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Research review

Extracorporeal membrane oxygenation for resuscitation of deceased cardiac donor livers for hepatocyte isolation

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ABSTRACT

Background: Deceased cardiac donors (DCDs) have become a useful source of organs for liver transplantation; nevertheless, there are concerns about the longevity of these grafts. The aim of this study was to evaluate the use of extracorporeal membrane oxygenation (ECMO) to resuscitate DCD porcine livers as a preclinical model using hepatocyte isolation and viability as a marker to assess whole-graft preservation.

Materials and methods: We randomized Landrace pigs into three groups after cardiac death and 30 min of warm ischemia: group 1, peritoneal cooling with intravascular cooling for 2 h; group 2, ECMO for 2 h; and group 3, control (conventional intravascular cooling and retrieval). We then reperfused group 1 and 2 livers for 2 h on an *ex vivo* reperfusion circuit and isolated hepatocytes.

Results: After reperfusion, hepatocyte viability was significantly improved in the ECMO group compared to the cooling groups, as measured by trypan blue, methylthiazolyldiphenyl-tetrazolium bromide, and seeding efficiency. Glycogen and reduced glutathione content were significantly used in the ECMO group both before and after reperfusion compared with group 2. The adenosine diphosphate:adenosine triphosphate ratio showed an improved trend (lower) in the ECMO group compared with the cooling group but did not reach statistical significance either before or after reperfusion.

Conclusions: This preclinical study suggests that ECMO is a viable technique for liver preservation that gives an improved yield of hepatocytes when isolated from a DCD liver, suggesting improved liver preservation.

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1. Introduction

Like most other solid organ transplants, liver transplantation has become a victim of its own success, with an increasing number of patients on the waiting list during an era of a decreasing donor pool [1]. To overcome this restriction, various innovations have been developed, including split liver transplantation and living donor liver transplantation [2]. Extended criteria donors represent a potential pool of organs that could significantly increase the number of liver transplants and therefore decrease waiting list mortality [3]. Deceased cardiac donor (DCDs) could potentially provide the largest pool of extended criteria donors, but they are currently underused because of concerns about poor rates of graft survival.

The DCD liver is often regarded as a marginal organ, with a reduced graft survival rate of about 50% at 1 y and higher rates of primary nonfunction (PNF) [4]. Most clinical experience has been described using Maastricht category 3 controlled donors [5]; yet, a potentially larger pool would be to use category 2 uncontrolled donors, which have been evaluated more frequently in renal transplantation [6]. A few centers have reported outcomes after uncontrolled DCD liver transplants [7–9]. In 1995, Casavilla *et al.* [7] reported their experience of transplants in six patients; unfortunately, five of these failed because of postoperative complications. There was also a 50% rate of retransplantation.

A recent development in this area was a report of 20 uncontrolled DCD liver transplants after a period of extracorporeal membrane oxygenation (ECMO). This technique has the advantage of using normothermic recirculation of oxygenated blood while the donor team waits for permission from the family or relatives for organ donation [10]. Hepatocytes isolated from DCD livers have been well-described [11] but not after a period of ECMO. The aim of this study was to evaluate the use of ECMO to resuscitate DCD porcine livers in a preclinical model using hepatocyte isolation and viability as a marker to assess whole-liver graft function.

2. Materials and methods

2.1. Organ procurement

We premedicated a randomized series of 15 female white Landrace pigs (31.9 ± 4 kg) intramuscularly with 10 mg/kg ketamine and 1 mg/kg midazolam. We then cannulated an ear vein and induced anesthesia using 0.5 mg/kg propofol. Each animal was intubated using an extended endotracheal tube. We used pulse oximetry via a tail probe to assess oxygenation. After intubation, we inserted central lines into both the carotid artery and jugular vein to allow for collection of blood and fluid replacement and arterial pressure monitoring. Anesthesia was maintained with intravenous propofol (1 mg/kg/h) throughout the procedure. Animal care was in accordance with the project license accepted by the UK Home Office. We performed an initial laparotomy and inserted both arterial (aorta) and venous (IVC) catheters. We then closed the abdomen. A laparoscopic port was included in the abdominal closure adjacent to the umbilicus for intraperitoneal cooling to mimic the human situation [12,13]. Each pig was culled with 10 mL KCl and subjected to 30 min warm ischemia. After this, we infused 1 million units of streptokinase and 20 mg phentolamine and heparin 10,000 IU directly into the aorta [14]. Animals were then randomized to either ECMO or conventional intravascular cooling combined with peritoneal cooling/cold intravascular perfusion with histidine-tryptophan-ketoglutarate (HTK) (Custodial, Canada).

We subjected all groups to 30 min warm ischemia after cardiac death. Group 1 ($n = 5$) had 2 h of cold intraperitoneal cooling [12,13] combined with conventional intravascular cooling. Group 2 ($n = 6$) involved 2 h of ECMO bypass; and group 3 acted as a control group ($n = 4$) that had conventional intravascular cooling with HTK (Custodial), followed by standard liver retrieval.

2.2. Group 1: peritoneal cooling circuit–cold intravascular perfusion

We infused peritoneal dialysis fluid (2.5 L) into the peritoneal cavity via the gas inlet of the laparoscopic port. Once the peritoneal cavity was filled, we connected a 2-m length of flexible, inert silicone elasomer tubing (8 mm) to a stainless-steel coil. We connected another 2-m length of tubing to the other end of the stainless-steel coil, with the free end inserted into the lumen of the laparoscopic port, before introduced it to the pelvis to complete the circuit. The coil was then placed into ice and one length of the tubing was placed into a peristaltic pump (Watson Marlow, Falmouth, UK). We took care to vent air from the refrigeration unit and pump. The peritoneal dialysis fluid from the pelvis passed to the coil and chilled fluid, then irrigated the peritoneal cavity for a further 2 h [13]. Cold intravascular perfusion was performed simultaneously through the double-balloon aortic cannula with HTK and exsanguination through the distal IVC (Fig. 10).

2.3. Group 2: ECMO

The ECMO circuit consisted of a cardiopulmonary bypass pump. Flow was maintained with the addition of 1 L 0.9% normal saline. Arterial inflow was through the double-balloon aortic cannula and the venous outflow was through the IVC. We maintained oxygenation flow at 4–5 L/min with the temperature at 38°C throughout the 2-h period of ECMO. Venous oxygen saturation of circulating blood was maintained at 30%–40%.

2.4. Group 3

The control group underwent simple cold storage in which the organ was flushed with 5 L cold HTK solution. The intravascular cooling was carried out via a double balloon in the aortic cannula and venous exsanguination through the IVC cannulation. The livers were then retrieved and viability was assessed.

2.5. Liver retrieval and ex vivo liver reperfusion

After preservation, we performed a midline laparotomy. The hepatectomy was performed by standard procedure [14]. After