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 defined prominently by heterotypic cell–cell contacts and meeting high metabolic demands. In this preliminary study, we aimed to optimise heterotypic co-culture of either Human Umbilical Vein Endothelial Cells (HUVECs) or Endothelial Outgrowth Cells (EOCs) with hepatic C3A cells, using appropriate biomatrix scaffolds.

Methods Different ratios of HUVECs: C3A or EOCs:C3A to form co-cultures were studied using contrast/confocal microscopy and flow cytometry following appropriate immunostaining. Flow cytometry was used to study integrin expression (Cd49a, Cd49b, Cd49f, Cd49e), endothelial markers (CD31) and EpCam, as an hepatic marker. Fingerprint *unbiased* metabolomics analysis was used to assess function of co-cultured cells. Matrigel and MaxGel (Sigma) were tested as candidate bioscaffolds and were compared with standard 2D cultures on plastic and collagen for each cell line as controls.

Results A ratio 3:1 (HUVECs:C3A) was optimal for growth using endothelial culture medium (Lonza EGM-2 medium, UK). Cell phenotype was 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.

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 Diagnosis of overt hepatic encephalopathy (HE) in patients with cirrhosis often relies on subjective clinical examination. Arterial ammonia correlates poorly with HE grades and a blood marker for diagnosis and monitoring is lacking. Metabolic profiling by nuclear magnetic resonance (NMR) spectroscopy of blood correlates with Model for End Stage Liver Disease Score (MELD)[1] and may offer diagnostic biomarkers in overt HE.

Methods Thirty-seven patients with cirrhosis and differing grades of HE were identified. Arterial blood was drawn for arterial ammonia, blood gas analysis and NMR spectroscopy. HE was diagnosed and graded by West-Haven criteria and Trail Making (A and B) Tests. ^{1}H NMR spectroscopy was performed in a Bruker 600 MHz Avance spectrometer using a Carr-Purcell-Meiboom-Gill sequence prior to multivariate analysis using orthogonal partial least squares discriminant analysis (OPLS-DA).

Results 24 male and 13 female patients, 21 with alcohol-related cirrhosis, 5 viral hepatitis, 11 mixed/other causes, with median (range) age 57 (35–75) years made up the study cohort. Fifteen patients had no HE while 15 had grades 1–2 and 7 grades 3–4. Median MELD score was 14 (4–32) and median arterial ammonia level 106 (19–268 μmol/L). Ammonia level correlated weakly with HE grade (Kendall's τ=0.238, p=0.040) but not with MELD score (τ=0.010, p=0.118). No multivariate models using HE grade as a categorical (OPLSDA) or continuous (OPLS) variables resulted in validity (eg, OPLS model: R2(Y)=0.489, Q2(Y)=−0.091). A 2-component OPLS model using ammonia as a Y variable identified multiple metabolites correlating with arterial ammonia (overall R2 (Y)=0.566, Q2(Y)=0.394, cross-validated ANOVA p=0.0008). Metabolites associated with high ammonia levels included pyruvate, 3-hydroxybutyrate, glutamate, phenylalanine and an unassigned resonance at 2.43 ppm, while the resonances associated with lipoproteins correlated negatively with ammonia.

Conclusion Plasma ^{1}H NMR spectroscopy did not discriminate effectively between grades of HE in this cohort of patients with cirrhosis and overt HE. Multiple metabolites correlate with arterial ammonia level with some overlap from the metabolic pathways implicated in high MELD patients. Alternative metabolic profiling techniques such as mass spectroscopy may be required to assist in diagnosis in these patients.

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PTU-046 METABOLIC PROFILING OF PLASMA BY NMR SPECTROSCOPY ACCURATELY PREDICTS OUTCOME IN PATIENTS WITH DECOMPENSATED CIRRHOSIS AND ACUTE ON CHRONIC LIVER FAILURE

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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 serum 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 ^{1}H 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 $p=10^{-27}$). 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 95% 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 ^{1}H 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.

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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 use of non-invasive liver tests (NILTs) for assessing hepatic fibrosis carries fewer risks than liver biopsy. However, their clinical utility and cost-effectiveness, in patients with suspected alcohol-related liver disease, are unknown. This study explored whether the Enhanced Liver Fibrosis (ELF) test, FibroTest, and FibroScan are likely to be cost-effective in a UK setting.

Methods Systematic reviews of the clinical effectiveness of the three NILTs under study and of the morbidity/mortality associated with liver biopsy were undertaken and used, where possible, to populate a mathematical model constructed to simulate the experience of patients suspected of having alcohol-related liver injury. The model used the following strategies: (1) biopsy all; (2) use a NILT for triage and biopsy more selectively; and (3) use a NILT alone to determine diagnosis and subsequent management. Thirty-six separate scenarios were evaluated varying the type and sensitivity of the liver biopsy, the accuracy of the non-invasive tests, and the disutility associated with the biopsy procedure.

Results No clear conclusions could be drawn, following the systematic reviews, about the accuracy of these three NILTs in the diagnosis of alcohol-related liver disease because there was significant heterogeneity in the published studies, particularly in relation to drinking behaviour and diagnostic cut-offs. The assessment was also confounded by the paucity/absence of data on: (1) the sensitivity of the liver biopsy; and (2) the difference in costs and health between abstinent patients and those who continued to drink. There was wide variation in the threshold level for a decrease in abstinence rates using NILTs in both triage and replacement strategies. The maximum threshold value was 6.1 percentage points with the minimum value being zero percentage points. There was also wide variation in the threshold levels for the incidental gains associated with biopsy using NILTs in both triage and replacement strategies. The maximum value was 0.188 QALYs with the minimum value being zero QALYs.

Conclusion The clinical utility and cost-effectiveness of these three NILTs cannot be assessed, at the present time, primarily because information on the key variables needed to populate the model are lacking. Thus, caution should be exercised in their use, in patients with suspected alcohol-related liver disease, at the present time.

Competing interests None declared.

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 regimens.

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