Results Flow-based adhesion assays using human HSEC demonstrated that B cells were captured from flow and adhered to human HSEC but they had limited motility in comparison to T cells. B cells also underwent transmigration and CLEVER-1 blockade led to a reduction of B cells undergoing transmigration. Blockade of CLEVER-1, VAP-1 and ICAM-1 in combination had a cumulative effect on transmigration, suggesting that all three receptors contributed to B cell transmigration.
Conclusion Up to now most interest has focused on the role of T cells but hepatic infiltrates contains B cells and B cells have been directly implicated in models of liver disease and as drivers of liver fibrosis. This work demonstrates that CLEVER-1 is an adhesion molecule within the hepatic sinusoids and contributes to B cell transmigration. CLEVER-1 is a potential target for modulating B cell recruitment to the human liver.

P103 METABOLIC PROFILING OF THE RAT LIVER AFTER CHRONIC INGESTION OF α-NAPHTHYLISOTHIOCYANATE USING IN VIVO AND EX VIVO MAGNETIC RESONANCE SPECTROSCOPY

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Introduction Hepatobiliary injury, associated with intrahepatic cholestasis and biliary hyperplasia, is a commonly encountered adverse effect in man in response to certain drugs and toxins. Some human diseases affecting the biliary tree can be modelled in rats by ingestion of the hepatobiliary toxin, α-naphthylisothiocyanate (ANIT). The ability to detect biliary hyperplasia and associated hepatobiliary injury non-invasively, by longitudinal liver specific assessment, would be of value in the development of novel therapies and aid towards the understanding of hepatic pathophysiological processes.
Aim To investigate the use of in vivo hepatic phosphorus-31 (^{31}P) magnetic resonance spectroscopy (MRS) to provide potential biomarkers for hepatobiliary injury linked to biliary hyperplasia in the ANIT-fed rat model and to investigate longitudinal changes according to dose over a 2-week time period.
Method All experiments were performed in compliance with the UK Animals (Scientific Procedures) Act 1986. Chronic hepatobiliary dysfunction was investigated in rats fed a diet supplemented with ANIT at three doses (ANIT_0.025%, ANIT 0.04% and ANIT_0.05%) for 2 weeks using in vivo hepatic ^{31}P MRS. In vivo ^{31}P MRS data collected at baseline and weeks 1 and 2 for each of the three ANIT groups were compared to results from corresponding pair-fed controls (six groups of n=8 per group). Serum was collected for clinical chemistry and tissue for both histology and ex vivo ^{1}H magic angle spinning (MAS) MRS after sacrifice at 2 weeks.
Results In vivo ^{31}P MRS showed phosphodiesters (PDE), relative to total phosphorus signal (tPh), were significantly increased ($p<0.05$) after 1 and 2 weeks in both ANIT 0.05% and ANIT 0.04% groups relative to controls, but an increase in phosphomonesters (PME)/tPh was observed in the ANIT 0.05% group only. Clinical chemistry findings confirmed chronic liver injury to some extent at all ANIT dosages. Histological findings included a dose related increase in both severity of biliary hyperplasia and focal hepatocellular necrosis with increasing doses of ANIT. Ex vivo ^{1}H MAS MRS findings supported the in vivo MRS findings in that the peak assigned to glycerophosphocholine and phosphocholine (GPC+PC) was relatively increased in the ANIT 0.05% and ANIT 0.04% groups ($p<0.05$) relative to the respective control groups.
Conclusion ANIT-induced moderate hepatobilliary dysfunction was associated with a dose dependent increase in phosphodiesters in vivo and choline-containing phosphodiesters and phosphomonoesters ex vivo. Such data suggest a role for magnetic resonance spectroscopy techniques as a non-invasive way of investigating hepatobilliary dysfunction.

P104 COAGULATION PROTEINS IN LIVER FIBROSIS: A ROLE FOR TISSUE FACTOR AND FIBRIN/FIBRINOGEN

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Introduction Recent evidence suggests a role for the coagulation cascade in promoting liver fibrosis, but with the exception of thrombin the expression and role of individual coagulation proteins in the pathogenesis of liver fibrosis is poorly understood. Furthering our understanding of the role of specific coagulation proteins is essential when considering viable targets for anti-fibrotic therapies.
Aim To quantify and qualify the expression of tissue factor (TF) and fibrin/fibrinogen in both murine liver fibrosis and human hepatitis C (HCV) related liver fibrosis.
Method C57BL/6J mice (n=7), aged 8 weeks old, were treated with carbon tetrachloride by intraperitoneal injection for a period of 4 weeks to induce liver fibrosis. Animals were then culled and livers extracted and fixed in formalin. Mice injected with normal saline acted as normal controls. For human tissue, archived liver biopsy specimens (n=11) performed for the clinical staging of chronic HCV infection were used. An indirect immunohistochemical detection technique was employed with digital image analysis to qualify and semi-quantify expression of TF and fibrin/fibrinogen in tissue sections.
Results In murine liver tissue, TF and fibrin/fibrinogen were expressed in hepatic sinusoids, peri-fibrotic areas and fibrotic septa. Digital image analysis demonstrated significant upregulation of TF ($p=0.002$) and fibrin/fibrinogen ($p=0.009$) in fibrotic vs normal control liver tissue. In HCV human liver tissue, TF and fibrin/fibrinogen were expressed in hepatic sinusoids and fibrotic areas. Digital image analysis demonstrated a significant correlation between TF expression and both fibrosis grade ($r=0.71$; $p=0.015$) and inflammatory score ($r=0.79$; $p=0.004$). Fibrin/fibrinogen expression was significantly correlated with inflammatory score ($r=0.82$; $p=0.007$), with a borderline correlation with grade of fibrosis ($r=0.66$; $p=0.056$). A significant correlation between TF and fibrin/fibrinogen expression was demonstrated ($r=0.82$; $p=0.024$).
Conclusion The hepatic expression of TF and fibrin/fibrinogen is upregulated with fibrosis and inflammation. These findings suggest that activation of the coagulation cascade occurs in and may contribute to the generation of hepatic fibrosis. The therapeutic potential of targeted inhibition of specific coagulation proteins need to be evaluated in fibrotic liver disease.

P105 BILIARY DRAINAGE IN A RAT: A LONG-TERM CONTINUOUS DRAINAGE MODEL RESULTING IN LIVER DAMAGE AND AN ALTERED FXR PATHWAY, LIPID AND CHOLESTEROL METABOLISM

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Introduction We hypothesised that the development of intestinal failure associated liver disease (IFALD) caused by an enterocutaneous fistula in which the enterohepatic circulation is interrupted without obstructive cholestasis leads to reduced farnosoid X receptor (FXR) signalling, ultimately leading to liver damage. Previous studies have primarily been performed in models of bile duct obstruction.

Aim Therefore we aimed to develop a biliary diversion model in the rat with long-term continuous biliary drainage and determined its effect on hepatic function and lipid metabolism.

Methods In rats (n=7–9), the bile duct was cannulated and externalised to accomplish biliary drainage. Sham controls underwent laparotomy without cannulation. Rats were sacrificed after 3 and 7 days, and liver samples were collected for histological assessment (necrosis, inflammation, bile duct proliferation). Sirius red staining and Oil Red O staining were used for analysis of fibrosis and steatosis, respectively. Quantitative PCR was performed for genes involved in the FXR pathway (FXR, SHP, BSEP, CYP7A1 and CYP8B1), lipid and cholesterol metabolism (FAS, SREBF, ACACA, PPARg, SCD1, LDLr and HMG-CoA reductase). AST, ALT, AP, GGT and bilirubin (total and direct) were assessed.

Results Continuous biliary diversion resulted in increased hepatic inflammation and necrosis at day 3 and day 7 ($p<0.05$) and was accompanied by bile duct proliferation (absent in controls). Fibrosis was only seen at day 7 ($p<0.05$). A trend towards decreased lipid droplets was apparent in the experimental group. Hepatic expression of FXR was markedly decreased in the experimental group at day 3 (1.11 ± 0.19; $p<0.05$) compared to sham (1.76 ± 0.18) and at day 7 (0.58 ± 0.16 vs 1 ± 0.09; $p<0.05$). As expected, SHP expression was downregulated at day 7 (0.29 ± 0.15; $p<0.05$) compared to sham (1.38 ± 0.53). CYP7A1 was upregulated in the drainage group at day 3 (1.59 ± 0.45 vs 0.32 ± 0.11; $p<0.05$) which suggests stimulation of bile acid biosynthesis. CYP8B1 also showed increased expression, although not significant. BSEP expression was decreased at day 3 (0.84 ± 0.09 vs 1.34 ± 0.24; $p<0.05$) and at day 7 (0.58 ± 0.16 vs 1 ± 0.09;$p<0.05$), suggesting impaired canulicular bile acid transport. Genes involved in the fatty acid metabolism were downregulated in biliary diverted rats. Fatty acid synthase (0.65 ± 0.24) and acetyl-CoA carboxlyase (0.82 ± 0.21) were decreased at day 7 ($p<0.05$). HMG-CoA reductase expression was upregulated at day 3 (0.69 ± 0.12;$p<0.05$) compared to sham (0.35 ± 0.06), suggesting stimulated cholesterol synthesis. AST, ALT, AP, GGT and bilirubin (total and direct) were significantly increased at day 7 ($p<0.05$).

Conclusion Biliary diversion in rats induced hepatic inflammation, necrosis, fibrosis and bile duct proliferation. Biliary diversion had evident hepatic effects with respect to FXR signalling and lipid metabolism. This model is suitable to assess long-term effects of continuous bile drainage to test therapeutic interventions aimed to reduce IFALD.