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

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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

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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