Keynote Speakers:

Dr Catherine Hall & Prof Holly Shiels

Manchester Institute of Biotechnology
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Organising Committee:

Dr Sophie Saxton, University of Manchester
Dr Claire Wilson, University of Liverpool
Dr Miriam Lettieri, University of Manchester
Dr Alex Njegic, Queen Mary University of London

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Programme

9:00 – Registration, coffee & pastries

9:50 – Welcome from the ECCS 2022 Committee

10:00 – **Genetics of Cardiovascular Diseases session**. Chairs: Dr Claire Wilson, University of Liverpool & Dr Richard Monaghan, University of Manchester

Caglar Gok, University of Glasgow: *"Peptide Modulation of Cardiac Na+/Ca2+ Exchanger (NCX1) Activity".*

Kirsty Wadmore, University of Liverpool: *"Long QT Syndrome-Associated Mutations D130V and E141K Affect the Structure-Function Relationship of Calmodulin".*

Michal Zulcinski, University of Leeds. *"Transcriptome analysis of temporal artery biopsies to identify genes and pathogenic pathways related to patterns of arterial inflammation in Giant Cell Arteritis".*

10:45 – **Vascular Health & Disease Keynote speaker:** Dr Catherine Hall, University of Sussex: *"Regional differences in neurovascular function and their relevance for disease".*

11:30 – Coffee & Posters

12:00 – **Vascular Health & Disease session.** Chairs: Dr Sophie Saxton, University of Manchester & Dr Calum Wilson, University of Strathclyde.

Melissa Scholefield, University of Manchester: *"Glucose Metabolism is Altered in the Alzheimer's and Parkinson's Disease Dementia Brain".*

Azziza Zaabalawi, Manchester Metropolitan University: *"Liposomal delivery of the CYP1B1 inhibitor, Tetramethoxystilbene, restores endothelial function ex vivo in human arteries from hypertensive coronary artery bypass graft patients".*

Matthew Lee, University of Strathclyde: *"Endothelial cells operate on a small-world, scale-free network to control vascular function".*

Nura Mohammed, University of Bradford: "*Therapeutic resolution of PAH by established drugs".*

(Cardiac Physiology) Chitaranjan Mahapatra, University of Paris-Saclay: "*Quantitative analysis of sodium ion channel-based glucose sensing to study abnormal electrical activities in sinoatrial cell".*

13:15 – Lunch & Posters

14:15 – **Beyond the Research**. Chair: Dr Miriam Lettieri, University of Manchester.

Dr Maggy Fostier – "*Improving the sustainability of our laboratories*".

Prof Rachel Cowen – *"Prioritising Diversity and Inclusion in STEM".*

Prof Elizabeth Cartwright – *"Fellowship applications – maximising your chance of success"*

15:15 – Coffee break

15:45 – **Cardiac Physiology session.** Chairs: Dr Alex Njegic, Queen Mary University of London & Dr Rehan Junejo, Manchester Metropolitan University.

Alice Whitley, University of Manchester: *"Dynamic cardiac microtubules are required for transverse (t)-tubule growth and homeostasis".*

Amy Foster, University of Salford: "*The effects of anthracyclines on calcium handling and contractility in sheep ventricular myocytes".*

16:15 – **Cardiac Physiology Keynote speaker:** Prof Holly Shiels, University of Manchester: "*The heart of the world's longest living vertebrate, the Greenland shark".*

17:00 – Closing remarks & prizes

17:30 – Drinks, nibbles and networking at Brewdog Manchester Outpost.

Special thanks to our co-chairs and judges:

Dr Calum Wilson, University of Strathclyde

Dr Richard Monaghan, University of Manchester

Dr Yasina Somani, Liverpool John Moores University

Dr Samantha Borland, University of Manchester

Dr Natasha Hadgraft, Manchester Metropolitan University

Dr Ahmed Aburima, Hull York Medical School

Dr Alena Shantsila, University of Liverpool

Dr Blanca Tardajos Ayllon, University of Sheffield

Dr Abigail Lay, University of Manchester

Dr Rehan Junejo, Manchester Metropolitan University

Dr Siyu Tian, University of Sheffield

Dr Catherine Hall is a senior lecturer in Psychology at the University of Sussex, UK. Catherine is interested in how the brain balances energy supply and demand. During her PhD (with John Garthwaite, UCL), she studied nitric oxide (NO) consumption by brain tissue. As a post-doctoral researcher (with David Attwell, UCL), she investigated how NO alters how the brain uses oxygen, and what processes of neuronal transmission use the most oxygen. She then studied how NO and other signalling molecules interact to control the brain's energy supply by regulating the tone of capillary pericytes, and how this regulation is impaired after stroke. In 2014 she became a Senior Lecturer at the University of Sussex, and now studies how the regulation of the brain's energy supply (neurovascular coupling) varies during different brain states, across different brain regions and at the onset of conditions such as Alzheimer's disease and obesity. You can see more about current projects on her lab website. She is a member of the Sussex Neuroscience Steering Committee, the University Senate, convenes the core first year module "Psychobiology" and lectures on topics relating to basic neuroscience, neurovascular function and dementia.

The Heart of the World's Longest Living Vertebrate, the Greenland Shark. The life span of the Greenland shark is at least 272 years and may be as long as 500 years making this animal the longest living vertebrate on the planet. This extreme longevity is interesting with respect to the heart, because in humans, heart disease is synonymous with age. What allows the heart of this shark to beat for hundreds of years? This seminar will explore the cellular mechanism that may be involved in extreme longevity including mitochondrial function, genomic stability and cardiac regeneration.

Holly Shiels is a Professor of Integrative Physiology at the University of Manchester, UK. Her lab studies the impact of the environment on animal physiology. They explore molecular and cellular mechanisms that impact cardiac function in response to environmental change and link these with altered organismal metabolism, locomotion and behaviour to determine the intersection of the cardiovascular system and the environment on fitness. Her work on the Greenland shark stems from 3 International Expeditions led by Prof John Steffensen, U of Copenhagen. More information on the Greenland Shark can be found in his pages and also on Holly's blogs.

Early Career Researcher abstracts – Oral Presentation

Peptide Modulation of Cardiac Na+/Ca2+ Exchanger (NCX1) Activity

Caglar Gok, Will Fuller

School of Cardiovascular and Metabolic Health, University of Glasgow

NCX1 controls bidirectional Na+/Ca2+ exchange in an electrogenic manner, therefore Ca2+ flux in numerous tissues. Exchanger activity is regulated in several ways through its large intracellular loop: activated by Ca2+ and PIP2 while inhibited by Na+ and Exchanger Inhibitory Peptide (XIP). To inactivate NCX1, the XIP domain interacts with the XIP binding domain (XBD) situated between 709-728aa residues. Herein we used custom-made cell penetrating peptides mimicking XIP and XBD as a pharmacological tool to modify NCX1 activity, thus cellular physiology in different settings. We engineered several cell lines stably expressing tetracycline-inducible NCX1: wild-type (WT-NCX1), non-functional XIP domain (K229Q and ∆229-232), missing XBD (∆XBD), and missing XIP (∆XIP). FRET sensors were prepared for all mutated NCX1 lines to study intermolecular structural changes between NCX1 dimers. We measured NCX1 activity by measuring intracellular Ca2+, and Na+-induced-Ca2+-intake in engineered cell lines and rat mesenteric Vascular Smooth Muscle Cells (VSMCs) using Fluo-4 dye. CellOPTIQ platform was used to study cardiac contractility in cultured Neonatal Rat Ventricular Myocytes (NRVMs). Interaction between XIP and XBD stabilized the NCX1-NCX1 dimer, and was required to promote NCX1 inactivation. Disrupting NCX1 inactivation either at XIP or XBD elevated intracellular Ca2+ levels in engineered cell lines. An exogenous peptide with same aa-sequence as XBD increased intracellular Ca2+ and Na+-induced-Ca2+-intake in VSMCs. XBD peptide (but not scrambled control) reduced contraction-duration and accelerated relaxation in NRVM, consistent with increased NCX1-mediated Ca2+-efflux. Taken together, our results paves the way for developing peptidomimetic compounds that targets XIP, thus activates NCX1 in multiple cell types.

Long QT Syndrome-Associated Mutations D130V and E141K Affect the Structure-Function Relationship of Calmodulin

Kirsty Wadmore[1], Caroline Dart[2], Nordine Helassa[1]

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Introduction

Long QT syndrome (LQTS) is a life-threatening cardiac arrhythmia syndrome affecting approximately 1:2000 births. Recently, mutations in the calcium (Ca2+) sensing protein calmodulin (CaM) have been linked to LQTS. However, the molecular mechanisms remain unclear. CaM's main function is to regulate the activity of a variety of proteins, including the Ca2+ channels, RyR2 and Cav1.2, involved in cardiac muscle contraction. This project aims to investigate the impact of the CaM mutations D130V and E141K on CaM's structure-function relationship, and how these may cause the LQTS phenotype.

Methods

Thermostability and secondary structure conformation of CaM variants were explored through circular dichroism. The affinity of CaM variants for peptides of RyR2 and Cav1.2 (IQ and NSCaTE domains) was investigated using isothermal titration colorimetry (ITC). The effect of the mutations on ion channel activity was determined using patch-clamp electrophysiology and Ca2+ imaging.

Results

The LQTS-associated mutants D130V and E141K did not show altered thermostability compared to WT CaM. α-helical content was significantly reduced in the CaM mutants compared to WT CaM, from 64% to approximately 40%. ITC showed that Ca2+/CaM binding to RyR2 was reduced up to 3-fold for the LQTS-associated variants. The affinity for the mutants was reduced up to 3-fold and 2-fold for the Cav1.2-IQ and Cav1.2-NSCaTE domains respectively.

Conclusion

Overall, these data demonstrate that LQTS-associated variants have an impact on the secondary structure of CaM, and its ability to interact and modulate the activity of ion channels relevant to cardiac muscle contraction (RyR2 and Cav1.2).

Transcriptome analysis of temporal artery biopsies to identify genes and pathogenic pathways related to patterns of arterial inflammation in Giant Cell Arteritis

Michal Zulcinski[1], Gary Reynolds[2], Lubna Shafi[1], Arundhati Chakrabarty[1], Mark M. Iles[1], Ann W. Morgan[1]

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[2] *Translational and Clinical Research Institute, Newcastle University, NE1 7RU, Newcastle upon Tyne, UK*

Giant cell arteritis (GCA) is the most common form of vasculitis in people over 50 years old. Better understanding of the underlying genetics and molecular mechanisms driving GCA is needed to discover alternative treatment options, currently limited to high-dose steroids on disease onset. Our study integrates bulk and single-cell transcriptomes generated from temporal artery biopsies to reveal the immune cell landscape of inflammatory infiltrates in GCA and to identify transcripts and pathways associated with histological phenotypes. Patients from the UK-GCA Consortium (UKGCA; n=79) and Newcastle and North Tyneside registry (NNT; n=9) were selected for the study. Bulk RNA-sequencing data was generated for each UKGCA case (all GCA positive), while all NNT cases (5 positive and 4 negative) were subjected to single-cell RNA-sequencing. Various clinical and histological features were recorded for each patient. Deconvolution of bulk RNA-seq data was performed using several different software with single-cell dataset as reference panel. Differentially expressed genes were identified for each histological phenotype revealing that the histological phenotypes showing the greatest transcriptional changes between the groups were the presence of media destruction (5159 genes; FDR-corrected p-value<0.01) and the extent of inflammation in the adventitia and media (3503 and 3333 genes respectively; all FDR-corrected p-values<0.01). The deconvoluted bulk data revealed the proportions of distinct cell populations in the biopsy samples and showed that the myofibroblast population had the strongest association with transcriptomic profiles confirming the importance of vascular remodelling. Further analyses are underway to determine the functional and biological significance of identified genes and related pathways.

Glucose Metabolism is Altered in the Alzheimer's and Parkinson's Disease Dementia Brain

Melissa Scholefield, Stephanie J. Church, George Taylor, David Knight, Richard D. Unwin, and Garth J. S. Cooper

University of Manchester

Despite their high prevalence, the causative factors leading to the development of dementia diseases such as Alzheimer's disease (AD) and Parkinson's disease dementia (PDD) remain unclear. Among other possible contributory factors, vascular insults have been strongly associated with these diseases, including type II diabetes mellitus, hyperlipidaemia, and hypertension. However, knowledge regarding the metabolic profile of the brain in these diseases is limited. As a follow-up to previous studies in AD, we performed a metabolomics analysis of the PDD brain in order to investigate cerebral changes that may be related to the vascular risk factors for this disease. A semi-targeted liquid chromatography–mass spectrometry (LC–MS) metabolomics analysis was carried out on nine PDD cases vs nine controls across nine different brain regions. Concentrations of 64 metabolites were compared between cases and controls, and findings were compared to those previously identified in AD. Of the 64 analytes, 47 were found to be altered in at least one region of the PDD brain. These included several metabolites involved in glucose metabolism, with widespread increases in fructose and more region-specific alterations identified in fructose-6-phosphate, glucose, glucose-6-phosphate, mannose-6-phosphate, and ribose-5-phosphate. These findings mirror those seen in AD and may present a link between vascular risk factors and these two diseases. AD and PDD share cerebral alterations in glucose metabolic pathways that may present a potential pathogenic mechanism by which vascular risk factors contribute to disease development. As such, lifestyle changes may be an important approach in tackling the increasing prevalence of these age-related conditions.

Liposomal delivery of the CYP1B1 inhibitor, Tetramethoxystilbene, restores endothelial function ex vivo in human arteries from hypertensive coronary artery bypass graft patients.

Azziza Zaabalawi[1], Lewis Renshall[2], Adam Lightfoot[1], Hans Degens[1], Yvonne Alexander[1], Ragheb Hasan[3], Haris Bilal[3], Lynda K Harris[2], May Azzawi[1].

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Hypertension is a significant risk factor for cardiovascular disease morbidity and mortality, characterised by reduced nitric oxide (NO) bioavailability and increased reactive oxygen species (ROS) generation. We have previously demonstrated that inhibition of the cytochrome P450 enzyme CYP1B1, using 2,3',4,5'-Tetramethoxystilbene (TMS) restores rat aortic vasodilation in an acute hypertensive environment by potentiating NO release [1]. We aimed to determine if TMS has a similar positive effect in human internal mammary arteries (IMAs) harvested from hypertensive coronary artery bypass graft (CABG) patients, and assess mechanisms involved.

Endothelial-dependent (Acetylcholine, ACh 10-12 –10-3M) and independent (Sodium nitroprusside, SNP) dilator responses were assessed in isolated IMA rings taken from 45 CABG patients (45-75 years; IRAS approval 255023), in the presence/absence of TMS-liposomes (1nM), and dilator pathway inhibitors; nitric oxide synthase inhibitor Nω-nitro-L-arginine, potassium channel blockers Apamin and TRAM-34, and cyclooxygenase inhibitor Indomethacin. Mitochondrial ROS, cytosolic superoxide, and intracellular NO were measured using MitoSOX, Dihydroethidine and DAF-FM, respectively.

Liposomal delivery of TMS improved its stability (compared to TMS solution; 0.129±0.02ng/mL vs 0.086±0.01ng/mL at 4h; $P<0.05$), and alleviated attenuated ACh-induced dilation in diseased IMAs (@ACh 10-4 M: 56.9±5.1%; n=8 vs 12.7±7.8%; n=6; $P<0.01$) for TMS-liposomes vs blank liposomes, respectively), via inhibition of CYP1B1, reduction in ROS moieties and stimulation of NO synthesis. Responses were significantly attenuated following incubation with the dilator inhibitors, alone or in combination. Our findings have important implications for the future implementation of complementary anti-hypertensive treatment strategies.

Endothelial cells operate on a small-world, scale-free network to control vascular function

Matthew D Lee, Calum Wilson, John G McCarron

University of Strathclyde

The endothelium is the innermost layer of all blood vessels and it controls a host of cardiovascular functions including vascular contractility, haemostasis and inflammation. The importance of the endothelium is clear since changes in the behaviour of this single layer of cells underlies almost all cardiovascular disease. To control each cardiovascular function, the endothelium processes and responds to endless streams of information that originate from multiple sources (i.e. blood cells, hormones or neighbouring endothelial cells). However, how the endothelium processes all this information, which can often arrive simultaneously, is not fully understood. Here, by examining the Ca2+ response in thousands of endothelial cells in intact resistance arteries, we show that the endothelium utilises spatially-distinct subpopulation of cells that are primed to detect specific agonists. Additionally, cells that are primed to detect each agonist form clusters and by using cross-correlation analysis we show, that the Ca2+ response in active cells within a cluster are highly-correlated. However, how these spatially-distinct clusters communicate across the endothelium to coordinate vascular function is not fully understood. Using Network analysis, we show that the connections between active cells exhibit unexpectedly short path-lengths. This high clustering and short path-length reveal an endothelial network with a ‘small-world’ configuration. Small-world networks confer particularly dynamic properties including high signal-propagation speed, stability, and a high degree of synchronizability. This network organization explains how coordinated cell activity occurs across large regions of endothelium, despite sensing being distributed on spatially-distinct populations of cells.

Therapeutic resolution of PAH by established drugs

Nura Mohammed

University of Bradford

Pulmonary arterial hypertension (PAH) is a vascular disorder characterised by an imbalance in pulmonary arterial smooth muscle cell proliferation and death, caused by both inherited and environmental factors. Due to the constricting of blood arteries in the lungs, PAH causes right heart failure and early mortality in the absence of treatment. There are currently no treatments, emphasizing the need to find new therapeutic approaches. Here, two established compounds were investigated whether they can be reproposed for the treatment of PAH. In this study, it has been shown that these two compounds (A and B) promote BMPRII receptor-mediated signalling pathway in luciferase-based reporter assay at low concentrations in HEK293T cells. Compound B has also shown to inhibit TGFBRII receptor in the presence of TGFβ1 receptor via the TGFβ signalling pathway. These effects were validated using western blotting analysis in mutant Pulmonary arterial smooth muscle cells (mPASMCs) (bmpr2 +/-) cells, evidencing compound A shows a higher level of SMAD1/5 phosphorylation. Cell proliferation is an important factor contributing to vascular remodelling, therefore both compounds were experimented in mPASMCs (bmpr2 +/-) in the presence of TGFβ1 ligand to determine their effects where compound B inhibits cell proliferation. These findings indicate that both compounds modulate both BMP and TGFβ signalling pathways and indicate the benefits of reproposing these compounds in the treatment of PAH.

Quantitative analysis of sodium ion channel-based glucose sensing to study abnormal electrical activities in sinoatrial cell

Chitaranjan Mahapatra

University of Paris-Saclay

The glycaemia or the glucose concentration in the blood has been recognized as an important contributor for causing sudden cardiac arrest (SCA), which leads to sudden cardiac death (SCD). The SCD is very common to diabetic people and it is very challenging to investigate by the clinicians. Several non-clinical research groups are investigating to quantify the putative relationship between the blood glucose level and the cardiac electrophysiological output. We have predicted a hypothesis that the cellular glucose sensing mechanisms in the cardiac tissue might be an ideal candidate for understanding this relationship. We built a single compartmental in-silico model of the human sino-atrial node (SAN) action potential (AP) by adapting several published computational models. The glucose sensing mechanism is incorporated with the voltage gated sodium ion channel by using some ordinary differential equations. The differential equation parameters are adapted from the published experimental studies to mimic the effects of glucose concentration on the SAN AP firing patterns. After doing the simulation using voltage clamp and current clamp protocols, we showed that the higher glucose concentration reduced the voltage-gated sodium ion channel current and then the frequency of the SAN AP. In summary, this model has explored a promising cellular explanation for the blood sugar level induced bradycardia and SCD.

Dynamic cardiac microtubules are required for transverse (t)-tubule growth and homeostasis

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Introduction

Transverse (t)-tubules facilitate rapid, synchronous Ca2+-release and contraction in cardiac myocytes. In our ovine heart failure (HF) model, atrial t-tubules are lost alongside perturbed Ca2+-handling, but termination of tachypacing ensues t-tubule restoration and normalised Ca2+-transient. Understanding t-tubule homeostasis in disease is therefore therapeutically advantageous. Cardiac microtubules (MTs) are highly dynamic, interact with the tabulating protein BIN1 at the dyad and in human HF, undergo pathophysiological changes. We hypothesised that microtubules influence t-tubule development and homeostasis.

Methods

Neonatal rat ventricular myocytes (NRVMs) were co-transfected with BIN1-mKate/EMTB-3xGFP to induce t-tubule formation/label microtubules. Nocodazole or taxol treatment prior-to or post-BIN1 transfection disrupted or stabilised MTs during t-tubule development or maintenance. Sheep were surgically implanted with a pacemaker, and subject to rapid ventricular pacing (210bpm) until end-stage HF. Left atrial cells were isolated then fixed for immunocytochemistry.

Results

Microtubule disruption with nocodazole prior to t-tubule development decreased t-tubule density ($p=0.008$) and t-tubule length ($p=0.0077$). Post-t-tubule development, disrupting MTs decreased t-tubule density 2-fold ($p=0.008$), shortened t-tubule length ($p=0.004$), and increased t-tubule number by 25% ($p=0.015$), indicating fragmentation. Attenuating MT dynamics with taxol decreased maintained t-tubule density ($p=0.044$) and t-tubule length ($p=0.002$). Similarly, MT densification occured in HF sheep atrial myocytes ($p<0.0001$), concurrent with significant t-tubule loss ($p=0.02$), complementing the taxol findings.

Conclusion

MTs are likely important for t-tubule development, elongation and maintenance. Pathological and taxol-induced inhibition of MT dynamics in HF and NRVM experiments both coincide with t-tubule loss, emphasising the importance of dynamic MTs in t-tubule regulation, potentiating novel microtubule-directed treatments for HF.

The effects of anthracyclines on calcium handling and contractility in sheep ventricular myocytes

Amy Foster & Dr David Greensmith

University of Salford

Anthracyclines are highly effective against childhood cancers but can produce cardiotoxicity leading to heart failure later in life. To understand why, previous studies have measured the effects of anthracyclines on cardiac intracellular calcium handling using a variety of small animal models and techniques. While providing detailed information, we are not aware of any integrative studies in a large animal model. Our experiments sought to address this. Primary sheep ventricular myocytes were isolated in accordance with the Animals (Scientific Procedures) Act, UK, loaded with fura-2AM then field simulated at 0.5 Hz. Calcium dynamics and sarcomere length were measured using photometry and sarcomere detection respectively. 1 nM doxorubicin (DOX) irreversibly reduced the Ca transient amplitude and systolic shortening by 39.13 ± 3 % and 42.46 ± 3.74 % respectively. Diastolic Ca and sarcomere length were unaltered. The amplitude of caffeine-evoked Ca transients was reduced by 33.07 ± 5.16 %. The rate of decay of the systolic Ca transient was reduced by 16.56 ± 3.02 % while that of the caffeine evoked Ca transient increased by 64 ± 25.52 %. Calculated kSERCA decreased by 34.07 ± 8.49 %. Phase plane analysis revealed the slope of the relationship between sarcomere length and intracellular Ca increased. Our findings suggest DOX increases NCX activity and decreases SERCA activity leading to reduced SR Ca content thence systolic Ca and contractility. Interestingly DOX may alter myofilament sensitivity. Our next experiments will investigate the effect of DOX on membrane Ca fluxes and further explore the effect on myofilament sensitivity.

Early Career Researcher abstracts – Poster Presentation

POSTER 1: PAR2 regulates vascular tone in resistance arteries

Xun Zhang, Matthew D. Lee, Charlotte Buckley, Morley D. Hollenberg, Calum Wilson & John G. McCarron.

University of Strathclyde

Protease activated receptor-1 & 2 (PAR1 and PAR2) are expressed widely in cardiovascular tissues including endothelial and smooth muscle cells. PAR1 and PAR2 may regulate vascular tone via endothelial Ca2+ signalling but the mechanisms are incompletely understood. To explore PAR1 and PAR2 regulation of the endothelium, the PAR1 activating peptide, TFLLR-NH2 (TFLLR) and PAR2 activating peptide, 2-Furoyl-LIGRLO-amide (2fLI) were used together with the agonist thrombin (PAR1) and trypsin (PAR2). PAR2 activation (2fLI) evoked Ca2+ waves that propagated across multiple endothelial cells. The Ca2+ waves were temporally-distinct from those generated by muscarinic receptor activation. PAR2 activation was also primarily restricted to different clusters of endothelial cells from those of muscarinic receptor activation. These observations suggest the endothelium spatially segregates functions to distinct clusters of cells. PAR2-evoked Ca2+ signals were blocked by IP3 receptor inhibitors (2-APB or caffeine) and a phospholipase C (PLC) inhibitor (U73122). PAR2 is therefore a PLC coupled receptor which evokes Ca2+ release from the IP3 sensitive store in endothelial cells. PAR2 activation also evoked endothelium-dependent dilation of arteries. On the other hand, PAR1 activation (thrombin or TFLLR) did not trigger Ca2+ responses in native mesenteric endothelial cells or dilate or contract mesenteric arteries. Nor did PAR1 activators alter the response to PAR2 or muscarinic receptor activation. These results suggest that PAR2 but not PAR1 regulates vascular tone in arteries by evoking Ca2+ release from the internal store via IP3 receptors in endothelial cells.

POSTER 2: The Relationship between Indoxyl Sulfate and Oxidative Stress in Cardiomyocytes: A Meta-regression Analysis

Haadya Sidra, Natasha Hadgraft

Manchester Metropolitan University

Background: Indoxyl Sulfate (IS), a potent uremic toxin, accumulates within chronic kidney disease patients and contributes to cardiotoxic effects, particularly arrhythmias. IS inhibits uncoupling protein-2, which increases reactive oxygen species (ROS) and ultimately induces oxidative stress (OS). Although current literature has discerned this association, disparities exist in investigated IS concentrations.

Aim: To establish the relationship between IS concentrations at 0.1-1000uM and OS within cardiomyocytes.

Methods: Articles examining IS's effects upon OS in cardiomyocytes were identified in PubMed, Scopus, and Web of Science. These were critically appraised with risk-of-bias assessment and funnel plots. The mean fold change in ROS and NADPH oxidase-2 (NOX2) from control documented at various IS concentrations was extracted. Meta-regression analysis was performed on normally distributed data presented as mean ± S.D.

Results: The individual data points obtained demonstrated a strong positive correlation between mean fold change in ROS from control and IS concentrations (r=0.759, P=0.004). However, the significance of this association was masked with meta-regression analysis (r=0.898, P=0.102), possibly attributable to variability in ROS generated at lower IS concentrations. Although IS and NOX2 demonstrated a moderately strong positive relationship (r=0.564), this was deemed insignificant (P=0.322).

Conclusion: IS induces OS as exhibited by increased ROS formation in cardiomyocytes. As this impact did not correspond to NOX2 formation, IS likely exerts greater influence upon other sources of ROS. Exposure time may impact the relationship between ROS and IS, as this enables endogenous antioxidants to limit accumulation. Additional research emphasising IS's activities in cardiomyocytes is requisite to further elucidate findings.

POSTER 3: Left ventricular dysfunction and heart failure risk factors in de novo pacemaker recipients and those requiring pacemaker generator replacement

NH. Abdul Samad, JE. Lowry, CA. Cole, S. Straw, J. Gierula, KK. Witte, MF. Paton

University of Leeds

Aims

Right ventricular pacing burden (RVP%) can lead to left ventricular systolic dysfunction (LVSD) and subsequently increased risk of heart failure hospitalisation (HFH) and mortality. The purpose of this study was to compare the predictors of LVSD, HFH and mortality in patients receiving newly implanted pacemaker (NI) and pacing generator replacement (PGR).

Methods and results

This is an observation cohort study comparing NI and PGR patients. Clinical demographic, echocardiographic, and pacing measures, medication and past medical history were collected at baseline and follow up of 12-months. 514 NI patients (mean age 76 (±11) years; 66% male) were recruited and compared to 491 PGR patients (mean age, 76 (±12); 56% male). Age (hazard ratio (HR), 1.06; 95% confidence interval (CI), [1.03,1.09], $p < 0.001$ vs HR 1.06; 95% CI [1.03,1.09], $p < 0.001$), respectively), atrial fibrillation (AF) (HR 2.03; 95% CI [1.29, 3.22], $p = 0.002$ vs HR 1.77; 95% CI [1.16, 2.72], $p = 0.008$, respectively) and serum creatinine (HR 1.00; 95% CI [1.00, 1.01], $p = 0.013$ vs HR 1.01; 95% CI [1.00, 1.01], $p < 0.01$, respectively) were significant predictors of HFH and mortality at both NI and PGR. RVP% (odds ratio (OR) 2.61; 95% CI [1.59, 4.29], $p < 0.001$ vs OR 3.93; 95% CI [2.19, 7.04], $p < 0.001$) was the only significant predictor of LVSD at both NI and PGR.

Conclusion

Despite efforts to reduce unnecessary RV pacing through new manufacture algorithm, RVP% remains an important predictor of LVSD at both NI and PGR.

POSTER 4: Exploring the structural basis of Protease activated receptor 4 (PAR4) protein interactions by proteomic analysis.

Marco Bonfanti, Lisa Van Den Driest, Margaret Cunningham

University of Strathclyde

Protease activated receptor 4 (PAR4) is a G-protein coupled receptor (GPCR) found on platelets, which becomes activated by proteolytic cleavage of its N-terminus. On the extreme end of its C tail (S381 SLLQ), there may be a potential short linear motif (SLiM) resembling a PDZ binding motif. PDZ proteins are molecules that can engage the receptor to regulate GPCR localisation, trafficking, and signalling. In this study we sought to characterise the network of PAR4 interacting partners and investigate how removal of this region affects PAR4-protein interaction. Stable isotope labelling of amino acids in cell culture (SILAC) proteomics was performed in metabolically labelled HEK293 cells transiently transfected with YFP, WT PAR4-YFP or PAR4ΔSSLLQ-YFP. Following GFP-trap affinity purification (Chromotek), transfected samples were pooled (1:1:1) prior to tryptic digestion and Orbitrap mass spectrometry (LC MS/MS) processing (n=5). Datasets were filtered and network analysis was performed to assess enrichment and to determine differential regulation of interacting partners between WT PAR4-YFP or PAR4ΔSSLLQ-YFP datasets. Out of the 10,380 proteins identified across the datasets, only 577 exceeded the threshold of a 95% confidence interval. No PDZ containing proteins were found to be related to the proposed SLiM region. However, 304 proteins from this list were common to those expressed in the human heart. Clusters of mitochondrial and ribosomal proteins were found, highlighting toles in cellular and metabolic processes with predominant molecular functions being binding and catalytic activity. These proteins were mainly involved in translation, metabolism of amino acids, mitochondrial calcium ion transport, and cellular responses to stress.

POSTER 5: Sex-dependent mitochondrial function in right ventricle in an experimental model of pulmonary arterial hypertension

Chelbi Coyle, Prof. Margaret L. MacLean and Dr. Lian Tian

University of Strathclyde

Background: Pulmonary arterial hypertension (PAH) has a female predominance with a ~4:1 female to male ratio. However, female PAH patients exhibit better right ventricular (RV) compensation in the face of increased pulmonary arterial pressures and thus better survival than the males. Though RV function is the major determinant of survival, current therapies target the pulmonary vascular disease, rather than the RV directly. Impaired mitochondrial function has been shown to contribute to RV dysfunction, but its sex differences are unknown. This study aimed to determine sex difference in mitochondrial function in RV in PAH in order to identify novel therapeutic targets.

Methods: Male and female Sprague-Dawley rats were injected with Sugen (25 mg/kg) or vehicle and exposed to hypoxia for 3 weeks followed by 5 weeks in normoxia. RV haemodynamics were measured via pressure-volume loop measurement. Confocal imaging was performed on fresh RV to examine mitochondrial properties. Expression of protein was measured by western blot.

Results: Sugen/Hypoxia caused significant increases in RV systolic pressure and hypertrophy in both sexes, but reduced RV-pulmonary artery coupling in males only. Sugen/Hypoxia caused significant decreases in mitochondrial membrane potential and mitochondrial fusion and a trend of decrease in mitochondrial superoxide in males only. The protein levels of mitochondrial transcription factor A and nuclear factor erythroid 2-related factor 2 were decreased in Sugen/Hypoxia male RVs only, associated with impaired mitochondrial properties in males.

Conclusions: Mitochondrial function in RV displayed sex dimorphism in Sugen/Hypoxia rat model of PAH. Further investigations into the associated mitochondrial pathways could help unravel potential therapeutic targets for RV.

POSTER 6: Effects of nociceptive and mechanosensitive afferents sensitization on central and peripheral hemodynamics following exercise-induced muscle damage

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This study aims to test the separated and combined effects of mechanoreflex activation and nociception through exercise-induced muscle damage (EIMD) on central and peripheral haemodynamics before and during single passive leg movement (sPLM). Eight healthy young males undertook four experimental sessions, in which a sPLM was performed on the dominant limb while in each specific session the contralateral was: a) in a resting condition (CTRL), b) stretched (ST), c) resting after EIMD called delayed-onset-muscle-soreness (DOMS) condition, or d) stretched after EIMD (DOMS+ST). EIMD was used to induce DOMS in the following 24-48h. Femoral blood flow (FBF) was assessed using doppler ultrasound while central haemodynamics were assessed via finger photoplethysmography. Leg vascular conductance (LVC) was calculated as FBF/MAP. RR-interval were analyzed in the time (RMSSD) and frequency domain (LF/HF). Blood samples were collected before each condition and gene expression analysis showed increased fold changes for P2X4 and IL1β in DOMS and DOMS+ST compared with baseline. Resting FBF and LVC were decreased only in the DOMS+ST condition (-26ml/min and -50ml/mmHg/min respectively) with decreased RMSSD and increased LF/HF ratio. MAP, HR, CO, and SV were increased in ST and DOMS+ST compared with CTRL. Marked decreases of delta peaks and AUC for FBF (∆: -146ml/min and -265ml respectively) and LVC (∆: -8.66ml/mmHg/min and ±1.7ml/mmHg/min respectively) all $p<.05$. These results suggest that combination of mechanoreflex and nociception resulted in decreased vagal tone and concomitant rise in sympathetic drive that led to increases in resting central hemodynamic with reduce limb blood flow before and during sPLM.

POSTER 7: Prevalence of pre-eclampsia and adverse pregnancy outcomes in women with pre-existing cardiomyopathy: a multi-centre retrospective cohort study.

Laura Ormesher, Sarah Vause, Suzanne Higson, Anna Roberts, Bernard Clarke, Ed Johnstone, Jenny Myers.

University of Manchester

Background: Pre-eclampsia is associated with postnatal maternal cardiovascular dysfunction, however the temporal relationship remains unknown. This study aimed to compare the prevalence of pre-eclampsia in women with pre-existing cardiac dysfunction with the background population.

Methods: This was a multicentre retrospective cohort study across 13 sites in the UK and Australia. Pregnancy and echocardiography data were collected from women with impaired left ventricular ejection fraction (LVEF) <55%. Prevalence of adverse pregnancy outcomes were compared against quoted population prevalence, using equality of proportions test. Multivariable regression analyses explored factors associated with pregnancy outcome, having adjusted for confounders.

Results: In this cohort of 282 pregnancies, pre-eclampsia prevalence was not increased (4.6% versus 4.6%, p=0.99); 12/13 women with pre-eclampsia had additional risk factors. Prevalence of preterm pre-eclampsia (<37 weeks) and fetal growth restriction (FGR) were increased (1.8% versus 0.7%, p=0.03; 15.2% versus 5.5%, p<0.001, respectively). Neither systolic nor diastolic function correlated with pregnancy outcome. Antenatal ß blocker exposure (n=116) was associated with a lower birthweight Z-score (adjusted difference -0.31 [95% C.I. -0.61- -0.01], p=0.04).

Conclusion: This study demonstrated a modest increase in preterm pre-eclampsia and significant increase in FGR in women with cardiac dysfunction. The absence of dose-effect demonstrated by lack of correlation between severity of cardiac dysfunction and pregnancy outcome does not support a causal relationship between cardiac dysfunction and pre-eclampsia, especially accounting for the background risk status of the population. The mechanism underpinning the relationship between cardiac dysfunction and FGR merits further research but could be influenced by concomitant ß blocker use.

POSTER 8: Developing MRI sequences to Assess maternal cardiac and Placental function in women with HyperteNsion in prEgnancy (DAPHNE)

Duhig K, Naish J, Pleva L, Miller C, Myers J.

University of Manchester

Background Hypertensive disorders of pregnancy (HDP) are a leading cause of maternal and infant morbidity and mortality. Studies suggest HDP includes a variety of maternal cardiovascular phenotypes, such as early-onset preeclampsia demonstrating a raised peripheral vascular resistance (PVR)/low cardiac output (CO) phenotype, and later-onset preeclampsia with high CO and volume overload. Stillbirth risk is elevated across gestations in HDP, thus the maternal and fetal phenotypes may arise from unique combinations of cardiovascular and placental impairments. Antenatal assessment to identify these subtypes may limit maternal and fetal harm. Objectives To develop a novel cardioplacental MRI protocol to assess maternal cardiac structure and function, alongside structural and functional assessment of the placenta to improve clinical phenotyping in HDP. Methods Prospective observational cohort study for novel cardioplacental MRI sequence development. Results Ten women with successful completion and development of cardioplacental MRI protocol. Maternal cardiac protocol includes: volumes, ejection fraction, strain, fibrosis (T1), oedema (T2), perfusion (IVIM) and aortic distensibility. Placental protocol comprises: volumes, microstructure (diffusion), oxygenation (T2* and T1-weighted oxygen enhanced) and fibrin deposition (T2-weighted). All women found the scan protocol acceptable, which is completed in under 1 hour. Conclusion A joint cardioplacental MRI scan protocol is both feasible and acceptable to pregnant women with HDP. Further work is underway to upscale this protocol and gather fine-grained diagnostic and prognostic information on maternal and placental vascular disease. Cardiac and placental MRI biomarkers may assist in disentangling the complex etiology of hypertension-associated placental disease and facilitate improved clinical phenotyping to enhance pregnancy risk-stratification.

POSTER 9: Dimethylarginine dimethylaminohydrolase 2 (DDAH2) as a possible therapeutic target for inflammation in atherosclerosis

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Study rationale: Atherosclerosis remains one of the most common causes of morbidity and mortality around the world. Asymmetric dimethylarginine (ADMA) is an independent cardiovascular disease risk factor and its increase has been associated with many cardiovascular diseases most likely through its competitive inhibition of nitric oxide synthase. ADMA is metabolized by dimethylarginine dimethylaminohydrolase (DDAH). DDAH2 is the isoform expressed in the immune system. A deeper understanding into ADMA metabolism, especially in macrophages, will help identify potential new therapeutic agents in atherosclerosis. This study aims to investigate the role of DDAH2 in the inflammatory response via high throughput RNA sequencing analysis.

Methodology: In-silico analysis of RNA sequencing data derived from peritoneal macrophages from wildtype and macrophage specific-DDAH2 knockout mice (DDAH2Mφ-/-) were compared in basal and inflammatory conditions. Further mechanistic insights were established in both bone marrow derived macrophages treated with ADMA, the NOS inhibitor L-NAME and cells derived from DDAH2Mφ-/- mice.

Results: 4966 genes were significantly differentially expressed in wildtype macrophages following LPS stimulation that did not change in stimulated DDAH2Mφ-/- cells. Thus these genes may be DDAH2 dependent and appear to be required for normal immune response. More than 200 reactome pathways appear to be enriched with cytochrome c-mediated apoptotic response being the most enriched (Fold Enrichment 3.71, FDR 4.53-02).

Conclusions: RNA Sequencing data analysis has identified DDAH2 dependent genes required for a normal immune response, genes known to play a role in atherosclerosis and genes involved in apoptosis. Therefore, DDAH2 may be a potential therapeutic target for inflammation in atherosclerosis.

POSTER 10: Doxorubicin mediated impairment of vascular tone and recovery by NRF2 antioxidant pathway activator CDDO.

Avnish Verma, Parveen Sharma, Richard D. Rainbow

University of Liverpool

While extensive research has highlighted DOX-induced cardiotoxicity, insight into vascular toxicity remains more elusive. Recent evidence suggests anthracyclines can abrogate phenylephrine mediated contraction, induce ex-vivo vascular stiffness, and impair endothelium-dependent relaxation. Understanding alterations in vascular function mediated by DOX is important as the vasculature is the primary tissue exposed to its toxic effects culminating in progressive vascular dysfunction, an antecedent to the development of future cardiovascular disease. We aimed to investigate the effects of DOX on vascular function. Contractile responses to Angiotensin II were reduced compared to untreated vessels ($p<0.01$) and restored in vessels co-treated with CDDO-me. Contractile responses in DOX treated vessels to cumulative concentrations of phenylephrine, UTP and endothelin I were attenuated and rescued by DOX/CDDO-me co-treatments. Dilatation by P1075, a potent KATP channel activator (vasodilator) remained unchanged in DOX and DOX/CDDO-me co-treatments. Both, DOX and DOX/CDDO-me ($p<0.01$ vs. untreated) abrogated contractile desensitization. The results demonstrate that DOX attenuates contractile response and desensitization, with DOX/CDDO-me mediating contractile recovery in response to agents of known vasoconstriction and abrogated desensitization. Neither DOX nor DOX/CDDO-me mediated relaxation of tone, suggesting the effects of DOX at least in part for vasodilation may be driven through mechanisms other than those governed by potassium channels. The vasotoxic effects of DOX in driving altered and persistent contractions are indicative of an altered balance in vascular tone (towards vasoconstriction) as seen in a hypertensive phenotype, with potential for progressive cardiac dysfunction owing to pressure overload and thus cardiotoxicity seen in patients several years after anthracycline treatment.

POSTER 11: Do plasma levels of interleukin-6 and -10 correlate to cardiac dysfunction in coronary artery disease?

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Coronary Artery Disease (CAD) is a leading cause of morbidity and mortality in the United Kingdom. In CAD, inflammation is a key pathogenic factor and elevated levels of inflammatory markers such as cytokines are associated with decreased cardiac function1. However, clinical studies examining this are limited2. In this preliminary study, we measured plasma levels of a pro- and anti-inflammatory cytokine in a CAD patient cohort then correlate to indices of cardiac function.

The study was conducted in accordance with local and IRAS ethical approval (IRAS ID: 247341). Preoperative serum samples were collected from patients scheduled for routine coronary revascularisation surgery. Serum interleukin-6 (IL-6) and interleukin-10 (IL-10) concentrations were measured using high-sensitivity ELISA kits (Abcam, United Kingdom. Invitrogen, Massachusetts).

Average IL-6 and IL-10 concentrations were 16.02 ± 0.01 pg/mL and 4.06 ± 0.01 pg/mL respectively. Interestingly, IL-6 and IL-10 levels did not correlate with LVOT obstruction or stroke volume in our patient cohort ($n = 9$ - 19). However, IL-6 levels significantly correlated with ejection fraction ($n = 25$, $p < 0.01$), arterial systolic pressure (PASP) ($n = 8$, $p = 0.03$), end systolic volume (ESV) ($n = 9$, $p = 0.01$) and end diastolic volume (EDV) ($n = 9$, $p = 0.02$). IL-10 only significantly correlated with ESV ($n = 11$, $p = 0.02$).

This preliminary data indicates that IL-6 and IL-10 may be of use as diagnostic markers in CAD patients. We now aim to increase the study power and identify key correlates.

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POSTER 12: Understanding the relationship between dysferlin, transverse (t)-tubules & arrhythmias

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Transverse (t)-tubules are invaginations of the sarcolemma that facilitate EC-coupling in cardiac myocytes. T-tubule damage correlates with aberrant Ca2+ handling, reduced contractility and arrhythmias in heart failure, but our understanding of how t-tubules are regulated is limited. Dysferlin is a membrane repair protein known to regulate t-tubules and EC coupling in skeletal myocytes. It is emerging that dysferlin may regulate t-tubules and Ca2+ handling in the heart, but these mechanisms are poorly understood. We compared ventricular t-tubule characteristics from young wild type (WT) and global DYSF knock-out (KO) mice. We measured dyad width using electron microscopy and also investigated ventricular arrhythmias in WT, heterozygous mutant and DYSF KO Langendorff hearts using programmed electrical stimulation (PES). We observed a reduction in t-tubule density (P = <0.001) and connectivity (P = <0.01) within DYSF KO hearts compared to WT controls. Decreased t-tubule density was associated with more numerous (P = <0.05) yet shorter (P = <0.01) tubules, indicating increased fragmentation. There was no change in t-tubule orientation (P = 0.71). Dyad width was less in DYSF KO cells compared to WT controls (P = <0.01). Finally, in response to PES we observed ventricular tachycardia and/or ventricular fibrillation in 25% of WT hearts, in 75% of heterozygous hearts, which express half of WT dysferlin and in 100% of DYSF KO hearts where dysferlin is lost completely. Dysferlin regulates the healthy t-tubule network and dyad structure and a reduction or loss of dysferlin expression correlates with an increase in ventricular arrhythmias in Langendorff hearts.

POSTER 13: Carotid body mitochondria become dysfunctional in heart failure sheep.

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Carotid body (CB) is the main peripheral oxygen sensor which contributes to the worsening of heart failure (HF) due to its enhanced oxygen sensitivity. Previous studies suggested that unusually low complex IV oxygen affinity and mitochondrial reactive oxygen species (mitoROS) may play an important role in CB chemotransduction cascade. As such, any changes in mitochondrial function could influence the overall CB oxygen sensitivity in HF. This study therefore sought to describe the electron transport chain function, mitoROS production and complex IV oxygen affinity in control and HF CB. CB and left ventricle were collected from control and tachypaced HF sheep. Substrate-uncoupler-inhibitor-titration protocols were employed to measure mitochondrial oxygen consumption (OCR; normalised to citrate synthase activity), H202 production rate and the kinetics of mitochondrial oxygen affinity by high-resolution respirometry. Mitochondrial content in each sample was estimated by using citrate synthase assay. In comparison to the heart, CB mitochondria have low aerobic capacity, high complex IV oxygen affinity and H2O2 production rate. In HF, mitochondrial content was statistically increased as well as OCR during leak respiration with complex I substates. Respiratory control ratio was decreased, while H202 production rate remained unchanged. Furthermore, oxygen affinity of complex IV was lower in HF, albeit non-significantly. These results suggest that the CB mitochondria become dysfunctional and less efficient at producing ATP in HF animals. A lack of change in complex IV oxygen affinity and H202 production rate in HF CB mitochondria suggests that the oxygen sensing mechanism may be affected by extra-mitochondrial factors.

POSTER 14: Increased red cell distribution width is a predictive biomarker for atrial fibrillation post-cardiac surgery

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Atrial fibrillation (AF) is a cardiac arrhythmia closely linked to cardiovascular, thromboembolic and inflammatory conditions. Postoperative AF (POAF) after cardiac surgery is a common adverse outcome and can lead to life-threatening complications. Biomarkers, such as red cell distribution width (RDW), have been used to identify the risk of AF in cardiovascular disease, however, their use as predictive biomarkers in cardiac surgery has not been established despite their routine use as a preoperative test. Identification of biomarkers capable of predicting the risk of developing POAF after cardiac surgery would allow improvement in clinical care and could reduce the incidence of adverse outcomes related to AF. This systematic review and meta-analysis set out to investigate whether RDW can be used as a predictive biomarker for AF post-cardiac surgery. PubMed, Scopus and Web of Science databases were utilised to identify studies reporting preoperative RDW (%) and incidence of POAF after cardiac surgery procedures, including coronary artery bypass graft (CABG), cardiac valve repair or replacement, and aorta surgeries. Review of the literature identified four studies, totalling 708 participants, 174 of which developed POAF and 534 who did not. Combined analysis of the four studies demonstrated, despite moderate heterogeneity between the studies, patients with increased preoperative RDW were at a higher risk of developing POAF after cardiac surgery (OR [odds ratio] 1.45; 95% CI [confidence interval], 1.13-1.86; p=0.004). We demonstrate via meta and combined analysis of 4 studies, raised preoperative RDW to be indicative of an increased incidence of atrial fibrillation post-cardiac surgery. Suggesting RDW could be used as a predictive biomarker for POAF after cardiac surgery. Further work is required to identify the RDW values that represent the at risk range for POAF.

POSTER 15: p-Cresyl Sulphate Concentration Changes with Chronic Kidney Disease and Contribution to Cardiovascular Dysfunction; A Meta-Analysis

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p-cresyl sulphate (pCS) is a protein-bound uremic toxin implicated in cardiovascular dysfunction such as left ventricular hypertrophy (LVH). Uremic toxins are known to increase with chronic kidney disease (CKD) progression. The aim of this study was to determine how pCS levels change between moderate and end-stage CKD, as the latter is when cardiac dysfunction is known to occur. A meta-analysis was performed, including studies measuring pCS concentration in patients with CKD stage 3 and stage 5. As expected, pCS concentrations elevated with CKD progression, with a mean increase of 12.87 mg/L [95% CI -17.37, -8.37] from CKD stage 3 to stage 5 ($Z = 5.61$, $p < 0.00001$). There was more variability in pCS concentration in studies of elderly patients with CKD, whereby pCS concentration increased by 21.33 mg/L [96% CI -38.86, -3.81] from CKD stage 3 to stage 5 ($Z = 2.39$, $p = 0.02$). Elevated pCS concentration may contribute to cardiovascular dysfunction in CKD, and therefore may be a target for therapeutic intervention; this is supported by literature suggesting pCS is involved in the pathogenesis of LVH in CKD patients. Further study is required to understand the role of pCS in development of LVH and to identify effective therapeutics to limit the effects of pCS accumulation. Through understanding how pCS concentration changes with CKD progression, and the influence of age, this provides relevant concentration ranges for future studies into cardiovascular dysfunction in CKD.

POSTER 16: Does imipramine cause detubulation of adult rat ventricular cardiomyocytes resulting in contractile failure and a phenotype akin to drug-induced heart failure?

Lauren McGuinness, Martyn Mahaut-Smith (University of Leicester), Richard Rainbow

University of Liverpool

Heart failure constitutes insufficient cardiac output that fails to meet systemic metabolic demands, arising from impaired heart muscle. T-tubules are key structures involved in efficient contractile function within cardiomyocytes by facilitating synchronous calcium release from sarcoplasmic reticulum stores, aiding in immediate interaction with sarcomere units to generate effective contractile force. Recent evidence suggests the anti-depressant imipramine can act as a detubulating agent through breakdown of T-tubule integrity. The research presented aimed to establish the functional impact of imipramine on freshly isolated cardiomyocytes and their contractile behaviour.

Cardiomyocytes were isolated from adult male Wistar rats and perfused with either normal Tyrode's solution to act as control conditions or imipramine (30, 100 or 300 μM). Contractile force generated was determined by measuring the length of contracting cardiomyocytes using an edge detection system, whilst corresponding intracellular calcium changes under both control and imipramine conditions were determined using fluo-4 AM dye loaded into cardiomyocytes.

At the lowest concentration observed, imipramine resulted in reduced contractile behaviour across the cardiomyocytes, with many losing contractile ability all together (mean AUC ***$p<0.001$). Such observations were synonymous with blunted and inhomogeneous calcium transients (mean AUC **$p<0.01$).

These data suggest inefficient intracellular calcium signalling underpins the dysfunctional contractile activity observed. Imipramine is suspected to interfere with the T-tubule network via PI(4,5)P2, causing a breakdown of its integrity. This hinders the T-tubules ability to facilitate contraction that effectively mimics heart failure in-vitro and may provide insight into the clinical manifestation of pathophysiological heart failure.

POSTER 17: Pim kinase is a novel regulator of endothelium driven thrombosis.

Eima Karim, Amelia Drysdale, Sophie Nock, Sarah Jones, Amanda Unsworth

Manchester Metropolitan University

Atherothrombosis, the development of an occlusive clot in an artery, is triggered by atherosclerotic plaque rupture/erosion, and is the consequence of a complex interaction between multiple cell types in the blood and vasculature, with endothelial cells and platelets playing significant roles. The healthy endothelium prevents thrombosis whilst an activated or damaged endothelium favours thrombosis. Simultaneously targeting platelets and the endothelium could provide an effective anti-thrombotic therapeutic approach. Pim kinases (Pim-1, -2, and -3), have been shown to modulate platelet function, and whilst shown to be expressed in endothelial cells, the role of Pim kinase in the thrombotic properties of the endothelium remains unknown. The regulatory role for Pim kinase in endothelial cell control of thrombus formation in response to cigarette smoke extract (CSE) and TNFa, initiators of endothelial cell damage were therefore determined, using techniques including immunofluorescence microscopy, qPCR, Western Blotting, and ELISA. We confirmed mRNA expression of all three Pim kinase isoforms, and protein expression of Pim-1 in HUVECs. HUVECs treated with CSE and TNFa demonstrated a decrease in eNOS levels, a protective mediator of cardiovascular homeostasis. To investigate whether Pim kinase plays a role, HUVECs were treated with AZD1208, a pan Pim kinase inhibitor, and a decrease in expression of VWF, a pro-coagulant mediator, and release of inflammatory markers, IL-6, and IL-8 were observed. Collectively, these findings identify a potential role for Pim kinase in atherothrombosis, and indicates that Pim kinase inhibitors could be repurposed for use alongside other anti-thrombotic agents for the prevention of cardiovascular-related events.

POSTER 18: Sex-specific impact in offspring exposed to in-utero angiotensin II in a model of superimposed preeclampsia

Sol Olivera, Hannah Fulton, Dilys Freeman, Delyth Graham

University of Glasgow

Preeclampsia (a hypertensive disorder of pregnancy) is a leading cause of maternal and neonatal deaths but its causes are still not understood. Thus, the development of an animal model of preeclampsia is needed. The aim of this study was to investigate sex differences in the cardiovascular impact of an adverse in-utero environment in the offspring of a rat model of superimposed preeclampsia. Pregnant stroke-prone spontaneously hypertensive rats (SHRSP) were infused with 0.9% saline (control) (n=6 male, n=6 female) or >650ng/kg/min angiotensin II (ANGII) (n=6 male, n=5 female) at gestational day 10.5. Neonates were weighed and echocardiography was carried out regularly between 5 and 17 weeks of age (W5-17). Tissues were harvested at sacrifice (W18-19). ANGII-exposed offspring showed significant growth restriction between 1-7 days of age with no sex differences (7.5±2.7 (control) v. 6.1±1.8g (ANGII), $P<0.05$). ANGII-exposed males showed an increase in left ventricular mass (normalised to tibia length) during early stages (W5-W13) (0.66±0.40 (control males) v. 1.30±0.42 (ANGII males), $P<0.05$) in addition to an increase in the E/A ratio (1.32±0.16 (control males) v. 1.87±0.26 (ANGII males), $P<0.05$). In contrast, ANGII-exposed females showed a late increase in the E/A ratio at W17 (1.24±0.23 (control females) v. 2.07±0.13 (ANGII females), $P<0.05$). Moreover, a female-specific increase in gonadal fat was observed in the offspring exposed to an ANGII environment (1.8±0.1 (control females) v. 2.2±0.1g (ANGII females), $P<0.05$). These results suggest an early cardiovascular deterioration in the males in contrast with a late cardiometabolic dysregulation in the females after intrauterine exposure to ANGII.

POSTER 19: Investigating the cardiotoxicity of particulate matter air pollution using a mouse models.

Sana Yaar, David Bechtold, Luigi Venetucci, Holly Shiels.

University of Manchester

Cardiovascular disease (CVD) accounts for more than half of the 6.7 million premature deaths attributed to air pollution. Studies have found particulate matter (PM), a subgroup of pollutants, are associated most strongly with CVD; particularly due to the ability of smaller particles (PM2.5), such as polyaromatic hydrocarbons (PAHs), to enter the systemic circulation. Most research in this field is done in fish - in this study, using a mouse model, we show that exposure to a single PAH, Phenanthrene (Phe), can directly alter cardiac activity.

Acute exposure (15 minutes) to Phe led to a significant reduction in heart rate and prolongation of the RR interval both *ex-vivo* (25uM Phe, 17% reduction, P=0.022, n=5) in isolated hearts and *in-vivo* (50ug/kg Phe, 10% reduction, P=0.0043, n=7) in anaesthetised mice, suggesting Phe has some direct effects on the heart, which are unaffected by peripheral factors. *Ex-vivo* exposure to 25uM Phe also lead to a significant reduction in conduction velocity (n=8, P=0.0087) and prolongation of the ventricular action potential duration (n=6, P=0.0042), again highlighting the direct effects of Phe on the heart. To investigate the effects of prolonged Phe exposure, wild-type mice (10-weeks) were exposed to either 30ug/kg Phe (n=7), 3ug/kg Phe (n=6) or vehicle only (DMSO, n=6) for a 28-day period, followed by *in-vivo* electrocardiograph and echocardiograph recordings and tissue collection. Exposure to 30ug/kg Phe had a significant impact on *in-vivo* HR and lead to prolongation of the QTc interval (n=7, $P<0.05$), suggesting the pro-arrhythmic effects of Phe previously seen in fish, may also occur in mammals. Echocardiography data showed exposure to 3ug/kg Phe only significantly reduced end-diastolic volume by almost 50% (n=5, $P<0.05$). This data suggests Phe is also affecting the ability of the heart to fill, a phenotype not previously studied in fish. A significant increase in heart weight was observed in animals exposed to 3ug/kg Phe (n=10, P=0.002), however molecular analysis found no changes in the RNA levels of common hypertrophic markers (ANP and BNP). Using Gas-chromatography-mass-spectrophotometry (GCMS) we found no differences in the concentration of Phe in cardiac tissue, suggesting this level of exposure does not lead to Phe accumulation.

This study is the first to show that exposure to a single pollutant, Phe, can have significant effects on the mammalian cardiovascular system. This study is also the first to investigate prolonged exposure to Phe in mice, showing actions on both cardiac electrophysiology and ventricular filling. More detailed investigations of Phe exposure, both acute and chronic, in mouse models of health and disease could reveal key mechanisms of actions for common pollutants such as Phe. As the global levels of pollution continue to rise, it is vital we understand how these pollutants are affecting us, only then can we manage and mitigate this avoidable cause of disease and death.

POSTER 20: Targeting arrhythmias via modulating the cyclic guanosine monophosphate pathway; a translational role for the clinically available sGC activators?

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Background

Dysregulated cellular calcium (Ca^{2+}) cycling is a key driver for arrhythmias, which contribute to ~50% of heart failure (HF) deaths. Evidence suggests modulating cGMP signalling is antiarrhythmic. However studies have augmented cGMP via inhibiting the cGMP specific phosphodiesterase - PDE5. Here, effectivity relies upon unchanged PDE5 levels in HF. Our objective was to assess whether modulating cGMP, via activating soluble guanylate cyclase (sGC) with the clinically available drug Cinaciguat, is antiarrhythmic.

Methods/Results

An integrative cellular to whole heart approach was employed to assess Cinaciguat's efficacy (CIN;10μM):

Single sheep ventricular myocytes were field stimulated and Ca^{2+} transients recorded under baseline and pro-arrhythmic conditions (hypokalaemia (2mM) and dofetilide (I_{Kr} blocker;5μM)). CIN increased Ca^{2+} transient amplitude ($p=0.02$) and accelerated the Ca^{2+} transient rate of decay ($p=0.002$). CIN reduced delayed ($p=0.0005$) and early afterdepolarisation incidence ($p=0.02$). PKG inhibition (KT-5823;1μM) abolished CIN's effect on Ca^{2+} transient parameters and afterdepolarisation incidence. Experiments were repeated in sheep HF ventricular myocytes where CIN exerted the same effects.

In whole mouse hearts, arrhythmias were elicited by isoprenaline (100nM) and programmed electrical stimulation. Voltage and Ca^{2+} were measured via epicardial optical mapping. Arrhythmias were scored using the Clasen method. CIN reduced arrhythmia score ($p=0.012$) whereas vehicle control had no effect. CIN reduced Ca^{2+} transient ($p<0.0001$) and action potential duration ($p<0.004$).

Conclusions/Future

A clinically available sGC activator can modulate Ca^{2+} handling and is antiarrhythmic at the cellular and organ level, giving confidence for translation. Future work will involve in vivo CIN administration and then gain access to run clinical evaluations on sGC activators.

POSTER 21: A vascular smooth muscle potassium channel dysfunction underlies small vessel disease of the brain in both hypertension and Alzheimer's disease

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Patients with both Alzheimer's disease (AD) and hypertension-related vascular dementia (VaD) often show extensive small vessel disease (SVD) of the brain post-mortem. One phenotypic character of SVD is a reduction in cerebral blood flow (CBF). The large-conductance calcium-activated potassium (BK) channel controls CBF by reducing arterial contractility. The BK channel is activated via small, localised calcium release events from ryanodine receptors (RyR), known as calcium sparks. We wished to examine whether defects in calcium spark to BK channel coupling contributes to the SVD phenotype in these diseases.

Male spontaneously hypertensive (BPH/2) mice and normotensive controls (BPN/3) were used at 8 months of age to study VaD. To study AD we used 18-20 month old male APP23 mice that have significant amyloid-beta accumulation. During this study we used pressure myography, patch clamp and spinning-disc confocal microscopy to investigate vascular ion channel function within the cerebral microvasculature.

Pressure-induced constriction from cerebral pial arteries was significantly greater in both the BPH/2 and APP23 animals compared to controls. Pressure myography and electrophysiology studies revealed that in both models there was a reduction in BK channel activity. In the APP23 model, this reduction in BK channel function was due to a decrease in calcium spark frequency. However in the BPH/2 mice, separation of the BK channel and RyR resulted in BK channel dysfunction.

Our data indicates that vascular BK channel dysfunction is a common mechanism underpinning reduced CBF in SVD. Therefore, improving vascular BK channel function could provide new therapies for both AD and VaD.

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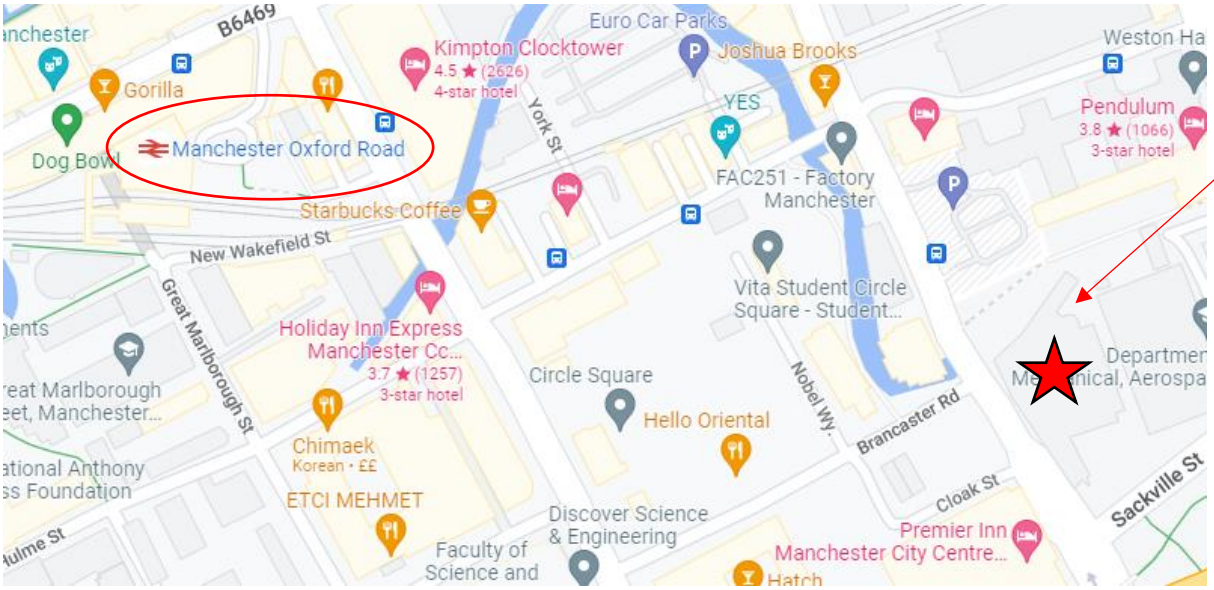
Where to find us



Entrance is here, take steps from the main road

Directions to the evening reception