and IL-10) cytokines, were analysed. Immunohistochemical markers of activated microglia (ED1, OX6 and Iba-1), astrocytes (GFAP), inflammatory responses (IL-1ß and iNOS), cellular stress (HSP-25) and the predominant antiinflammatory and alternative microglial activation marker TGF-ß (using RT-PCR), were also analysed.

Results Compared to Shams, arterial and brain ammonia ($p<0.001$, respectively), bilirubin (from day-1) and the cytokines, TNF-a and IL-6 were significantly increased by 4 weeks BDL ($p<0.001$), which induced mild brain oedema ($p=0.07$). There was no evidence of classical microglial activation or cellular infiltration (indicated by negative OX6 staining and few perivascular ED1 positive cells evident in all groups), with observable resting microglia (Iba-1 positive staining) in all groups. Protein expression for iNOS and IL-1ß increased using Western blotting. Increased GFAP staining indicated significant astrocyte activation, primarily in the corpus callosum in all BDL groups. Hsp 25 (cellular stress indicator) peaked at 2 weeks post-BDL and was located mostly in the corpus callosum similar to activated astrocytes. TGF-ß was significantly upregulated by 3-fold in all BDL groups.

Conclusion Regional astrocyte activation and cellular stress (indicated by increased HSP-25 expression), are early features of BDL. These events are associated with increased brain proinflammatory cytokine production and iNOS expression. This proinflammatory response is not due to obvious microglia activation, but consequent upon activation of astrocytes, possibly related to hyperammonemia and/or associated cell swelling. However, an observed TGF-ß response may reflect compensatory antiinflammatory microglial responses, designed to limit the effect of astrocyte activation; with interventions targeting its brain expression potential novel therapies for hepatic encephalopathy.

OP09 THE DEVELOPMENT AND TESTING OF THE UNIVERSITY COLLEGE LONDON LIVER SUPPORT DEVICE (UCL-ARSENEL): IMPROVEMENT OF SURVIVAL IN PARACETAMOL-INDUCED ACUTE LIVER FAILURE PIGS

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Introduction An effective liver support device to remove metabolic toxins is an unmet clinical need. We have demonstrated that in liver failure, albumin function is irreversibly damaged, preventing detoxification processes, and that bacterial endotoxins induce neutrophil dysfunction causing indiscriminate damage and immune failure. Previous generations of artificial devices were designed to remove toxins from patients' plasma, but did not address albumin damage and/or inflammation. An albumin replacement system with a novel endotoxin ligation (ARSeNEL) component was developed to selectively filter plasma to adsorb endotoxin and replace damaged albumin.

Method The device consists of three components; plasmapheresis, endotoxin and high cut-off (100 kDa) cartridges; with fresh frozen plasma replacing ultrafiltered plasma. We tested the device in a model of acetominophen-induced acute liver failure (ALF). Sixteen female landrace pigs (3 sham, 8 ALF, 5 ALF+UCL-ARSeNEL) were studied. ALF was induced via intra-gastric acetaminophen administration, confirmed by deranged clotting function (ca. 20hrs to ALF). Animals were treated with UCL-ARSeNEL or haemofiltration control within 2 h of ALF confirmation. Endpoints were: survival; ICP; standard biochemistry; cytokines; albumin damage; and plasma endotoxin levels.

Results Survival post ALF was significantly increased using UCL-ARSeNEL (ALF 15.8±2.4 h vs UCL-ARSeNEL 23.8±1.9 h; $p=0.02$). No haematological or biocompatibility issues were observed. Endotoxin reduced by a quarter (1.99±0.18 Eu/ml vs 1.42±0.21 Eu/ml) in the device group at 16hrs. The changes in ICP index (1.7±0.07 vs 1.4±1.58), INR (16.6±6.6 vs 6.8±0.5), ischaemia-modified albumin ratio (0.45±0.166 vs 0.35±0.108), ammonia (177±131 vs 153±84 μM) and mean arterial pressure (71±7.6 vs 87±6.0 mm Hg) showed marked improvement in the UCL-ARSeNEL group.

Conclusion The results of the study confirm that UCL-ARSeNEL is safe and effectively improves survival in ALF pigs by addressing the key pathophysiological derangements such as albumin dysfunction and endotoxinaemia; which impact upon end-organ function. The results justify a clinical trial, which is being planned.

OP10 RELAXIN INHIBITS HUMAN MYOFIBROBLAST CONTRACTILITY AND MODULATES PORTAL HYPERTENSION IN VIVO

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Introduction The peptide hormone relaxin (H2-RLN) has a broad range of biological activities including antifibrotic, anti-inflammatory and haemodynamic effects in a range of target tissues. Increased intrahepatic vascular resistance in cirrhosis is due to mechanical factors related to scarring, but also a dynamic component mediated by myofibroblast (MFB) contractility. We hypothesised that H2-RLN could modulate the dynamic attribute of portal hypertension (PHT) via a direct effect on hepatic stellate cell (HSC)-MFB mediated vasoconstriction.

Aim To determine whether H2-RLN could target the contractile phenotype of activated HSC-MFBs in vitro, counterbalance the contractile response in vivo in a model of sinusoidal portal hypertension, and explore the mechanisms underlying its portal hypotensive effect.

Method Gene expression in culture activated human HSCs was analysed by qRT-PCR and Western blotting. Collagen gel contraction assays were used to assess HSC-MFB contractility. Nitric oxide (NO) production was measured by Griess assay. Cirrhosis and portal hypertension was induced in age matched male Sprague–Dawley rats by 8 weeks bi-weekly i.p. CCl4, before randomisation to treatment with recombinant human H2-RLN or placebo delivered by s.c. osmotic minipump for 72 hrs (n=10 per group). Portal pressure was measured by direct cannulation. Serum H2-RLN levels were quantified by immunoassay. Liver fibrosis was measured after Sirius red staining by calculation of collagen proportionate area by digital image analysis. Finally, systemic arterial and portal pressure was measured simultaneously in groups of cirrhotic and control rats randomised to acute i.v. H2-RLN or placebo.

Results Treatment of culture activated HSCs with H2-RLN down-regulated expression of the major cytoskeletal protein, alpha-smooth muscle actin (alpha-SMA), in a dose dependent manner. In collagen gel contraction assays, H2-RLN inhibited HSC-MFB contraction, an effect shown by RNA interference to be mediated via RXFP-1 receptor signaling and increased NO production. Rats treated with CCl4 for 8 weeks developed micronodular cirrhosis, splenomegaly and portal hypertension. Treatment with s.c. H2-RLN for 72 h achieved physiologically relevant serum concentrations and decreased portal pressure by 24% (mean 12.8±0.8 mm Hg vs 9.8±0.3 mm Hg; $p=0.002$), whereas placebo had no significant effect. The observed haemodynamic response was independent of fibrosis regression. Hepatic expression of alpha-SMA and other intermediate filament proteins was markedly inhibited by H2-RLN, while eNOS activity was increased. Infusion of i.v. H2-RLN in cirrhotic rats rapidly depressed portal pressure (mean 28%±6) but