151 NOX5 INDUCES VASCULAR DYSFUNCTION AND ARTERIAL REMODELLING INDEPENDENTLY OF BLOOD PRESSURE ELEVATION IN ANG II-INFUSED NOX5-EXPRESSING MICE

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10.1136/heartjnl-2017-311726.150

Nox5 is a unique Ca^{2+}-sensitive Nox isoform that is expressed in human vascular smooth muscle cells (VSMC). Although Nox5 has been implicated in diabetic nephropathy, its role in vascular function and development of hypertension remain unclear. Nox5 is not expressed in rodents, and accordingly we generated humanised Nox5 mice with Nox5 expressed in a VSMC-specific manner (Nox5SM22). Control (wild-type) and Nox5SM22 mice were infused with Ang II (600 ng/Kg/day). Blood pressure (BP) was assessed by tail-cuff. Vascular function and structure of resistance arteries were measured by myography. Ang II increased BP in WT and Nox5SM22 mice with no significant differences. Arteries from Nox5SM22 mice exhibited reduced endothelium-dependent relaxation versus WT controls (%ACh relaxation: 55.1±4 vs ctl: 81.6±7%). Fasudil (Rho kinase inhibitor)-induced relaxation was reduced in Nox5SM22 mice versus controls (%Fas relaxation: 111.3±11 vs ctl: 166.6±8%) ($p<0.05$). Ang II increased the maximal contraction to U46619 (thromboxane A2 mimetic) in WT (115.8±2 vs untreated: 101.4±2%) and Nox5SM22 (121.3±3 vs untreated: 99.1±2) ($p<0.05$) and induced endothelial dysfunction in all groups. Fasudil-induced relaxation was impaired by Ang II in WT (102.7±6 vs untreated: 166.6±8%, $p<0.05$) but not further impaired in Nox5SM22 mice (114.9±6 vs untreated: 111.3±11%). Ang II increased cross-sectional area (CSA) and lumen diameter) while in Nox5SM22 mice, Ang II increased wall thickness, wall-to-lumen ratio, CSA and decreased lumen diameter, with associated increased vascular stiffness. Our findings indicate that in mice expressing human Nox5 in VSMCs, endothelium-dependent relaxation is impaired, fasudil-mediated vasodilation is attenuated and vessels undergo exaggerated hypertrophic inward remodelling with increased stiffness; processes that occur independently of BP elevation. These data suggest an important role for Nox5 in Ang II-induced vascular dysfunction and remodelling, but not in the development of hypertension. Moreover, we identify Rho kinase as a putative target for Nox5-induced vascular injury. We provide novel insights into Nox5 vascular biology and demonstrate that vascular Nox5 actions are dissociated from BP effects.

152 THE ROLE OF ADAMTS-5 IN EXTRACELLULAR MATRIX REMODELLING OF THORACIC AORTIC ANEURYSMS

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10.1136/heartjnl-2017-311726.151

Introduction Thoracic aortic aneurysms (TAA) are common in patients with bicuspid aortic valve (BAV). ADAMTS-1 (a disintegrin and metalloproteinase with thrombospondin motifs) has recently been implicated in TAA formation (*Oller et al*, Nat Med, 2017). The contribution of other ADAMTS proteases to TAA is currently unknown.

Method Using proteomics, we compared the extracellular matrix (ECM) composition in the greater (i.e. the aneurysm-prone area) and lesser curvatures of TAA in BAV patients. Our findings in patients were complemented by studies in ADAMTS-5 deficient mice.

Results In BAV patients with TAA, the large aggregating proteoglycan versican was the most differentially regulated ECM protein in the aneurysm-prone area. In mice, ADAMTS-5 is the main versican-degrading member of the ADAMTS family. Hence, a model of aortic dilatation by angiotensin II (AngII) infusion was adopted in mice lacking the catalytic domain of ADAMTS-5 (Adamts-5^{cd}). AngII treatment raised blood pressure in wild-type (WT) mice; this response was attenuated and associated with increased dilation of the ascending aorta in Adamts-5^{cd} mice. Concomitantly, versican accumulation and reduced versican degradation products were observed in Adamts-5^{cd} aortas compared to WT controls. The presence of other ADAMTS members, including ADAMTS-1, was not sufficient to maintain versican processing and prevent aortic dilation in Adamts-5^{cd} mice.

Conclusion Our results support the emerging role of ADAMTS proteases in TAA. ADAMTS-5 rather than ADAMTS-1 is the key protease for versican regulation in murine aortas. Further studies are needed to define the ECM substrates of the different ADAMTS proteases and their contribution to TAA formation.

153 METABOLIC DISORDER-INDUCED GLOBAL NOX2 ACTIVATION AND ENDOTHELIAL DYSFUNCTION IN AGEING

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10.1136/heartjnl-2017-311726.152

Ageing is an independent risk factor of cardiovascular diseases which involves oxidative stress derived from a Nox2-containing NADPH oxidase. However, the mechanism of endothelial Nox2 activation in normal ageing process remains unclear. In this study, we investigated the therapeutic potential of targeting Nox2 in improving global metabolism and endothelial function at old age by using age-matched wild-type and Nox2 knockout mice at 3–4 months (young); 11–12 months (middle aged) and 21–22 months (ageing). Compared to young mice, middle-aged and ageing wild-type mice had significantly higher blood pressure, hyperglycaemia, hyperinsulinaemia. These were accompanied by oxidative stress in multiple organs including the lung, the liver, the heart and vessels. The vessel motor function was examined in an organ bath using aortas isolated from these mice. Endothelium-dependent vessel relaxation to acetylcholine was significantly impaired in aortas of wild-type ageing mice, and this was accompanied by increased expressions of Nox2 and markers of inflammation, activation of MAPK and Akt and decreased insulin receptor expression and function. However, these aging-associated disorders in the aortas were significantly reduced by knocking out Nox2 in mice. In response to high glucose plus high insulin challenge, coronary microvascular endothelial cells isolated from wild-type mice displayed significantly increased Nox2 expression, oxidative stress and cell senescence, e.g. increase p53 expression

and β²–galactosidase activity. However, these responses were absent or significantly reduced in endothelial cells isolated from Nox2 knockout mice. In conclusion, metabolic disorders in particular hyperglycaemia and insulin resistance play an important role in mediating Nox2 activation and oxidative stress in multiple organs in ageing. Nox2 is involved in normal ageing process-associated vascular inflammation and oxidative damage of endothelial dysfunction.

154 MYOCARDIAL NOX2 ACTIVITY REGULATES ATRIAL FIBRILLATION SUSCEPTIBILITY

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10.1136/heartjnl-2017-311726.153

Background Gp91-containing NADPH oxidases (NOX2) are a significant source of reactive oxygen species (ROS) in the human atrial myocardium. An increase in NOX2 activity accompanies atrial fibrillation (AF) induction and electrical remodelling in animal models and predicts incident AF in humans; however, whether an increase in atrial NOX2 activity is necessary to create a substrate for AF remains to be demonstrated. The purpose of this study is to determine whether an increase in NOX2-derived ROS directly contributes to the development of AF and to identify the molecular changes that occur downstream of NOX2 activation in atrial myocytes.

Methods and Results Mice with myocardial NOX2 overexpression (NOX2-Tg) showed a 2-fold increase in NADPH-stimulated superoxide production (2-hydroxyethidium by HPLC) in both the left and right atria, without any other electrophysiological or structural abnormalities (assessed by surface ECG and echocardiography); however, AF susceptibility assessed *in vivo* by transesophageal pacing was significantly higher in NOX2-Tg mice compared with their wild-type (WT) littermate controls (AF probability: 16.9±1.8% vs. 10.2±1.6% respectively, n=25–26/genotype, $p<0.01$). Moreover, oral supplementation with atorvastatin (30 mg/kg/day), an inhibitor of NOX2 activity, substantially reduced atrial NADPH-stimulated ROS and AF susceptibility in NOX2-Tg mice. *Ex vivo* high-resolution optical mapping of di-4-ANEPPS-stained atrial preparations revealed no differences in the action potential duration of left and right atria of NOX2-Tg mice compared to controls, suggesting that electrical arrhythmogenic remodelling does not occur with NOX2 overexpression. However, abnormalities in intracellular Ca^{2+} handling have also been linked to AF; intriguingly, western blot analyses of atrial tissues revealed a 28% reduction (n=11–23/genotype, $p=0.0032$ with un-paired students t-test) in the phosphorylation status of the ryanodine receptor (RyR) at Ser2814 in the right, but not the left atria of NOX2-Tg mice. Our ongoing experiments are now aimed at determining whether NOX2-derived ROS alters diastolic calcium leak from the sarcoplasmic reticulum in isolated atrial myocytes due to altered RyR gating.

Conclusions Myocardial NOX2 overexpression and related increase in NADPH-stimulated ROS production are associated with increased AF susceptibility *in vivo* and reduced RyR phosphorylation at Ser2814. These findings suggest increased NOX2 ROS may promote arrhythmogenesis by modulating atrial calcium handling.

155 HIGHER IGM ANTI OXIDISED LDL ANTIBODIES POINT TO FAVOURABLE PLAQUE CHARACTERISTICS AS DETERMINED BY RADIO FREQUENCY INTRAVASCULAR ULTRASOUND (RF-IVUS) AND NEAR INFRARED SPECTROSCOPY (NIRS) IN THE INTEGRATED IMAGING AND BIOMARKER STUDY 3 (IBIS-3)

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10.1136/heartjnl-2017-311726.154

Background Malondialdehyde (MDA)-LDL is one of the main oxidation products involved in atherosclerosis pathogenesis. IgM antibodies against MDA-LDL have been generally regarded as protecting from atherosclerosis. In contrast, the role of IgG antibodies is less clear. How antibody levels relate to coronary atherosclerosis plaque characteristics still needs to be determined.

Hypothesis We tested the hypothesis that low IgM anti-MDA antibody levels in serum are associated with plaques with larger lipid-laden necrotic cores.

Method IBIS-3 was a prospective cohort study that was designed to determine the ability of rosuvastatin to decrease necrotic core volume in coronary atherosclerosis. Patients undergoing coronary artery grafting or percutaneous intervention for (un)stable angina pectoris or myocardial infarction were eligible. After the standard procedure, radiofrequency intravascular ultrasound (RF-IVUS) and near-infrared spectroscopy (NIRS) measurements were performed in a non-culprit coronary artery with a diameter stenosis<50%. After a median of 386 days of high dose rosuvastatin treatment, imaging was repeated on the same segment. Antibodies against MDA-LDL, as well as total serum IgM and IgG, were measured by ELISA. Associations between antibodies and the RF-IVUS and NIRS parameters were assessed using linear regression models with quartiles as independent variables and with further adjustments for age, sex, diabetes, smoking, LDL and HDL cholesterol and previous use of statins.

Results A total of 143 patients of the included patients had both blood samples and RF-IVUS measurements available, and NIRS was also performed on 90 of these. Mean age was 59.6 (9.0) years (84.6% male) and 94.4% were already on a regular statin before inclusion. At baseline, IgM anti MDA-LDL antibody levels had a strong independent inverse relationship with lesional necrotic core volume ($p=0.027$) and percentage ($p=0.011$) as well as with lipid core burden index (LCBI) ($p=0.024$) in the worst 4 mm segment. This relationship was partially dependent on total serum IgM, as higher IgM levels also reflected a favourable necrotic core percentage on RF-IVUS. There was no correlation between any imaging parameter and IgG antibodies, total serum IgG, HDL-cholesterol or LDL-cholesterol. Although total atheroma burden increased over one year, there was no change in necrotic core volume.