**PMO-128**

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

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1J Macnaughton,* 1J Soeda, 1A Mouralidaran, 2S Sandeman, 1c Howell, 2S Milhalovsky, 3O Kozynchenko, 3S Tennissen, 1N Davies, 2R Moskerjee, 1J Oben, 1R Jalan. 1Hepatology, UCL, London, UK; 2Centre for Biomedical and Health Science, University of Brighton, Brighton, UK; 3MastCarbon, Guildford, UK

**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 leptin-/lep− null and 10 heterozygote male mice were randomised to receive powder with 5% (w/v) 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 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 leptin−/lep− mice, oral carbon treatment was associated with a significant reduction in ALT 389±250 IU/ml to 405±42 IU/ml (p<0.05). Total KC population was found to be increased in leptin+/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 leptin+/lep− mice (p<0.05) compared to untreated leptin+/lep− controls. A significant reduction in the F4/80+, CD68+, CD11b+ cell sub-population in leptin+/lep− in the presence of carbon treatment group was also observed (p<0.05). Moreover, hepatic TLR-4 expression was reduced in carbon-treated leptin+/lep− mice compared to non-treated controls. Finally, we observed a trend towards reduction in final body weight in carbon-treated leptin+/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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J Fallowfield,* V Snowdon, R Aucott, T Gordon-Walker, A Pellicoro, J Iredale. MRC Centre for Inflammation Research, University of Edinburgh, Edinburgh, UK

**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 BASIL 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. CC4, 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 immunassay. Relative levels of Ser473 phosphorylated Akt (p-Akt) and Ser1177 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 (Thr382). Portal pressure (PP) and mean arterial pressure (MAP) were measured under general anaesthesia by direct cannulation.

**Results**

Rats treated with CC4 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±0.5 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 hypertensive effect of H2-RLX was abrogated by co-administration of the NOS inhibitor L-NAME (11.4±0.5 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 hypertensive 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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1K Lockman,* 2X E Burgess, 1P Lee, 1A Pyde, 1P C Hayes, 1C Filippi, 1J N Plowрис. 1Hepatology, University of Edinburgh, Edinburgh, UK; 2The Scottish Metabolomics Facility, University of Glasgow, Glasgow, UK; 3MRC Scottish Regenerative Medicine, University of Edinburgh, Edinburgh, UK

**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 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 FFA biosynthesis, three were bile acids and three were the availability by cGMP immunoassay. Relative levels of Ser473 phosphorylated Akt (p-Akt) and Ser1177 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 (Thr382). Portal pressure (PP) and mean arterial pressure (MAP) were measured under general anaesthesia by direct cannulation.

**Results**

Rats treated with CC4 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±0.5 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 hypertensive effect of H2-RLX was abrogated by co-administration of the NOS inhibitor L-NAME (11.4±0.5 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 hypertensive effect of H2-RLX, suggesting that it could represent a novel liver-specific NO donor in cirrhotic PHT.

**Competing interests**

None declared.

**Posters**

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