BAS/BSCR39 CIRCULATING CHEMICAL AND CELLULAR INJURY/REPAIR RESPONSES ARE LINKED TO CARDIAC DYSFUNCTION AND REMODELLING IN HUMAN MYOCARDIAL INFARCTION

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[1,2]R J McGeoch, [1]A McGarrity, [1,2]A R Payne, [2]S Watkins, [2]T Steedman, [2]A Davie, [2]W S Hillis, [2]M M Lindsay, [2]S D Robb, [3]E Rhaume, [3]J C Tardif, [2]K G Oldroyd, [1,2]C Berry. *[1]BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, Scotland, UK; [2]Golden Jubilee National Hospital, Clydebank, UK; [3]Montreal Heart Institute, Montreal, Canada*

Background Endogenous regenerative pathways may contribute to cardiovascular repair following ischaemic injury. Based on recent results in experimental studies, we investigated candidate endogenous chemical and cellular injury/repair responses in human myocardial infarction (MI).

Methods Circulating injury (eg, platelets) and repair (circulating CD34+ progenitor cells, serum vascular endothelial growth factor (VEGF) and thymosin β4, and urine acetyl-Ser-Asp-Lys-Pro (AcSDKP)) responses were quantified 2 days and 3 months after acute MI. Progenitor cells in whole blood were quantified using flow cytometry, and cytokines were measured by immunoassay. An automated analyser was used for haematological measurements. Invasive measures of coronary artery microvascular resistance and collateral supply were measured acutely using coronary thermodilution techniques. Cardiac function and remodelling were quantified by magnetic resonance imaging.

Results 35 consecutive patients with MI (mean±SD age 58%± 10 years) were included. AcSDKP measured 2 days post-MI negatively predicted left ventricular ejection fraction (R^2=0.43; p=0.024) and positively predicted left ventricular end-systolic volume index (R^2=0.56; p=0.011) at 3 months. At follow-up, CD34+ count negatively predicted myocardial infarct mass (R^2=0.29; p=0.015) and left ventricular end-systolic volume index (R^2=0.20; p=0.02). In multivariable analyses, haemoglobin concentration measured 2 days post-MI negatively predicted coronary collateral supply (p=0.006), whereas red cell distribution width (p=0.004) and platelet count (p=0.0001) positively predicted coronary collateral supply. Serum VEGF at 3 months and change in VEGF were negative multivariable predictors of left ventricular end-diastolic volume index at 3 months (p=0.021 and p=0.006, respectively).

Conclusion Circulating chemical and cellular responses participate in myocardial injury and repair and represent targets for therapeutic development.

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BAS/BSCR40 CORONARY HEART DISEASE RISK ASSOCIATED WITH THE HOMOZYGOUS MINOR ALLELE FOR ENDOTHELIAL PROTEIN C RECEPTOR SER219GLY

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[1]H A Ireland, [1]J A Cooper, [1]P J Talmud, [2]A D Hingorani, [2]M Kivimki, [2]M Kumari, [3]R Morris, [4]I Tzoulaki, [5]J Price, [5]F G Fowkes, [1]S E Humphries. *[1]Division of Medicine, UCL, London, UK; [2]Division of Epidemiology and Public Health, UCL, London, UK; [3]Division of Primary Care and Population Health, Royal Free Campus, UCL, London, UK; [4]Department of Epidemiology and Biostatistics, Imperial College London; London, UK; [5]Centre for Population Health Sciences, University of Edinburgh, Edinburgh, UK*

Rationale for the study Endothelial protein C receptor (EPCR) is involved in the regulation of thrombin generation and inflammation. PROCR gene variant rs867186 codes for an amino acid substitution (Ser219Gly) within the membrane spanning region of EPCR. The minor allele of the variant has been identified as functional, and associated with increased shedding of EPCR from the endothelial surface.[1] Previous analysis has also shown increased coagulation activated markers in those with the Gly allele1.[2] Furthermore, while heterozygotes for this variant may be protected from coronary heart disease (CHD), homozygotes had a threefold elevation in CHD risk in a prospective cohort (NPHSII).[1] In a case–control study of CHD (HIFMECH), there was a suggestion that the EPCR genotype interacts with factors present in metabolic syndrome, to increase CHD risk.[1]

Methodology NPHSII was analysed to further assess the CHD risk associated with EPCR Ser219Gly in those who have been identified as diabetic, or as having 'metabolic syndrome', during 15 years' follow-up.

Results Individuals who were Gly/Gly and had 'metabolic syndrome' had a fourfold increase in CHD risk compared with Ser/Ser (HR=4.72, CI 1.69 to 13.4) p=0.006; or an eightfold increase in risk above those who were Ser/Ser without metabolic syndrome (HR=8.02, CI 2.89 to 22.2) p=0.006. No Gly/Gly individuals without metabolic syndrome had a CHD event.

Conclusions Homozygotes for the Gly allele are present at a frequency of ~1%. If the findings in the study can be replicated, the variant would constitute a considerable CHD risk in 1% of diabetics. Replication studies are under way in three further, large prospective cohorts.

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BAS/BSCR41 MODULATION OF EXTRACELLULAR MATRIX PROTEIN EXPRESSION BY INTERLEUKIN 1 IN HUMAN CARDIAC MYOFIBROBLASTS: REGULATION BY P38 MAP KINASE

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N A Turner, P Warburton, K E Porter. *Division of Cardiovascular and Neuronal Remodelling, Multidisciplinary Cardiovascular Research Centre (MCRC), University of Leeds, Leeds, UK*

The proinflammatory cytokine interleukin 1 (IL-1) elicits catabolic effects on the myocardial extracellular matrix (ECM) early after myocardial infarction but there is little understanding of its direct effects on cardiac myofibroblasts (CMF), a key cell type involved in the regulation of myocardial remodelling. We used a focused RT-PCR microarray to investigate the effects of IL-1 on expression of 41 ECM genes in CMF cultured from different patients, and explored the regulatory role of the p38 MAPK signalling pathway. IL-1 (10 ng/ml, 6 h) had only a minimal effect on mRNA expression of structural ECM proteins, including collagens, laminins, fibronectin and vitronectin. However, IL-1 induced marked increases in expression of several ECM proteases, including matrix metalloproteinases MMP-1 (collagenase-1), MMP-3 (stromelysin-1), MMP-9 (gelatinase-B) and MMP-10 (stromelysin-2). Conversely, IL-1 reduced mRNA expression of ADAMTS-1, a metalloproteinase that suppresses neovascularisation. IL-1 stimulated a small increase in expression of tissue inhibitor of metalloproteinases (TIMP)-1, but not TIMP-2. Data for MMPs 1, 2, 3, 9 and 10 and ADAMTS-1 were confirmed by quantitative real-time RT-PCR. IL-1 strongly activated the p38 MAPK pathway in human CMF, as determined by immunoblotting with phospho-specific antibodies. A p38 MAPK inhibitor (SB203580) reduced IL-1-induced mRNA expression of MMP-3, but did not affect expression of any of the other MMPs studied. SB203580 also markedly reduced ADAMTS-1 mRNA expression.

In summary, IL-1 induces a distinct pattern of ECM protease expression in human CMF, in part regulated by p38 MAPK,

affirming the key role of IL-1 and CMF in postinfarction cardiac remodelling.

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BAS/BSCR42 EFFECT OF P38-α GENE SILENCING ON CYTOKINE AND MATRIX METALLOPROTEINASE EXPRESSION BY HUMAN CARDIAC MYOFIBROBLASTS

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J K Sinfield, A Das, K E Porter, N A Turner. *Division of Cardiovascular and Neuronal Remodelling, Multidisciplinary Cardiovascular Research Centre (MCRC), University of Leeds, Leeds, UK*

Cardiac myofibroblasts (CMF) have a key role in the remodelling of the heart that occurs following a myocardial infarction. This remodelling can be initiated by increased myocardial levels of proinflammatory cytokines (eg, interleukin (IL)-1), that can stimulate cardiac myofibroblasts to express other proinflammatory cytokines and matrix metalloproteinases (MMPs). The p38 mitogen-activated protein kinase (MAPK) signalling pathway is also known to be detrimental in the myocardial remodelling process. There are four known p38 MAPK subtypes (α, β, γ and δ) and CMF express the α, γ and δ subtypes. The aim of this study was to determine the role of individual p38 subtypes in mediating IL-1-induced increases in proinflammatory cytokine and MMP expression in cultured cardiac myofibroblasts from different patients. Pharmacological inhibitors of p38-α/β (SB203580) and p38-α/β/γ/δ (BIRB-0796) inhibited IL-1-induced IL-6 and MMP-3 mRNA expression to similar extents, suggesting a key role for p38-α. Neither inhibitor affected IL-1-induced IL-1β or MMP-9 mRNA levels. Gene silencing with p38-α siRNA oligonucleotides selectively reduced p38-α protein expression by >95% and prevented consequent phosphorylation of the downstream substrate MAPKAPK2. However, p38-α silencing did not markedly inhibit phosphorylation of the MAPKAPK2 substrate HSP27. Furthermore, p38-α gene silencing did not reduce IL-1-induced expression of IL-6 or MMP-3 (or IL-1β or MMP-9). Thus, in contrast to results with pharmacological p38 MAPK inhibitors, gene silencing of p38-α in human cardiac myofibroblasts did not inhibit IL-1-induced IL-6 and MMP-3 expression. This raises interesting questions about pharmacological versus molecular strategies for inhibiting p38 MAPK subtypes in the remodelling heart.

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BAS/BSCR43 X-BOX BINDING PROTEIN 1 SPLICING IS CRUCIAL IN ENDOTHELIAL CELL PROLIFERATION

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Lingfang Zeng, Qingzhong Xiao, Hongling Li, Andriana Margariti, Yanhua Hu, Qingbo Xu. *Cardiovascular Division, King's College London BHF Centre, London, UK*

The X-box binding protein 1 (XBP1) is an endoplasmic reticulum stress response transcription factor. Our previous study showed that sustained activation of XBP1 splicing led to atherosclerosis development (Zeng *et al.* PNAS 2009). However, the function of XBP1 expression and splicing in endothelial cells and angiogenesis remains unclear. To study this issue, we generated XBP1 knockout mice by deletion of exons 1 and 2 of the gene. XBP1-null (XBP1−/−) embryos at E12.5 showed growth retardation and pale colouration phenotype. The average body weight of XBP1−/−embryos was 40% less than that of wild-type (XBP1+/+) animals. The blood vessel density in XBP1−/− embryos was significantly reduced, owing to a smaller number of CD31+ and Flk1+ cells. In in vitro culture of whole embryonic cells, XBP1−/− cells grew significantly more slowly and lost response to VEGF stimulation. To study the mechanism of XBP1-mediated cell growth, human umbilical vein endothelial cells were treated with VEGF that transiently activated IRE1α phosphorylation at Ser724. The mRNA movement inhibitor, cycloheximide, ablated VEGF-induced IRE1α phosphorylation and XBP1 splicing. Co-immunoprecipitation assay showed that there was interaction among KDR, IRE1α and unspliced XBP1 (XBP1u), which could be increased by VEGF treatment. Further experiments demonstrated that the C-terminal region of KDR and the kinase domain of IRE1α are responsible for their interactions. MTT and BrdU incorporation assays indicate that transient activation of XBP1 splicing increased while long-term activation decreased endothelial survival and proliferation. Knockdown of XBP1 or IRE1α ablated VEGF-induced proliferation in endothelial cells. Immunofluorescent staining and TOP Flash reporter assay showed that overexpression of XBP1s increased β-catenin translocation into the nucleus. Thus, this study demonstrated for the first time that XBP1 is crucial for endothelial growth and angiogenesis, in which VEGF-stimulated IRE1α/XBP1 splicing system and interaction with β-catenin are key elements, indicating a potential target of XBP1 for protecting endothelial integrity.

BAS/BSCR44 AKT ISOFORMS IN ISCHAEMIC PRECONDITIONING

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S P Kunuthur, D J Hausenloy, D M Yellon. *The Hatter Cardiovascular Institute, University College London, UK*

Akt/protein kinase B is a key mediator of protection against myocardial ischaemia-reperfusion (I-R) injury. Previous studies have shown that Akt is activated in response to cardioprotective ischaemic preconditioning (IPC) stimulus. However, there are three different Akt isoforms, of which Akt1 and Akt2 are the most abundant in the heart, and it is unclear which isoform is crucial for IPC-induced cardioprotection. The aim of this study was to identify the Akt isoform which is essential for mediating IPC-induced cardioprotection.

Mice deficient in Akt1 or Akt2 were subjected to in vivo regional myocardial ischaemia for 30 min followed by reperfusion for 2 h with or without a prior IPC stimulus, comprising 5 min of ischaemia followed by 5 min of reperfusion. Infarct size and area at risk were determined by differential staining with tetrazolium chloride and Evans Blue dye. Mice lacking either single or both alleles for Akt1 were not amenable to IPC (see table 1). In contrast, only mice lacking both alleles for Akt2 were resistant to IPC, whereas mice heterozygous for Akt2 were still amenable to IPC (see table 1).

Table 1 Infarct sizes expressed as a percentage of the area at risk

	+/+	+/−	−/−
Akt1			
I-R	45.5%±2.6%	45.3%±5.1%	47.2%±7.2%
I-R+IPC	28.9%±1.4%*	40.5%±8.0%	37.9%±4.0%
Akt2			
I-R	41.8%±4.0%	46.2%±4.7%	46.4%±5.6%
I-R+IPC	30.2%±2.3%[1]	20.7%±2.0%[1]	35.9%±5.0%

*$p<0.05$ versus respective I-R group.

In conclusion, it appears that Akt1 but not Akt2 is essential for cardioprotection conferred by ischaemic preconditioning.