Platelets are individually encapsulated in monodisperse (CV of 1–4%) water-in-oil droplets with a mean volume of 14 pL and a diameter of 30 Î¼m. Droplets are produced with a throughput of 4 kHz, with droplets containing a single platelet produced at a rate of 0.25 kHz (following a Poisson distribution). With this method an intrinsic variation in the platelet response to convulxin is observed, that is unrelated to the size of the platelet.

The research entailed the development of a method capable of measuring the intrinsic variation in platelet function. The method will be used to compare the sensitivity profiles of people with known risk factors for arterial thrombosis with the profiles of people with low risk. This presents the possibility to identify a novel prognostic biomarker. Furthermore, a better understanding of the functional heterogeneity of platelets could be used to identify new targets to aid the rational design of new therapeutics. Ideally, this would target only hyperactive platelets, while preserving normal haemostasis.

191 ATHEROPRONE FLOW ALTERS ATP-INDUCED CALCIUM SIGNALLING IN THE ENDOTHELIUM

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Introduction The development of the atherosclerotic plaque occurs at distinct points in the vasculature, with areas such as bends and branch points susceptible to plaque formation. The endothelium at these sites is influenced by a disturbed blood flow with low wall shear stress. Numerous studies have shown that stimulation of endothelial cells with this flow pattern induces several inflammatory signalling pathways, but the mechanisms in which this flow pattern is sensed remain unclear.

ATP is released extracellularly from endothelial cells in response to shear stress. The endothelium expresses the ATP-gated cation channels P2X4 and P2X7, which have been previously shown to respond to shear stress induced ATP release. Their expression is also increased in the endothelium following inflammatory stimulation. Therefore, we hypothesise that P2X receptors are involved in sensing atheroprone flow and inducing inflammatory signalling, thereby promoting development of atherosclerosis.

Methods Human Umbilical Vein Endothelial Cells (HUVECs) were cultured under flow for 72 h using an ibidi flow pump system. Atheroprotective flow was applied using +13 dyn/cm^2 and ± 4 dyn/cm^2 (0.5hz) was used for atheroprone flow. After flow conditioning, HUVECs were then loaded with a fluorescent calcium dye (CAL-520) and their response to 300Î¼M BzATP was measured.

Results Endothelial cells preconditioned with atheroprone flow showed an enhanced calcium response after BzATP stimulation. This enhancement was dependent on extracellular calcium as no increase was detected when performed in calcium free extracellular buffer. The expression level of P2X4 and P2X7 receptors was significantly enhanced in HUVECs preconditioned with atheroprone flow compared to atheroprotective. Moreover the expression of the cell surface ATPase CD39, which breaks down extracellular ATP, was increased under atheroprotective flow. Chemical inhibition of CD39 with ARL-67156 also significantly enhanced the calcium response to BzATP in cells under atheroprotective, but not atheroprone flow.

Conclusions We have identified an enhanced ATP induced calcium response and expression of the ATP-gated P2X4/7 receptors in HUVEC under atheroprone flow conditions. We observed enhanced expression of the cell surface ATPase CD39 in endothelial cells under atheroprotective flow suggesting that extracellular ATP is more rapidly hydrolysed to adenosine under such conditions. We therefore propose that shear stress induced ATP plays a role in specifically activating cells under atheroprone flow and induces inflammatory signalling pathways, underlying atherogenesis susceptibility in vulnerable regions of the vasculature.

192 HYPOXIA ENHANCES THE REPARATIVE EFFECT OF TISSUE PROTECTIVE ERYTHROPOIETIN AND ITS NON-ERYTHROPOIETIC PEPTIDE ANALOGUE IN AN ENDOTHELIAL CELL INJURY MODEL

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Background Endothelial injury is a critical feature in the early stages of vascular disease. Inflammation and hypoxia are often associated with endothelial injury, stimulating the expression of several cytokines that include erythropoietin (EPO). Endothelial cell-derived EPO appears to be important for protecting the endothelium against ischaemic injury. A non-erythropoietic analogue of EPO; pyroglutamate helix B surface peptide (pHBSP) retains these protective properties of EPO without possessing its erythropoietic effects. The aim of our study was to assess the effects of these molecules in a model of endothelial injury under normoxic and hypoxic conditions.

Method The reparative effects of EPO and pHBSP were assessed under hypoxia (1% O_2) and normoxia (21% O_2) in an *in vitro* model of endothelial injury (scratch assay). A monolayer of bovine aortic endothelial cells (BAECs), grown to confluence in a multi-well plate, was scratched and the closure of the injured endothelial monolayer was assessed over 24 h. The effects of EPO and pHBSP on BAEC proliferation, chemotaxis and apoptosis were assessed under similar hypoxic conditions in separate experiments. The potential molecular mechanisms of these effects were also explored.

Results Both EPO and pHBSP enhanced scratch closure under hypoxic conditions by 13 ± 2.6%, and 10 ± 1.69% respectively ($p < 0.01$) compared to normoxic conditions (3.2 ± 0.9% and 2.9 ± 0.3% for EPO and pHBSP respectively $p > 0.05$). These effects appeared to be by promoting cell proliferation and migration of BAECs ($p < 0.05$). EPO also protected BAECs from staurosporine-induced apoptosis under hypoxic conditions. The priming effect of hypoxia was associated with stabilisation of HIF-1Î ±, EPO receptor (EPOR) up-regulation and decreased phosphorylation of endothelial nitric oxide synthase (eNOS) at the Ser-1177 residue. The effect of hypoxia on the latter was rescued by EPO. Hypoxia was associated with a reduction in nitric oxide (NO) production as assessed by its oxidation products nitrite and nitrate, and this was consistent with the oxygen requirement for the endogenous production of NO by NO synthase (NOS). Whilst EPO did not affect NO formation in normoxia, it markedly increased NO production under hypoxic conditions, in a NOS-dependent

manner; its effects were inhibited by NOS inhibitors (e.g. L-NAME).

Conclusion and implication The tissue-protective properties of EPO-related cytokines are likely to be mediated by NO in pathophysiological settings associated with poor oxygenation. Further work should be directed towards an understanding of the cellular redox status and some of the signalling events down-stream of the emerging EPO/EPOR/NO axis that underpin its beneficial biological effects. These findings may be particularly relevant to atherogenesis and post-angioplasty restenosis.

193 LACK OF FIBROBLAST GROWTH FACTOR-23 (FGF23) PRESERVES CARDIAC FUNCTION IN A MURINE MODEL OF ACUTE MYOCARDIAL INFARCTION

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Myocardial infarction (MI) is a major cause of death worldwide. We recently showed that intact circulating fibroblast growth factor-23 (FGF23) is profoundly up-regulated after experimental MI in mice and rats. FGF23 is a bone-derived hormone involved in systemic phosphate homeostasis and vitamin D metabolism. Although the pathophysiological mechanisms remain to be identified, clinical studies have shown a strong association between FGF23 and left ventricular hypertrophy, atrial fibrillation and cardiac systolic dysfunction. Here, we explored the hypothesis that FGF23 may be causally linked to progression of cardiac dysfunction post-MI, using a mouse model lacking both *Fgf23* and a functioning vitamin D receptor (VDR). Surgery was performed on 3-month-old, male, wild-type (WT), VDR and *Fgf23*$^{-/-}$/VDR$^{\hat{I}"/\hat{I}"}$(Fgf23/VDR) compound mutant mice on a C57BL/6 background. To normalise mineral homeostasis in VDR-ablated mice, all mice were kept lifelong on a rescue diet enriched with calcium, phosphorous and lactose. MI was induced by permanent ligation of the left descending coronary artery. Sham-operated (Sham) mice served as a control. One week after MI, cardiac function was assessed by echocardiography and electrocardiography (ECG). Intracardial pressure monitoring was performed by catheterization as a terminal procedure, 2 weeks post-MI. Echocardiography confirmed left ventricular infarction, and ECG recordings revealed comparable ST depression in MI mice of all genotypes. However, Fgf23/VDR compound mutant MI mice showed improved fractional shortening, relative to WT and VDR MI controls. In addition, Fgf23/VDR compound mutant MImice were more resistant against the impairment of ventricular contraction and relaxation observed in WT and VDR MI mice, as measured by left ventricular dP/dt_{max}, dP/dt_{min}, and relaxation time tau, 2 weeks post-MI surgery. Our data indicate that lack of Fgf23 improves cardiac contractile function following experimental MI. This finding underscores the potential importance of the heart-bone-axis and of the new field of cardio-osteology because the levels of circulating intact FGF23 may influence cardiac recovery after MI.

194 FERRITIN OXIDISES LOW DENSITY LIPOPROTEIN AT LYSOSOMAL PH

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Background Many lines of evidence support the role of oxidised low density lipoprotein (LDL) as the main culprit in atherogenesis. Our laboratory has previously shown that iron is involved in the oxidation of LDL in lysosomes, a possible mechanism for the progression of atherosclerosis. Ferritin is an iron-storage protein which might enter lysosomes by autophagy and be involved in lysosomal LDL oxidation. The aim of this study was to test the hypothesis that ferritin can catalyse the oxidation of LDL at lysosomal pH and that antioxidants protect against this oxidation.

Method LDL (50 ug protein/ml) was oxidised by ferritin (0.05–0.2 uM) at 37°C and pH 4.5, the formation of conjugated dienes was monitored spectrophotometrically at 234 nm up to 1200 min. The effect of pH on LDL oxidation was tested by comparing the oxidation of LDL by ferritin (0.1 and 0.2 uM) at pH 4.5 or pH 7.4. The iron released from ferritin was monitored spectrophotometrically using the ferrous iron chelator bathophenanthroline. Ferritin (0.1 uM) was incubated at 37°C at pH 4.5 and pH 7.4. Bathophenanthroline (30 uM) was added at different time intervals up to 24 h and the amount of ferrous complex was measured at 535 nm. Iron release was also measured by ultrafiltration followed by atomic absorption spectrophotometry.

Results LDL was oxidised effectively by ferritin (0.05–0.2 uM). The oxidation was much faster at lysosomal pH 4.5 than at pH 7.4, which could be attributed to our finding that more iron was released from ferritin at pH 4.5. EDTA and diethylenetriamine pentaacetate inhibited the oxidation, but did not inhibit it entirely. The water-soluble lysosomotropic drug cysteamine (5 uM-10 mM) inhibited the initial oxidation of LDL in a concentration-dependent manner, although the lower concentrations exhibited a delayed prooxidant effect which was less marked with the higher concentrations. Concentrations above 1 mM had no prooxidant effect. Cysteamine was shown, using the ferrous iron chelator bathophenanthroline, to release iron from ferritin and this might explain the prooxidant effect. The lipid-soluble antioxidant N, N′-diphenyl-p-phenylenediamine (5 and 10 uM) inhibited the oxidation of LDL by ferritin without any prooxidant effect.

Conclusion These findings support the possible involvement of ferritin in lysosomal LDL oxidation and the use of appropriate antioxidants to prevent this oxidation in atherosclerosis.

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