P31 PATTERN AND SIGNIFICANCE OF BIOCHEMICAL ABNORMALITIES IN REFERRALS TO A NAFLD CLINIC

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Introduction Non-alcoholic fatty liver disease (NAFLD) is the commonest cause of referral to liver clinics, and accounts for around 11% of new patients. Despite this little is known of the typical pattern of liver biochemical abnormalities in this group. Many algorithms primarily use transaminases as their primary biochemical analyte. The aim of this project was to clarify common patterns and to determine their clinical significance.
Method This was a single-centre, retrospective database study of all patients referred to a specialist NAFLD clinic between May 2006 and May 2009. Patients with any co-existing cause of liver disease, a history of excess alcohol or HOMA less than three were excluded from subsequent data analysis. For the 143 patients who remained a database search for contemporaneous blood results and clinical history was performed.
Results Mean age at the time of referral was 55.8 years (SD 15.9). M/F=1:1.04. 54% were diabetic; 62% had hyperlipidaemia and 58% had a history of hypertension. 23 (16%) were deemed to be cirrhotic in the initial series of investigations. In this cohort elevated GGT was the commonest biochemical abnormality, present in 76.2% of referrals. GGT was elevated above 100 U/L in 38.5% of patients. In contrast, elevated ALT (>50U/L) was present in 58.0% of referrals; and was above 100U/L in 10.5%. Mixed ALT and GGT abnormalities were the commonest pattern of LFT abnormalities, present in 46.9%. A cholestatic pattern (here defined as an abnormal GGT but normal ALT) was present in 30.1% of referrals; elevated transaminases as an isolated abnormality were present in only 11.9% of referrals. On univariate analysis the only factors associated with elevated GGT was female gender (p=0.009) and the presence of cirrhosis (p=0.003). GGT >100 was associated with cirrhosis and increasing age on univariate analysis, however on multivariate analysis only the presence of cirrhosis remained statistically significant (p=0.005). Elevated ALT had no such association with cirrhosis and was negatively associated with the presence of diabetes and increasing age.
Conclusion To date emphasis has been placed on serum transaminases in screening for NAFLD. However, in our cohort an elevated GGT was the commonest biochemical abnormality and, unlike transaminases, this was associated with the presence of cirrhosis. Biochemical profiles that omit GGT will be inadequate in screening for NAFLD.

P32 GUT STERILISATION WITH NORFLOXACIN MODULATES CEREBRAL INFLAMMATION IN CIRRHOSIS AND PREVENTS DETERIORATION IN BRAIN OEDEMA AND DELAYS COMA IN CIRRHOTIC RATS

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Introduction In a bile duct ligated (BDL) rodent model of cirrhosis, superimposed infection/inflammation leads to multiorgan failure including cerebral inflammation, worsening oedema and coma. The mechanism of why cirrhotic animals are predisposed to the effect of LPS is not clear. Toll like receptors, the ubiquitous receptors for bacteria trigger an inflammatory cascade through NFkB. We hypothesised that endotoxemia in cirrhosis primes the brain leading to an activation of TLR4 pathway which can be modulated by selective gut decontamination.
Aim The aims of the study were to determine whether (1) cirrhosis is associated with altered expression of TLR4 (endotoxin receptor), NFkB and cytokines in the brain and whether (2) selective decontamination of gut with norfloxacin would attenuate TLR4 expression and therefore protect against the development of accelerated brain oedema following LPS administration.
Method Six groups of Sprague–Dawley rats were studied (n=6 each). Animals underwent sham operation or bile-duct ligation (BDL) and were studied 4 weeks later. The animals were studied at coma stages after administration of LPS (1 mg/Kg) or vehicle. Study groups: Sham operated, Sham-operated+LPS; BDL (4 weeks), BDL +LPS; BDL+norfloxacin and BDL+LPS (norfloxacin 20 mg/kg daily for 10 days; PO). Ammonia (measured spectrophotometrically), brain cytokines (ELISA) were measured. Protein expression of Brain TLR4 and NFkBp65 was assessed with Western blot and the frontal cortex for brain water (dry weight method).
Results TLR4 and NFkBp65 protein expression was significantly higher in BDL rats (p=0.04 and p=0.03), this increased further on administration of LPS (p=0.02 and p=0.01) respectively. Norfloxacin reduced the expression of TLR4 and NFkBp65 to sham levels (p=0.03, p=0.02) respectively. Selective decontamination of gut with norfloxacin led to a reduction in ammonia (p=0.04, p=0.01), brain water (p=0.04, p=0.04) and brain TNF (p=0.02, p=0.02) in BDL and BDL rats treated with LPS respectively. Pre-treatment of the BDL animals with norfloxacin prior to administration of LPS significantly delayed occurrence of coma and improved survival (p<0.002).
Conclusion Our data provide strong evidence indicating an important pathogenic role of TLR4 in mediating susceptibility of cirrhotics to worsening coma following an infection/inflammation. Selective gut decontamination with norfloxacin attenuates the TLR4 expression thereby modulating the inflammatory milieu in the brain, delays coma and improves survival in cirrhotic animals administered LPS. Selective inhibition of TLR4 and/or selective gut decontamination may be therapeutic targets in hepatic encephalopathy.

Basic science

P33 TREATMENT WITH TOLL-LIKE RECEPTOR 4 ANTAGONIST RESTORES THE INFLAMMATORY CELLULAR IMMUNE DYSFUNCTION IN ACETAMINOPHEN INDUCED ACUTE LIVER FAILURE MICE

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Introduction Acute liver failure (ALF) is a rare, catastrophic syndrome commonly caused by acetaminophen (APAP) that results in the death of about 40% patients. In addition to liver dysfunction, its outcome is critically dependent upon the severity of inflammation. Paradoxically, infection complicates the outcome of patients with ALF. Toll-like receptor 4 (TLR4) plays an important role in the innate immune response recognising and mediating pro-inflammatory cytokine release.
Aim The aims of this study were to characterise the cellular immune response in APAP induced ALF in mice and to determine whether treatment with a specific TLR4 antagonist (STM28) restores cellular immune dysfunction.
Method CD1 (n=18) mice were studied (Sham, n=6; APAP (500 mg/kg IP), n=6; and APAP (500 mg/kg IP) plus STM-28, 20 ug prior to and after 6 h, n=6). The mice were sacrificed after 8 h. Peripheral blood was stained with fluorochrome-labelled antibodies specific for peripheral blood myeloid cells, dendritic cells (plasmacytoid and myeloid, expression of CD86 in myeloid); monocytes (resident and inflammatory); total granulocytes and neutrophils; CD4/CD8 T-cells and the expression of CD25 in CD4T cell populations; B cells

and NK cells. Flow cytometric analysis was performed on a FACS Canto II. Biochemistry was measured spectrophotometrically.
Results Compared with sham animals, ALF was associated with widespread and profound abnormalities in cellular immune responses which were significantly improved in the group treated with TLR4 antagonist after APAP administration; significant improvement was observed in (STM28 vs APAP): total myeloid cells (39% vs 57%, p=0.0002), neutrophils (21% vs 37%, p=0.005), total granulocytes (28% vs 48%, p=0.0016), monocytes (6% vs 11%, p=0.0009) and resident monocytes, which are able to differentiate into macrophage (7% vs 15%, p=0.01). No significant differences were observed in subtypes of myeloid and plasmocytoid dendritic cells (54% vs 56% and 15% vs 10%, respectively), T-lymphocytes (16% vs 14%), B cells (7% vs 6%) and NK cells (1% vs 1%). These decrease in cellular inflammatory response was associated with a significant reduction in markers of liver injury (ALT: p<0.001; Ammonia: p<0.01).
Conclusion The results of this study suggest profound cellular immune dysfunction in APAP induced ALF mice which have a predominant pro-inflammatory phenotype. This dysfunction can be significantly improved by treatment with a TLR4 antagonist. This restoration of immune dysfunction is associated with significantly less liver injury indicating that TLR4 antagonism may have important therapeutic potential in APAP induced ALF.

P34 THE MECHANISM BEHIND SYNERGISTIC ACTION OF L-ORNITHINE AND PHENYLACETATE TO REDUCE AMMONIA IN BILE-DUCT LIGATION RATS

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Aim This study was designed to test the hypothesis that OP has additional actions on the key ammonia regulating enzymes glutamine synthetase (GS) and glutaminase (GA), which results in the observed ammonia lowering effect of OP in cirrhotic rats.
Method 11.53 g: 4 sham operated, and 11 BDL. 5 BDL's received OP (5 days, IP 0.6 g/kg), 5 BDL's received ornithine (5 days, IP 0.6 g/kg), 5 BDL's received phenylacetate (5 days, IP 0.6 g/kg) and six received saline (IP). We measured plasma levels for: ammonia and standard biochemical markers. Expressions of GS, GA and ornithine amino transferase (OAT) were determined by Western blot (expressed as a % of sham values) and activity by end-point methods in liver, kidney, gut, muscle and lung.
Results Plasma ammonia was decreased in BDL-OP rats vs BDL-saline (58.97±6.02 vs 106.2±20.56 μmol/l). BDL-OP rats showed increased GS expression in liver (66% BDL-OP vs 55% BDL-saline; p<0.01) and showed further increased levels in the muscle (153% BDL-OP vs 142% BDL-saline). OP prevents the BDL related increases in glutaminase expression (124% vs 163%; p<0.05) and activity (0.45±0.16 mIU/mg protein BDL-OP vs 1.14±0.046 mIU/mg protein BDL-saline; p<0.01) in gut. We demonstrated that this prevention is due to effect of ornithine in glutaminase activity (0.46±0.17 mIU/mg protein BDL-O vs BDL-saline; p<0.05) and not to phenylacetate. OP treatment increased OAT expression in muscle (142 %BDL-OPvs.114% BDL-saline; p<0.01) and lung (103%BDL-OP vs 127%BDL-saline; p<0.01).
Conclusion OP treatment in BDL rats increased the conversion of glutamate to glutamine by stimulation of OAT and GS in the muscle and also resulted in normalisation of glutaminase expression and activity in the gut, indicating that OP effectively restricts the production of in vivo ammonia in a cirrhotic model explaining the lack of stoichiometry between ammonia reduction and excretion of phenylacetylglutamine. In summary, the mechanism by which OP reduces ammonia in cirrhosis is by increasing glutamine synthesis (action of "O") and its excretion as phenylacetylglutamine (action of "P") and concomitantly normalising gut glutaminase activity (action of "O"), demonstrating synergistic effect of "O" and "P".

P35 TREATMENT WITH AN ALPHA 2A ADRENORECEPTOR ANTAGONIST MODULATES HEPATIC INFLAMMATION, MARKEDLY REDUCES PORTAL PRESSURE, AND IMPROVES ARTERIAL PRESSURE AND HEPATIC BLOOD FLOW IN CIRRHOTIC RATS

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Introduction Inflammation plays a pivotal role in modulating the severity of intrahepatic resistance in cirrhosis. Our studies have shown a close relationship between the activation of the sympathetic nervous system, inflammatory response and severity of portal hypertension. Stimulation of alpha 2a adrenergic (ADRA2a) receptors results in inflammation and vasodilation in resistance vasculature, and its antagonism has shown benefit in models of sepsis.
Aim The aim of the study was to test the hypothesis that treating bile duct ligated rats (BDL) with an ADRA2a antagonist reduces hepatic inflammation and improves the haemodynamic abnormalities associated with cirrhosis.
Method Male Sprague-Dawley rats (N=46) were studied 4-weeks after BDL surgery (N=29) or sham operation (N=17) and randomised to two doses of placebo or ADRA2a antagonist (BRL 44408, Sigma, UK, 10mg/kg s.c 24 hours prior to study). Portal vein and hepatic arterial blood flow, mean arterial (MAP) and portal pressure were measured directly. Plasma biochemistry was measured by colorimetry. ADRA2a and NFkB protein expression were determined by western blotting and immunohistochemistry (ADRA2a).
Results BDL rats had significantly increased hepatic protein expression of ADRA2a compared with sham operated rats and this was mostly shown to be located on hepatocytes by immunohistochemistry. Following treatment with ADRA2a antagonist there was a significant increase in the MAP (p<0.05) and a significant reduction in portal pressure as compared to the placebo treated group (11.4±3.4 vs. 18.0±3.7 mmHg, p<0.001). The hepatic arterial blood flow was markedly increased in the treated group without significant change in the portal venous blood flow resulting in a significant reduction in intrahepatic resistance post treatment (1.1±0.2 vs. 0.5±0.1 mmHg/ml/min, p<0.05). Biochemical analysis showed a significant reduction in plasma lactate (p<0.05), AST (p<0.05) and a trend towards reduction in creatinine in treated animals. Hepatic phosphorylated NFκB expression was increased in BDL animals and this reduced significantly with ADRA2a antagonist treatment (p<0.05).
Conclusion The results of this study show for the first time that modulating ADRA2a-mediated sympathetic tone and hepatic inflammation with an ADRA2a antagonist significantly improves systemic haemodynamics and reduces portal pressure, whilst also increasing hepatic blood flow. Our data provide the rationale for evaluating an ADRA2a antagonist in the treatment of portal hypertension.

P36 BLOCK OF INTERFERON γ AND CO-CULTURE WITH SMDCS ENHANCE ANTIGEN-SPECIFIC T-REG SUPPRESSION ABILITY IN AUTOIMMUNE HEPATITIS TYPE 2

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Introduction CD4posCD25high regulatory T-cells (T-regs), central to immune homeostasis, are impaired in autoimmune hepatitis type 2