Results 5αR1 expression in human livers was parenchymal and greater in non-alcoholic steatohepatitis (NASH) livers compared to normal liver and was associated with greater lobular inflammation. 5αR1 gene expression was not affected by NASH severity. ALIOS induced steatohepatitis (6 m) and significant fibrosis (F3 at 12 m) in WT and 5αR1 KO mice. Compared with WT mice, 5αR1 KO mice fed the ALIOS diet demonstrated significantly greater liver weight, steatosis score (median 3 vs 1), and hepatic triglyceride accumulation by 6 months (93.1 vs 62.4 nmol/mg p=0.002). mRNA expression of CPT1α, a key enzyme in hepatic fatty acid β oxidation, was reduced in $5\alpha R1^{-/-}$ mice. There was a trend (p=0.1) towards worse inflammation (Kleiner lobular inflammation/qPCR for hepatic TNFa) at 6 months in 5αR1 KO mice, but no difference in inflammation or fibrosis at 12 months despite the presence of greater hepatic steatosis. The number of panCK-positive cells observed in WT mice fed ALIOS diet increased significantly with longer duration (0 m 0.15%, 6 m 0.44%, 12 m 0.94%, p=0.028) and at 12 months was significantly greater than mice fed normal chow (0.94% vs 0.18% p=0.03). Despite the absence of cirrhosis 5/10 WT mice developed dysplasia/hepatocellular carcinoma after ALIOS for 12 months vs 0/5 5αR1 KO mice. 5aR1 KO mice had lower numbers of panCK-positive cells (0.62% vs 0.94%) compared to its WT.
Conclusion ALIOS diet induces the entire spectrum of NAFLD from simple steatosis to advanced NASH with fibrosis and HCC, with a mounting oval cell response to increasing duration of diet. 5αR1 KO promotes hepatic steatosis in the absence of worsening fibrosis, attenuates the oval cell response and exerts a protective effect on hepatocarcinogenesis thereby demonstrating the role of 5αR in NAFLD pathogenesis.

P87 VASCULAR ADHESION PROTEIN-1 PROMOTES INCREASES IN LIVER INFILTRATING CD4+ T CELLS AND NKT CELLS AND INDUCTION OF FIBROGENESIS IN STEATOHEPATITIS

doi:10.1136/gutjnl-2011-300857a.87

[1]L C Claridge, [1]C J Weston, [1]E L Haughton, [2]N Westerlund, [1]G M Reynolds, [1]P F Lalor, [3]J W Tomlinson, [2]D J Smith, [4]C P Day, [1]D H Adams. *[1]NIHR Biomedical Research Unit and Centre for Liver Research, University of Birmingham, UK; [2]Biotie Therapies Corp., Turku, Finland; [3]Centre for Obesity Research, University of Birmingham, UK; [4]Institute of Cellular Medicine, Newcastle University, Newcastle, UK*

Introduction Vascular Adhesion Protein-1 (VAP-1) is an adhesion molecule and membrane-bound amine oxidase. Our group has previously demonstrated that VAP-1 is able to support lymphocyte recruitment across human hepatic sinusoidal endothelium in vitro. Soluble VAP-1 (sVAP-1) is released into the circulation from adipose tissue and the hepatic vascular bed, has insulin like effects and is capable of inducing and propagating oxidative stress.
Aim To investigate the role of VAP-1 in the progression of steatosis to steatohepatitis and cirrhosis.
Method Human hepatic VAP-1 expression was determined using immunofluorescent labelling and multicolour confocal microscopy. Serum sVAP-1 levels were measured by ELISA in 144 patients with histologically graded NAFLD and 74 controls matched for age and metabolic phenotype. Two murine models of steatohepatitis were studied; (1) a methionine choline deficient diet was administered for 6 weeks in wild-type (WT) mice, WT mice treated with anti-VAP-1 antibody, and VAP-1 null mice (n=6 per group), 2) A western lifestyle model incorporating high trans-fat diet and fructose supplemented drinking water was administered to WT and VAP-1 null mice for 6, 9 and 12 months (n=10 per group). Control animals remained on normal chow. Liver infiltrating lymphocytes were identified and quantified using flow cytometry. Fibrosis was assessed by immunohistochemistry and qRT-PCR for aSMA and collagen I.
Results (1) Human studies: We report increased hepatic expression of VAP-1 in NAFLD associated with elevated serum levels of sVAP-1 compared with controls (946±468 ng/ml vs 265±78 ng/ml, p<0.0001). Multiple regression modelling reveals sVAP-1 to be the best predictor of histological fibrosis in our cohort. We detected strong VAP-1 expression in fibrotic septa co-localised with activated liver myofibroblasts (aLMF). In vitro human aLMF expressed and released sVAP-1 which promoted lymphocyte migration through a novel H_2O_2-mediated enzyme-dependent mechanism. (2) Experimental steatohepatitis: VAP-1 null and/or antibody treated mice developed fewer inflammatory foci and delayed onset of fibrosis in two murine models of NASH. In both models there was a specific failure to expand the intrahepatic CD4+ and NKT cell populations in VAP-1 null mice compared with 2 to 3-fold increases in WT mice. Three WT animals developed hepatocellular carcinoma by 12 months in the western lifestyle model but no tumours were found in VAP-1 null mice.
Conclusion Our results implicate an important role for VAP-1 in steatohepatitis, in both humans and mice. The ability to target VAP-1 with antibodies or small molecule inhibitors makes it an attractive therapeutic target.

P88 VASCULAR ADHESION PROTEIN-1 (VAP-1) MODULATES GLUCOSE AND LIPID UPTAKE IN NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

doi:10.1136/gutjnl-2011-300857a.88

[1]S Karim, E Liaskou, [1]J Youster, [2]Fei-L Lim, [2]K MacAulay, [3]S Jalkanen, [1]D H Adams, [1]P F Lalor. *[1]University of Birmingham; [2]Unilever Discover; [3]University of Turku*

Introduction NAFLD is characterised by steatosis, chronic inflammation and fibrosis. The underlying mechanisms include insulin resistance, increased free fatty acid flux from de-novo lipogenesis and decreased lipid oxidation. Vascular Adhesion Protein—1 (VAP -1), is an adhesion molecule with semicarbazide- sensitive amine oxidase activity (SSAO), which is also expressed as a soluble protein in serum (sVAP-1) and elevated in inflammatory liver diseases such as NAFLD. VAP-1 has been shown to modulate glucose and lipid uptake in muscle and adipose tissue and thus we investigated whether it may contribute to glucose and lipid homeostasis in human liver tissue.
Method We have used precision cut liver slices (PCLS) from normal and diseased human liver specimens and cultured human sinusoidal endothelium (HSEC) and hepatocyte cells in combination with VAP-1 substrates (200 μM methylamine or benzylamine) and inhibitors (400 μM bromoethylamine) to perform standard ex vivo radiolabelled glucose uptake and fatty acid uptake assays using oil red O quantification following exposure of cells to Oleic and Palmitic acid (PA). Immunohistochemical staining and qPCR were performed using standard techniques and for confirmatory experiments HSEC were transfected with enzymatically active/inactive VAP1.
Results QPCR confirmed upregulation of VAP-1 mRNA (ΔΔCT =1.144 p=0.03) in NASH vs normal liver and also changes in FABP1, -4, -5, FATP3, -4 (p≤0.05 for all) and GLUT-1, 2, 3, 5, 8, 9 and 12 in NAFLD compared to normal individuals. Results were confirmed using immunohistochemical staining. Exposure of human PCLS to sVAP-1 and methylamine typically resulted in a 38—54% increase in PA uptake (p≤0.01 for all) and a 20% increase in hepatocyte glucose uptake in vitro which could be inhibited using bromoethylamine. Transduction of HSEC with enzymatically active VAP-1 also increased glucose uptake which was prevented in the absence of enzyme activity. Interestingly methylamine treatment of human liver resulted in decreased expression of mRNA for glucose transporters and an increase in some lipid transporters including FABP6, FATP and LRP8, and H_2O_2 produced by SSAO activity increase lipid uptake by hepatic cells.