagulopathy (3.3±3.3%). Extra arteries occurred in 26.4±2.6% ($p<0.001$ by ANOVA cf. uninjected controls) of embryos injected with 3 ng of TFPI MO. To further define the role of TFPI in vascular function, RNAi-mediated knockdown of TFPI was performed in human endothelial cells (EC). Knockdown of TFPI resulted in enhanced EC tube formation on Matrigel and EC migration in injury model associated with increased phosphorylation of Vascular Endothelial Growth Factor Receptor-2.



Abstract 141 Figure 2

Conclusion These data represent the first demonstration of TFPI expression in zebrafish and the first description of a unique phenotype following TFPI knockdown. They support a model in which TFPI acts a molecular break to angiogenesis both in vivo, during early vertebrate embryogenesis, and in vitro in mature human endothelial cells, secondary to constitutive regulation of VEGF signalling.

142 ATRIAL SOURCES OF REACTIVE OXYGEN SPECIES VARY WITH THE SUBSTRATE AND DURATION OF ATRIAL FIBRILLATION: IMPLICATIONS FOR THE ANTIARRHYTHMIC EFFECT OF STATINS

doi:10.1136/heartjnl-2011-300198.142

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Background Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia and is associated with altered nitric oxide (NO)-redox balance. The molecular mechanisms and implications of this phenomenon in the management of patients with AF are poorly understood. Statins improve NO-redox imbalance and decrease the occurrence of postoperative AF but are less effective in the secondary prevention of AF, suggesting that the sources of reactive oxygen species might vary with the substrate and duration of AF.

Methods and Results We investigated atrial tissue from 130 patients undergoing cardiac surgery (26 with permanent AF, 32 who developed AF post-operatively and 72 who were in normal sinus rhythm before and after surgery), and from goats in sinus rhythm (SR, n=19) with or without atrial structural remodelling secondary to surgical AV block (AVB, n=10) or after 2 weeks (2W, n=15) or 6 months (6M, n=10) of pacing-induced AF. Atrial NADPH oxidase activity (chemiluminescence and 2-OH ethidium, Abstract 142 figures 1 and 2), NOX2 & p22phox protein level were increased after 2W-AF and in patients who developed AF post-operatively (n=32). In contrast, the increased superoxide production in atrial tissue from goats with AVB or 6M-AF was exclusively driven by mitochondrial oxidases and uncoupled NOS (secondary to a reduction in atrial BH4 level and an increase in arginase activity). These findings were recapitulated in the right atrial appendage of patients. Increase in basal superoxide production in postoperative AF was associated with an apocynin-reversible increase in NADPH oxidase activity and protein level of the NOX2 and p22phox subunits. NOS activity remained coupled despite the increase in superoxide production. In line with this, atrial BH4 content was unaltered. In contrast, in patients with permanent AF, increased superoxide production was not reversed by apocynin, and was maintained by mitochondrial oxidases and uncoupled NOS (secondary to BH4 deplition). Ex-vivo inhibition of HMG-CoA reductase with atorvastatin (20 μMol/l) inhibited NADPH oxidase activity (via reducing activity of Rac1 and membrane translocation of cytosolic subunit p47phox and p67phox of NADPH oxidase) and caused a mevalonate-reversible reduction in

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

doi:10.1136/heartjnl-2011-300198.143

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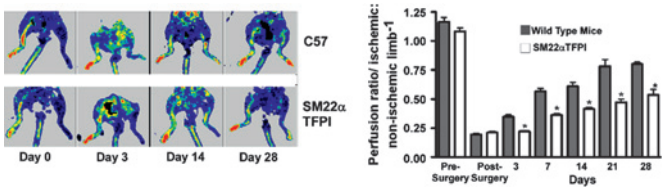
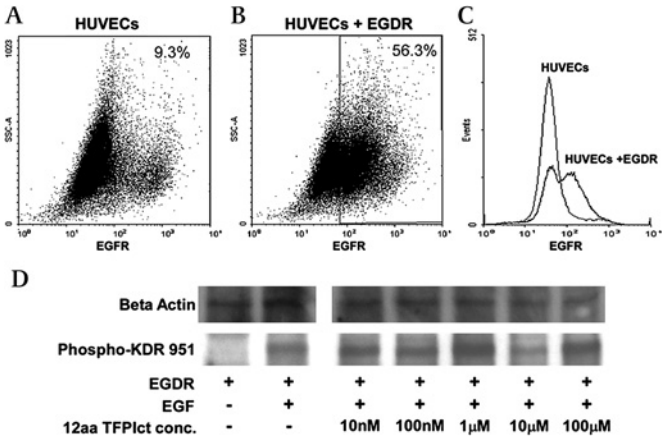
Introduction The biological systems of coagulation and angiogenesis show considerable interdependence. Proteases and inhibitors within the tissue factor (TF) pathway of coagulation have emerged as potential regulators of angiogenesis. Tissue factor pathway inhibitor (TFPI), as the primary physiological 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

doi:10.1136/heartjnl-2011-300198.144

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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