PMO-128 EFFECTS OF ORAL NANOPOROUS CARBON THERAPY IN LEPTIN NULL MICE AS A MODEL OF NON-ALCOHOLIC STEATOHEPATITIS

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Introduction Endotoxaemia is implicated in the pathogenesis of non-alcoholic fatty liver disease. Modulation of intra-luminal factors driving bacterial translocation may have the capacity to impact on the natural history of the disease. Nanoporous carbons are non-absorbable, synthetic materials which are safe with porosity manipulated for adsorption of middle and high molecular weight molecules and surface chemistry modified to alter adsorption capacity for biological molecules. We sought to determine their biological effects in leptin null mice, which are hyperphagic and obese with evident steatohepatitis and to ascertain whether nanoporous carbons can reverse established non-alcoholic steatohepatitis (NASH) in these animals.

Methods 10 lep^{-}/lep^{-} null and 10 heterozygote male mice were randomised to receive powdered chow ± carbon (0.4 g/100 g body weight/day) for 4 weeks. Extent of liver injury was assessed by serum levels of ALT. Additionally, non-parenchymal cells were isolated and the Kupffer cell (KC) population characterised by flow cytometry as those cells expressing F4/80, CD68 and CD11b. Reactive oxygen species (ROS) production by isolated KCs was also assayed. Hepatic TLR-4 expression as a surrogate of endotoxaemia was determined by immunohistochemistry.

Results In lep^{-}/lep^{-} mice, oral carbon treatment was associated with a significant reduction in ALT 889±280 IU/ml to 408±42 IU/ml ($p<0.05$). Total KC population was found to be increased in lep^{-}/lep^{-} mice compared to heterozygote control with a significant reduction observed with carbon treatment ($p<0.05$). A significant reduction in KCs ROS production was also observed in carbon treated lep^{-}/lep^{-} mice ($p<0.05$) compared to untreated lep^{-}/lep^{-} controls. A significant reduction in the F4/80+, $CD68^{-}$, CD11b+ cell sub-population in lep^{-}/lep^{-} in the presence of carbon treatment group was also observed ($p<0.05$). Moreover, hepatic TLR-4 expression was reduced in carbon-treated lep^{-}/lep^{-} mice compared to non-treated controls. Finally, we observed a trend towards reduction in final body weight in carbon-treated lep^{-}/lep^{-} mice compared to untreated controls group ($p=0.095$).

Conclusion Oral nanoporous carbon through modulating endotoxaemia and KC function may be a promising therapy in NASH.

Competing interests None declared.

PMO-129 RELAXIN REDUCES PORTAL HYPERTENSION THROUGH STIMULATION OF HEPATIC NITRIC OXIDE PRODUCTION

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Introduction We have previously reported that the multifunctional hormone relaxin (RLX) downregulated the activation state and contractility of hepatic myofibroblasts and reduced portal hypertension (PHT) in cirrhotic rats (Fallowfield J *et al* BASL 2010). RLX has been shown to induce a range of haemodynamic effects in different organs and species, largely through effects on nitric oxide (NO). In cirrhosis, there is hepatic NO deficiency and hyporesponsiveness. We postulated that the effects of RLX on PHT were, at least in part, mediated by activation of the NO pathway.

Methods Cirrhosis and PHT was induced in age-matched male Sprague-Dawley rats by 8 weeks biweekly i.p. CCl4, before randomisation to the following groups: (1) recombinant human H2-relaxin (H2-RLX) s.c. for 72 h; (2) placebo s.c. for 72 h; (3) H2-RLX s.c. + L-NAME p.o. for 72 h; (4) placebo s.c. + L-NAME p.o. for 72 h; $n=5–10$/group. NO levels in serum were determined by quantitative immunoassay for total nitrite and hepatic NO bioavailability by cGMP immunoassay. Relative levels of Ser^{473} phosphorylated Akt (p-Akt) and Ser^{1179} phosphorylated eNOS (p-eNOS) protein in whole liver extracts were quantified by Western blotting. Rho-kinase activity was assessed by phosphorylation of the endogenous Rho-kinase substrate moesin (Thr^{558}). Portal pressure (PP) and mean arterial pressure (MAP) were measured under general anaesthesia by direct cannulation.

Results Rats treated with CCl4 for 8 weeks developed micronodular cirrhosis, splenomegaly and PHT. There was no difference in mean serum nitrite levels between H2-RLX and placebo treated rats. However, H2-RLX increased hepatic cGMP production ($p<0.01$) and upregulated expression of p-Akt ($p<0.05$) and p-eNOS ($p<0.05$) protein. In contrast, there was no difference in p-moesin levels. H2-RLX treated animals had a lower mean PP than placebo controls (11.6±0.3 mm Hg [95% CI 10.97 to 12.81] vs 9.2±0.6 mm Hg [7.66 to 10.7]; $p=0.008$) and decreased spleen size ($p=0.01$). The portal hypotensive effect of H2-RLX was abrogated by co-administration of the NOS inhibitor L-NAME (11.42±0.35 mm Hg [10.44 to 12.4]; $p=0.004$ vs H2-RLX). MAP was comparable in RLX and placebo treated animals that also received L-NAME.

Conclusion A reduction in NO bioavailability is considered to be a major factor increasing intrahepatic vascular tone in cirrhosis. Our data indicate that H2-RLX was capable of stimulating intrinsic (but not systemic) NO generation in fibrotic liver by activating the Akt/eNOS/cGMP pathway. Furthermore, inhibition of this axis with L-NAME ablated the portal hypotensive effect of H2-RLX, suggesting that it could represent a novel liver-specific NO donor in cirrhotic PHT.

Competing interests None declared.

PMO-130 ALTERED ACETYL-COA METABOLISM IN HEPATIC MITOCHONDRIAL IMPAIRMENT IN IN VITRO MODELS OF HEPATIC CELLULAR STEATOSIS

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Introduction Increased ketogenesis, in the presence of unaltered β-oxidation, is a feature of human steatohepatitis. This is thought to be attributable to decreased acetyl-coA entry to tricarboxylic acid cycle with mitochondrial impairment. In this study, we examined the diversion of acetyl-coA towards free fatty acid (FFA) biosynthesis and mevalonate pathways (including vitamin D3, steroids hormones and bile acids) in the presence of mitochondrial dysfunction and triglyceride accumulation.

Methods Human hepatoblastoma C3A cells were treated with; oleate or various combinations of octanoate (O), lactate (L), pyruvate (P) and ammonia (N) for 72 h. Metabolites that correspond to the intermediates of FFA biosynthesis, mevalonate pathways were measured using metabolomics study.

Results We have previously shown that LPON treatment, but not oleate, affected mitochondrial function as evidenced by decreased respiration and ROS formation with concomitant enhanced ketogenesis despite the similarities in triglyceride accumulation. Using metabolomics analysis, we identified three metabolites that correspond to FFA biosynthesis, three were bile acids and three were the

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derivatives of steroid hormones and vitamin D3 synthesis. We also identified mevalonate and 7-dehydrodemosterol, the intermediates of cholesterol biosynthesis. The concentrations of FFA biosynthesis intermediates were higher with LPON compared with oleate (3-oxo-tetradecanoate (p=0.005) and 3-oxo-hexadecanoate (p=0.02)). Although mevalonate (p=0.37) and 7-dehydrodesmosterol (p=0.46) levels were higher with oleate than that seen with LPON, these differences did not reach statistical significance. In contrast, bile acids were significantly elevated with oleate than LPON ((taurocholate (p=0.002), glycocholate (p=0.001), (6RS)-22-oxo-23,24,25,26,27-pentanorvitamin D3 6,19-sulphur dioxide adduct (p=0.04) and 1,25-dihydroxy-2,4-dinor-1,3-secovitamin D3 (p=0.0006).

Conclusion These data suggest that, aside from enhanced ketogenesis, impaired mitochondrial function is also associated with acetyl-coA diversion towards FFA synthesis, but not mevalonate pathways. These differences are likely to reflect cellular demand in the presence of decreased ATP formation with mitochondrial dysfunction.

Competing interests None declared.

PMO-131 FGF INDUCIBLE PROTEIN 14 IS UPREGULATED IN NEOVESSELS DURING CHRONIC INFLAMMATORY LIVER DISEASE AND PROMOTES INTRAHEPATIC ENDOTHELIAL CELL ANGIOGENESIS IN VITRO FOLLOWING STIMULATION VIA TWEAK

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Introduction TWEAK and Fn14 members of the TNF superfamily of ligands and receptors collectively regulate a diverse range of immune, inflammatory and regenerative responses. Recent studies indicate a potential role of TWEAK and Fn14 in tissue repair following liver injury where they may promote angiogenesis and neovessel growth. TWEAK/Fn14 could therefore facilitate inflammatory cell recruitment and promote portal associated lymphoid tissue development during inflammation.

Aims (1). To investigate TWEAK/Fn14 expression in human liver tissue during chronic liver disease. (2) To study Fn14 expression in isolated intra-hepatic endothelial cells (IHEC). (3) To determine angiogenic responses of IHEC to TWEAK.

Methods Tissue sections from explanted human livers at the time of hepatobiliary surgery, including normal donor; normal tissue adjacent to malignant lesions; HCV; ALD; NASH; Chronic Allograft Rejection; PSC and PBC, were subjected to immunohistochemistry or dual immunofluorescence using antibodies to TWEAK, Fn14 and phenotypic markers CD31 and CD68. Isolated IHEC were cultured with combinations of TNFα, IFNg, FGF, and assessed for TWEAK/Fn14 expression using flow cytometry. In addition IHEC were incubated with recombinant TWEAK in presence ± TNFα and assessed using a matrigel angiogenesis assay for vessel formation and branching.

Results Fn14 expression was low in normal tissue in portal vessels and sinusoids, whereas in disease portal neovessels (CD31 +ve) were highly positive for Fn14. TWEAK expression was low in normal tissue but highly expressed in CD68+ve monocytic cells surrounding areas of neovascularisation and inflammatory cell aggregation. Fn14 expression significantly up-regulated on isolated IHEC when stimulated with the TNFα. Confocal imaging showed that the expression of Fn14 was predominantly cytoplasmic unless stimulated with TNF which enhanced cell surface expression. IHEC were consistently negative for TWEAK and TWEAK stimulation increased IHEC angiogenesis, where a change in cell morphology and enhanced branching of capillary like structures was observed. Pre-incubation with Fn14 antagonistic mAb completely abrogated vessel formation and branching.

Conclusion These new data show that Fn14 activation stimulates neovessel branching in IHEC and that TNF-α promotes mobilisation of cytoplasmic Fn14 to the cell surface suggesting an important regulatory role for TWEAK/Fn14 in neovascularisation and portal lymphoid aggregation during hepatic inflammation.

Competing interests None declared.

PMO-132 HISTONE VARIANT MACROH2A1 KNOCK-OUT WORSENS HIGH FAT DIET-INDUCED SYSTEMIC INSULIN RESISTANCE, GLUCOSE INTOLERANCE AND HEPATIC STEATOSIS IN MICE

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Introduction Hepatic steatosis is a major risk factor for the development of severe liver damage, including fibrosis, cirrhosis and hepatocellular carcinoma. It often exists as a co-morbidity factor with diabetes type I/II and with other manifestations of the metabolic syndrome. Recent studies highlight the importance of an epigenetic basis for the development of steatosis based on macroH2A1. MacroH2A1 is a histone variant of histone H2A, which possesses an additional protein domain called macro. When incorporated into the chromatin of hepatocytes, macroH2A1 regulates gene expression. Two alternatively spliced isoforms of macroH2A1 exist, which have been shown to be markers of breast, skin and lung cancer. Whole-body knock out of macroH2A1 in mice induces glucose intolerance and changes in genes regulating hepatic lipid metabolism. However, overt hepatic steatosis was not observed and the significance of these findings is unclear. We hypothesised that macroH2A1 could be involved in the pathogenesis of hepatic steatosis induced in the high fat-diet mouse model of metabolic syndrome.

Methods Wild type and homozygous knock-out male mice were placed on a high fat diet (Western diet, 42% of energy intake from saturated fats) for 8 weeks. At the end of the treatment, measurement of insulin sensitivity and glucose tolerance (ITT and GTT) were performed. Mice were sacrificed, and plasma and liver tissue harvested for further assays, including protein, gene expression and immunohistochemistry analyses.

Results Deterioration in insulin resistance and glucose intolerance induced by high fat diet was observed in macroH2A1 knock-out mice, compared to wild type littermates. Moreover, lack of macroH2A1 in mice significantly worsened the increase in circulating non-esterified fatty acids (NEFA) and the hepatic intracellular content of tryglicerides induced by high fat diet. Gene expression studies unveiled an increase in the hepatic expression of lipoprotein lipase (Lpl) and fatty acid transporter CD36 in knock-out mice.

Conclusion MacroH2A1 is a regulator of hepatic fat accumulation and its absence worsens hepatic steatosis and systemic imbalances upon a high fat regimen in mice. This could be due to a direct effect of macroH2A1 on chromatin structure and on the expression of key genes involved in liver lipid metabolism.

Competing interests None declared.

REFERENCES

1. **Changolkar LN,** Costanzi C, Leu NA, *et al*. Developmental changes in histone macroH2A1-mediated gene regulation. *Mol Cell Biol* 2007;**27**:2758–64.
2. **Boulard M**, Storck S, Cong R, *et al*. Histone variant macroH2A1 deletion in mice causes female-specific steatosis. *Epigenetics Chromatin*. 2010;**3**:8.

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