TRACK 7: BASIC SCIENCES

Abstract no: 83

Fetal and neonatal ductus arteriosus is regulated with ATP-sensitive potassium channels

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Background: The fetal patency and neonatal closure of the ductus arteriosus (DA) is regulated with oxygen and prostaglandins. The proposed oxygen sensors of fetal and neonatal DA include P450-endothelin and the Kv channel. We hypothesised that the ATP-sensitive potassium channel (K_{ATP} channel) is another oxygen sensor.
Methods: Fetal and neonatal DA were studied in Wistar rats using sulfonylurea drugs, including tolbutamide, chlorpropamide, gliclazide, glimepiride and glibenclamide (K_{ATP} channel inhibitors), diazoxide and pinacidil (K_{ATP} channel openers: KCOs), and rapid whole-body freezing.
Results: Tolbutamide, chlorpropamide and gliclazide easily passed across the placenta, and dose-dependently constricted fetal DA following orogastric administration to near-term pregnant rats. The fetal DA constricted 30% with clinical doses of sulfonylurea drugs, and closed completely with larger doses. Glimepiride and glibenclamide passed across the placenta minimally, and only mildly constricted the fetal DA after maternal administration, but constricted and closed the fetal DA dose-dependently with direct fetal injection. Fetal DA closure was associated with hydrops and fetal death. Diazoxide and pinacidil delayed DA closure following neonatal injection immediately post-natally, and dilated the closed the DA with injection 30 minutes post-natally.
Conclusions: All tested sulfonylurea drugs constricted fetal DA dose-dependently and with complete closure at large doses. KCOs dilated the neonatal DA. These results indicate physiological regulation of fetal and neonatal DA with K_{ATP} channels. This study has several clinical implications. Sulfonylurea-associated fetal death was 1st reported in South Africa 50 years ago. The mechanism of death remained unclear prior to this study. Sulfonylureas may be useful for closing patent DA in premature neonates. The recently reported reopening of neonatal DA associated with the use of diazoxide for hyper-insulinaemic hypoglycaemia has been proved experimentally. The DA-dilating effect of KCO drugs may be useful as a bridge to surgery in neonatal DA-dependent congenital heart diseases.

Abstract no: 163

Comparison of contractile performance of patients with and without mucopolysaccharidosis undergoing cardiac valve replacement

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Objective: Progressive valve pathology is the most prominent cardiac manifestation in patients with mucopolysaccharidosis (MPS). Valve and papillary muscle thickening and short chordae lead to valve regurgitation and are associated with systolic and diastolic dysfunction. There is some evidence that differences in venous return might cause decreased preload and reduced end-diastolic volume and ventricular pressure, but the underlying mechanism remains unclear.
Methods: Tissue from 6 patients (3 MPS, 3 non-MPS) undergoing aortic and/or mitral valve replacement was obtained from the right auricle, transported in an oxygenated Krebs-Henseleit solution and skinned with Triton-X. We performed 3 experiments on each patient (n=18). The fibres were exposed to a gradual increase in calcium concentration and the corresponding force was measured and recorded.
Results: (1) Calcium-induced contraction was statistically significantly different between patients with and without MPS (p=0.03); (2) The non-MPS male fibres showed significantly higher force values compared to males with MPS (p=0.0002); and (3) Female fibres did not show significant differences in contractile behaviour between MPS and non-MPS fibres.
Conclusion: Our data showed statistically significantly different contractile behaviour of male and female fibres with and without MPS. A lowered calcium sensitivity, which leads to a deficiency in the Frank-Starling mechanism might be the physiological correlation for this observation and may be a clinical indication for optimised pre-operative treatment of these patients.

Abstract no: 164

Impact of gender on contractile behaviour of skinned papillary muscle of female and male patients with mucopolysaccharidosis

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Background: Mucopolysaccharidosis (MPS) are a group of metabolic disorders caused by the absence of lysosomal ensymes which cause dermatan and heparin sulphate to accumulate in the tissue. There is a lack of information regarding the impact of gender on these lysosomal storage diseases.
Methods: We examined right atrial tissue of 3 patients with MPS I or II (n=9, 3 preparations per patient) and significant stenosis of the mitral (Δp mean = 17±11.5mmHg) and aortic valves (Δp mean = 40±21mmHg) and undergoing valve replacement. Human tissue was obtained from the right atrium before implementation of ECC, and it was prepared and stimulated with a stepwise increase in calcium-containing solution. The results were recorded.
Results: All fibres developed the greatest force (mean: 1.71±0.75mN) at the highest calcium concentration (pCa 4.5). Male MPS fibres developed statistically significantly less force than female fibres (p=0.016). The contractile behaviour of the female fibres differed significant from the male fibres (p=0.02 for MPS male 1, and p=0.03 for MPS male 2). Calcium sensitivity, i.e. pCa^{2++}_{50} was also statistically significantly different for male and female fibres (pCa 5.5, female vs male fibres, p=0.01).
Conclusion: The data showed differences in contractile performance in these fibres and imply a different sensitivity in male and female MPS patients. We feel it is justified to report these limited results because this is an extremely rare kind of disease. It may help to consider specific peri-operative treatment for patients with MPS.

Abstract no: 198

Mesenchymal stem cell-mediated reversal of bronchopulmonary dysplasia and associated pulmonary hypertension

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Background and aims: Clinical trials have failed to demonstrate an effective preventative or therapeutic strategy for bronchopulmonary dysplasia (BPD), a multifactorial chronic lung disease in pre-term infants, which is frequently complicated by pulmonary hypertension (PH). Mesenchymal stem cells (MSCs) and their secreted components have been shown to prevent BPD and pulmonary fibrosis in rodent models. We hypothesised that treatment with conditioned media (CM) from cultured mouse bone marrow-derived MSCs could reverse hyperoxia-induced BPD and PH.
Methods: Newborn mice were exposed to hyperoxia (FiO2 = 0.75) for 2 weeks, then treated with 1 intravenous dose of CM from either MSCs or primary mouse lung fibroblasts (MLFs), and placed in room air for 2 - 4 weeks. Histological analysis of the lungs harvested at 4 weeks of age was performed to determine the degree of alveolar injury, blood vessel number and vascular remodelling. At 6 weeks of age, pulmonary artery pressure (PA acceleration time) and right ventricular hypertrophy (RVH; RV wall thickness) were assessed by echocardiography, and pulmonary function tests were conducted.
Results: When compared to MLF-CM, a single dose of MSC-CM-treatment: (1) Reversed the hyperoxia-induced parenchymal fibrosis and peripheral PA devascularisation (pruning); (2) Partially reversed alveolar injury; (3) Normalised lung function (airway resistance, dynamic lung compliance); (4) Fully reversed moderate PH and RVH; and (5) Attenuated peripheral PA muscularisation associated with hyperoxia-induced BPD. A micro-RNA expression analysis on the hyperoxia-induced BPD lungs ±MSC-CM intervention is underway.
Conclusion: To the best of our knowledge this is the 1st evidence that reversal of key features of hyperoxia-induced BPD and its long-term adverse effects on lung function can be achieved by a single intravenous dose of MSC-CM in vivo, thereby pointing to a new therapeutic intervention for chronic lung diseases, including pulmonary hypertensive vascular disease.

Abstract no: 210

Remote ischaemic preconditioning with, but not without, metabolic support protects against ischaemia-reperfusion injury in the newborn piglet in-vivo

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Introduction: While remote ischaemic preconditioning (rIPC) protects the mature heart against ischemia-reperfusion (IR) injury, the effect of rIPC on the neonatal heart is controversial. The neonatal heart relies almost solely on carbohydrate metabolism, which is modified by rIPC in the mature heart. Glucose-insulin (GI) infusion provides myocardial substrate supplementation, which may compensate for adverse metabolic effects induced by rIPC in the immature heart.
Hypothesis: rIPC combined with GI improves cardiac function and reduces infarcts size compared to control or rIPC alone after IR injury in neonatal piglet in-vivo.
Methods: Thirty two newborn (1 - 4 days old) piglets were randomized into 4 groups: control, rIPC, GI and GI+rIPC. GI and GI+rIPC groups received insulin in 20% glucose at a rate corresponding to 100mU/kg/h continuously from 40 minutes prior to ischemia. rIPC and GI+rIPC groups underwent 4 cycles of 5 minutes limb ischemia followed by 5 minutes reperfusion. Myocardial IR was induced by 40 minutes occlusion of the left anterior descending artery followed by 2 hours reperfusion. Left ventricular pressure was measured using 3F Millar microtip catheters. Interstitial lactate was measured using microdialysis and infarct size measured using triphenyltetrazolium chloride staining.
Results: Systolic recovery (dP/dtmax as % of baseline) after 2 hours reperfusion was improved in GI+rIPC (84.7±5.3%) compared to control (71.2±4.9%, $p<0.05$) and rIPC (33.9±12.9%, $p<0.01$) but not different from GI (82.9±8.1%, ns). Lactate levels (% of baseline) were lower in GI+rIPC (85.5±4.9%) compared to control (125.1±9.1%, $p<0.01$) and rIPC (233.9±31.1%, $p<0.01$). Infarct size relative to area at risk was 12.7±1.1% in GI+rIPC compared to 16.4±1.5% in control ($p=0.06$), 18.1±0.8% in rIPC ($p<0.01$) and 24.1±2.1% in GI.
Conclusion: rIPC+GI, but neither rIPC nor GI alone protects the neonatal porcine heart against IR injury in-vivo. rIPC alone appears to have detrimental metabolic and functional effects that are compensated by simultaneous metabolic support with GI infusion.

Abstract no: 251

Iron homeostasis played a critical role in the process of cardiomycocyte hypertrophy during left ventricular retraining for children with transposition of the great arteries

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Background: The left ventricle (LV) regresses after the neonatal period in patients with transposition of the great arteries (TGA) with an intact ventricular septum (IVS) or restrictive ventricular septal defect (VSD). Pulmonary artery banding (PAB) can induce cardiomyocyte hypertrophy for the subsequent arterial switch operation. We screened the altered plasma proteins after PAB and explored the implications for LV retraining.

Methods: Eight late-referral children with TGA/IVS or small VSD were enrolled in part I of the study. The plasma was collected 30 minutes before and 48 hours after PAB. Differential gel electrophoresis (DIGE) proteomics was used to identify the altered proteins. The significantly changed proteins were then confirmed by ELISA. In part II of the study, children with TGA (n=16) undergoing PAB and matched children undergoing open-chest surgery without cardiopulmonary bypass (n=12) were enrolled and the changed level of the chosen proteins was measured. In part III of the study, cell size and mRNA expression of the biomarkers for cardiac hypertrophy were evaluated in a neonatal cardiomyocyte culture model with the associated protein.
Results: Proteomic analysis revealed significant change in 25 proteins. Furthermore, ELISA analysis showed 3 differential proteins, including ceruloplasmin (CP), transferrin (TF) related to iron ion homeostasis, and parvalbumin (PVALB) related to heart development, were regulated 1.37-, 1.33-, 1.38-fold, respectively. These changes were confirmed in part II of the study to exclude the involved inflammatory response during open-chest surgery. The in vitro study showed that after 48 hours' incubation with TF, the size of cardiomyocytes increased 1.94 times. Meanwhile, the expression of natriuretic peptide precursor A and B and PVALB was significantly enhanced.
Conclusions: Augmented levels of CP and TF indicated iron homeostasis played a critical role in the process of immature cardiomyocyte hypertrophy during LV retraining. TF could directly promote cardiomyocyte hypertrophy and hold therapeutic/prognostic potential in clinical practice.

Abstract no: 272

Endogenous sulphur dioxide regulates monocrotaline-induced pulmonary vascular collagen remodelling in rats

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Background: Mechanisms for pulmonary hypertension remain unclear. Sulphur dioxide was recently discovered to be generated endogenously in vascular tissue and to have important vascular functions; however, the role of the endogenous sulphur dioxide pathway in the pathogenesis of pulmonary vascular collagen remodelling has not been defined.
Aim: To explore the role of sulphur dioxide, a new gaseous signal, in the regulation of pulmonary vascular collagen remodelling induced by monocrotaline and its regulatory mechanisms.
Methods: A rat model of monocrotaline-induced pulmonary hypertension was developed and administered with an L-aspartate-ß-hydroxamate and sulphur dioxide donor to evaluate the effects of sulphur dioxide on pulmonary vascular collagen remodelling. The endogenous sulphur dioxide pathway and collagen metabolism were examined. Transforming growth factor-$ß_1$-stimulated cultured pulmonary arterial fibroblasts were used to further the study.
Results: The results showed significant pulmonary hypertension and pulmonary vascular collagen remodelling in association with the augmented sulphur dioxide pathway. L-aspartate-ß-hydroxamate further increased pulmonary artery pressure and aggravated pulmonary vascular collagen remodelling, accompanied by decreased sulphur dioxide production. However, sulphur dioxide donor treatment decreased pulmonary artery pressure, attenuated pulmonary vascular collagen remodelling with inhibited collagen synthesis and augmented collagen degradation, and decreased transforming growth factor-$ß_1$ of the pulmonary arteries. Furthermore, sulphur dioxide could prevent activation of the p38 signalling pathway as well as abnormal collagen synthesis in the fibroblasts.
Conclusions: Up-regulation of the endogenous sulphur dioxide pathway played a protective role in pulmonary artery collagen remodelling induced by monocrotaline. The mechanisms may involve inhibition of the transforming growth factor-$ß_1$ expression and activation of the p38 signalling pathway.

Abstract no: 278

Effect of sulphur dioxide on pulmonary artery pressure in rats with experimental abdominal aorta an inferior vena cava shunting

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Objective: To explore the effect of sulphur dioxide on the pulmonary artery pressure of rats with abdominal aorta and inferior vena cava shunting.
Methods: Twenty five male Sprague Dawley rats were randomly divided into a shunting group; a shunting with sulphur dioxide group; and a control group. Abdominal aorta and inferior vena cava shunting was produced in rats in the shunting group and in the shunting with sulphur dioxide group. After 8-week shunting, the pulmonary artery pressure of each rat was evaluated using the right cardiac catheterisation procedure.
Results: Compared with the control group, the mean, systolic and diastolic pulmonary artery pressures were raised in the shunt rats (72.88, 58.33 and 43.35%, respectively). Compared with the shunting group, the systolic pulmonary artery pressure of rats in the shunting with sulphur dioxide group decreased by 22.12% ($p<0.05$), the mean pulmonary artery pressure decreased by 20.63% ($p<0.05$) but the diastolic pulmonary artery pressure did not change ($p<0.05$).
Conclusion: Sulphur dioxide decreased the mean and systolic pulmonary artery pressures of rats with abdominal aorta and inferior vena cava shunting.

Abstract no: 291

Ischaemia and ischaemic preconditioning in the hypertrophic and failing right heart

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Aim: To investigate the response to ischaemia and ischaemic preconditioning in the hypertrophic and failing right heart.

Methods: Male Wistar rats (n=74) were subjected to a sham operation (n=24), moderate pulmonary trunk banding (mPTB, n=24) or severe pulmonary trunk banding (sPTB, n=26). Four weeks after the operation, the right ventricle (RV) weight and function was evaluated. Hearts were quick-frozen (n=28) or isolated and perfused in Langendorff apparatus (n=46) with Krebs-Henseleit buffer. The perfused hearts were randomised to IPC (2 x 5 minutes global ischaemia) or no preceding ischaemia (CON), followed by 40 minutes of global ischaemia and 120 minutes of reperfusion. Measurement of the infarct size/area-at-risk (IS/AAR) ratio and post-ischaemic RV function was used to evaluate the effect of IPC on the right ventricle. The quick-frozen hearts were used to evaluate key components of the GPCR/NPR-AKT-eNOS-PKG and RISK pathways.

Results: The mPTB procedure caused compensated RV hypertrophy and the sPTB procedure caused RV hypertrophy with failure. Hypertrophy of the RV caused an increase in infarct size in hearts from mPTB and sPTB animals compared to sham rats (IS/AAR: 66.5±3.4; 59.3±2.4 vs. 35.6±2.9% respectively, $p<0.0001$). Cardioprotection by IPC was possible in sham and mPTB hearts, measured by a decrease in IS/AAR and improved haemodynamic recovery of RV contractile function. The mPTB procedure did not cause an increase in RV cGMP. In sPTB hearts with hypertrophy and failure, IPC did not improve IS/AAR or haemodynamic recovery and an increase in RV cGMP was observed.

Conclusion: Right ventricular hypertrophy increased infarct size, and when failure was present, abolished cardioprotection by ischaemic preconditioning in the right ventricular myocardium of the rat. The abolition of cardioprotection was followed by an increase in myocyte cGMP. It will be investigated whether there was a causal connection.

Abstract no: 337

Mutations in calmodulin and ventricular tachycardia, syncope and sudden cardiac death

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Background: Primary arrhythmias such as long QT syndrome (LQTS) and catecholaminergic polymorphic ventricular tachycardia (CPVT) may cause syncope and sudden cardiac death. CPVT might be suspected with a history of exercise/emotion-related syncope, sudden cardiac death, normal QTc on ECG and normal structure of the heart.

Methods: A genome-wide linkage analysis of a large 4-generation Swedish family with CPVT-like disease but without mutations in the cardiac ryanodine receptor gene (RYR2) was performed. A candidate gene involving calcium homeostasis was analysed for mutations using sequencing. The functional consequences of the identified mutation were determined using a calcium-binding assay.

Results: A novel locus for a severe dominantly inherited CPVT-like disease was identified on chromosome 14q31 - 32. Sequencing revealed a heterozygous mutation (Asn5311e) in CALM1 gene encoding for calmodulin, the intracellular calcium sensor and signalling protein. When screening a collection of 61 arrhythmia samples negative for RYR2 mutations, a second de novo missense mutation (Asn97Ser) in an Iraqi patient with CPVT-like disease was found. Both mutations demonstrated compromised calcium binding and the Asn97Ser mutation elicited a defective interaction with RYR2, exclusively occurring at a low calcium concentration.

Conclusion: Missense mutations in calmodulin have been demonstrated. The molecular mechanism of a defective calmodulin-RYR2 interaction may lead to arrhythmias. Calmodulin mutations can be tolerated but might cause CPVT-like disease with syncope and sudden cardiac death.

Abstract no: 470

Contractile properties of human lymphatic vessels in vitro: A new perspective on protein-losing enteropathy following Fontan operation

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Background: Protein-losing enteropathy (PLE) is a devastating late complication after the Fontan operation for univentricular hearts. Although the pathophysiology is not fully understood, high venous pressure is often present and may impair lymph flow, leading to intestinal oedema and PLE. Lymphatic vessels are divided into segments by unidirectional valves and the muscular vessel wall generates phasic contractions pumping lymph centrally. In our study we investigated the contractile properties of human lymphatic vessels to provide insight into the complex pathophysiology of PLE, with a view to future pharmacological treatment.

Methods: Thoracic ducts were harvested with informed consent from 26 patients undergoing oesophageal cancer surgery. Isolated vessel segments were mounted in a wire myograph for isometric force measurements. The diameter-tension relationship was established and the following pharmacological protocols tested: dose-response curves for norepinephrine; endothelin-1 and thromboxane analogue U46619; phasic contractile activity in the presence of L-NAME; and indomethacin.
Results: The diameter-tension relationship revealed the vessels generating maximal contractions at a transmural pressure of 21mmHg. The active curve was flat, indicating the vessels can contract at a wide range of diameters, with peak pressures of approximately 60mmHg. Phasic contraction frequency was 1.39±0.35 min-1. Blocking the NO production with L-NAME, and subsequently prostaglandins with indomethacin, increased the frequency 2- and 5-fold, respectively. Norepinephrine, endothelin-1 and U46619-induced contractions in a dose-dependent manner with maximal contractions of 40 - 60mmHg: High doses of all substances turned the phasic contractions into small oscillations, almost resembling a ventricular fibrillation.
Conclusion: We have shown that the thoracic duct from humans generates phasic contractions and that this activity can be modulated in several ways. Furthermore, the human thoracic duct has the capacity to overcome high venous pressures. Based on our results, we propose a novel approach to treating PLE by pharmacologically increasing lymphatic pumping.

Abstract no: 502

An in vivo cardiac assay to determine the functional consequences of putative long QT syndrome mutations

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Background: Genetic testing for long QT syndrome (LQTS) is now a standard and integral component of clinical cardiology. A major obstacle to the interpretation of genetic findings is the lack of robust functional assays to determine the pathogenicity of identified gene variants in a high-throughput manner. Since zebra fish have cardiac electrophysiology similar to that of humans, the goal of this study was to design and test an in vivo high-throughput cardiac assay to distinguish between disease-causing and normal KCNH2 (hERG1) variants using the zebra fish as a model organism.
Methods: We tested the ability of previously characterised LQTS hERG1 mutations and polymorphisms to restore normal repolarisation in the kcnh2 knockdown embryonic zebra fish. Fertilised zebra fish eggs were injected with kcnh2-morpholino with or without hERG1 mutant cRNAs. The cardiac phenotypes of embryos (48-hour post-fertilisation) were visually assessed under light microscopy to determine the degree of repolarisation. Results of the zebra fish assay were compared with the current benchmark in vitro assay.
Results: The cardiac assay correctly identified a non-disease-causing variant in 9/10 cases (negative predictive value 90%) while correctly identifying a disease-causing variant in 40/40 cases (positive predictive value 100%).
Conclusions: The in vivo zebra fish cardiac assay is as precise as the current benchmark in vitro assay for the detection of disease-causing mutations and far superior in terms of throughput rate. Together with emerging algorithms for interpreting a positive LQTS genetic test, the zebra fish cardiac assay provides an additional tool for the final determination of pathogenicity of gene variants identified in LQTS genetic screening.

Abstract no: 522

Hydrogen sulphide upregulated haeme oxygenase 1 expression in rats with volume overload-induced heart failure

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Background: Chronic heart failure (CHF) is a common complication of left-to-right shunt congenital heart disease. The mechanism responsible for CHF is not fully understood. Hydrogen sulphide (H2S), a newly found gasotransmitter, has been reported to play an important pathophysiological role in the cardiovascular system. The present study was designed to determine the role of H2S in CHF induced by left-to-right shunt, leading to volume overload.
Methods: Thirty male Sprague-Dawley rats were randomly divided into 4 groups: shunt group (n=8), sham group (n=8), shunt+sodium hydrosulphide (NaHS) group (n=8), and sham+NaHS group (n=6). Chronic heart failure was induced in the rats by abdominal aorta-inferior vena cava puncture. Rats in the shunt+NaHS and sham+NaHS groups were injected intra-peritoneally with NaHS (H2S donor) at 56μmolkg-1d-1, and at the same time, rats in the shunt and sham groups were injected with the same volume of physiological saline. Eight weeks after surgery, left ventricular HO-1 mRNA expression was measured by real-time PCR. Protein expression of HO-1 was evaluated by Western blots.
Results: 8 weeks after surgery, protein expression of HO-1 was significantly decreased in the shunt group compared with that in the sham group (0.54±0.11 vs. 1.04±0.20, $p<0.05$). Protein expression of HO-1 was significantly increased in the shunt+NaHS group compared with that in the shunt group (1.06±0.10 vs. 0.54±0.11, $p<0.05$). HO-1 mRNA expression was significantly increased in the shunt+NaHS group, compared with that in the shunt group (5.86±0.61 vs. 1.86±0.29, $p<0.01$).
Conclusions: H2S may play a protective role in volume overload-induced heart failure by up-regulating protein and mRNA expression of HO-1.

Abstract no: 541

Hydrogen sulphine ameliorates volume overload-induced ventricular remodelling by matrix metalloproteinases (MMP-8, MMP-13) and their tissue inhibitor (TIMP-a) in rats

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Background: Chronic heart failure is a common complication of left-to-right shunt congenital heart disease, and ventricular remodelling is an important pathophysiological basis of volume overload-induced chronic heart failure. Hydrogen sulphide (H2S) is a newly found gasotransmitter in the cardiovascular system. But the effect of H2S on ventricular remodelling induced by volume overload is unknown.
Methods: This study used 30 male Sprague-Dawley rats (120 - 140g), which were randomly divided into 4 groups: shunt group (n=8), sham group (n=8), shunt+sodium hydrosulphide (NaHS) group (n=8), and sham+NaHS group (n=6). An animal model of volume overload was induced by abdominal aorta-inferior vena cava puncture in the rats. Eight weeks after surgery, left ventricular matrix metalloproteinase-8 (MMP-8), MMP-13 and tissue inhibitor of metalloproteinase-1 (TIMP-1) expressions were measured by real-time PCR, Western blots and immunohistochemistry, respectively.
Results: Eight weeks after surgery, in the shunt group, MMP-8, MMP-13 and TIMP-1 mRNA expression and the ratio of MMP-13/TIMP-1 were significantly increased compared with those in the sham group (all $p<0.05$). MMP-13 and TIMP-1 mRNA expression and the ratio of MMP-13/TIMP-1 were significantly decreased in the shunt+NaHS group compared with those in the shunt group (all $p<0.05$). Protein expression of MMP-8, MMP-13, TIMP-1, and the ratios of MMP-8/TIMP-1 and MMP-13/TIMP-1 were significantly increased in the shunt group compared with those in the sham group (all $p<0.05$). Protein expression of MMP-8, MMP-13, TIMP-1 and the ratio of MMP-8/TIMP-1 were significantly decreased in the shunt + NaHS group compared with those in the shunt group (all $p<0.05$).
Conclusions: H2S might play a protective role in volume overload-induced ventricular remodelling by regulating protein and mRNA expression of MMP-8, MMP-13 and TIMP-1.

Abstract no: 588

Inotropic effects of iloprost in the hypertropic and failing right heart

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Background: To investigate whether iloprost has a direct inotropic effect in the pressure-overloaded hypertrophic and failing right ventricle (RV).
Methods: Rats were subjected to pulmonary trunk banding (PTB) (n=8) or sham operations (n=8). PTB enables evaluation of the direct effect of iloprost in the right heart, excluding the influence from pulmonary vasodilation. After the development of RV hypertrophy and failure, measurements were made at baseline and after intravenous administration of placebo, iloprost 10ng/kg/min (Ilo10) and iloprost 100ng/kg/min (Ilo100). Cardiac output, systemic blood pressure and RV function were evaluated by MRI, echocardiography and invasive pressure measurements.
Results: Animals subjected to PTB developed significant RV hypertrophy and failure. RV weight/tibia length ratio was elevated and tricuspid annular plane systolic excursion was markedly decreased compared to the sham animals. Iloprost caused a decrease in mean arterial blood pressure (MAP). In both groups of animals, infusion of Ilo100 induced an increase in stroke volume (placebo vs. Ilo100±SEM: PTB 0.19±0.008 vs. 0.21±0.01ml, $p<0.01$, sham 0.25±0.01 vs. 0.28±0.01ml, $p<0.05$) as well as in dP/dtmax (placebo vs. Ilo100±SEM: PTB 4730±451 vs. 5338±605mmHg/sec, $p<0.05$, sham 2218±218 vs. 2521±386mmHg/sec, $p<0.05$). Additionally, an elevation in cardiac output (placebo vs. Ilo100±SEM: 63.0±5 vs.71.4±5ml/min, $p<0.001$) and RV systolic pressure (placebo vs. Ilo100±SEM: PTB 110±6 vs. 121±6mmHg, $p<0.01$) were observed in the PTB group. Infusion of nitroprusside, titrated to cause the same level of decrease in MAP as iloprost, did not increase any of the measured parameters.
Conclusion: Our results suggest that the prostacyclin analogue iloprost has inotropic properties, directly improving ventricular function in the hypertrophic and failing as well as in the healthy right heart.

Abstract no: 613

Vascular histopathological reaction to Gore-Tex strips used for pulmonary artery banding in an in vivo porcine experimental model

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Objective: Pulmonary artery banding (PAB) is used as a surgical palliation to reduce excessive pulmonary blood flow caused by congenital heart defects. Due to the lack of histological studies dealing with tissue remodelling caused by contemporary PAB materials, our aim was to analyse tissue reaction to the presence of Gore-Tex strips fixed around the porcine pulmonary artery.
Methods: Gore-Tex strips were used for PAB in a growing porcine model. After 5 weeks, histological samples with PAB (n=5) were compared to healthy pulmonary arterial segments distal to the PAB or from sham-operated animal (n=1). Using stereology, we quantified the density of vasa vasorum and the area fraction of elastin, smooth muscle actin, macrophages and nervi vasorum within the pulmonary arterial wall.

Results: PAB samples had higher amounts of macrophages, lower amount of nervi vasorum, and a trend towards decreased smooth muscle content when compared with samples without the PAB strips. There was no destruction of elastic membranes, no medionecrosis, no pronounced inflammatory infiltration or foreign body reaction, and no vasa vasorum deficiency following the PAB. All the histopathological changes were limited to the banded vascular segment and did not affect distal parts of the pulmonary artery.
Conclusion: Our results suggest that Gore-Tex strips used contemporarily for PAB in a clinical setting do not cause as severe histological damage to the pulmonary arterial wall after 5 weeks in a growing porcine PAB model compared with previously published series using different PAB material.

Abstract no: 615

Remote ischaemic preconditioning modulates metabolism and impairs recovery in the neonatal heart subjected to ischaemia reperfusion injury

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Background: Remote ischaemic preconditioning (rIPC) reduces cardiac injury in older children and adults after cardiac surgery. Dialysed plasma from mature rabbits undergoing rIPC protects isolated mature rabbit hearts in a Langendorff model, but the effect on the neonatal heart is unknown. The neonatal heart relies almost solely on carbohydrate metabolism, known to be modified by rIPC in the mature heart. We used microdialysis combined with targeted metabolomics to profile metabolism in the immature rabbit heart.
Hypothesis: Treatment of neonatal rabbit hearts prior to ischaemia-reperfusion injury with either in vivo rIPC or perfusion with dialysate from adult rIPC-treated rabbits alters myocardial function and metabolism and may be detrimental.
Methods: Fifteen newborn (1 - 4 days old, 49 - 72g) rabbits were randomised into 3 groups (n=5 in each group): control, in vivo rIPC and adult rIPC dialysate group. Plasma from the rIPC-treated adult rabbits was dialysed, added to the buffer and administered to the dialysate group. Hearts were mounted in a Langendorff model and perfused for 55 minutes stabilisation, 40 minutes global ischaemia and 120 minutes reperfusion. A microdialysis probe was inserted intramurally in the LV and samples (10µl) were analysed using UPLC-MS/MS, targeting lactate and purine metabolites.
Results: During the final 10 minutes of ischaemia, interstitial lactate concentration almost doubled in the rIPC (7.8±1.0mM/l) and dialysate group (9.3±0.6mM/l) compared with the controls (4.6±0.1mM/l) ($p<0.05$). Simultaneously, a marked increase in inosine concentrations was measured for the intervention group: rIPC (49.2±4.0µM/l) and dialysate group (52.5±9.6µM/l) compared to the controls (17.2±6.0µM/l) ($p<0.05$). Functional recovery (dP/dt min) during 30 minutes reperfusion was impaired in the rIPC (-329.4±34mmHg) and the rIPC dialysate (-270.3±65mHg) groups compared to the controls (-683.8± 56mmHg) ($p<0.05$).
Conclusions: In vivo rIPC and rIPC dialysate increased cytosolic energy expenditure during ischaemia and attenuated functional recovery in the neonatal isolated rabbit heart.

Abstract no: 731

Platelet function and morphology in idiopathic pulmonary hypertension

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Background: Thrombosis and proliferation are the two important pathological features of IPAH. Theoretically, both these processes may be initiated by platelets. A barrage of activated platelets in the pulmonary circulation, similar to that happening in the nailbed causing clubbing, may lead to all the known pathological changes seen in IPAH.
Methods: Nine consecutive patients of IPAH [median age of 24 years (14 - 47 years)] were included in the study. Nine patients with rheumatic heart disease with pulmonary hypertension [median age of 42 years (22 - 66 years)] were recruited as controls. Blood samples were taken from superior vena cava, pulmonary artery, left ventricle and femoral artery. Collagen and Adenosine-di-phosphate were utilised for the assessment of platelet reactivity and platelet morphology was analyzed under electron microscopy.
Results: There was no difference in the proportion of active platelets between iPAH group and the control group at various sites. In the iPAH group, the number of active platelets was significantly higher in pulmonary artery as compared to that of femoral artery ($p=0.01$). In contrast, there was no difference in the number of active platelets between various sites in patients with RHD with iPAH. Platelet reactivity at the various sites did not differ significantly between iPAH and RHD with PAH groups. There was no significant difference in platelet reactivity measured biochemically by collagen and ADP between pulmonary artery and the rest of the sites.
Conclusions: We found an increased existence of active platelets in pulmonary circulation as compared to systemic circulation by electron microscopy in iPAH patients. We found no difference in the level of platelet reactivity between pulmonary and systemic circulation biochemically. Patients with iPAH did not show significantly high platelet reactivity as compared to the patients with RHD with moderate to severe PAH.

Abstract no: 770

Reversible pulmonary trunk banding: Wall stress-associated activation of myocardium glucose-6-phosphate dehydrogenase is normalised by intermittent systolic overload in young goats

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Background: Increased myocardial glucose-6-phosphate dehydrogenase (G6PD) activity has been demonstrated in young goats submitted to traditional pulmonary artery banding (PAB). This biochemical alteration drives superoxide anion generation and elevates oxidative stress by elevated G6PD-derived NADPH in the failing heart. This study sought to assess the myocardial mechanics and kinetics of G6PD activity during intermittent systolic overload of the sub-pulmonary ventricle in a young animal model.

Methods: Thirty young goats with comparable weights were separated into 5 groups according to the study period duration (0, 24, 48, 72, and 96 hours). A 12-hour systolic overload of the right ventricle (RV) was alternated with a 12-hour resting period with an adjustable PAB. Systolic overload was adjusted to achieve a 0.7 RV/aortic pressure ratio. Echocardiographic and haemodynamic evaluations were performed every day post-operatively. After completing the training programme of each group, the animals were humanely killed for morphological and G6PD activity assessment.

Results: A 130.8% increase occurred in the RV mass of the 96-hour group, compared with the 0-hour group ($p<0.0001$). Increased RV volume/mass ratio and wall stress observed in the 24-, 48-, and 72-hour groups were associated with increased RV G6PD tissue activity (Pearson correlation, 0.77 and 0.87; $p = 0.05$ and 0.03, respectively). A full recovery of these parameters was observed in the 96-hour group, compared to baseline values. No significant changes were observed in the G6PD activity of the ventricular septum and left ventricle.

Conclusions: G6PD tissue activity is associated with changes in ventricular volume and RV wall stress. This study suggests that intermittent systolic overload for sub-pulmonary ventricle retraining in young goats may improve the altered cardiac energy substrate metabolism and decrease the formation of reactive oxygen species, thus preventing cardiac deterioration post-PAB.

Abstract no: 831

The identification and verification of putative KCNE2-interacting proteins with relevance to Long QT syndrome

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Background: Long QT syndrome (LQTS) is a cardiac repolarisation disorder characterised by a prolonged QT interval on an echocardiogram (ECG). The symptoms of LQTS range from minor like dizziness and syncope to more severe such as seizures and sudden cardiac death (SCD). Clinical features of LQTS are a result of the precipitations of Torsades de Pointes, which is a form of polymorphic ventricular tachycardia. A number of genetic forms of LQTS have been identified with more than 900 mutations in 10 different genes leading to disease pathogenesis. However a large percentage of LQT affected patients have no mutations within the known LQT genes. Of these known mutated genes, KCNE2 is one that is associated with LQT6. This is a beta-subunit of potassium ion channels of which the mutations are mainly located in the C-terminal domain. Thus we hypothesise that genes encoding proteins that interact with KCNE2 might be identified as disease modifying genes and the present study aims to use a yeast 2 hybrid (Y2H) analysis in order to identify and verify putative interactors of the C-terminal of KCNE2.

Materials and methods: The C-terminal domain of KCNE2 was used as bait to screen a pre-transformed cardiac cDNA library using Y2H analysis. Putative interactors will be verified using 3D-colocalisation and Co-immunoprecipitation experiments.

Results: A number of putative KCNE2 interactors were identified by Y2H and are currently being verified. These include Filamin C (FLNC), protein tyrosine phosphatase (PTPRK), crystalline alpha B (CRYAB), voltage-dependent anion-selective channel protein 1 (VDAC1), titin (TTN) and cardiac actin (ACTC1).

Conclusion: The genes encoding verified interactors will be screened in our SA panel of LQT patients, to potentially identify novel LQT causative genes. Furthermore, the interactions verified in the present study may shed some light on the mechanism of pathogenesis of LQT causative mutations in KCNE2.

Abstract no: 861

Right ventricular myocardial performance index is paradoxically decreased with severe pressure overload hypertrophy in young rats

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Background: Although the myocardial performance index (MPI) is usually increased in the presence of RV dysfunction, debate continues over the correlation between right ventricular (RV) MPI and functional derangement in patients with congenital heart disease (CHD). To address this controversy, we took serial measurements of the RV MPI during the development of RV dysfunction induced by pressure overload.

Methods: RV pressure overload was induced by partial pulmonary arterial banding (PAB) in 3-week-old rats. The rats were divided into 2 groups: mild pulmonary stenosis (PS) group (20 - 40% stenosis, n= 20) and severe PS group (40 - 70% stenosis, n=28). Sham-operated animals (sham group, n=30) underwent the same

surgical procedure without PAB. Pressure-overloaded RV hypertrophy, which was documented by weighing the heart, evaluation of echocardiograms, and cardiac hypertrophy-associated gene expression were evaluated. The RV MPI was checked 1, 2, 3 5, and 8 weeks after PAB.
Results: The RV MPI of the mild PS group did not differ significantly from that of the sham group. The RV MPI of the severe PS group, however, was paradoxically lower than that of the sham group ($p<0.05$).
Conclusions: The RV MPI was paradoxically decreased in severe RV pressure overload hypertrophy induced by PAB.

Abstract no: 898
Glycogen kinase-3 inhibition: Good or bad for the heart?

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Background: Glycogen synthase kinase-3 (GSK-3) is a serine-threonine kinase that was discovered as a regulator of glycogen synthase and known as a role player in cardioprotection. Myocardial GSK-3 may: (1) Regulate expression of SERCA-2a, affecting contractility; (2) Phosphorylate and inhibit IRS-1, disrupting insulin signalling; and (3) Regulate growth via interaction with Wnt and hypertrophic signalling pathways. GSK-3 inhibitors are being developed for clinical use.
Aim: To determine whether myocardial GSK-3 and its substrate proteins are dysregulated in obesity and pre-diabetes, and to study the effects of GSK-3 inhibition on the hearts of obese, pre-diabetic rats.
Methods: Pre-diabetic Wistar rats [induced by a diet causing hyperphagia (DIO) for 16 weeks] were compared to age-matched controls. Half of each group was treated with the GSK-3 inhibitor (CHIR118637 - 30mg/kg/day) for 4weeks (weeks 12 - 16 of the diet period). After 16 weeks, echocardiography was performed and glucose tolerance was established, biometric and biochemical parameters were determined, myocardial performance was verified by ex vivo perfusion, and protein expression was ascertained in snap-frozen hearts by Western blotting and specific antibodies. Ca2+ATPase activity was determined spectrophotometrically and cardiomyocytes were used to determine cell size and localisation of NFATc3 and GATA4.
Results: Treated and untreated DIO gained more body weight and intra-peritoneal fat. GSK-3 inhibition improved glucose tolerance and echo parameters in DIO. CHIR had no effect on GSK-3 expression but increased phosphorylation in CHIR. CHIR was associated with increased NFATc3 and GATA4 nuclear translocation. CHIR elevated IRS-2 expression but had no effect on IRS-1 and SERCA-2a.CHIR increased PKB/Akt and phospholamban phosphorylation in DIO rats.
Conclusion: GSK-3 protein may play a role in glucose homeostasis and regulation of IRS-2 expression but its inhibition did not enhance IRS-1 or SERCA-2a expression. CHIR reversed cardiac hypertrophy in DIO rats but it caused hypertrophy in the controls.

Abstract no: 907
Genetic polymorphisms associated with allogeneic red blood cell transfusion requirements in paediatric patients undergoing cardiac surgery

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Background: Patients undergoing repair of congenital heart disease require allogeneic blood transfusions, mainly to allow cardiopulmonary bypass and to offset blood loss from bleeding. Clinical factors associated with increased bleeding risk, include coagulation system activity, platelet reactivity, use of deep hypothermic circulatory arrest, and surgery duration and complexity. We sought to determine whether genetic polymorphisms known to be associated with the coagulation/fibrinolytic system or with platelet function are associated with red blood cell transfusion requirements associated with paediatric cardiac surgery.
Methods: A total of 625 cardiac surgeries in 383 patients were reviewed. Ninety six SNPs on 53 genes involved in the coagulation/fibrinolysis pathways were assayed using the Illumina GoldenGate® custom SNP panel; genotyping was successful for >99% of SNPs. Associations between SNPs and red blood cell transfusions within 48 hours of surgery (adjusted for age at surgery, surgical complexity and pre-operative oxygen saturation) were assessed in regression models adjusted for repeated measures. Bootstrap resampling (1 000 samples) was used to offset multiple comparison bias and exclude SNPs with very low minor allele frequencies.
Results: Median red blood cell requirement was 114ml/kg (interquartile range: 73 - 174ml/kg). Coagulation factor polymorphisms associated with increased red blood cell requirements included factor VIII rs100873005 CC/CG SNPs [+40 (14) ml/kg, p=0.004] and factor XI rs2036914 CC SNPs [+51 (16) ml/kg, p=0.001]. Additional SNPs associated with a higher volume of red blood cell requirements included alpha-2-macroglobulin precursor rs669 GG SNPs [+91 (23) ml/kg, p<0.001], guanine nucleotide-binding protein ß3 rs5443 CC SNPs [+35 (18) ml/kg, p=0.05] and chemokine receptor 2 rs1799864 AA/AG SNPs [+42 (16) ml/kg, p=0.008]. Both alpha-2-macroglobulin precursor rs669 and factor XI rs2036914 were also found to be associated with low pre-operative antithrombin activity, a key marker of heparin resistance and increased bleeding volumes.
Conclusions: Patients with congenital heart disease have substantial transfusion requirements during and immediately after paediatric cardiac surgery. While clinical factors are critical in determining the required amount of transfusions, genetic polymorphisms also have a key role in this process.

Abstract no: 908

Assessing the interleukin-6 174 G/C single nucleotide polymorphism and coronary artery disease

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Background: Interleukin 6 (IL6) is a pro-inflammatory cytokine involved in the pathogenesis of chronic inflammatory diseases such as coronary artery disease (CAD). The -174 IL6 G/C promoter polymorphism influences mRNA and protein levels and is implicated in CAD. This polymorphism has been investigated but limited data are available on South African Indian (SAI) and black (SAB) population groups, despite high disease prevalence. This study aimed to assess the -174 IL6 G/C polymorphism in SAI subjects with CAD.
Methods: Polymorphic variants were assessed by polymerase chain reaction–restriction fragment length polymorphism, and IL6 levels were measured by ELISA.
Results: The -174 IL6 C allele was found at a higher frequency in the total SAI subjects (23%) compared to SABs (2%), irrespective of disease status ($p<0.0001$, OR = 0.0503, 95% CI: 0.0183 - 0.1388) and in healthy SAI (29%) and SAB (2%) controls ($p<0.0001$, OR=0.0507, 95% CI: 0.0152-0.1699). A significant association between the -174 IL6 G allele and CAD in SAI was found (84 vs. 71% - SAI controls; $p=0.0431$, OR=0.468, 95% CI: 0.23-0.953). Circulating levels of IL6 were elevated in total (6.58±0.56pg/ml) and healthy (6.62±0.63pg/ml) SABs compared to total (1.80±0.22pg/ml) and healthy (2.51±0.57pg/ml) SAI groups, as well as CAD patients (1.46±0.36pg/ml, $p<0.0001$). Levels of IL6 were elevated in all groups with homozygous -174 IL6 C alleles but only significant in the healthy SAI group (GG: 3.73±0.94pg/ml vs. GC/CC: 0.89±0.5pg/ml, $p=0.0001$).
Conclusion: The presence of the IL-6 -174 G allele influences levels of IL-6 and increases the risk of CAD in South African Indians.

Abstract no: 922

Autophagy upregulation in cardiotoxicity: Pharamacological vs. genetic manupulation

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Background: Cardiotoxicity is a well-known side effect of anthracyclines such as doxorubicin (DXR), resulting in substantial morbidity. The most widely accepted hypothesis for their mechanism of action is oxidative stress, which leads to the induction of cell death as a direct consequence of DNA damage and/or interference with DNA repair. Autophagy, a major catabolic process, has been shown to play a vital role in cardiac homeostasis. This process is often elevated following various forms of cardiovascular stress. However, whether autophagy participates as a pro-survival or pro-death pathway remains to be determined. This study therefore aimed to determine whether pharmacological or genetic manipulation of autophagy alleviates DXR-induced toxicity.
Methods: H9C2 rat cardiac myoblasts were treated with rapamycin (50nM-CR) or siRNA (mTOR-CM) for 24 hours to up-regulate autophagy. This was followed by treatment with DXR alone (3μM-CD) or in combination with rapamycin (RD) or siRNA (MD) for a further 24 hours, where after cell viability, apoptosis, mitochondrial morphology and DXR localisation was assessed.
Results: Assessment of cell viability indicated that groups CM and CD significantly reduced viability [75.48±1.81% ($p<0.001$) and 65.58±2.25% ($p<0.01$)] vs. the control. Group RD significantly improved viability [78.93±10.85% ($p<0.05$)] vs. CD. Caspase activity was also significantly elevated in group CD [444.60±29.33% ($p<0.001$)] vs. the control, whereas group RD significantly reduced (78.86±7.14%) caspase activity. Normal mitochondrial morphology was not adversely affected in groups CM, CR and RD. However groups CD and MD displayed abnormal mitochondria that were shorter, fragmented and discontinuous.
Conclusions: These results indicate a prospective role for rapamycin against DXR-induced cardiotoxicity and highlight rapamycin as a plausible adjuvant therapy to counteract and improve the life-threatening impediment of DXR's actions in clinical practice.

Abstract no: 928

Grape seed proanthocyanidin extract limits cardiac damage

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Background: Heart failure is a leading cause of mortality worldwide. To date, many pharmaceutical agents have been used to treat cardiac hypertrophy and early stages of heart failure. These therapies have proven reasonably effective; however, there is a need for alternative therapeutic strategies, more specifically, natural therapies that are cost effective and safe, to prevent or reverse hypertrophy before it develops into heart failure. Proanthocyanidolic oligomer supplementation (PCO), a grape seed extract, was shown to quicken muscle recovery and reduce inflammation in a skeletal muscle model of injury. Considering the positive effects on muscle recovery, as well as results suggesting that resveratrol, another member of the polyphenol family, could limit the occurrence of cardiac hypertrophy, the possibility exists that PCO might also be beneficial.
Methods: An osmotic mini-pump containing isoproterenol (2mg/kg/day), a dual $ß_1$ - $ß_2$-adrenergic receptor agonist, was used to induce hypertrophy. Male Wistar rats (280 - 320g) were orally gavaged with either PCO (20mg/kg/day) or distilled water for 2 weeks prior to the subscapular implantation of the pump containing isoproterenol or ascorbate (vehicle). After 7 days, the rats were killed and the heart isolated. Paraffin wax-embedded hearts were sectioned (2μm) and stained with Picro Sirius for fibrosis. A macrophage marker, ED1, was used to determine the infiltration of immune cells.
Results: Results indicated that the isoproterenol groups lost significant amounts of weight one day ($p<0.05$) after implantation. PCO treatment reduced hypertrophy, as measured by a lower heart weight to tibia length and heart weight to body weight ratio. Histological analysis showed more damage, inflammation and fibrosis in the isoproterenol group receiving placebo treatment (I-PLA) compared to the group receiving PCO supplementation (I-PCO). Both groups displayed significantly more fibrosis than their respective controls.
Conclusion: The cost-effective, over-the-counter PCO supplement resulted in better recovery after isoproterenol infusion.

Abstract no: 932

Glucose and insulin improve recovery after de novo acute heart failure by stimulating the sinus node

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Background: Glucose-insulin-potassium (GIK) infusions improved recovery from sinus node dysfunction in patients. GIK infusions given in the ambulance to patients with acute coronary syndrome reduced hard endpoints in the immediate study, but effects on acute heart failure (AHF) are still unknown.
Methods: We therefore tested GI therapy on isolated rat hearts perfused retrogradely by a modified Krebs-Henseleit solution in the Langendorff system subject to de novo AHF. In this model the initial stabilisation phase hearts were perfused at 100cmH_2O with glucose (11.1mM) as sole substrate. Thereafter AHF was induced by under-perfusion at 20cmH2O. We added adrenaline 10^{-8}M to induce a hyper-adrenergic stimulus while reducing perfusate glucose to 2.5mM and adding high free fatty acids (FFA) to the buffer (1.3mM, 0.1mM BSA). In the recovery phase the hearts recovered incompletely although the perfusion pressure was restored to 100cmH_2O with the continued presence of high FFA, and with increased glucose (11.1mM). Only half of the hearts in this phase received insulin (0.3mU).
Results: Glucose coupled with insulin in the recovery phase increased the heart rate (168.5±34.5 vs. 36.7±25.0beats/min, $p<0.01$). LV developed pressure was unchanged in both groups (31.9±7.2 vs. 38.9±16.9mmHg). We attribute this cardioprotective increase of heart rate to the electrophysiological effects of glucose and insulin on sinus node function.
Conclusions: Our data suggest that glucose and insulin improve the heart rate of the acutely failing isolated heart by improving sinus node recovery after de novo AHF.

Abstract no: 960

Anti-senescent effect of statin therapy in coronary artery disease

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Introduction: Atherosclerosis is a premature-ageing syndrome associated with senescence of vascular endothelial and smooth muscle cells. Premature cellular senescence is induced by extrinsic risk factors that damage the structure of DNA. Experimental evidence and emerging clinical data suggest that the HMG CoA reductase inhibitors may exert their beneficial effects in patients with coronary artery disease (CAD) by reducing cellular senescence and preventing apoptosis.
Aim: We investigated the effect of prior statin therapy on leukocyte telomere length and the interaction with markers of inflammation, oxidative stress and cellular apoptosis.
Methods: Consecutive patients <45 years admitted to a tertiary cardiac unit were recruited following confirmation of obstructive CAD on angiography. A population-based, randomly selected, matched, healthy control group who were statin naïve were also studied. Apoptotic activity in peripheral blood mononuclear cells was determined using the Caspase-Glo® 3/7, 8 and 9 assays. Oxidative stress was assessed with athiobarbituric acid assay, which measures the lipid peroxidation end-product malondialdehyde. Leukocyte telomere length was measured with a quantitative PCR-based technique and calculated as the ratio of telomere repeats to single-copy gene copies (T/S ratio).
Results: The mean duration of statin therapy in patients was 31 months. Lipid peroxidation was significantly elevated in patients compared to controls [median/interquartile range 0.0060 (0.0030 - 0.0140) cases, 0.0035 (0.0025 - 0055) controls, $p<0.009$]. Caspase 8, an initiator of apoptosis activated by the extrinsic pathway, was significantly reduced in the cases. Telomere length was significantly longer in the cases [cases 0.71 (0.69 - 0.73), controls 0.67 (0.63 - 0.70), $p<0.001$]. There was no significant difference in LDL cholesterol and hsCRP levels between the groups.
Conclusion: Unrelated to the lipid-lowering, anti-inflammatory and anti-oxidant effects, chronic statin therapy was associated with longer telomere length, a marker of vascular ageing. This anti-senescent effect of statin therapy may emerge as a new strategy in the treatment of atherosclerosis.

Abstract no: 980

Regulation of cardiac hormones during asphyxia in neonates: Studies in a piglet model

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Background: The natriuretic peptides (ANP and BNP) are expressed in the myocardium and related to cardiac dysfunction. In adults they are up-regulated in response to cardiac ischaemia and increased wall stress, as seen in acute coronary syndromes and cardiac failure. In neonates, the peptides are also pursued as cardiac biomarkers. However, the role of a global hypoxic-ischaemic insult in the newborn period on cardiac natriuretic peptide expression has not been investigated. We investigated this in a 72-hour piglet model of hypoxic ischaemic encephalopathy.
Methods: 18-hour-old piglets were randomly divided into a control and intervention group. The piglets in the intervention group were anaesthetised and exposed to global hypoxia (45 minutes) verified by EEG-depression and arterial pH<7.0, whereas control piglets were only anaesthetised. The piglets were awakened and intensively cared for during a total of 72 hours. Blood samples were drawn after intubation but prior to any hypoxia and again after 72 hours. Natriuretic peptide concentration was analysed. The piglets were euthanised and regional myocardial biopsies were obtained. These biopsies were analysed for cardiac expression of natriuretic peptides and natriuretic peptide receptors by real-time PCR and Western blot.
Results: The biochemical analyses are being performed and results are pending.
Conclusion: In order to validate whether natriuretic peptides can be used as specific biomarkers of cardiac compromise in neonates, it will be of value to conclude whether the natriuretic peptide expression occurs as a result of global hypoxia per se or not. Furthermore, it is of great interest to convey whether the natriuretic peptide expression is regional and corresponds to plasma values in the same manner as seen in adults to verify whether parallels can be drawn from the vast knowledge of natriuretic peptides in adult cardiac disease.

Abstract no: 985

Does cardiac muscle restoration of the left ventricle differ from the right ventricle in the normal canine heart? A passive stress-strain relationship in vitro study

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Background: Early deterioration of diastolic ventricular function usually precedes systolic ventricular abnormalities. Normal cardiac muscle restoration is important for adequate relaxation and may depend on muscle fibre orientation. The passive stress-strain relationship as an indicator for cardiac muscle restoration has been established.
Objective: The goal of the study was to compare and characterise the in vitro passive stress-strain relationship of the left and right ventricular muscle in the normal canine heart.
Methods: Cardiac muscle tissue from 4 normal canine hearts was studied. Samples were taken from the ventricular free wall, ventricular septum and papillary muscles of both left and right ventricles. Each sample was divided into 3 blocks and was studied in 3 different orientations: longitudinal, radial and circumferential. Each sample underwent compression under 4 different strain rate conditions, ranging from 10 - 40/sec. Relaxation of the cardiac muscles was recorded by multiple sensors for each sample, and stress-strain loops were calculated from force and displacement data.
Results: Stress-strain relationships of the left and right ventricles were significantly different. Stress of the left ventricular free wall and septum, when measured in the radial and circumferential direction, was significantly higher than for the same amount of strain applied on right ventricular muscle. LV stress=40.5 - 60.2Pa (mean=48.2); RV stress=20.1 - 38.9Pa (mean= 1.2) ($p<0.001$). When the longitudinal direction was compared, right ventricular stress was significantly higher than left ventricular stress under the same strain conditions, RV stress=40.7 - 80.6Pa (mean=49.1); LV stress=20.4 - 40.3 (mean=31.2) ($p<0.001$). Papillary muscle stress-strain relationship was similar for both ventricles in the longitudinal direction.
Conclusions: Cardiac muscle restoration is different between the left and right ventricles in the normal canine heart. LV muscle is more efficient in the longitudinal direction, while RV muscle is superior in the radial and circumferential orientations. Measurements of cardiac restoration may provide new insight on diastolic function.

Abstract no: 1193

Changes of caspase 3, BCL2 and vascular endothelial growth factor gene after human umbilical cord blood-derived mesenchymal stem cell transfusion in pulmonary hypertension in rat models

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Objective: Pulmonary arterial hypertension (PAH) is difficult to treat and is characterised by increased pulmonary arterial pressure, right heart failure and death. PAH has been shown to be refractory to most of the conventional pharmacological therapies. Human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) are regarded as an alternative source of bone marrow-derived mesenchymal stem cells. hUCB-MSCs have recently been studied for evaluation of their potential as a source of cell therapy. The purposes of this study were to investigate changes of pulmonary pathology, haemodynamics and gene expression of caspase 3, Bcl2, vascular endothelial growth factor (VEGF), interleukin (IL)-6, and tumour necrosis factor (TNF)-α in monocrotaline (MCT)-induced PAH rat models after hUCB-MSCs transfusion.
Methods: The rats were grouped as follows: Control group (C group), subcutaneous injection of saline; M group, subcutaneous injection of MCT (60mg/kg); and hUCB-MSCs transfusion (U group). hUCB-MSCs (3×10^6/ml/cm^2) were transfused by intraperitoneal injection 1 week after MCT injection.
Results: The mean right ventricular pressure (RVP) significantly decreased in the U group compared with the M group in weeks 2 and 4. RV weight and the ratio of RH/LH+septum significantly decreased in the U group compared to the M group. The number of muscular pulmonary arterioles significantly decreased in the U group compared with the M group in weeks 2 and 4. Medial wall thickness of the pulmonary arteriole significantly decreased in the U group compared to the M group in week 2. Gene expressions of caspase-6, Bcl-2, VEGF, IL-6 and TNF-α significantly decreased in week 4 in the U group compared with the M group.
Conclusion: After hUCB-MSCs transfusion there was improvement of RVH, mean RV pressure and survival rate. Decreases in several gene expressions were observed. Additional research on the dose and frequency of hUCB-MSCs infusion is needed to determine the optimal parameters for PAH treatment.

Abstract no: 1194

Changes in caspase 3, BCL2 and vascular endothelial growth factor after bone marrow cell transfusion in rats with monocrotaline-induced pulmonary hypertension

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Background: Pulmonary arterial hypertension (PAH) is difficult to treat and is characterised by increased pulmonary arterial pressure, right heart dysfunction, lung vascular remodelling and death. Bone marrow-derived mesenchymal stem cell therapy has provided an alternative treatment for ailments of various organs by promoting regeneration at the site of pathology. The purposes of this study were to investigate changes of pulmonary pathology, haemodynamics and gene expressions of Caspase 3, Bcl2, and vascular endothelial growth factor (VEGF) in monocrotaline (MCT)-induced PAH rat models after bone marrow cell (BMC) transfusion. The rats were grouped as follows: control group; M group, subcutaneous injection of monocrotaline (MCT); BMC transfusion (B group). BMC were transfused by intravenous injection in the tail 1 week after MCT injection.

Results: The mean right ventricular pressure (RVP) significantly decreased in the B group compared with the M group in weeks 2 and 4. RV weight significantly decreased in the B group compared to the M group in weeks 2 and 4. The ratio of medial thickening of the pulmonary artery was significantly decreased in the B group compared with the M group in week 2. The number of muscular pulmonary arterioles significantly decreased in the B group compared with the M group in week 4. The number of muscular pulmonary arterioles increased in the M group compared with the C group in weeks 2 and 4. Gene expressions of caspase 3, Bcl2, and VEGF significantly increased in the M group compared with the C group and significantly decreased from week 2 in the B group compared with the M group.
Conclusion: After BMC transfusion, there was improvement of RVH and mean RV pressure. Decreases in several genes were observed. Additional research on the dose and frequency of BMC infusion is needed to better determine the optimal parameters for PAH treatment.

Abstract no: 1218
Complementary roles of the novel biomarker ST2 and NT-pro-BNP in African hypertensive subjects

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Background: Although NT-pro brain natriuretic peptide (NT-proBNP) levels have been shown to differentiate hypertensive left ventricular hypertrophy (LVH) without heart failure (HF) from hypertensive HF due to systolic and/or diastolic dysfunction, it has not been very helpful in differentiating hypertensive subjects with LVH from those without. We therefore decided to see the complementary role of soluble ST2, a novel biomarker of biomechanical strain.
Methods: This was a prospective cohort study. Echocardiography was performed on all subjects. LVH was considered present when left ventricular mass, indexed for height$^{2.7}$, was >46.2 g/m$^{2.7}$ in women and >49.2 g/m$^{2.7}$ in men. Plasma ST2 and NT-proBNP was measured using electrochemiluminescence-type immunoassay.
Results: Of 210 subjects studied, 42.9% were female, and the mean age of the study cohort was 50.3±11.3 years. Hypertensive subjects with LVH had higher concentrations of ST2 compared to those without LVH (23.0±8.3 vs. 14.5±4.9ng/ml, p=0.001) and those with hypertensive HF had higher levels compared with those with hypertensive LVH (134.7±57.3 vs. 23.0±8.3ng/ml, p=0.000). There was however no significant difference between NT-proBNP levels when hypertensive subjects with LVH were compared with those without LVH (p=0.68) but those with heart failure had significantly higher NT-proBNP levels compared with hypertensives with LVH (p<0.000). ST2 has a stronger correlation with clinical and echocardiographic parameters compared to NT-proBNP. Serum ST2 also correlated well with NT-proBNP (r=0.41, p<0.000). In the assessment of the hypertensive heart disease spectrum, ST2 correlates well with NT-proBNP and has proven to be a better marker.
Conclusions: Plasma ST2 levels appear to be a very useful marker in differentiating the different spectra of hypertension-hypertensive heart disease and may hold a future role in this regard.

Abstract no: 1226
Gene expression profiles in engineered cardiac tissues respond to mechanical loading and inhibition of tyrosine kinases

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Background: Several formulations for engineered cardiac tissue (ECT) have emerged that incorporate stem cells or immature cardiomyocytes into three-dimensional (3-D) constructs. These ECTs mature in vitro, acquire the features of mature cardiac muscle, appear to involve the p38MAP kinase (p38MapK) pathway, and respond to mechanical load with increased proliferation and maturation. We hypothesised that global ECT gene expression patterns are sensitive to mechanical loading conditions and tyrosine kinase inhibitors.
Methods: We generated 3-D ECTs from immature rat embryo heart cells, as previously published, and then treated constructs after 5 days in culture for 48 hours with mechanical stretch (5%, 0.5Hz) and/or the following selective inhibitors (birb796 for p38MapK, CI10404 for ERK1/2, or SP60025 for JNK). RNA was isolated from 3 sets of experiments and assayed using a standard Agilent rat 4 x 44k V3 micro-assay. The Ingenuity Systems Pathway analyser was used to analyse data from individual experiments, pooled within groups and between groups.
Results: Changes in gene expression in response to mechanical stretch and/or inhibitors were recorded. As anticipated, top pathways altered in response to these stimuli included cellular development, cellular growth and proliferation; tissue development; cell death, cell signalling and small-molecule biochemistry, as well as numerous other pathways.
Conclusion: ECTs display a broad spectrum of altered gene expression in response to mechanical load and/or tyrosine kinase inhibition, reflecting the complex regulation of proliferation, differentiation and architectural alignment during ECT maturation.

Abstract no: 1381

Cardioprotective effect of ACE 2 activator on left ventricular dysfunction secondary to pressure overload in the rat

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Objectives: The RAS (renin-angiotensin system) is activated after myocardial infarction, and RAS blockade with angiotensin converting enzyme inhibitors or angiotensin receptor blockers slows but does not completely prevent progression to heart failure. In contrast, angiotensin converting enzyme 2 (ACE 2)/angiotensin-(1 - 7) [Ang-(1 - 7)]/Mas is recognised as a counter-regulatory axis. Little is known about the role of ACE 2 in cardiac dysfunction secondary to pressure-overload.

Hypothesis: In pressure overload-induced cardiac dysfunction, we hypothesised that cardiac expressions of ACE 2 and Ang-(1 - 7) are down-regulated, and an ACE 2 activator can attenuate the development of left ventricular dysfunction through ACE2/Ang-(1 - 7)/receptor mas axis.

Methods and Results: In the Wistar rats subjected to ascending aortic banding (AOB), starting 29 days after banding, banded rats were treated with DIZE (ACE 2 activator) at a dose of 15mg/kg/day intra-peritoneally or vehicle for 14 days. Subsequently, there was down-regulated cardiac expression of Ang-(1 - 7) in AOB for 42 days compared to sham-operated rats. DIZE could significantly decrease the mean pulmonary arterial pressure and mean left atrial pressure, and attenuate left ventricular remodelling, respectively, when compared with the vehicle controls. In addition, DIZE caused up-regulated expression of ACE 2, receptor mas and endothelial nitric oxide synthase in 42-day banded rats.

Conclusions: These results indicate that activation of ACE 2 may provide preventive potential for attenuating the development of left ventricular dysfunction secondary to pressure overload. Further translational study, including oxidative stress in humans, is needed to substantiate the findings.

Abstract no: 1422

Arg72 variant of p53 codon 72 functional polymorphism and risk of coronary artery disease in a South African population

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Introduction: Atherogenic stimuli induce DNA damage through increased oxidative stress. DNA damage leads to increased expression of p53, a pro-apoptotic gene whose principal function is to protect cells from malignant transformation. p53 is thought to contribute to vascular disease by increasing apoptosis of macrophages and smooth muscle cells in advanced atherosclerotic lesions, rendering them vulnerable to rupture. More recently, p53 has been shown to regulate genes involved in lipid and carbohydrate metabolism. Polymorphisms of the p53 gene have been associated with increased susceptibility to coronary artery disease (CAD).

Methods: A common polymorphism in the p53 gene, Pro72Arg (rs1042522), results in the substitution of arginine (Arg) for proline (Pro) at codon 72 in the amino acid sequence of the protein. The Arg72 has been reported as a more potent inducer of apoptosis than the Pro72 variant. 100 young (mean age 37.5 years, range 24 - 45) male Asian Indian patients with CAD confirmed at angiography were compared with 100 healthy control subjects matched for age, gender and ethnicity. Polymorphic variants were assessed by polymerase chain reaction-restriction fragment length polymorphism.

Results: The frequency of p53 codon 72 genotypes were 28% Arg/Arg, 48% Arg/Pro and 24% Pro/Pro in CAD patients compared to 30, 61 and 9%, respectively in the control group. A significantly higher frequency of the p53 Arg72 allele was found in CAD patients compared to the p53 Pro72 allele (52 vs. 40%, $p<0.0121$, OR = 1.659, 95% CI: 1.116 - 2.467). Lipid and glycaemic indices were not significantly influenced by the p53 genotypic variants.

Conclusion: The p53 Arg72 allele is associated with increased risk of CAD in this cohort of Asian Indian male patients with premature CAD.

Abstract no: 1459

Regulatory B cells in humans: Identifying the regulatory capacity and interleukin-10 production of regulatory B cell phenotypes

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Background: In mice, CD1d+CD5+ B cells have regulatory properties associated with interleukin-10 (IL - 10) production. In humans, this phenotype is up to 10 times more frequent in infants than in adults. Infants show better heart transplant outcomes than older recipients, including acceptance of ABO-incompatible grafts. However, they also show increased severity of infection with polysaccharide-encapsulated bacteria. We hypothesised that CD1d+CD5+ B cells contribute to the altered immune response during infancy, particularly towards polysaccharides, including ABO-antigens and bacteria capsules.

Methods: CD1d+CD5+ B cells were FACS-sorted from paediatric splenocytes and cultured parallel to non-CD1d+CD5+ B cells using T-dependent (TD; α-IgM+CD40L) and T-independent (TI; CpG or α-IgM+crosslinker) B cell stimuli to measure IL-10 in supernatants by ELISA. The regulatory impact of CD1d+CD5+ B cells on other cells was assessed through proliferation of CFSE-stained; (1) Peripheral blood mononuclear cells ($PBMC^{original}$); (2) PBMC to which CD1d+CD5+ B cells were added to double the original proportion ($PBMC^{double}$); and (3) PBMC from which CD1d+CD5+ B cells were depleted ($PBMC^{depleted}$) after stimulation with B-cell stimuli or T-cell stimuli (α-CD3+CD28).

Results: IL-10 levels were higher with TI than with TD stimulation, however, little difference was observed between CD1d+CD5+ B cells and non-CD1d+CD5+ B cells. The mean percentage of dividing B cells stimulated with CpG was 21.9 lower in $PBMC^{double}$ than in $PBMC^{original}$ (p=0.018). When stimulated with α-IgM+CD40L, it was 31.9 higher in $PBMC^{depleted}$ than in $PBMC^{original}$ (p=0.042). The percentage of dividing T cells was 26.2 lower in $PBMC^{double}$ than in $PBMC^{original}$ (p=0.088).
Conclusions: These results indicate that CD1d+CD5+ B cells in humans inhibit the proliferation of B and T cells. Since IL-10 production was also found in non-CD1d+CD5+ B cells, these markers do not uniquely identify regulatory B cells in humans. Further analyses to determine the phenotype of IL-10-producing B cells are underway.

Abstract no: 1546
Effects of melatonin treatment on cardiac function in a model of pulmonary arterial hypertension

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Background: Pulmonary arterial hypertension (PAH) is a disorder characterised by elevated pulmonary arterial pressure, which leads to cardiac hypertrophy and ventricular dysfunction. Current treatments are only marginally effective and additional therapies are required. Melatonin is a natural product that has been shown to be cardioprotective against hypertension and myocardial infarction. We therefore propose that a chronic melatonin treatment may be cardioprotective in a rat model of monocrotaline (MCT)-induced PAH.
Methods: Male Long Evans rats (150 - 175g) received a single subcutaneous injection of MCT (80mg/kg), which induced PAH after 28 days. Melatonin was given in the drinking water (4mg/kg/day) for the 28-day period. Cardiac hypertrophy was confirmed with a ratio of the right ventricle weight over heart weight (RVW/HW). Cardiac functional parameters were assessed at zero and 28 days using isolated heart perfusion and/or echocardiography. These parameters included right ventricular systolic (SP) and diastolic pressure (DP), ejection fraction (EF) and fractional shortening (FS).
Results: MCT increased RVW/HW, reduced EF (92.84±1.33% vs. 60.53±4.23%, p<0.0003), FS (28.23±2.68% vs. 61.03±2.89%, p 0.0002) and increased SP and DP. Chronic administration of melatonin in MCT-treated rats improved EF (60.5±4.2% vs. 84.1±1.7%, p<0.0008), FS (28.2±2.7% vs. 48.7±2.1%, p<0.0005), SP and DP.
Conclusions: Our data demonstrate that chronic melatonin improved cardiac function in MCT-induced PAH and suggest a cardioprotective role of melatonin in PAH.

Abstract no: 1561
The role of novel protein-protein interactions in the function and mechanism of the sarcomeric protein, myosin-binding protein H (MyBPH)

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Introduction: Left ventricular hypertrophy is a major risk factor for cardiovascular morbidity and mortality and is a feature of common diseases, such as hypertension and diabetes. It is therefore important to understand the underlying mechanisms influencing its development. Hypertrophic cardiomyopathy (HCM) has been viewed as a model disease in which to study the causal molecular factors underlying isolated cardiac hypertrophy. HCM is described as a disease of the sarcomere, and one of the regions of the sarcomere that has been identified as playing a key role in the regulation of contractility is the C-zone. Identifying binding partners of one of the C-zone proteins, myosin-binding protein C (MyBPC), has led to insights into the function of this protein. However, myosin-binding protein H (MyBPH) is another member of the myosin-binding protein family located within this region of which very little is known. Given the sequence homology and similarity in structure between MyBPC and MyBPH, we proposed that MyBPH may play critical roles in the cardiac sarcomere and possibly in HCM pathogenesis.
Methods: The present study reports the identification and verification of interacting partners of MyBPH with the aim of identifying the role of this protein in the sarcomere using yeast 2-hybrid (Y2H) analysis.
Results: Twelve interacting partners were identified, of which 3 [SUMO-conjugating enzyme UBC9, alpha cardiac actin (ACTC), and myosin 7 (Myh7)] were considered putative physiological interactors based on the plausibility of the interactions as assessed in silico. Putative interactors UBC9, ACTC and Myh7 proved to co-localise with MYBPH in differentiated rat cardiomyocyte cells. Furthermore, co-immunoprecipitation confirmed the interaction between MYBPH and UBC9, ACTC1 and MYH7.
Conclusion: The results of this study provide important clues to the function of MyBPH and, in so doing, improve our knowledge and understanding of this protein's role in the cardiac sarcomere.

Abstract no: 1627
Glucocorticoid-induced cardioprotection: A novel role for autophagy?

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Background: Ischaemic heart disease is a leading cause of death worldwide, therefore better treatment or prevention of ischaemia-reperfusion (I/R)-induced stress in the heart necessitates a better understanding of the molecular pathways and mechanisms of cell death. The well-established anti-inflammatory and immunosuppressive properties of glucocorticoids have led to their investigation as possible therapeutic agents to reduce ischaemia-reperfusion-induced stress in the heart. However, influences of glucocorticoids on cardiovascular disease and cell death are complex and often contradictory. I/R-induced stress leads to three types of cell death, which include apoptosis, autophagy and necrosis. Although autophagy is foremost a survival mechanism activated during cellular stress, it can

also lead to cell death under certain conditions. Many signalling pathways interlink with the autophagic machinery and are activated during I/R-induced stress in the heart, such as the mitogen-activated protein kinase family, which include p38-MAPK. These kinases are subsequently dephosphorylated by appropriate phosphatases. MAPK phosphatase-1 (MKP-1), a dual specificity phosphatase, inactivates the MAPKs by dephosphorylating specific Thr/Tyr residues. Up-regulation of MKP-1 during I/R-induced stress in the heart has been shown to be cardioprotective; however, little information exists regarding the role of autophagy in GC-induced protection in the heart. Therefore, the aim of this study is to describe some of the major signalling pathways activated during I/R-induced stress and the potential role of autophagy in GC-induced cardioprotection. By dissecting out the roles of autophagy and glucocorticoids with regard to shared metabolic effects and signalling pathways in cardiac injury, it is hoped to provide a framework for improved treatment of cardiovascular disease.

Abstract no: 1704

Differentiating transmural from transanastomotic graft endothelialisation through an isolation loop-graft model

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Background: The absence of a physiological intima is the primary reason for low patency in small to medium-sized synthetic vascular conduits. Despite incomplete endothelial surface coverage by trans-anastomotic outgrowth, vascular graft models have yet to distinguish this form of healing from trans-mural capillary sprouting. We have developed an isolation loop-graft model that clearly separates these distinctly different events.
Methods: Trans-anastomotic outgrowth was measured by implanting expanded polytetrafluoroethylene (ePTFE; ID 1.7mm, IND 15-25µm) for 2.4 and 6 weeks (n=6 per time point) in the abdominal aorta of Wistar rats. High-porosity polyurethane (PU; ID 1.7mm, 150-µm pore) grafts were then interposed between the ePTFE for 2, 4, 6 and 8 weeks (n=6 per time point). Looping the interposition grafts increased their length to 8cm and they were implanted for 6, 8, 12 and 24 weeks (n=8 per time point). Grafts were analysed by light, immunefluorescence (CD31) and scanning electron microscopy. Endothelialisation was expressed as maximal outgrowth (Imax) and segment graft coverage (GSE).
Results: Six-week proximal and distal trans-anastomotic growth rate did not differ (Imax = 0.3 ± 0.3 vs 0.3 ± 0.2mm/week, NS). The composite straight-graft ePTFE zones were too short to isolate trans-mural ingrowth; only 8% of the grafts had mid-graft endothelial coverage without trans-anastomotic breach. All six- and eight-week straight composite grafts had trans-anastomotic encroachment. This outgrowth edge never traversed the endothelium-free isolation zone in the loop grafts (23.6±10.1mm at 6 weeks and 10.5±45.7mm at 24 weeks), which separated it from trans-mural mid-graft endothelium. Trans-mural mid-graft endothelialisation reached pre-confluence by six weeks (GSE = 55±45%) and confluence between week 12 and 24 (GSE = 95.0±10.0% and 84.0±30.13%). The sub-intimal thickness stayed constant with a non-significant trend towards regression (91.8 ± 93.9 mm vs 71.4 ± 59.4mm at six and 24 weeks, respectively; NS).
Conclusion: Trans-mural endothelialisation can be clearly distinguished from trans-anastomotic outgrowth in a high-throughput rat model. A looped interposition-graft model provides sufficient isolation length to separate the 2 events for up to half a year, and does not result in an increase in intimal hyperplasia.