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

doi:10.1136/gut.2010.223362.60

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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

doi:10.1136/gut.2010.223362.61

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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

doi:10.1136/gut.2010.223362.62

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