

Normothermic extracorporeal human liver perfusion following donation after cardiac death

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Liver transplantation is the best treatment for patients with end-stage liver disease, selected patients with hepatocellular carcinoma and patients with fulminant liver failure. However, demand for liver transplantation continues to exceed organ supply.^{1,2} The shortage of available and suitable organs has led to increasing consideration of organs procured by donation after cardiac death (DCD). Unfortunately, DCD livers are particularly sensitive to the combination of warm and cold ischaemic injury during the period of cardiac death and organ procurement. Such injury leads to a high risk of ischaemic cholangiopathy³ and primary graft failure,⁴ and DCD remains a limited source of transplantable livers. To improve the ability to transplant DCD livers, investigators have begun to investigate novel techniques of organ preservation.⁵⁻¹⁰ These techniques have included animal studies of hypothermic machine perfusion⁵ and normothermic machine perfusion with oxygenated blood.⁶⁻¹⁰ Similar approaches have been used to improve the viability and transplantability of heart, kidney and lung grafts. To further explore the short-term feasibility and functional efficacy of normothermic extracorporeal liver perfusion (NELP) of DCD livers, we performed a proof-of-concept experiment using a human liver obtained in a case of DCD.

Methods

Ethics approval for the study was obtained from the human research ethics committees of the Austin Hospital (Austin HREC H2012/04549) and the Australian Red Cross Blood Service (host organisation of DonateLife Victoria). Written informed consent was obtained from the patient's family to use the procured liver for the experiment.

Donor

The donor was a 67-year-old male with no previous history of liver disease, who underwent DCD following withdrawal of life-sustaining therapy after a severe high spinal cord injury. Mechanical ventilation was withdrawn in the context of routine palliative care provided by the treating clinician, who had no role in the discussions, consent or any other aspect of the DCD process. The patient was pronounced dead 20 minutes after extubation. Lack of cardiac function

ABSTRACT

- Liver transplantation is a major life-saving procedure and donation after cardiac death (DCD) has increased the pool of potential liver donors.
- However, livers procured after DCD are at increased risk of primary graft dysfunction and biliary tract ischaemia. Normothermic extracorporeal liver perfusion (NELP) may increase the ability to protect, evaluate and, in future, transplant DCD livers.
- We conducted a proof-of-concept experiment using a human liver procured by DCD (deemed not suitable for liver donation) to assess the short-term (3 hours) feasibility, histological effects and functional efficacy of NELP.
- We used an extracorporeal membrane oxygenation circuit with separate hepatic artery and portal vein perfusion to achieve physiological perfusion pressures, and coupled this with parenteral nutrition and an insulin infusion. We achieved NELP with evidence of liver function (bile production, paracetamol removal and control of ammonia, bilirubin and lactate levels) for 3 hours. There was essentially normal liver and biliary tract histology after 8 hours of perfusion.
- Our experiment justifies further investigation of the feasibility and efficacy of human DCD liver preservation by NELP.

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was observed for the required period, and the patient was transported from the intensive care unit to the operating theatre for organ procurement.¹¹ At the time of laparotomy, the liver was deemed unsuitable to use clinically for liver transplantation.

Perfusion circuit

An extracorporeal perfusion circuit was constructed using a hollow-fibre oxygenator (Affinity CB511, Medtronic), a centrifugal pump (CBBP-80 Plus, Medtronic), a 1900 mL soft shell reservoir (MVR, Medtronic), polyvinyl chloride tubing (3/8 inch internal diameter, Medtronic), a gate clamp, pressure transducers (ITL Healthcare) and flow probes (DP-38, Medtronic).

Table 1. Changes in liver biochemistry and paracetamol levels during NELP

Parameter	Time after commencement of NELP (mins)			
	0	60	120	180
Total bilirubin ($\mu\text{mol/L}$)	3	3	3	2
ALP (U/L)	57	59	43	35
ALT (U/L)	6719	8197	7909	6447
GGT (U/L)	70	80	66	54
Ammonia ($\mu\text{mol/L}$)	169	140	152	147
	Time after addition of paracetamol (mins)			
	0	60	120	180
Paracetamol ($\mu\text{mol/L}$)	268	16	<8	<8

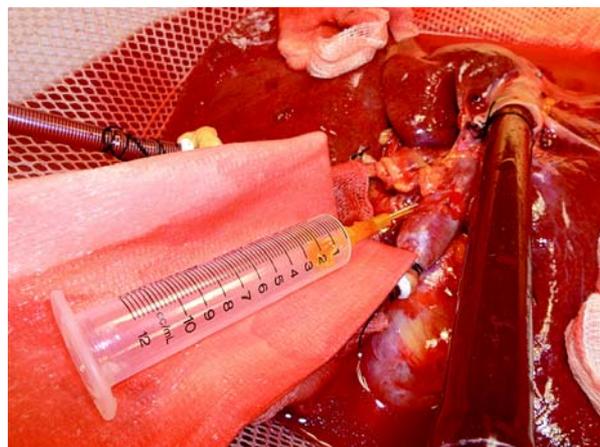
NELP = normothermic extracorporeal liver perfusion.
 ALP = alkaline phosphatase. ALT = alanine aminotransferase.
 GGT = γ -glutamyltranspeptidase.

After the centrifugal pump, an additional roller pump (COBE Cardiovascular) was also added to the circuit to perfuse the hepatic artery. Thus, two different pumps provided perfusion of the hepatic artery and the portal vein separately. The circuit was primed with 2 L of autologous blood obtained from the donor by cannulation of the inferior vena cava at the time of liver procurement, and with Hartmann's solution 500 mL. The oxygenator was attached to a heat exchanger to maintain blood temperature at 38°C.

Nutrition was provided to the reservoir and delivered to the liver during NELP using a 1000 mL bag of parenteral nutrition solution (Kabiven G19%, Fresenius Kabi) in which 200 units of insulin were added throughout the period of liver perfusion. The infusion was delivered at 5 mL/hour.

Before cessation of circulation, 25 000 units of heparin were administered to the donor intravenously. After a midline abdominal incision, the aorta was cannulated and in-situ perfusion by gravity through the aorta with cold (4°C) histidine–tryptophan–ketoglutarate (HTK) solution (NordMedica) was commenced. Aortic perfusion started 16 minutes after the donor blood pressure decreased to < 50 mmHg. Portal venous perfusion was not performed. The inferior vena cava was cannulated and 2 L of blood were collected into empty intravenous infusion bags via gravity drainage. The blood was then used for circuit priming. A clamp was placed across the supraceliac aorta. The abdominal organs were surrounded with iced saline slush. The liver was then removed and transferred on to the back table in a container filled with ice-cold solution.

Back-table perfusion with gravity-fed HTK was undertaken, with 500 mL through the portal vein, 200 mL through

Figure 1. Liver perfused by normothermic extracorporeal circuit, with oxygenated blood entering the hepatic artery and portal vein, and deoxygenated blood exiting via the inferior vena cava**Figure 2. Liver perfused by normothermic extracorporeal circuit with bile production**

the hepatic artery and 200 mL through the bile duct. Preparation before perfusion included ligation of possible bleeding vessels, identification of the bile duct and dissection of vessels for subsequent perfusion. This was 46 minutes after cessation of circulation. The time taken from a systolic blood pressure of 50 mmHg to perfusion was 29 minutes.

NELP support

The preservation solution was flushed with 500 mL of Hartmann's solution at 4°C through the portal vein. The common hepatic artery (Sarns 20 gauge cannula, Terumo), inferior vena cava stump (32 gauge bullet-tipped cannula,

Figure 3. Contrast-enhanced ultrasound imaging of macrovascular and microvascular perfusion during normothermic extracorporeal liver perfusion

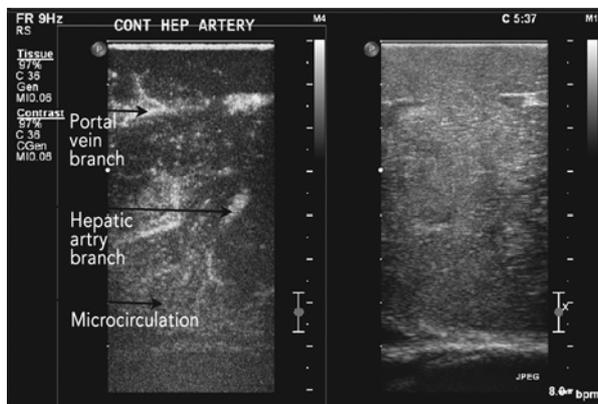
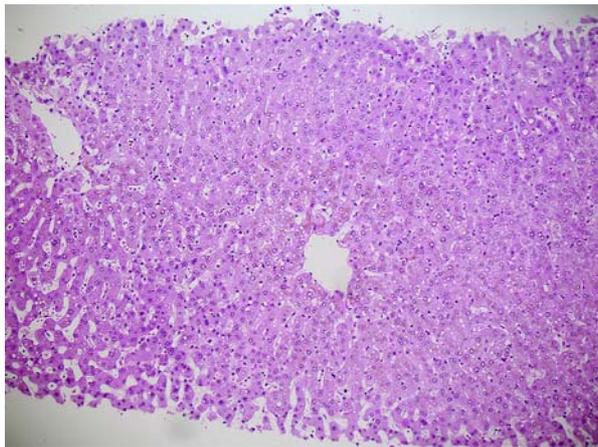


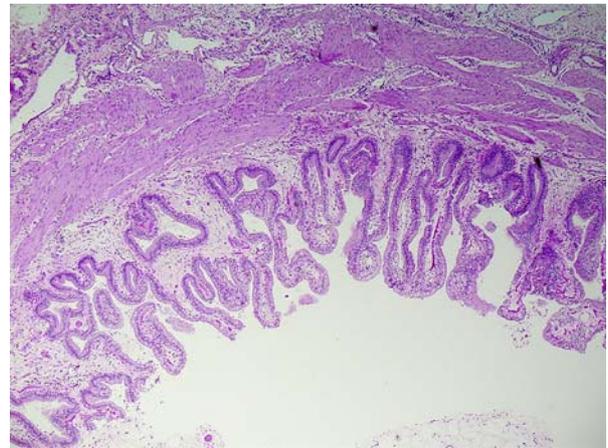
Figure 4. Histological section from core liver biopsy, obtained 4 hours after initiation of normothermic extracorporeal liver perfusion, showing no histological evidence of injury



Medtronic) and portal vein (EOPA 22 gauge cannula, Medtronic) were cannulated. The liver was suspended in a net and placed in a container filled with Hartmann's solution so that it could float and was not subject to pressure areas. At 167 minutes after cardiac death, NELP perfusion was started and immediately the liver became uniformly perfused (Figure 1).

The portal vein flow was maintained at 750–1000 mL/minute at a pressure of around 5–15 mmHg, and the hepatic artery flow was maintained at 200–300 mL/minute at a perfusion pressure of 40–50 mmHg. After 10 minutes of full perfusion, prostacyclin 1.5 mg (Flolan, Glaxo Wellcome) was added to the reservoir for endothelial protec-

Figure 5. Common bile duct section, obtained after almost 8 hours of normothermic extracorporeal liver perfusion, showing minimal epithelial changes and no evidence of necrosis



tion, and nutrition was started. Perfusate samples were taken during the perfusion every 60 minutes for the first 3 hours, and oxygen flow and air flow to the oxygenator were adjusted to maintain a P_{aO_2} of 80–100 mmHg and a P_{aCO_2} of 30–50 mmHg. Parenchymal (14G core) and common bile duct (2 mm long transection) biopsies were performed hourly.

Because the liver had limited but continued bleeding from small arterial bleeding points in the porta hepatis or the sites of liver biopsies, extra Hartmann's solution was added to the reservoir to maintain extracorporeal circuit flow.

Assessment of hepatic function after institution of NELP

After 60 minutes of NELP, paracetamol was administered as a 200 mg bolus into the reservoir to investigate the liver's metabolic capacity.

After the first 3 hours of formal assessment, perfusion continued for another 5 hours, but due to continued bleeding from the biopsy sites and a limited supply of donor blood, the haemoglobin level progressively fell to 10 g/L and no further functional assessments were performed.

Microvascular perfusion assessment

Perfusion at a microvascular level was qualitatively assessed using echo contrast ultrasonography. We performed all measurements using an IU22 ultrasound system (Philips) with an L9 probe (3 MHz). A contrast-specific mode with a low mechanical index (MI) (0.06) (R1) was used. Receive gain and scanning depth was kept constant for all measurements.

The ultrasound contrast agent (Sonovue, Bracco Diagnostics) was continuously infused at a rate of 1 mL/minute using a syringe pump (VueJect, Bracco Imaging). After the start of the infusion, we allowed a 2-minute equilibration period and performed five destruction–reperfusion sequences. Contrast microbubble destruction was achieved by applying five pulses at a high MI (flash:MI ratio, 1.24) and refilling observed with a low MI (15 seconds refilling time). We ascertained full destruction of contrast agent in the scan plane before performing the procedure.

Histological assessment

Bile duct, liver core biopsies and the whole liver were fixed in 10% neutral buffered formalin, processed and stained with haematoxylin and eosin.

Results

Biochemical and bile generation assessment

The lactate concentration decreased from 19 mmol/L at baseline to 14.9 mmol/L, 5.7 mmol/L and 2.7 mmol/L after 1, 2 and 3 hours of NELP, respectively. Glucose concentrations remained elevated (>25 mmol/L) despite the injection of additional insulin (150 units) into the circuit. The evolution of liver enzyme, bilirubin and ammonia levels are shown in Table 1. Bile production was maintained (Figure 2), and microvascular perfusion was demonstrated by contrast-enhanced ultrasonography (Figure 3).

Drug clearance assessment

The paracetamol concentration declined to nearly undetectable or undetectable levels within 60 minutes of injection and was undetectable after that (Table 1).

Histological assessment

The liver core biopsies taken at 1, 2 and 4 hours showed minor abnormalities with mild subcapsular ischaemia in the 2-hour biopsy, but an otherwise normal appearance (Figure 4). Focal mild centrilobular ischaemic changes were seen in the 6-hour biopsy. No intrahepatic bile duct damage, portal tract inflammation or oedema were seen in these biopsies, and the biopsies from the common bile duct from 1 to 6 hours were normal.

Mild epithelial swelling, vacuolation and reactive changes, and very mild mucosal inflammation were seen only in the whole liver specimen (taken at 8 hours). In this specimen, the portal tracts showed mild lymphoplasmacytic inflammation, with occasional mild infiltration of ducts, which were otherwise normal. The bile ducts of all calibres were viable and intact even at the conclusion of the experiment (Figure 5).

Discussion

Key findings

We conducted a proof-of-concept experiment, using NELP after DCD, to assess the short-term feasibility and efficacy of such treatment of a human liver over an extended ex-vivo perfusion period. We found that, using NELP, parenteral nutrition, separate hepatic artery and portal vein perfusion and physiological perfusion pressures, we achieved successful restoration and maintenance of function for 3 hours. During this period, the liver was able to normalise lactate levels, metabolise paracetamol, produce bile, decrease liver enzymes, maintain normal bilirubin levels and prevent escalation in ammonia levels. Histological assessment showed no evidence of ischaemic infarction and most of the parenchyma appeared normal. Importantly, there was no evidence of intrahepatic or extrahepatic biliary ischaemia.

Comparison with previous studies

One significant advantage of NELP is that it confers the ability to assess real-time liver viability during preservation. Butler et al reported successful ex-vivo normothermic extracorporeal porcine liver perfusion for 72 hours.⁹ During this period, the isolated livers maintained acid–base balance, electrolytes, protein synthesis (complement and factor V) and bile production. Normothermic perfusion preservation has also been shown to be superior to conventional cold storage in terms of such viability analyses.^{6,7} Xu et al delivered normothermic machine perfusion, after 2 hours of cold storage in preserving solution, to livers obtained after 60 minutes of warm ischaemia in pigs, and found restoration of metabolic and functional parameters after 4 hours.¹² Fondevila et al studied normothermic perfusion and oxygenation in pig livers, but only for a limited period before transplantation, only after donor treatment with ECMO, and they performed no functional assessment.¹³ Finally, Gravante et al perfused livers after a minimal ischaemic time and after cold protection and transportation from the abattoir to the laboratory, and they performed no functional tests.¹⁴ We recently reported acceptable liver function and histology in a model of porcine DCD.¹⁵

No human NELP has yet been reported in the medical literature, but a recent report in the lay press¹⁶ indicates that at least two human livers have now been treated with a form of NELP. Although no details are available, it appears that such livers were likely to be from brain-dead donors, because they were successfully transplanted after such perfusion. Thus, to our knowledge, this is the first report of NELP immediately after DCD and warm ischaemia in a human liver, with maintained perfusion for several hours and functional and pharmacological assessment. Further confirmation of the reproducibility of such technology and

the technique, and improvement in the management of bleeding from the perfused liver are important to the evolution of DCD liver protection, preservation and possibly subsequent transplantation methods in humans.

Significance of study findings

To our knowledge, this is the first successful experimental preservation of a human DCD liver using NELP. The results of our experiment provide further support for the notion that NELP is a viable technique. In the future, NELP may help expand the donor pool by increasing the use of viable livers. In addition, it may be used to provide liver-replacement therapy in conjunction with conventional extracorporeal blood purification techniques for patients with fulminant hepatic failure.

Conclusions

Our experiment demonstrates the ability to maintain good liver function in a human DCD liver for 3 hours, with a normal histological appearance after 8 hours of perfusion, using NELP delivered with parenteral nutrition, separate hepatic artery and portal vein perfusion and physiological perfusion pressures. This technique offers promise as a protection from ischaemic injury for DCD livers and a way of enabling their extended assessment and possible subsequent use for transplantation.

Competing interests

None declared.

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