PMO-123 GENE TRANSFER OF DIMETHYLARGININE DIMETHYLAMINOHYDROLASE-1 REDUCES PORTAL PRESSURE IN A RODENT MODEL OF CIRRHOSIS

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G Mehta,* V Sharma, A Habtesion, V Balasubramaniyan, N A Davies, R Jalan, V Budhram-Mahadeo, R P Mookerjee. *UCL, London, UK*

Introduction Portal hypertensive bleeding is a grave complication of cirrhosis. Asymmetric dimethylarginine (ADMA), an endogenous eNOS inhibitor, is elevated in cirrhosis, relates to degree of portal hypertension, and is prognostic in acute-on-chronic liver failure. Dimethylarginine-dimethylaminohydrolase-1 (DDAH-1), a key enzyme metabolising hepatic ADMA, is reduced in cirrhosis. Therapies which indirectly increase hepatic DDAH-1, such as anti-TNFα therapy or FXR agonists, lead to reduced hepatic ADMA, increased NO and lowered portal pressure. Therefore, there is accumulating evidence for DDAH-1 as a therapeutic target, but specific evidence for DDAH-1 reconstitution is lacking. The aim of this study was to adopt a DDAH-1 gene therapy strategy in portal hypertension.

Methods Hydrodynamic injection leads to hepatic gene transduction by causing turbulent, retrograde venous flow, permeation of hepatic parenchymal cells and consequent plasmid expression. Human DDAH-1 cDNA was cloned into the pCMV-Sport6 expression plasmid. Sprague-dawley rats (n=9) underwent bile duct-ligation, and after 4 weeks were injected with 800 μg of either pCMV-Sport6-DDAH1 (n=5) or non-expressing control plasmid (n=4). After 72 h, rats underwent direct portal pressure assessment under anaesthesia and were then sacrificed. Plasma ALT was measured by Cobas-Integra analyser. Transgene expression was measured by quantitative PCR, with Taqman probes specific for human DDAH-1 to distinguish rodent DDAH-1. Protein expression was measured by western blot.

Results Highly effective gene transfer (between 20 and 120-fold increase) of human DDAH-1 was seen in 3 out of 5 treated animals. None of the animals treated with control plasmid expressed human DDAH-1. The three "responders" to gene therapy also had highly significantly increased DDAH-1 protein expression compared with "non-responders" or controls ($p<0.05$). There was no difference in ALT between the groups. Portal pressure was significantly lower in "responders" to gene therapy than "non-responders" or animals treated with control plasmid ($p<0.05$).

Conclusion This study demonstrates that DDAH-1 is a specific molecular target for portal pressure reduction. Hydrodynamic injection is variable in efficiency of gene delivery due to the nature of the technique. However, despite these limitations, this study clearly shows proof of concept for efficient vector-based DDAH-1 gene therapy in lowering portal pressure and preventing bleeding in cirrhosis.

Competing interests None declared.

PMO-124 OXIDATIVE STRESS INDUCES ALTERNATIVE SPLICING AND NUCLEAR TRANSLOCATION OF KRUPPEL LIKE FACTOR 6

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G Patman,* K Y Hui, Q M Anstee, H L Reeves. *Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, UK*

Introduction Progression of simple steatosis to NASH is attributed to inflammation and oxyradical overload ("oxidative stress"). Expression of the zinc finger transcription factor, KLF6, which up-regulates p21 and glucokinase, is increased in whole liver as disease progresses. KLF6 is regulated by alternative splicing and we propose that the balance of KLF6 alternative splice forms in different cellular compartments within the liver may influence the rate of NAFLD progression. We have therefore studied the impact of oxidative stress on KLF6 splice isoform expression.

Methods HepG2 cells were treated with either tertiary butyl hydrogen peroxide or angiotensin II, in the presence/absence of the anti-oxidant N-acteyl-L-cysteine (NAC). Reactive oxygen species were quantified by DCFDA and lucigenin chemiluminescence. KLF6 isoform expression and subcellular localisation was determined using variant specific real-time PCR/western blotting and immunofluorescence (IF).

Results While mRNA expression of both KLF6 full length (KLF6-FL) and its SV1/SV2 variants was markedly increased (10–50-fold) after exposure to oxidative stress, this occurred at 8 h and was unaffected by NAC. Expression of the little characterised KLF6-SV3 variant, however, was dramatically increased by over 30-fold at just 15 min. Furthermore, this increase was significantly attenuated by 50% in the presence of NAC. Western blotting confirmed protein accumulation of both KLF6-FL and KLF6-SV3 at 30 min, falling after 4 h, with a sixfold increase in the KLF6 target gene, p21, at 4–8 h. KLF6 IF studies confirmed that both KLF6-FL and KLF6-SV3 in HepG2 cells translocate to the nucleus after exposure to oxidative stress. In human NAFLD, a modest 1.5–2-fold increase in KLF6-FL mRNA contrasted sharply with a dramatic ninefold increase in KLF6-SV3 mRNA ($p<0.01$) in tissues with inflammation (n=16) compared to those without (n=7).

Conclusion While KLF6-FL clearly accumulates in the nucleus of HepG2 cells in response to oxidative stress, it is the little characterised KLF6-SV3 isoform which is redox sensitive at the level of transcription and which is dramatically increased in association with inflammation in NAFLD. This variant retains the NLS and the first zinc finger of the DNA binding domain. Further characterisation of its functions and targets will help us to understand its role in NAFLD progression.

Competing interests None declared.

PMO-125 NEUTROPHIL INTRACELLULAR TOLL-LIKE RECEPTOR (TLR) 9 EXPRESSION SERVES AS A BIOMARKER THAT DETERMINES PRESENCE AND SEVERITY OF ENCEPHALOPATHY IN ACUTE LIVER FAILURE AND CIRRHOSIS

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[1]G K Manakkat Vijay,* [1]R D Abeles, [1]S Ramage, [2]A Riva, [1]J M Ryan, [1]N J Taylor, [1]J A Wendon, [2]S Chokshi, [1]Y Ma, [1]D L Shawcross. *[1]Institute of Liver Studies and Transplantation, King's College London School of Medicine at King's College Hospital, Denmark Hill, London, UK; [2]Foundation for Liver Research, London, UK*

Introduction There is a marked propensity for patients with acute liver failure (ALF) and cirrhosis to develop sepsis and inflammation which may hasten the development of hepatic encephalopathy (HE) and cerebral oedema. Neutrophil dysfunction is an important biomarker of poor prognosis in liver failure and neutrophil TLR9 expression upregulates with ammonia exposure. There is a paucity of understanding regarding the relationship between neutrophil dysfunction and the development of HE, moreover there is a lack of predictive/prognostic biomarkers that differentiate ALF patients that will go on to develop HE. The aim of this study was to investigate the relationship between neutrophil TLR9 and development of HE.

Methods In healthy controls (n=12) and patients with ALF (n=12) and cirrhosis (n=50) we investigated neutrophil TLR9 expression using fluorochrome-conjugated monoclonal antibodies [CD16 (PE), CD11b (APC-Cy7) and TLR9 (APC) (after cytofixation/permeabilisation)] by flow cytometry in determining responses to endotoxin

and bacterial challenge at baseline, and following 2 h stimulation with lipopolysaccharide (LPS) and ammonia. Pro- and anti-inflammatory cytokines were determined by CBA.

Results Baseline neutrophil TLR9 expression was significantly higher in patients with HE (ALF: Grade 3/4 vs controls: $p<0.02$, vs grade 0–2: $p<0.03$) (Cirrhotics: Grade 3/4 vs controls: $p<0.03$, vs grade 0–2: $p<0.05$). Moreover their baseline TLR9 expression was associated with severity of HE and higher IL6 and IL8 levels. CD16 expression was downregulated by a median of 45% (range 25%–85%) in ALF patients with grade 3/4 HE compared to controls and in cirrhotics by a median of 88% (range 5%–90%) (Grade 3/4 vs controls: $p<0.05$). Baseline CD11b expression did not differ between controls and patients. Exposure to LPS and ammonia upregulated TLR9 and CD11b and downregulated CD16.

Conclusion Neutrophil TLR9 expression in patients with ALF and cirrhosis serves as a useful biomarker that differentiates those who develop high grade HE from those who do not. High baseline TLR9 expression and low CD16 expression promote a pro-inflammatory cytokine milieu that may help to explain the propensity to develop infection and why inflammation hastens the development of HE. TLR9 antagonists may be of therapeutic value in restoring neutrophil activity.

Competing interests None declared.

PMO-126 THE ROLE OF VASCULAR-ADHESION-PROTEIN 1 (VAP-1) IN MEDIATING MONOCYTE MIGRATION ACROSS INFLAMED HEPATIC SINUSOIDAL ENDOTHELIUM

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H Zimmermann,* C J Weston, S M Curbishley, D H Adams. *NIHR Biomedical Research Unit and Centre for Liver Research, University of Birmingham, Birmingham, UK*

Introduction There is compelling evidence that accumulating monocyte-derived macrophages are pivotally involved in driving liver fibrogenesis. It remains unclear which molecules mediate transmigration of these cells across hepatic sinusoidal endothelial cells (HSEC). VAP-1 is an atypical adhesion molecule with enzymatic monoamine oxidase activity that is predominantly expressed in the liver microvasculature. It possesses key function in the recruitment of various lymphocyte subsets. The aim of this study was to decipher VAP-1 contribution to monocytic transendothelial transmigration.

Methods Primary human HSEC were isolated from explanted and grown to confluence in flow chambers. After activation with TNF-α/IFN-γ for 24h HSEC were treated with VAP-1 antibody and enzyme inhibitors. Monocytes were enriched from peripheral blood by using OptiPrep gradient. Monocyte subsets ($CD14^{++}CD16^{-}$, $CD14^{++}CD16^{+}$, $CD14^{+}CD16^{++}$) were isolated by FACS-sorting. Isolated monocytes were perfused over HSEC monolayers under constant flow simulating physiological shear stress (0.05 Pa). Adhesion and transmigration was studied using phase contrast microscopy. Transwell assays were used to study the phenotype of transmigrated monocytes by flowcytometry.

Results HSEC pretreatment with VAP-1 antibody (TK8-14) or enzyme inhibitor Semicarbizide equally reduced monocyte transmigration by ~50%. VCAM-1 blockade had a similar but redundant effect whereas CLEVER-1-antibody or LOX-inhibitor (β-APN) did not alter monocyte transmigration. VAP-1 antibody acted in a time-dependent manner with influence on monocyte adhesion only after short-term application (15 min). Inhibiting VAP-1 led to profound reduction of proinflammatory *nonclassical* $CD14^{+}CD16^{++}$ monocyte transmigration but also affected *classical* $CD14^{++}CD16^{-}$ whereas the *intermediate* $CD14^{++}CD16^{+}$ subtype was not affected. Under static conditions VAP-1 enzymatic or antibody inhibition was significantly blunted suggesting flow to be a mandatory prerequisite for the biological function of VAP-1 on monocytes. Increased expression of HLA-DR and the M2 macrophage marker CD206 on monocyte subsets after endothelial transmigration was not altered by VAP-1 inhibition.

Conclusion Endothelial VAP-1 differentially modulates monocyte recruitment under flow conditions in a time-dependent fashion and . favours transmigration of a proinflammatory monocyte subset. The critical role of VAP-1 enzyme function renders small molecules as a promising therapeutic approach in combating liver inflammation and subsequent fibrosis.

Competing interests None declared.

PMO-127 BIOLOGICAL EFFECTS OF ORAL NANOPOROUS CARBON IN BILE DUCT LIGATED RATS

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[1]J Macnaughtan,* [1]J Soeda, [1]A Mouralidarane, [2]S Sandeman, [2]C Howell, [3]O Kozynchenko, [3]S Tennison, [2]S Mikhalovsky, [1]N Davies, [1]J Oben, [1]R Mookerjee, [1]R Jalan. *[1]Hepatology, UCL, London, UK; [2]Centre for Biomedical and Health Science Research, University of Brighton, Brighton, UK; [3]Mast Carbon, Guildford, UK*

Introduction Gut-derived bacterial products and associated dysregulated inflammatory response play a central role in the pathogenesis of cirrhosis. Therapeutic options to target these factors are currently limited to long-term antibiotics. Nanoporous carbons are non-absorbable, synthetic materials which are safe with porosity manipulated for adsorption of middle and high molecular weight molecules and surface chemistry modified to alter adsorption capacity for biological molecules such as cytokines and endotoxin. We sought to determine their biological effects in bile-duct ligated rats as a model of cirrhosis.

Methods 131 male Sprague-Dawley rats underwent bile duct-ligation or sham biliary surgery. Animals were pair fed with or without oral carbon therapy 2 weeks from surgery until completion of the experiment at 4–5 weeks. Intra-peritoneal lipopolysaccharide (LPS) was administered to four subgroups 3.5 h prior to completion of the study. The following groups were studied: Sham (n=16), Sham + carbon (n=15), Sham + LPS (n=11), Sham+LPS+carbon (n=10), BDL (n=27), BDL + carbon (n=26), BDL+LPS (n=10), BDL+LPS +carbon (n=16). Portal haemodynamics were performed on 93 rats and Kupffer cell (KC) numbers and Reactive oxygen species (ROS) production assessed by flow cytometry in a sub-group of animals. Liver biochemistry and portal venous cytokines were performed.

Results A significant reduction in portal pressure was observed in BDL+LPS (mean 18.05±0.88 mm Hg untreated, 10.17±1.07 mm Hg with carbon, $p<0.05$) and BDL (mean 12.57±0.43 mm Hg untreated, 11.02±0.28 mm Hg with carbon, $p<0.05$) groups following carbon treatment. A significant reduction in ALT was observed in the carbon treated BDL+LPS ($p<0.05$) and BDL groups ($p<0.05$). Carbon treatment in BDL rats was associated with a significant reduction in LPS-induced ROS production. A trend towards reduction in portal venous IL-4 and IL-10 was observed in carbon-treated BDL rats. No significant difference in portal venous TNF-α was observed. Finally, a significant increase in body mass was observed in the BDL carbon-treated group ($p<0.05$).

Conclusion Oral nanoporous carbon therapy results in a significant reduction in portal pressure and liver biochemistry associated with a reduction in endotoxin-induced KC ROS production. We postulate therefore, that the effect of nanoporous carbon is possibly via a reduction in endotoxin induced KC stimulation.

Competing interests None declared.