undergoing liver transplantation. Significant variations exist among liver transplant centres in the risk of post-transplant biliary disease that cannot be accounted for by differences in recipient, donor or graft characteristics.

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Clinical hepatology

OP16 EVIDENCE FOR COMPARTMENTALISED ENDOTOXAEMIA AND ITS EFFECT ON NEUTROPHIL FUNCTION IN THE PORTAL CIRCULATION IN CIRRHOSIS

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Introduction Bacterial translocation and endotoxaemia are important in the pathogenesis of neutrophil dysfunction in cirrhosis. At present, it is not clear whether systemic endotoxaemia occurs as a consequence of a defect at the gut barrier interface or diminished hepatic function and associated portosystemic shunting.
Aim The aims of this study were (1) to quantify the degree of portal endotoxaemia and the contribution of the liver in the regulation of systemic endotoxin (ET) levels, (2) to determine intestinal production of cytokines and adhesion molecules and (3) to determine whether the portal and hepatic milieu modulates neutrophil function.
Method 12 patients with cirrhosis (54±3.2 yr, Pugh 10.2±1.0, eight male, four female) were studied prior to and 1-h after TIPSS insertion. Blood was sampled from the artery, hepatic vein (HV), portal vein (PV) and its tributaries. PV blood was sampled before insertion of the TIPSS. Endotoxin (ET) (LAL assay), LBP, BPI, IL-6, IL-10, TNFR55, TNFR75, sICAM and sELAM (using ELISA) were measured. Neutrophil respiratory burst and phagocytosis was measured using FACS in neutrophils derived from the HV and PV, prior to and following cross-incubation with PV and HV plasma, respectively.
Results TIPSS insertion resulted in a significant increase in arterial ET levels from 0.08±0.02 to 0.19±0.02 EU/ml (p=0.0001). Mean ET levels in the PV and HV pre-TIPSS insertion were 0.22±0.02 and 0.04±0.02 EU/ml respectively. Transintestinal (TI) and transhepatic (TH) ET fractional extraction (FE) rates were 2.7±0.7 and −0.5±0.06, respectively. HV neutrophil resting burst was significantly increased from 52±5.3 to 78±4.5% after incubation with PV plasma (p<0.0001). Conversely PV neutrophil resting burst was significantly reduced from 85±3.5 to 60±5.3% (p<0.0001) after incubation with HV plasma. Positive intestinal FE rates were observed most markedly with BPI, IL-6 and IL-10. Neutrophil phagocytosis was reduced post-TIPSS from 66±7.5 to 42±6.5% (p=0.0004) and resting burst increased from 62±5.6 to 87±2.8% (p=0.0001).
Conclusion This study provides direct evidence for portal endotoxaemia in cirrhosis. The data suggest that the liver is responsible for compartmentalising ET and associated neutrophil dysfunction within the portal circulation. Intestinal production of IL-6 and IL-10 was also demonstrated. TIPSS insertion disturbs this compartmentalisation and exposes the systemic circulation to portal endotoxaemia. Strategies to diminish portal endotoxaemia or enhance hepatic ET clearance capacity are important therapeutic targets to minimise neutrophil dysfunction in cirrhosis.

OP17 CLINICAL EFFECT OF ALBUMIN DIALYSIS IN PATIENTS WITH INTRACTABLE PRURITUS CORRELATES CLOSELY WITH CHANGES IN AUTOTAXIN ACTIVITY BUT NOT BILE SALT LEVELS

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Introduction About 5% of patients with severe cholestatic liver disease have intractable pruritus. Albumin dialysis is an effective treatment for these patients but the mechanism through which it reduces the severity of pruritus is not clear. Autotaxin (ATX) is a 125 kD protein which cleaves a choline group of lysophosphatidylcholine (LPC) thereby forming the biological active lysophosphatidic acid (LPA). Increased serum ATX activity has been described in patients with cholestatic pruritus (*Gastroenterology*, 2010; in press).
Aim The aim of the study was to determine the effect of albumin dialysis using molecular adsorbents recirculating system (MARS) on autotaxin activity and bile salts and their relationship to the severity of pruritus.
Method 15 patients (11F/4M, PBC 10, PSC 2, other 3) with severe pruritus that was resistant to medical therapy were treated in 31 sessions (each consisting of 3, 8-h treatments; median follow-up of 12.8 months) with MARS. The intensity and severity of itch was quantified using an itch severity scale (ISS) and the visual analogue scale (VAS) pre and post treatment and then weekly for up to 12 weeks. ATX activity was measured in diluted serum samples and albumin dialysate before and after MARS treatments using a fluorescence assay. ATX protein levels were determined by Western blotting. Bile salts were measured by LCMS.
Results MARS treatment was associated with immediate, significant and complete response (R) in 11 patients, two patients had a partial response (PR) and two patients had no response (NR). Median ATX activities were not different between responders and non-responders (R=216.5; NR=306.6). A mean reduction of ATX activity of 27.6±4.13% was seen in R, 10.7±10.3% in PR and 1.5±1.48% in NR. This change in ATX activity was directly correlated with the reduction in ISS (r2=0.59; p<0.005) and VAS (r^2=0.47; p<0.02). The change in serum ATX activity correlated closely with the change in serum ATX protein level (r^2=0.5; p<0.01). Expectedly, no ATX activity was measureable in the albumin dialysate. ATX levels returned to pre-treatment values with relapse of itching which occurred in all responders between 6 weeks and 4 months. No significant changes in serum bile salts were observed.
Conclusion Our study suggests an important role for ATX activity in modulating the severity of pruritus in cholestatic patients providing novel insights into the pathogenesis of itch in cholestatic disease. The reduction is not due to removal of circulating ATX into the dialysate suggesting that MARS treatment either reduces the production of ATX or enhances its hepatic clearance. Alternatively, or in addition, either a substrate or co-factor of ATX is extracted by the MARS system.

OP18 ABNORMAL LIVER HISTOLOGY IN PATIENTS TAKING METHOTREXATE CORRELATES POORLY WITH DOSAGE OR DURATION OF THERAPY AND REFLECTS ESTABLISHED RISK FACTORS FOR STEATOHEPATITIS

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Introduction Chronic liver injury has been described in patients taking low dose methotrexate (MTX) for psoriasis or rheumatological

disorders. Liver biopsies are often requested on the basis of elevated serum levels of procollagen III peptide (P3NP) or cumulative dosage (CD). However, the Roenigk histological grading system of MTX-associated liver injury is barely distinguishable from the spectrum of non-alcoholic steatohepatitis (NASH), a disorder not controlled for in the original association studies. We hypothesised that MTX usage would correlate poorly with histological abnormalities and that these may instead reflect coincidental risk factors for NASH.

Aim To identify clinical and laboratory parameters predictive of abnormal liver histology in patients taking methotrexate.

Method 41 patients (60% male, mean age 56 years) receiving MTX were identified from a prospective liver biopsy database over a 6-year period in a single centre. Liver histology was reviewed by a single, blinded pathologist and independently scored according to both the Roenigk MTX injury score and the modified Kleiner/Angulo NASH grading systems. Clinical data were used to calculate BMI, Child-Pugh score, MELD, AST:ALT ratio, APRI score, NAFLD fibrosis index and FIB-4 score.

Results There was a high prevalence of obesity (median BMI 31) and 46% of livers had a fatty appearance on pre-biopsy ultrasonography. Elevated mean P3NP levels (mean 5.91 ug/l) were the commonest indication for biopsy, followed by high CD. The median weekly MTX dose was 15 mg with a mean cumulative dosage of 4200 mg (range 360–10300) over a median treatment duration of 60 months.

Macrovesicular steatosis was found in 90% of biopsies and 28% had evidence of steatohepatitis. However, mild fibrosis was present in only 28%, with moderate fibrosis in just 5% and no specimens demonstrated cirrhosis. Applying a CD cutoff of 4 g MTX did not influence the biopsy findings. Serum P3NP levels as well as the duration and total CD of MTX use all correlated poorly with the grade of liver injury. Non-invasive predictors of NAFLD fibrosis such as Angulo score, APRI index and FIB-4 were more accurate in predicting histology. Liver biopsy findings led to a change of MTX dosage in only 5% of cases. During a median follow-up period of 50 months (range 12–114), no patients developed overt chronic liver disease despite continued MTX use. Paired biopsies were available from eight additional patients and demonstrated no histological progression over a mean interval of 38 months (20–53).

Conclusion Obesity and other risk factors for NASH are highly prevalent in patients taking methotrexate for psoriasis. In our cohort, the liver histology correlated poorly with the duration and total dosage of methotrexate therapy and did not progress with further exposure. Elevated serum P3NP levels were an unreliable indicator of liver fibrosis. In contrast, using clinical parameters and non-invasive fibrosis scoring systems could significantly reduce unnecessary liver biopsies in these patients.

Basic science

OP19 DIFFERENTIAL EXPRESSION OF MICRORNAS DURING HEPATIC STELLATE CELL ACTIVATION AND THEIR ROLE IN THE REGULATION OF HEPATIC STELLATE CELL PROLIFERATION AND APOPTOSIS

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Introduction Activation and proliferation of myofibroblastic hepatic stellate cells (HSC) is a pivotal event in liver fibrogenesis. MicroRNAs (miRNAs) are implicated in the regulation of a large number of important cellular functions including cell proliferation, differentiation and apoptosis.

Aim To characterise changes in miRNA expression during HSC activation and to investigate the effect of silencing candidate miRNAs on HSC proliferation and apoptosis.

Method Expression of all known miRNAs was determined in quiescent (day 1) and culture-activated (day 10) rat HSC by microarray. Expression of selected, differentially regulated miRNAs was verified by real-time PCR at multiple time-points during culture-activation. Putative target genes of up- and down-regulated miRNA were organised into hierarchical categories based on their gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) classification. Activated HSC were transfected with chemically modified, single stranded nucleic acid miRNA inhibitors by electroporation. HSC proliferation and apoptosis were determined by [^{3}H]-thymidine incorporation and acridine orange staining, respectively.

Results A total of 21 and 17 miRNA were up- or down-regulated >1.5-fold during HSC culture-activation. The level of expression of eight selected miRNAs identified by microarray was confirmed by real-time PCR, with up to 170-fold change in expression observed between day 1 and day 10 (Abstract OP19 Table 1). Multiple GO terms and KEGG pathways were functionally enriched amongst the targets of up- and down-regulated miRNAs. Inhibition of mir-143 in activated myofibroblastic HSC inhibited proliferation by 33.5% (p=0.001) and increased serum-deprivation induced apoptosis by 68.3% (p=0.027).

Abstract OP19 Table 1 Results

Up-regulated miRNA	Fold increase	p Value
mir-125b	130	0.002
mir-199a	73	0.020
mir-145	40	0.041
mir-143	25	0.024
Down-regulated miRNA	Fold decrease	p value
mir-126a	170	0.030
mir-155	7.7	NS
mir-30a	2.9	NS
mir-26a	1.3	NS

Conclusion Activation of rat HSC was accompanied by marked up- and down-regulation of multiple miRNAs with potential to influence many cellular functions. Inhibition of mir-143 is pro-apoptotic and anti-proliferative in HSC, suggesting an important pro-fibrotic role for this miRNA in HSC and identifying a potential novel target for anti-fibrotic therapy in the liver.

OP20 HEPATOCYTES AND CHOLANGIOCYTES DO NOT HAVE SIGNIFICANT TELOMERE SHORTENING WITH INCREASING CHRONOLOGICAL AGE IN NORMAL LIVERS

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Introduction Telomeres, which cap and protect chromosomal DNA, shorten with each cell division reaching a critical point eventually when the cell is arrested in G1 phase and enters a state of cellular senescence. This process has been demonstrated in both normal ageing and chronic disease in diverse tissues. Murine studies demonstrated that telomere shortening within the liver predisposes to cirrhosis. Few studies have examined the effect of increasing age on telomere length within healthy human liver. Measuring telomere length in liver by Southern blotting assumes that results are representative for hepatocytes. However, liver comprises a diverse group of cells including hepatocytes, Kupffer cells, stellate cells, lymphocytes and cholangiocytes. A large volume four colour quantitative fluorescent in situ hybridisation (Q-FISH) technique was developed to measure telomere length within each cell type.