Conclusion In conclusion, we demonstrate for the first time global alterations in cellular expression of glucose and lipid transporter proteins in human NAFLD. We confirm that VAP-1 is elevated in disease and that SSAO activity of VAP-1 results in enhanced hepatic lipid and glucose uptake and changes in transporter expression. Thus we propose that bioactive metabolites of SSAO activity contribute to the metabolic derangement evident in fatty liver disease.

P89 OSTEOPONTIN PROMOTES LYMPHOCYTE RECRUITMENT IN STEATOHEPATITIS

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Introduction Steatohepatitis is the critical step in the progression to fibrosis, and is characterised by increased inflammatory cell recruitment from the circulation. The cytokine Osteopontin (OPN) is intricately involved in cell-recruitment and tissue-repair, and we reported that OPN is significantly upregulated during non-alcoholic steatohepatitis (NASH).
Aim Thus, we hypothesised that OPN promotes steatohepatitis by supporting leucocyte migration across hepatic sinusoidal endothelium.
Method Wild-type mice were fed chow or methionine-choline deficient (MCD) diet to induce NASH. After 4 weeks, mice were sacrificed; severity of disease assessed by serum aminotransferase (AST), hepatic OPN quantified by QRTPCR and immunohistochemistry, serum OPN measured by ELISA. In separate experiments, MCD-fed mice were treated with anti-OPN or IgG, and flow cytometry used to quantify numbers of liver infiltrating lymphocytes (LIL). Primary human hepatic sinusoidal endothelial cells (HSEC) were stimulated with recombinant (r) OPN (0–1000 ng/ml), and expression of adhesion molecules (ICAM-1, VCAM-1, CD31) quantified by western blot. To assess lymphocyte transendothelial migration, lymphocytes were perfused over rOPN- or vehicle-treated-HSEC, with or without TNFa (20 ng/ml) + IFNa (100 ng/ml). In separate experiments, TNFa+IFNa stimulated-HSEC were treated with sham or OPN-aptamers and total lymphocyte adhesion recorded. Human livers with NASH were immunostained for OPN, and FACS used to quantify LIL isolated from control or NASH-cirrhotic patients.
Results In mice, diet-induced NASH upregulated expression of hepatic OPN by threefold ($p<0.05$), serum OPN by twofold ($p<0.05$), and increased intrahepatic CD4 by 2.2-fold, CD8 by 4.5-fold, and NKT cells by 3.2-fold ($p<0.05$). MCD-fed mice treated with anti-OPN accumulated fewer CD3, CD4, CD8 and NKT cells ($p<0.05$), and exhibited attenuated injury (ALT: threefold reduction; $p<0.02$). rOPN induced expression of ICAM-1, VCAM-1 and CD31 on human HSEC, enhanced lymphocyte recruitment under conditions of flow (41%), and amplified recruitment capacity of TNFα +IFNγ stimulated HSEC (23%), while OPN neutralisation with RNA-aptamers reduced lymphocyte recruitment by 50% (all $p<0.05$). In humans, expression of OPN was significantly upregulated in NASH; livers from NASH-cirrhosis harboured twofold more CD4 and threefold more CD8 and NKT cells ($p<0.05$) than normal.
Conclusion OPN is upregulated during steatohepatitis in mice and humans, and promotes lymphocyte recruitment across HSEC. Neutralisation of OPN significantly reduces lymphocyte recruitment and liver injury. Our results suggest that OPN is a promising anti-inflammatory target in steatohepatitis.

P90 α-1 ANTITRYPSIN (A1AT) POLYMERS CAUSE EXTREME HEPATOCYTE AGEING

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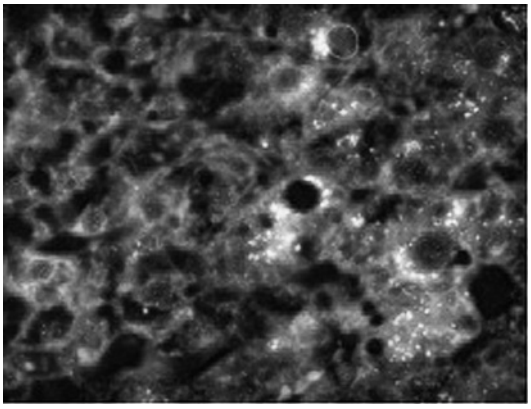
Introduction a1AT, synthesised predominantly in the liver, is the archetypal inhibitor of the serpin protein family. a1AT deficiency is a common inherited disorder; Glu342Lys substitution causes abnormal folding of mutant protein, which may polymerise and aggregate in the endoplasmic reticulum. a1AT aggregates are the histological hallmark of a1AT-related liver disease but it is unclear how aggregates induce liver injury.
Aim To determine whether hepatocytes containing polymerised-a1AT (pa1AT) had accelerated ageing manifest as shortened telomeres.
Method Liver biopsy sections were studied from 60 patients with a1AT- related liver disease with a broad spectrum of fibrosis, recruited from the Cambridge metabolic liver clinic (30 were homozygous and 30 heterozygous). Comparison was made with sections from 20 age and sex matched time zero biopsies obtained at liver transplant. Mean hepatocyte telomere length, a reflection of age, was measured by quantitative fluorescent in situ hybridisation (QFISH) with a PNA Cy5 probe. Nuclei were identified with DAPI, hepatocytes with antibody against hepar-1 and pa1AT with specific mouse monoclonal antibody (2C1). Images were obtained and analysed using the Olympus ScanR software system (Abstract P90 figure 1). Statistical analysis used Graph Pad Prism.
Results Hepatocyte nuclei were larger in patients with both homozygous and heterozygous a1AT deficiency ($p=0.002$) and had shorter telomeres ($p<0.0001$) than age and sex matched controls. Homozygous patients had shorter hepatocyte telomeres than heterozygous patients ($p=0.003$). Hepatocyte nuclei in both homozygous and heterozygous a1AT deficiency were larger in cells with pa1AT compared to neighbouring cells without pa1AT ($p=0.002$). Hepatocyte telomeres were far shorter in cells that contained pa1AT than neighbouring hepatocytes without pa1AT ($p<0.0001$, Abstract P90 figure 2). Hepatocytes with pa1AT showed additional telomere shortening with increased age ($p=0.0002$). Fibrosis stage was related to telomere shortening- telomeres shortened as the stage of fibrosis increased.



Abstract P90 Figure 1 QFISH image which highlights the a1AT polymers (shown as bright white speckles), surrounding some of the affected hepatocytes.