subsequent tissue damage from release of proteolytic enzymes and reactive oxygen species, contributing to ongoing organ failure. Neutrophil function in ALF has not been previously interrogated.
Aim This ongoing longitudinal study aims to characterise neutrophil function in patients with ALF admitted to King's College Hospital.
Method Neutrophils were isolated from a cohort of age/sex matched patients with ALF (n=22), healthy volunteers (n=9) and septic controls (n=5). Serial samples were taken on admission and every 3–4 days following ITU admission until death/discharge. Phagocytosis was analysed by flow cytometry using FITC-labelled *E coli* and oxidative burst (OB) was determined by the percentage of CD16-Phycoerytherin labelled neutrophils producing reactive oxygen species at rest and after stimulation with opsonised *E coli*. Physiological variables, biochemistry, arterial ammonia and microbial culture results were collected prospectively.
Results Within the ALF cohort 14 patients fulfilled poor prognostic criteria, of whom 8 underwent successful liver transplantation (LT) and 6 died without LT, 8 survived with medical management. Aetiology of ALF was acetaminophen n=5; acute viral hepatitis n=4; seronegative liver failure n=10; drug/other n=3. APACHE II and SOFA scores on admission were higher in patients with ALF compared to septic controls 21 (17–25) vs 12 (9–15) (p=0.08) and 16 (15–17) vs 5 (1–6) (p=0.01) respectively. Impaired neutrophil phagocytosis ($p<0.01$) and increased spontaneous OB (p=0.05) was observed on admission in all patients with ALF compared to both control groups. Admission neutrophil phagocytic dysfunction was associated with higher CRP*, MELD*, INR* and SOFA scores* (*all $p<0.05$). Spontaneous OB deteriorates further on days 4–8 in poor prognostic groups compared to spontaneous survivors (p=0.038). This was accompanied by decline in OB in response to *E coli* which was also observed in septic controls (p=0.045). The defects in neutrophil function showed a trend towards improvement (phagocytosis, spontaneous and stimulated OB all p=ns) during the first 72 h following successful LT.
Conclusion In conclusion, in patients with ALF fulfilling "poor prognostic criteria" neutrophils demonstrate early impairment of phagocytosis, increased baseline OB but decreased OB in response to bacterial stimulation. These observed defects are likely to contribute to ongoing cellular/organ dysfunction and the increased susceptibility to nosocomial sepsis seen in ALF.

P53 THE CONTRASTING EFFECT OF OCTANOATE AND OLEATE ON PHOSPHATASE AND TENSIN HOMOLOGUE EXPRESSION IN IN VITRO MODEL OF STEATOSIS USING HEPG2/C3A CELLS

doi:10.1136/gut.2010.223362.79

K A Lockman, N Plevris, C Pemberton, P Cowan, P Lee, A Pryde, P C Hayes, C Filippi, J N Plevris. *Hepatology Department, University of Edinburgh, UK*

Introduction The tumour suppressor phosphatase and tensin homologue (PTEN) is mutated or deleted in several human cancers including hepatocellular carcinoma. PTEN-deficient mice demonstrated triglyceride accumulation, steatohepatitis, progressing to liver fibrosis and hepatocellular carcinoma. Similarly, reduced PTEN expression with free fatty acid (FFA) oleate has been shown to promote hepatic steatosis. In other cancer, mitochondrial respiration defect with enhanced glycolysis and NADH formation has been suggested to be a key event in PTEN downregulation.
Aim Our aims were to examine whether i) medium chain FFA octanoate altered PTEN expression ii) PTEN downregulation with FFA was associated with hepatic mitochondrial dysfunction.
Method Human hepatoblastoma cell line HepG2/C3A was pretreated for 3 days with oleate (0.25 mM) or octanoate (2 mM). PTEN expression was determined using quantitative real time PCR. Mitochondrial function was measured using BDTM oxygen biosensor in the presence of 2,4 dinitrophenol. Lactate and pyruvate concentrations were measured in the supernatant to determine glycolytic activity and NADH/NAD+ ratio. Intracellular lipid accumulation was confirmed with triglyceride concentrations. Experiments were done in triplicate to n=3. Results are expressed in mean±SEM. Differences between groups were analysed by one-way ANOVA.
Results We have previously demonstrated that oleate and octanoate pretreatment resulted in a similar intracellular triglyceride accumulation. In this study, we have found that despite similarities in triglyceride concentration, PTEN expression was lower in octanoate pretreated cells (octanoate 0.84±0.06, oleate 1.18±0.12, untreated 1.19±0.12 fold change from b-actin, p=0.04). However, octanoate pretreatment was not associated with impaired respiration (octanoate 0.24±0.01, oleate 0.20±0.02, untreated 0.28±0.01 AFU/gTP (gram of total protein)/min). Nevertheless, reduced PTEN expression with octanoate was associated with increased glycolysis (octanoate 315.2±42.91, oleate 100.9±14.09, untreated 145.3±8.83 μmol/gTP/hr, p=0.0001) with raised NADH/NAD ratio (octanoate 17.3±1.4, oleate 13.8±2.9 untreated 17.3±1.4; p=0.007).
Conclusion To our knowledge, the effect of octanoate on PTEN expression has not been previously shown. In contrast to the previous finding, our data demonstrate that octanoate, not oleate, downregulates PTEN expression. Differences in glycolysis hence redox potential may have influenced the disparity in PTEN expression between these FFA. Octanoate has recently been proposed to be beneficial in weight loss and diabetes. However, our findings suggest that it may not have a favourable effect on the progression of nonalcoholic fatty liver disease.

P54 VASCULAR ADHESION PROTEIN-1: A KEY PLAYER IN THE MODULATION OF HEPATIC FIBROSIS

doi:10.1136/gut.2010.223362.80

C Weston, E L Haughton, N M Westerlund, L C Claridge, J Pravin, D J Smith, D H Adams. *Centre for Liver Research, University of Birmingham, UK*

Introduction Vascular adhesion protein-1 (VAP-1) is a membrane-associated amine oxidase present on sinusoidal endothelium that supports lymphocyte recruitment to the liver. We have recently detected increased expression of VAP-1 in cirrhotic livers, and reported that a circulating soluble form is elevated in patients with fibrotic liver disease leading us to propose that VAP-1 plays a broader role in hepatic fibrosis.
Aim To investigate the role of VAP-1 in the initiation, progression and resolution of hepatic fibrosis.
Method Human: The distribution and expression of VAP-1 was investigated using multicolour confocal microscopy of normal and diseased human liver. Hepatic stellate cells (aHSC) and activated liver myofibroblasts (aLMF) were isolated from human liver tissue. Cell proliferation was studied using CyQuant. Apoptosis was investigated using a caspase-3 flow cytometry approach and cell detachment assay. Cell spreading was evaluated using xCELLigence impedence measurements. Modified Boyden chambers were used to assess cell migration. Mouse: Liver fibrosis was induced by CCl4 administration in wild-type C57BL/6 mice, wild-type mice dosed with anti-"VAP"-1 antibody and VAP-1 null mice. The degree of fibrosis and inflammatory infiltrate was assessed during active fibrosis and subsequent resolution using immunohistochemistry and qRT-PCR.
Results VAP-1 was present on sinusoids and vascular endothelium in normal human liver but was markedly increased in expanded septa in fibrotic disease where it co-localised with markers of HSC and

aLMF (CD90, -SMA) and extracellular matrix (ECM). Increased VAP-1 levels also correlated with the accumulation of advanced glycation end products in the scar, suggestive of a link between amine oxidase activity and modification of ECM. Cultured human aHSC and aLMF expressed VAP-1 mRNA and produced enzymatically active VAP-1 protein. Purified, soluble VAP-1 was a potent promigratory signal for lymphocytes and aHSC in vitro, possibly through amine oxidase activity. Soluble VAP-1 did not induce aHSC apoptosis or proliferation but was associated with an increase in cell spreading.

A role for VAP-1 in CCl4-induced liver fibrosis was confirmed in vivo. Both wild-type mice treated with a blocking anti-VAP-1 antibody and VAP-1null mice showed significantly reduced fibrosis after 8 weeks CCL4 compared with wild-type, and had accelerated resolution of fibrosis after cessation of CCl4. Wild-type mice receiving CCl4 also showed a significant increase in VAP-1 and elastin mRNA levels, mature macrophages, CD45-positive infiltrate and serum VAP-1 levels above that observed for VAP-1 null animals or those receiving antibody.

Conclusion These data suggest a multifunctional role for VAP-1 in liver disease in which VAP-1 not only supports lymphocyte and HSC recruitment but also modulates ECM remodelling and fibrogenesis.

P55 SYNGENIC BONE MARROW TRANSFER STIMULATES HEPATIC PROGENITOR CELL EXPANSION VIA TWEAK/FN14 SIGNALLING: IMPLICATIONS FOR HUMAN AUTOLOGOUS CELL THERAPY

doi:10.1136/gut.2010.223362.81

T Bird, B Knight, L Boulter, S Gordon-Keylock, D Wjotacha, J Thomas, O Sansom, J Iredale, S Forbes. *University of Edinburgh, UK*

Introduction Autologous bone marrow cell (BMC) therapy for liver disease has shown increased liver regeneration in animal studies and in phase 1 clinical studies. As yet no clear mechanism accounts for these observations. BMCs do not directly form into hepatocytes but can improve liver regeneration and function. In chronic liver disease hepatic progenitor cells (HPCs) are a potential source of parenchymal regeneration. Whilst the transplantation of human HPCs is not currently a practical therapeutic option, manipulating endogenous HPCs in vivo represents a potential approach. HPCs exist in a specialised niche of leukocytes and mesenchymal cells, which secrete cytokines that are capable of regulating HPC behaviour. We therefore hypothesised that infusion of BMC into the liver may induce HPC expansion via paracrine signalling.

Aim To investigate the effect of autologous BMC infusion upon regeneration by hepatic progenitor cells.

Method 107 syngenic BMCs were infused into healthy mice by tail vein, we examined intrahepatic donor cell engraftment, cytokine expression, liver function tests, and HPC activation. Cell tracking utilised either GFP+ or male cells delivered into female wild type mice. Whole liver and specific cell fractions were analysed by immunocytochemistry, in-situ hybridisation, FACS, and qRT-PCR.

Results Following BMC transfer, a progressive and sustained expansion of HPCs was observed (mean±SEM 41.9±2.1 cells per field vs PBS control 23.5±4.1 at 21 days post infusion, p=0.003) with associated increase in liver/body weight ratio (BMC 0.0513±0.001 vs PBS control 0.0470±0.001, p=0.022). BMCs engrafted the liver adjacent to HPCs for up to 3 weeks and were mostly F4/80+ macrophages (81%). Transfer of F4/80+ macrophages alone into healthy mice recapitulated the HPC expansion. Cytokine gene expression analysis revealed one soluble signal in particular, TWEAK, increased in the recipient's liver following donor BMC engraftment (5.20-fold induction vs control at Day 3). Extracted donor derived BMCs expressed TWEAK, as do macrophages. TWEAK is known to be a direct mitogen to HPCs via the Fn14 receptor. When Fn14-/- mice were used as recipients to wild type BMC infusions the expansion of HPCs was entirely lost (mean±SEM 27.2±1.4 vs negative PBS control 30.6±2.4, p=0.128 vs positive wild-type control 42.7±2.3, p=0.0002), demonstrating that the HPC expansion is dependent on TWEAK-Fn14 paracrine signalling between BMCs and HPCs.

Conclusion These data describe a hitherto unknown mechanism by which infused autologous macrophages signal in a paracrine manner to HPCs via TWEAK. These observations suggest potential for the development of novel therapies to promote human liver regeneration.

P56 FACTOR XA INHIBITION SUPPRESSES THIOACETAMIDE INDUCED LIVER FIBROSIS

doi:10.1136/gut.2010.223362.82

A Dhar, Q Anstee, F Sadiq, A Levene, R Goldin, M Thursz. *Department of Hepatology, Imperial College London, UK*

Introduction In addition to its role in activating fibrinogen, thrombin mediates cellular activation of macrophages, platelets and hepatic stellate cells via the protease-activated receptor, PAR-1. Thrombin antagonists demonstrate anti-fibrotic properties. Factor Xa (FXa), a protease which is activated earlier in the coagulation cascade, promotes connective tissue growth factor, and activates fibroblasts via PAR receptors. Direct FXa inhibition has recently been shown to significantly reduce lung fibrosis, a paradigm for hepatic fibrosis, in a bleomycin mouse model. Specific inhibition of FXa may offer additional efficacy as an anti-fibrotic in models of chronic liver injury.

Aim To evaluate the impact of FXa inhibition and thrombin antagonism on hepatic fibrosis using a thioacetamide (TAA) mouse model.

Method 45 C57BL/6J mice were administered TAA (300 mg/l) via drinking water for a period of 8 weeks to induce liver fibrosis. A subset of these animals were given Rivaroxaban, a direct FXa inhibitor (n=15), or Dagibatran, a direct thrombin antagonist (n=15). Both drugs were administered daily by oral gavage at doses to achieve prolongation of the prothrombin time. The remaining animals (n=15) received no anticoagulation, and acted as the control group. At 8 weeks livers were extracted and liver sections stained with picorsirus red and visually scored for fibrosis using an adapted Ishak Modified Histology Activity Index by a blinded histopathologist. Digital image analysis was performed to calculate the mean percentage area of fibrosis per section.

Results In control mice the mean fibrosis score was 4.08 and the mean percentage area of fibrosis was 3.76%. In comparison mice treated with FXa inhibition had a mean fibrosis score of 2.46 (p=0.008) and mean percentage area of fibrosis of 2.02% (p=0.012). In contrast mice treated with thrombin inhibition had a mean fibrosis score of 3.25 (p=0.68 vs controls), and mean percentage area of fibrosis of 3.70% (p=0.68 vs controls). Factor Xa inhibition was significantly more effective than thrombin in reducing percentage area of fibrosis (p=0.031).

Conclusion FXa inhibition significantly decreased the rate of hepatic fibrosis in a TAA model of liver fibrosis. It is likely that direct thrombin inhibition is less effective than FXa inhibition because thrombin inhibitors fail to block PAR-mediated stellate cell activation by FXa. FXa inhibition is a potential novel anti-fibrotic approach and warrants further investigation in human studies.