**PTU-044**
HEPATIC/ENDOTHELIAL CELL CO-CULTURE: ESTABLISHING OPTIMAL CONDITIONS FOR LIVER TISSUE ENGINEERING

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**Introduction**

Development of 3D hepatic organoids utilising human cell derivatives for in vitro drug testing and bioartificial liver support systems is challenging. Tissue engineering thick, complex structures such as human liver organoids (micro-tissue; >500 mm) will require vascularisation of 3D cultures—coupled with biomatrix support scaffolds—to both maintain integrity and promote formation of endothelial channels (sinusoid-like structures) to facilitate oxygen and nutrient transfer to hepatocytes in 3D culture. Key challenges involve directing nascent microvessels into an appropriate environment, ensuring the formation of high metabolic demand. In this preliminary study, we aimed at optimising the co-culture of hepatic cells and vascular cells to form more sheet-like structures in co-culture. Metabolomics analysis was performed to assess function of co-cultured cells. Matrigel and MaxGel (Sigma) were used as candidate bioscaffolds.

**Methods**

Different ratios of HUVECs: C3A or EoCs:C3A to form co-cultures were studied using confocal microscopy and flow cytometry following appropriate immunostaining. Flow cytometry was used to study integrin expression (Cd49a, Cd49b, Cd49f, Cd49e), and CD31 expression on endothelial culture medium (Lonza EGM-2 medium, UK). Cell contact and cell morphology of C3A cells, but not EoCs.

**Results**

A ratio 3:1 (HUVECs:C3A) was optimal for growth using endothelial culture medium (Lonza EGM-2 medium, UK). Cell phenotypes were maintained for 7 days in co-culture with strong integrin (Cd49a, Cd49b, Cd49f, Cd49e), and CD31 expression on HUVECs and EpCam on C3A cells, while a reduction of HUVEC cell number was noted with a parallel increase of C3A cells by day 7—to form more sheet-like structures in co-culture. Metabolomics analysis of culture media showed enhanced urea cycle, lipid synthesis and amino acid utilisation of C3A cells in co-culture. Matrigel promoted formation of microvessel structures, with interconnected channels, in both EoCs and HUVECs; and was superior to MaxGel. MaxGel in 3D sandwich culture promoted differentiated (cuboidal) morphology of C3As, but not EoCs.

**Conclusion**

Optimisation of both cell ratios and cell numbers, as well as selection of appropriate culture media are critical factors in developing a successful hepatic co-culture system with the ability to form sinusoid-like/microvessel structures. This study represents an early step towards understanding the requirements of vascularised liver tissue for future clinical/pharmaceutical applications.

**Competing interests**

None declared.

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**PTU-045**
PROTON NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF PLASMA IN PATIENTS WITH CIRRHOSIS CORRELATES WITH ARTERIAL AMMONIA BUT NOT GRADE OF HEPATIC ENCEPHALOPATHY

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**Introduction**

Acute-on-chronic liver failure is associated with a high mortality and difficulty in outcome prediction. Neither liver specific
models such as the Model for End Stage Liver Disease (MELD) or intensive care prognostic schema accurately predict hospital mortality. Multivariate methods applied to nuclear magnetic resonance (NMR) spectroscopy of urine have demonstrated correlation with MELD score but whether metabolic profiling predicts outcome in these patients is unknown.

Methods 80 patients referred to Kings College Hospital with decompensated cirrhosis were studied with 20 healthy controls. Plasma was drawn on admission for subsequent 1H NMR spectroscopy using a Carr-Purcell-Meiboom-Gill sequence in a 600 MHz Bruker Avance spectrometer. Full resolution NMR spectra were used in an orthogonal projection to latent structures discriminant analysis (OPLS-DA) to predict hospital mortality.

Results Patients had a median (range) age of 55 (23–75) years with 51 (64%) male. Aetiology of cirrhosis was alcohol related in 40 (50%), autoimmune in 15, viral hepatitis in 10, non-alcoholic steatohepatitis (NASH) in 5, with haemochromatosis and cryptogenic cirrhosis the causes in the remainder. Median (range) MELD score was 14 (6–40) with 18 patients not surviving to hospital discharge. The OPLS-DA model accurately discriminated between patients and healthy controls (R2(X)=0.65, R2(Y) =0.84, Q2(Y) =0.76, cross-validated (leave-one-out) sensitivity and specificity 100%, CV-ANOVA p=10–25). Metabolites increased in patients were lactate, tyrosine, and glucose with LDL, VLDL and phosphocholines being increased in controls. OPLS-DA accurately discriminated between survivors and non-survivors (R2(X)=0.63, R2(Y)=0.64, Q2(Y)=0.37, sensitivity 100%, specificity 98% CV ANOVA p=10–6). Metabolites increased in non-survivors included lactate, tyrosine and phenylalanine with lipid and phosphocholine resonances reduced in non-survivors. Removing 1/3 of patients at random from the learning set, remodelling and predicting outcome for these patients resulted in an AUROC of 0.95 (95% CI 0.87 to 1.00, sensitivity 93%, specificity 86%) for predicting survival using NMR profiling in comparison with 0.78 for MELD (p<0.001).

Conclusion Plasma metabolic profiling with 1H NMR spectroscopy in patients with cirrhosis and acute-on-chronic liver failure can accurately describe a metabolic phenotype of non-surviving patients. Validation of these techniques in larger datasets is required.

Competing interests M McPhail: Grant/Research Support from: Wellcome Trust, UK, D Shawcross: None Declared, I Collart: None declared, E Want: None declared, K Veselkov: None declared, M Crossley: None declared, C Willars: None declared, G Auzinger: None declared, J O’Grady: None declared, W Bernal: None declared, E Holmes: None declared, J Wendon: None declared, S Taylor-Robinson: None declared.

PTU-047

THE CLINICAL UTILITY AND COST-EFFECTIVENESS OF NON-INVASIVE TESTS FOR DETECTING FIBROSIS IN PATIENTS WITH SUSPECTED ALCOHOL-RELATED LIVER DISEASE

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Introduction The non-absorbable antibiotic rifaximin has been used to treat hepatic encephalopathy (HE) since the 1980s. Interest in its use increased following a recent Phase III study showing that treatment with rifaximin over 6-months resulted in a relative reduction in the risk of breakthrough HE of 58% compared to placebo (Bass et al. 2010). The aim of this study was to evaluate the efficacy and safety of rifaximin for the treatment of all types of HE in patients with cirrhosis against a variety of comparative outcomes.

Methods A language unrestricted search for papers published between January 1983 and September 2011 was undertaken utilising 11 databases. All HE treatment trials were scrutinised and randomised trials identified. Raw data were obtained from six trials. Data were extracted on (1) the numbers of patients showing improvement overall; (2) the means (± 1SD) for mental state, asterixis, number connection tests (NCT), EEG and blood ammonia; and (3) adverse events. Fixed effect model meta-analyses were applied, subdivided by population type. Random effects analyses were used when statistically significant heterogeneity of results occurred. Overall relative proportions were calculated for binary outcomes and standardised mean differences (SMD) for quantitative outcomes.

Results 17 trials were identified comparing rifaximin to non-absorbable disaccharides (9 trials), other antibiotics (6 trials) or no

PTU-048

RIFAXIMIN FOR THE TREATMENT OF HEPATIC ENCEPHALOPATHY: A META-ANALYSIS OF RANDOMISED STUDIES

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Introduction The non-absorbable antibiotic rifaximin has been used to treat hepatic encephalopathy (HE) since the 1980s. Interest in its use increased following a recent Phase III study showing that treatment with rifaximin over 6-months resulted in a relative reduction in the risk of breakthrough HE of 58% compared to placebo (Bass et al. 2010). The aim of this study was to evaluate the efficacy and safety of rifaximin for the treatment of all types of HE in patients with cirrhosis against a variety of comparative outcomes.

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