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.

Aim To determine the effect of increasing age upon telomere length in healthy liver within hepatocytes, Kupffer cells, stellate cells, CD4 or CD8 lymphocytes and cholangiocytes.
Method Q-FISH was performed on paraffin-embedded tissue from 73 "time zero" liver biopsies obtained at liver transplantation from liver donors selected carefully for the absence of reperfusion injury and to include a wide age range (5–79 years). Hepatocytes, Kupffer cells, hepatic stellate cells, CD4 or CD8 lymphocytes and cholangiocytes were identified with monoclonal antibodies to Hepar-1, CD68, SMA, CD4, CD8 and CK-19, respectively; nuclei were counterstained with DAPI and telomeres identified with a specific Cy5-labelled PNA probe. Image acquisition and data analysis were performed with Olympus ScanR microscope and software. Mean telomere fluorescent intensity was measured and analysed using linear regression on GraphPad PRISM 5.0.
Results The Q-FISH methodology enabled large volume analysis of telomeres in various cell lineages within paraffin-embedded liver sections. A mean 40 000 hepatocytes, 30 000 Kupffer cells, 15 000 cholangiocytes and 4000 hepatic stellate cells, intrahepatic CD4 and CD8 lymphocytes were analysed per liver. Hepatic stellate cells had the longest telomeres at all ages whilst the others cell types had similar telomere lengths. Telomeres within Kupffer cells and stellate cells in healthy liver shortened, as might be expected, with increasing age ($p=0.024$ and $p<0.0001$, respectively). In contrast, telomeres in hepatocytes, cholangiocytes and lymphocytes in healthy livers did not shorten with increasing age.
Conclusion Cells within the healthy liver do not age equally. The disparity between age-related telomere shortening in Kupffer cells and stellate cells and maintained telomere length with age in cholangiocytes, hepatocytes and intrahepatic lymphocytes was unexpected but may reflect low turn over in hepatocytes and cholangiocytes. The absence of an effect of age on these cell lineages suggests that the potential for regeneration is maintained with age and has important implications for the choice of control tissues in studies of hepatic ageing.

OP21 THE ROLE OF TOLL LIKE RECEPTOR-4 IN THE PATHOGENESIS OF HEPATORENAL SYNDROME IN A BILE DUCT LIGATED MODEL OF CIRRHOSIS IN THE RAT

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Introduction Hepatorenal syndrome (HRS) is a frequent presenting complication in patients with cirrhosis and usually occurs with a superimposed infection/inflammation. It is associated with high morbidity and mortality. The pathogenesis of the HRS is not delineated. Toll like receptors (TLR's) play an important role in the recognition of molecules derived from microbes, which upon stimulation leads to activation of NFkB and pro inflammatory cascade. We hypothesised that cirrhosis associated endotoxaemia primes the kidney resulting in upregulation of TLR4 making the kidney susceptible to further endotoxaemia.
Aim The aims of this study were to determine whether (1) cirrhosis is associated with an upregulation of TLR4, NFkB and proinflammatory cytokines in the kidney and (2) whether selective decontamination would result in a reduction in TLR4 expression, attenuate NFkB and cytokines and make the kidneys less susceptible to further endotoxemic insult.
Method Six groups of Sprague–Dawley rats were studied; n=6 in each group (Sham operated, Sham-operated+LPS; BDL (4 weeks), BDL+LPS (1 mg/kg); BDL+Norfloxacin and BDL+LPS+Norfloxacin. The Norfloxacin groups were pretreated with Norfloxacin, 20 mg/kg administered orally daily for 10 days. The animals were studied 3 h after administration of placebo or LPS. Blood was for collected for biochemistry and cytokines. Kidney was collected for protein expression of TLR4 and NFkBp65 on Western blot and immunohistochemistry.
Results TLR4 and NFkBp65 protein expression was significantly upregulated in BDL rat kidney compared to sham ($p=0.03$ and 0.02), respectively, this increased further on LPS administration ($p=0.02$ and $p=0.01$). TLR4 was mainly localised in the proximal tubule and its brush border with ongoing apoptosis as evident by the presence of caspase-3 positivity on immunohistochemistry. Selective decontamination with Norfloxacin attenuated the inflammatory response by reducing the protein expression of TLR4 and NFkBp65 in BDL vs BDL group administered with LPS ($p=0.02$ and $p=0.01$). It also led to an improvement in the creatinine in BDL and BDL+LPS group administered Norfloxacin ($p=0.02$ and $p=0.03$), respectively. Norfloxacin ameliorated the kidney cytokine surge in BDL and BDL +LPS group (TNF $p=0.09$ and $p=0.04$, respectively).
Conclusion Our data provide strong evidence indicating an important pathogenic role of TLR4 in mediating susceptibility to the development of HRS in an experimental model of cirrhosis following inflammation/infection. Selective gut decontamination attenuates this pathological inflammatory process and prevents renal failure induced by LPS. TLR4 antagonist may be a therapeutic target for HRS.

OP22 A SIGNIFICANT REDUCTION IN "PRO-INFLAMMATORY" CD14LO/CD16HI MONOCYTES IN PARACETAMOL-INDUCED ACUTE LIVER FAILURE: ASSOCIATION WITH OUTCOME AND CCR2 EXPRESSION

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Introduction Acute liver failure (ALF) shares many clinical and immunological features with severe sepsis. In severe sepsis the relative proportion of "pro-inflammatory" CD14lo/CD16hi monocytes (MØ) is reduced, possibly due to recruitment into inflamed tissue. No data exist on monocyte subsets in paracetamol-induced ALF (PALF). Animal models of ALF suggest the importance of CC chemokine receptor (CCR) 2 mediated MØ recruitment and in human PALF high circulating levels and hepatic expression of CC chemokine ligand 2 have been observed.
Aim Investigate the relative composition of "pro-inflammatory" CD14hi/CD16lo, "intermediate" CD14hi/CD16hi and "classical" CD14lo/CD16hi MØ subsets and the association of CCR2 expression, in patients with PALF.
Method Blood was taken from five healthy controls and 20 patients with PALF within 24–48 hours of admission. Monoclonal antibodies against CD14, CD16 and CCR2 were used to determine MØ subsets and CCR expression. Results are expressed as the percentage proportion of total MØ and mean fluorescence intensity of CCR2 expression.
Results Compared to healthy controls, a lower proportion of CD14lo/CD16hi MØ was observed in PALF (2% vs 10%, $p=0.01$), CD14hi/CD16lo MØ tended to be more highly represented (95% vs 82%, $p<0.08$) but there were no differences in the proportion of CD14hi/CD16hi MØ (3% vs 8%). In PALF, CCR2 expression was up-regulated on CD14hi/CD16lo and CD14hi/CD16hi MØ subsets compared to controls (CD16lo: 811 vs 454; CD16hi: 3376 vs 855, $p<0.05$) but remained low at control levels on CD14lo/CD16hi M&Oslash.