Research Article

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Extracorporeal liver assist device to exchange albumin and remove endotoxin in acute liver failure: Results of a pivotal pre-clinical study

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Background & Aims: In acute liver failure, severity of liver injury and clinical progression of disease are in part consequent upon activation of the innate immune system. Endotoxaemia contributes to innate immune system activation and the detoxifying function of albumin, critical to recovery from liver injury, is irreversibly destroyed in acute liver failure. University College London-Liver Dialysis Device is a novel artificial extracorporeal liver assist device, which is used with albumin infusion, to achieve removal and replacement of dysfunctional albumin and reduction in endotoxaemia. We aimed to test the effect of this device on survival in a pig model of acetaminophen-induced acute liver failure.

Methods: Pigs were randomised to three groups: Acetaminophen plus University College London-Liver Dialysis Device (n = 9); Acetaminophen plus Control Device (n = 7); and Control plus Control Device (n = 4). Device treatment was initiated two h after onset of irreversible acute liver failure.

Results: The Liver Dialysis Device resulted in 67% reduced risk of death in acetaminophen-induced acute liver failure compared to Control Device (hazard ratio = 0.33, $p = 0.0439$). This was associated with 27% decrease in circulating irreversibly oxidised human non-mercaptalbumin-2 throughout treatment ($p = 0.046$); 54% reduction in overall severity of endotoxaemia ($p = 0.024$); delay in development of vasoplegia and acute lung injury; and delay in systemic activation of the TLR4 signalling pathway. Liver Dialysis Device-associated adverse clinical effects were not seen.

Conclusions: The survival benefit and lack of adverse effects would support clinical trials of University College London-Liver Dialysis Device in acute liver failure patients.

Keywords: Acute liver failure; Acetaminophen; Extracorporeal liver assist device; UCL-LDD; Albumin; Endotoxin; Toll-like receptor 4.

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Abbreviations: LT, liver transplantation; ALF, acute liver failure; APAP, acetaminophen; NAPQI, N-acetyl-p-benzoquinone imine; DAMP, damage-associated molecular pattern; HMGB1, high-mobility group box-1 protein; DNA, deoxyribonucleic acid; TLR4, toll-like receptor 4; Nalp3, nacht, leucine-rich repeat and pyrin domain-containing protein 3; IL, interleukin; HAS, human serum albumin; MARS, Molecular Adsorbent Recirculating System; ACLF, acute-on chronic liver failure; UCL-LDD, University College London-Liver Dialysis Device; PALF, porcine model of acute liver failure; ICP, intracranial pressure; CVP, central venous pressure; INR, international normalised ratio; CD, Control Device; P_aO_2, partial pressure of oxygen in arterial blood; APAP-UCL-LDD, group treated with APAP and UCL-LDD; APAP-CD, group treated with APAP and CD; Control-CD, group treated with placebo, water and CD; HMA, non-oxidised human mercaptalbumin; HNA-1, reversibly oxidised human non-mercaptalbumin-1; HNA-2, irreversibly oxidised human non-mercaptalbumin-2; ELISA, enzyme-linked immunosorbent assay; AST, aspartate amino transferase; ALP, alkaline phosphatase; SVRI, systemic vascular resistance index; CI, cardiac index; SVI, stroke volume index; LVSWI, left ventricular stroke work index; RVSWI, right ventricular stroke work index; PCWP, pulmonary capillary wedge pressure; P_aCO_2, partial pressure of carbon dioxide in arterial blood; RR, respiratory rate; P_{insp}, inspiratory pressures; SIRS, systemic inflammatory response syndrome; IL-1ra, IL-1 receptor antagonist; MAP, mean arterial pressure; HR, heart rate; P_aO_2/FiO_2, ratio of partial pressure of oxygen in arterial blood to percentage of oxygen in inspired gases; PEEP, positive end expiratory pressure.

Introduction

Liver transplantation (LT) is the only treatment proven to prolong survival in patients with acute liver failure (ALF), who fulfil criteria for poor prognosis, but LT remains a limited resource with alternative therapies being an unmet need [1]. In ALF, severity of liver injury and clinical progression of disease is in part consequent upon activation of the innate immune system [2–5]. Endotoxaemia in ALF contributes to this innate immune response [6]. Moreover in liver failure, the detoxifying function of albumin,

which is critical to recovery from liver injury, is irreversibly destroyed [7,8].

Acetaminophen (APAP) overdose is the leading cause of ALF in the UK and USA [1,9]. APAP toxicity results from its hepatic metabolism into toxic N-acetyl-p-benzoquinone imine (NAPQI). Subsequent formation of harmful NAPQI protein adducts causes mitochondrial oxidant stress and ultimately hepatocyte death [4]. However the severity of the ensuing liver injury and clinical syndrome of ALF is exacerbated by activation of the innate immune system by damage-associated molecular patterns (DAMPs) released by dying hepatocytes and likely other resident cells of the liver [3,5]. DAMPs implicated in ALF include high-mobility group box-1 protein (HMGB1), heat shock protein 70 and deoxyribonucleic acid (DNA) fragments [10–12]. HMGB1 has been shown to act via the toll-like receptor 4 (TLR4) pathway to exacerbate immune response [12–14] and DNA fragments may contribute to activation of the nacht, leucine-rich repeat and pyrin domain-containing protein 3 (Nalp3) inflammasome and subsequent activation of pro-inflammatory cytokines, Interleukin-1β (IL-1β) and IL-18 [11].

Endotoxin or lipopolysaccharide, a component of the cell wall of gram-negative bacteria, has been described as a "cofactor" in APAP-induced liver injury [6]. The liver plays a critical role in clearance of gut-derived endotoxin, which may or may not be associated with viable gram-negative bacteria. Data from APAP-induced rodent models of ALF suggest that endotoxin stimulates the innate immune response and produces liver injury through activation of hepatic Kupffer cells to increase pro-inflammatory cytokine production [14,15].

Albumin is the most abundant plasma protein and is synthesized in the liver. Besides maintaining plasma oncotic pressure, it also serves many detoxification, immune and circulatory functions [16]. Human serum albumin (HSA) infusion has been shown to reduce mortality in liver failure patients with spontaneous bacterial peritonitis and hepatorenal syndrome and following large volume paracentesis [16]. Molecular Adsorbent Recirculating System (MARS) is an extracorporeal liver assist device, based on the principle of albumin dialysis, which has been trialled extensively in patients with ALF and acute-on chronic liver failure (ACLF) but failed to show a survival benefit [17–19]. Prometheus, which is based on a similar principle, has been largely trialled in ACLF. Again, no survival benefit of this device could be demonstrated [18,20]. University College London-Liver Dialysis Device (UCL-LDD) was developed based on the hypothesis that the 'failure' of MARS, Prometheus and other such devices may be partly due to their inability to reduce activation of the innate immune system [21,22] and their inability to restore the function of circulating albumin molecules retained within the patient [7,8,23].

UCL-LDD is a novel artificial extracorporeal liver assist device, which includes a high cut-off filter for extraction of albumin, along with bound toxins, by haemofiltration and a selective endotoxin adsorption cartridge for selective endotoxin extraction by haemoperfusion. We hypothesised that UCL-LDD in combination with HSA infusion would prolong survival in ALF, not only by removal of water soluble and protein bound toxins, but also by selective reduction in endotoxemia, improvement in albumin detoxifying function and reduction in the innate immune response. Our aim was to interrogate our hypothesis by testing the effects of UCL-LDD compared to a Control Device delivering standard continuous renal replacement therapy in an APAP-induced porcine model of ALF.

Material and methods

Porcine model of acute liver failure (PALF)

A previously described pig model of APAP-induced ALF (PALF) was used in this study and has been described elsewhere [24]. Female, 26–36 kg, Landrace cross Large White pigs were used and all animal procedures complied with the animals (Scientific Procedures) Act 1986. Briefly, pigs were maintained under general anaesthesia with intermittent positive pressure ventilation. Pigs were instrumented for intravenous fluid and drug administration; arterial and venous blood sampling; and continuous monitoring of intracranial pressure (ICP), central venous pressure (CVP), direct arterial blood pressure, cardiac output and urine output. Critical care protocols for intravenous fluid therapy and maintenance of acid-base status, electrolyte balance, normoglycaemia, cardiovascular function and respiratory function were adhered to in order to maintain pre-defined physiological targets, detailed in Supplementary material [24]. However, albumin infusion was used according to a fixed protocol (see below).

Acute liver failure was induced in 'APAP pigs' with an aqueous APAP suspension, given via an oroduodenal tube. A loading dose of 0.25 g/kg APAP was followed by an hourly maintenance APAP dose, adjusted between 0.5 and 4 g to achieve toxic serum APAP concentrations of greater than 300 mg/L. APAP dosing was discontinued once the International Normalised Ratio (INR) exceeded three and this time point is hereafter referred to as 'ALF'. This value was chosen as, in the PALF model, an INR of three is associated with 100% mortality. Thereafter, APAP pigs were treated with UCL-LDD or a 'Control Device' (CD) until non-recoverable cardiorespiratory arrest or 20 h after ALF. At 20 h after ALF, surviving animals were terminated with sodium pentobarbitone. 'Control pigs' were administered water without APAP for 20 h and then supported for a further 20 h prior to termination with sodium pentobarbitone. The 20 h end point was a requirement of the local ethics and welfare committee.

For survival studies, "death" was defined as time of non-recoverable cardiorespiratory arrest or when at least two of the following criteria were met: haematocrit <10%; blood potassium >5.5 mmol/L; blood lactate >10 mmol/L; blood pH <7.25; partial pressure of oxygen in arterial blood (P_aO_2) <60 mmHg. These alternative parameters for "death" were based on pilot study data, which showed that cardiorespiratory arrest always occurred within 1 to 3 h of occurrence of two of these parameters and that use of these terminal phase criteria to define death reduced variability in survival times and therefore number of pigs required for survival studies.

UCL-LDD

All equipment and consumables were obtained from Gambro Dialysatoren GmbH, Rostock, Germany and used following the recommendations of this company. UCL-LDD incorporated two haemofilters: 'SepteX', a high cut-off filter for extraction of albumin, along with bound toxins, by haemofiltration and 'OXiris', a selective endotoxin adsorption cartridge for selective endotoxin extraction by haemoperfusion. Details of the UCL-LDD device are described in Supplementary material. UCL-LDD was compared to CD in which the two haemofilters of the UCL-LDD were replaced with standard continuous renal replacement haemofilters.

Albumin infusion

In early pilot experiments, a marked reduction in serum albumin concentrations, to values less than 10 g/L, were detected in pigs treated with APAP, likely due to excessive capillary leak in this species [24]. Hence a fixed protocol for albumin infusion was developed as part of the PALF model to compensate for this. At onset of APAP dosing, intravenous infusion of 20% HSA (Bio Products Laboratory Ltd, Hertfordshire, UK) was initiated at 1.6 g albumin/h, this was increased 12 h later to 16 g albumin/h and further increased at ALF to 20 g albumin/h. Control pigs were given 1.6 g albumin/h from onset of water (placebo) administration. In both APAP and Control pigs, two units of porcine fresh frozen plasma were given at ALF or equivalent in order to prevent bleeding. After onset of device treatment in APAP pigs albumin infusion was discontinued if serum albumin exceeded 20 g/L. This albumin infusion protocol ensured appropriate (targeted to serum albumin concentrations) albumin replacement with UCL-LDD treatment.

Study plan

Two studies, a pilot and main study, were performed sequentially.