

Young research workers' prize finalists

A THE ZEBRAFISH AS A NOVEL MODEL OF ARTERIOGENESIS

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Introduction: Collateral vessel formation (arteriogenesis) is poorly understood. Collateral vessels develop from existing endothelial communications in a nitric oxide (NO) dependent manner, but the role of ischaemia is unknown. Current models of arteriogenesis suffer many disadvantages. Angiography is very difficult in small mammals. Ischaemia and necrosis limit microarray or proteomic studies. These issues hamper the study of arteriogenesis. The transparent zebrafish embryo is an emerging tool in vascular biology. The gridlock mutant has an aortic coarctation resulting in an occluded proximal aorta. We evaluated this mutant for its suitability as a model of arteriogenesis.

Methods: Gridlock embryos expressing endothelial GFP were generated by crossing gridlock adults with transgenic Fl1-eGFP fish. To determine restoration of distal aortic blood flow, groups of 20–30 embryos were lightly anaesthetised and observed under a stereomicroscope. To determine the role of NO in restoration of distal aortic blood flow, embryos were incubated in L-NAME or L-arginine at the times and doses indicated. Confocal microangiography, digital motion analysis, and rt-PCR were performed as previously described.

Results: At 48 h post fertilisation, wildtypes have brisk aortic flow whereas no gridlock mutant had detectable distal aortic blood flow. By 120 h post fertilisation, however, 83 (SD 6) % of gridlock embryos had recovered distal aortic flow, via a variable pattern of collateral vessels, detected by digital motion analysis and confocal microangiography. When incubated in L-NAME (up to 1 mM) from 24–120 h post fertilisation there was a dose-dependent reduction in the percentage of gridlock embryos with distal aortic blood flow, reversed by co-incubation with 1 mM L-Arginine (Control 90 (2), 1 mM L-NAME 41 (10), 1 mM L-NAME and 1 mM L-arginine 80 (10), $p < 0.05$ L-NAME v control). This effect was maintained if embryos were removed from L-NAME for a 3 h washout, and was not apparent if 120 h post fertilisation embryos were treated with L-NAME for 5 h. We assessed endothelial GFP-expressing wildtype and gridlock mutants by serial confocal microscopy. We found no difference in vascular endothelial patterning between groups, indicating that collateral vessels arose from pre-existing endothelium. There was no difference in expression of hypoxia inducible factor 1- α in 4 or 5 dpf gridlock mutants compared with wild-type controls despite the occluded aorta (supporting the observation that embryos gain sufficient oxygenation via diffusion from the water).

Conclusions: The gridlock mutant restores blood flow to an occluded aorta by remodelling existing endothelial communications in a NO dependent manner, all hallmarks of mammalian arteriogenesis. The zebrafish therefore represents a novel model of arteriogenesis. Using this model, we have shown that arteriogenesis can proceed in the absence of ischaemia. This model allows an entirely novel approach to the study of arteriogenesis.

B THE SURVIVAL KINASES AKT AND ERK1/2 AND THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE AS A COMMON CARDIOPROTECTIVE PATHWAY

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Background: Our recent work has identified the survival kinases phosphatidylinositol-3-OH kinase (PI3K)-Akt, and p42/p44 extracellular signal-regulated kinases (Erk1/2), and the mitochondrial permeability transition pore (mPTP) as two potential targets of cardioprotection at the time of reperfusion. We hypothesised that the survival kinases Akt and Erk1/2 and the subsequent inhibition of mitochondrial permeability transition pore opening at time of myocardial reperfusion constitute a common cardioprotective pathway recruited by ischaemic preconditioning and postconditioning. We have demonstrated that inhibiting mPTP opening using cyclosporin-A [CsA] or sanglifehrin-A [SfA] for the first 15 min of reperfusion reduced myocardial infarct size in Langendorff-perfused male Sprague-Dawley rat hearts subjected to ischaemia-reperfusion. In addition, pharmacologically inhibiting mPTP opening at time of reoxygenation improved myocyte survival and recovery of contractile function in human atrial myocytes and atrial

trabeculae, harvested from patients undergoing cardiac surgery, subjected to hypoxia-reoxygenation.

Methods: Using models for detecting mPTP opening in rat mitochondria (using flow cytometry) and in adult rat myocytes (using confocal microscopy), we have demonstrated that CsA, SfA and hypoxic and pharmacological preconditioning (using diazoxide) inhibit mPTP opening. Using isolated Langendorff-perfused rat hearts, we have demonstrated that both IPC and postconditioning phosphorylate Akt and/or Erk1/2 at the time of reperfusion, and that inhibiting kinase phosphorylation using either LY294008 (a PI3K-Akt inhibitor) or PD98059 (a MEK1/2-Erk1/2 inhibitor) abrogated the reduction in infarct size.

Results: Using the model for detecting mPTP opening in myocytes, we have demonstrated that activating Akt using insulin inhibits mPTP opening. This inhibitory effect of insulin on mPTP opening was abolished in HL-1 cells expressing the dominant-negative Akt construct. In addition, HL-1 cells over-expressing Akt demonstrated inhibition of mPTP opening.

Conclusion: In conclusion, we have demonstrated that the survival kinases Akt and Erk1/2 and the mPTP constitute a common pathway for cardioprotection, and as such novel targets for myocardial protection. The use of pharmacological agents which activate these kinases and/or inhibit mPTP opening may be given as adjuvant therapy to current myocardial reperfusion strategies such as thrombolysis and primary percutaneous coronary intervention, thereby offering further cardioprotection over and above that provided by reperfusion itself.

C SKP2, A KEY PLAYER IN VASCULAR SMOOTH MUSCLE CELL PROLIFERATION IN VITRO AND IN VIVO, IS A MAJOR MEDIATOR IN CYCLIC-NUCLEOTIDE-RELATED GROWTH INHIBITION

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Background: Cyclic nucleotides inhibit vascular smooth muscle cell (VSMC) proliferation but the underlying molecular mechanisms are incompletely understood. We studied the role of S-phase kinase-associated protein-2 (Skp2), an F-box protein of SCF/Skp2 ubiquitin ligase responsible for polyubiquitylation and subsequent proteolysis of p27/Kip1, a key step leading to cell cycle progression.

Methods: Skp2 mRNA and protein were upregulated in mitogen-stimulated VSMCs, cultured human saphenous vein grafts and after balloon injury in rat carotid arteries, where the time-course and location of Skp2 expression closely paralleled that of proliferating cell nuclear antigen (PCNA).

Results: Skp2 siRNA reduced Skp2 expression, increased p27/Kip1 levels, and inhibited VSMC proliferation in vitro. On the other hand, adenovirus-mediated expression of rat Skp2 after filament injury of rat common carotid artery enhanced medial Skp2 expression, cell proliferation (BrdU incorporation), and importantly, the subsequent neointima formation. cAMP-elevating agents prominently inhibited VSMC proliferation and Skp2 expression through inhibiting Skp2 transcription as well as decreasing Skp2 stability. Adenovirus-mediated Skp2 expression reversed cAMP-induced p27/Kip1 upregulation and rescued cAMP-related S-phase entry inhibition up to 50%. 8-bromo-cGMP also moderately reduced Skp2 and cell proliferation when VSMCs were incubated with low serum concentration. After balloon injury of rat carotid arteries, local forskolin treatment significantly reduced Skp2 expression, VSMC proliferation and subsequent neointimal thickening.

Conclusion: These data demonstrate for the first time that Skp2 is an important factor in VSMC proliferation and its inhibition by cyclic nucleotides in vitro and in vivo.

D REMOTE ISCHAEMIC PRECONDITIONING REDUCES MYOCARDIAL INJURY AFTER ABDOMINAL AORTIC ANEURYSM REPAIR: A RANDOMISED TRIAL

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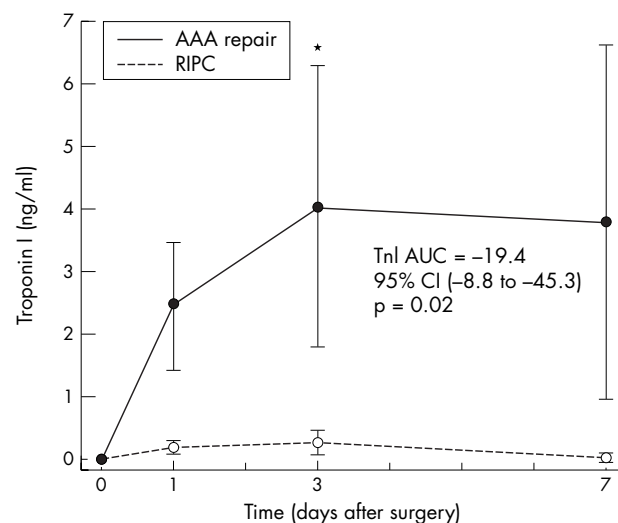
Background: Myocardial injury is a significant cause of perioperative morbidity and mortality following abdominal aortic aneurysm surgery. Subclinical myocardial injury, detected by a rise in serum cardiac troponin I (TnI), is common and is associated with decreased patient

survival. Remote ischaemic preconditioning (RIPC) is a phenomenon whereby a brief period of ischaemia followed by reperfusion prior to a prolonged ischaemic event can provide protection from cellular injury in distant organs. We investigated whether RIPC could prevent myocardial injury in patients undergoing elective abdominal aortic aneurysm repair (AAAR).

Methods: Between February 2003 to October 2005, 42 patients were randomised to AAAR alone (control) and 41 patients to AAAR with RIPC. Two cycles of intermittent cross clamping of the common iliac artery with 10 min ischaemia followed by 10 min reperfusion served as the IPC stimulus. Patients were screened for myocardial injury or infarction using symptomatic assessment, electrocardiography and TnI measurements taken preoperatively, 1, 3, and 7 days after surgery.

Results: There were no significant differences in baseline characteristics. RIPC significantly reduced the risk of myocardial infarction (MI) compared with AAAR (4.8% v 26%; $p=0.008$). Furthermore, RIPC significantly reduced the incidence of raised TnI by 38% (23/42 v 6/41; $p<0.001$). The mean TnI area-under-the-curve (AUC) was significantly less in the RIPC group (-19.4 ng/mL-days, 95% CI -8.9 to -45.4 ; $p=0.021$) compared with control AAAR. Logistic regression revealed the protective effect of rIPC was independent of other risk factors (OR 0.25, 95% CI 0.11 to 0.89; $p=0.011$). At a mean follow-up of 1.5 ± 0.9 years survival free of myocardial infarction was significantly higher in patients treated with RIPC compared to patients who underwent AAAR alone (85% v 57%; $p=0.005$).

Conclusion: In patients undergoing elective AAA repair intermittent lower limb ischaemia as a RIPC stimulus significantly reduces myocardial injury. This simple maneuver has potentially important clinical implications.



Abstract D

E EVIDENCE OF A DOMINANT BACKWARD-PROPAGATING "SUCTION" WAVE, RESPONSIBLE FOR DIASTOLIC CORONARY FILLING IN HUMANS, ATTENUATED IN LEFT VENTRICULAR HYPERTROPHY

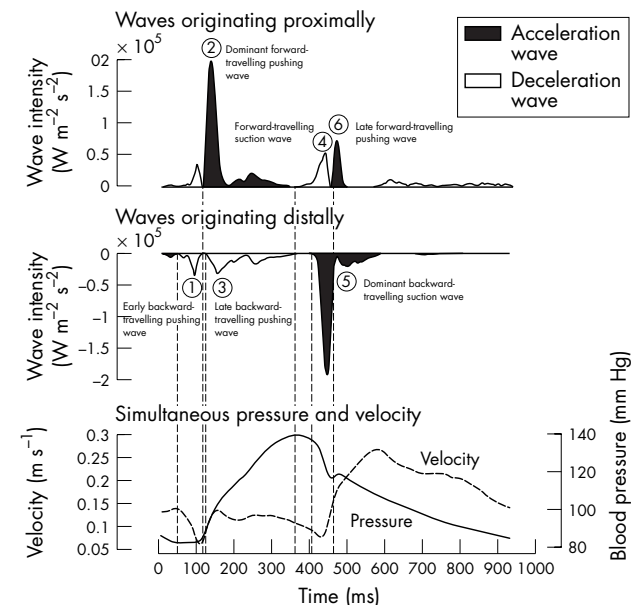
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Background: Coronary blood flow peaks in diastole when aortic blood pressure has fallen. Current models fail to completely explain this phenomenon. We present a new approach—using wave intensity analysis—to explain this phenomenon in normal subjects, and to evaluate the effects of left ventricular hypertrophy (LVH) on the coronary microcirculation.

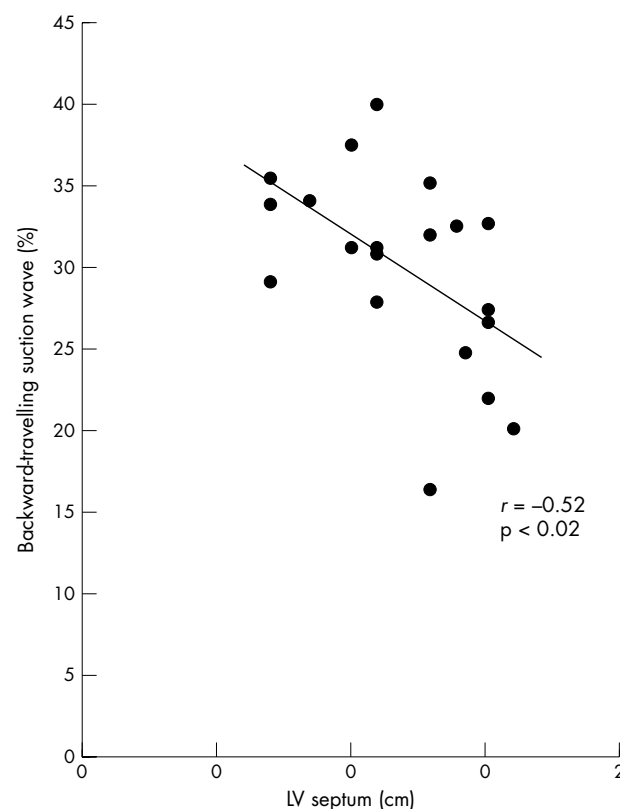
Methods: We measured simultaneous pressure and Doppler velocity with intracoronary wires in the left main stem, left anterior descending, and circumflex arteries of 20 subjects (mean age 54 (SD 10) years, 13 female) following a normal coronary arteriogram. Coronary wave speed was calculated using a new method we have recently developed. This value of wave speed was applied with measurements of pressure and

velocity to identify and quantify individual pressure and velocity waves, using wave intensity analysis, within the coronary artery circulation.

Results: A consistent pattern of six predominating waves was identified. 94% of wave energy, accelerating blood forward along the coronary artery, came from two waves: first a pushing wave caused by left ventricular ejection—the dominant forward-travelling pushing wave; and later a suction wave caused by relief of myocardial microcirculatory compression—the dominant backward-travelling suction wave. This suction wave (18.2 (SD 13.7) 10^3 $W m^{-2} s^{-1}$, 30%) was larger than the pushing wave (14.3 (17.6) 10^3 $W m^{-2} s^{-1}$, 22.3%, $p=0.001$) and was



Abstract E, figure 1.



Abstract E, figure 2.

associated with a substantially larger increment in coronary blood flow velocity (0.51 ± 0.14 m/s, $p < 0.001$). In LVH, the suction wave percentage was significantly decreased ($33.1 \pm 26.9\%$, $p = 0.01$) and inversely correlated with left ventricular septal wall thickness ($r = -0.52$, $p < 0.02$).

Conclusions: Six waves predominantly drive human coronary blood flow. These waves are unambiguously identified using wave intensity analysis, and provide a new tool for direct assessment of the coronary microcirculation. Peak coronary blood flow occurs in diastole because of the dominance of a "suction" wave, the dominant backward-travelling suction wave, generated by myocardial microcirculatory decompression. Left ventricular hypertrophy significantly alters the distribution of these waves, markedly reducing the dominant backward-travelling suction wave.

F A NOVEL ARRHYTHMOGENIC INDEX DERIVED FROM THE CRITICAL MASS THEORY PREDICTS OUTCOME AFTER ABLATION OF ATRIAL FIBRILLATION

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Background: The critical mass hypothesis predicts that a minimum mass of tissue is necessary to maintain fibrillation. Mathematical simulations based on a re-entrant model of atrial fibrillation (AF) suggest that myocardial conduction and refractoriness, by altering the wavelength of re-entry, can shift the probability curve for AF at a given atrial mass. We addressed the hypothesis that an Arrhythmogenic Index (AI) based on the atrial volume and wavelength would define the degree of atrial remodelling and thereby predict outcome after AF ablation procedures.

Methods: Patients undergoing clinically indicated left atrial procedures were recruited. Isochronal activation maps of both atria were created using CARTO software during sinus rhythm and pacing at 600 ms.

Conduction velocity, specifically in the direction of wavefront propagation, was determined by a novel algorithm based on principles of triangulation. Effective refractory periods were measured at three sites. Atrial volumes were determined by echocardiography and incorporated with wavelengths to define the AI (volume/wavelength) as a measure of vulnerability to AF. AF patients then underwent ablation procedures that either targeted focal triggers most likely responsible for initiating atrial fibrillation (pulmonary venous isolation/PVI) or modified the substrate (linear ablation). Recurrence of AF was assessed after 6 months, off all anti-arrhythmics.

Results: 64 patients (23 patients with left sided accessory pathways and no history of AF, 22 with paroxysmal AF, 19 with recurrent persistent AF) were studied. In patients with no history of AF, increasing age demonstrates a strong correlation with AI (range 141 to 759 mm², $r = 0.75$, $p < 0.01$), implying an AF-independent, pro-arrhythmic remodelling process. There was a stepwise increase in the AI with increasing burden of AF (controls 404 (SD 147), paroxysmal AF 680 (356), persistent AF 1045 (405) mm², $p < 0.0001$). Patients with recurrent AF after PVI had larger AI (943 (1047) \pm 437 (424) mm², $p = 0.02$). Therefore, in patients with a more arrhythmogenic substrate, ablation strategies aiming to isolate common sources of AF-initiating ectopy are less likely to prevent AF recurrence. For linear ablation, previous PVI ($p = 0.01$) but not AI was associated with successful outcome. This suggests that the substrate has been modified by linear ablation but AF is more likely to recur without isolating focal initiators of AF.

Conclusion: The process of electroanatomical remodelling can be quantified with the use of an arrhythmogenic index, which takes in account multiple pro-arrhythmic factors. Increasing age is associated with an AF-independent process of electroanatomical remodelling, which could account for the aging-related prevalence of AF. The arrhythmogenicity of the substrate is increased in AF and correlates with an increased clinical burden of AF. The degree of remodelling defined by the AI predicts recurrent AF after PVI.