Extended normothermic extracorporeal perfusion of isolated human liver after warm ischaemia: a preliminary report

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ABSTRACT

Background: Donation after circulatory death (DCD) livers are at markedly increased risk of primary graft dysfunction and biliary tract ischaemia. Normothermic extracorporeal liver perfusion (NELP) may increase the ability to transplant DCD livers and may allow their use for artificial extracorporeal liver support of patients with fulminant liver failure.

Objective: We conducted two proof-of-concept experiments using human livers after DCD to assess the feasibility and functional efficacy of NELP over an extended period.

Methods: We applied extracorporeal membrane oxygenation, parenteral nutrition, separate hepatic artery and portal vein perfusion and physiological perfusion pressures to two livers obtained after DCD.

Results: We achieved NELP and evidence of liver function (bile production, paracetamol removal and maintenance of normal lactate levels) in both livers; one for 24 hours and the other for 43 hours. Histological examination showed areas of patchy ischaemia but preserved biliary ducts and canaliculi.

Conclusions: Our experiments justify further investigations of the feasibility and efficacy of extended DCD liver preservation by ex-vivo perfusion.

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Liver transplantation is the best treatment for patients with end-stage liver disease, but demand for liver transplantation continues to markedly exceed organ supply.[1,2] This shortage of organs has led to increasing consideration of donation after circulatory death (DCD). DCD livers are particularly sensitive to warm ischaemic injury during the period of cardiac arrest before organ procurement. Such injury leads to a high risk of ischaemic cholangiopathy[3] and early graft loss.[4] Thus, DCD remains a limited source of transplantable livers.[5]

To improve the ability to transplant DCD livers, investigators have begun to assess novel techniques of organ preservation.[1] These techniques have included hypothermic machine perfusion[6] and normothemic machine perfusion with oxygenated blood.[6-11] We recently reported the feasibility of normothermic extracorporeal liver perfusion (NELP) of pig livers[12] and, for a few hours, of human livers.[13]

Another possible use of DCD livers is in the development of techniques for extracorporeal blood purification for the supportive treatment of patients with fulminant liver failure. For such therapy, the organ procured via DCD would have to remain functional for extended periods in order to provide sufficient detoxification. Whether such extended function with extracorporeal perfusion can actually be achieved with human livers remains unknown. To further explore the feasibility of extended (24 hours or greater) NELP of DCD livers, we conducted proof-of-concept studies using human DCD livers.

Methods

Ethics approval for the study was obtained from the Austin Health Human Research Ethics Committee (Austin HREC H2012/04549) and the Australian Red Cross Blood Service (host organisation of Donate Life, Victoria). Our study was funded by the Austin Tissue and Organ Donation Group, Austin Hospital, Melbourne, Australia, and the Austin Hospital Liver Transplantation Unit Research Fund. Written informed consent was obtained from the patients' families to use the procured livers for the experiments. The donors were a 67-year-old woman and a 64-year-old man whose livers were deemed unsuitable for transplantation.

Perfusion circuit

An extracorporeal perfusion circuit was constructed using a hollow-fibre oxygenator (Affinity CB511, Medtronic), a centrifugal pump (BP-50, Medtronic), a soft shell reservoir, polyvinyl chloride tubing (PVC ¼ to 3/16 inch internal diameter, Medtronic), a gate clamp, pressure transducers (ITL Healthcare) and flow probes (DP38P, Medtronic). After the centrifugal pump, an additional roller pump (Cobe) was added to the circuit to perfuse the hepatic artery. Thus, these two different pumps provided perfusion of the

Figure 1. Liver with cannulae in situ; liver is suspended in a net to avoid pressure injury

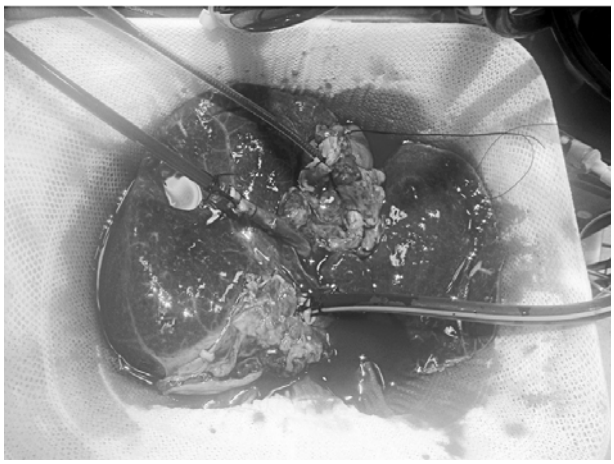
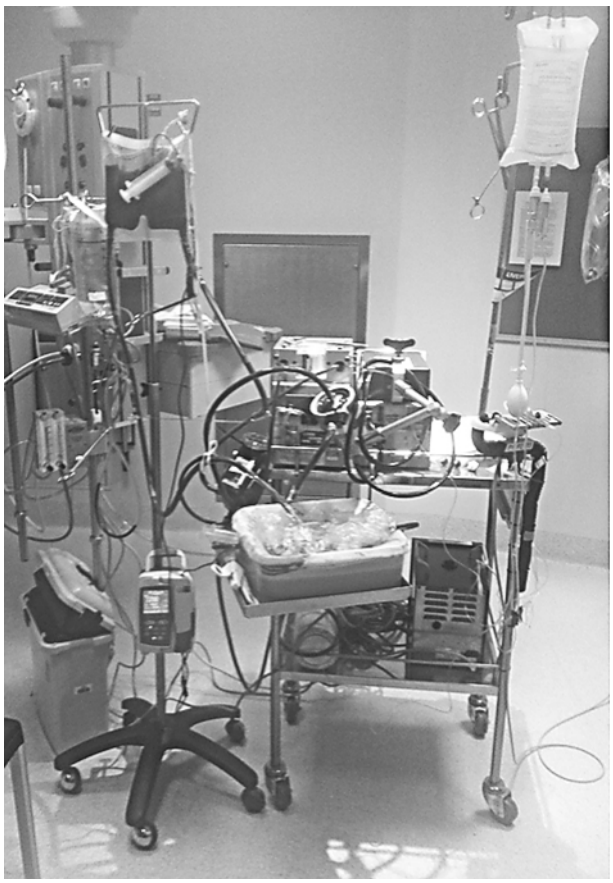


Figure 2. Normothermic extracorporeal liver perfusion circuit in operation; all components can be seen (reservoir, pumps, oxygenators, transducers and perfused liver)



hepatic artery and portal vein separately. The oxygenator was attached to a heat exchanger to maintain blood temperature at 39°C. Air and oxygen sources were used and flows and ratios adjusted to maintain near physiological partial pressures of carbon dioxide (CO_2) (between 20 mmHg and 50 mmHg) and oxygen (O_2) (between 60 mmHg and 160 mmHg).

Nutrition was provided to the reservoir and delivered to the liver during NELP using 100 mL of parenteral nutrition solution (Kabiven G19%, Fresenius Kabi) to which 200 units of insulin was added. The parenteral infusion was delivered at 5 mL/hour.

Before withdrawal of life support and after obtaining consent from the person responsible, 25 000 units of heparin were administered to the patient by intravenous (IV) injection. After circulatory death, the patients were transferred to the operating theatre. After a midline abdominal incision, the aorta was cannulated and in situ perfusion by gravity with cold (4°C) histidine–tryptophan–ketoglurate (HTK) solution (Normedica) was commenced. Portal venous perfusion was not performed. The inferior vena cava was cannulated and 2 L of blood was collected into empty IV infusion bags via gravity drainage. The blood was then used for circuit priming. A clamp was placed across the supracoeliac aorta. The abdominal organs were surrounded with saline slush. The liver was then removed and transferred to the back table in a container filled with ice-cold solution.

Back-table perfusion with gravity-delivered HTK solution was undertaken, with 500 mL through the portal vein, 200 mL through 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. For the first liver, the time from systolic blood pressure of 50 mmHg to perfusion was about 150 minutes and the time from ciculatory death to perfusion was 120 minutes. For the second liver, the time from systolic blood pressure of 50 mmHg to perfusion was about 215 minutes and the time from circulatory death to perfusion was 200 minutes.

NELP

The preservation solution was flushed with 500 mL of Hartmann solution at 4°C through the portal vein. The aortic stump with the common hepatic artery connecting it with the liver, the inferior vena cava stump and portal vein were cannulated with suitable cannulae varying from 22 to 32 Fr gauge in diameter (Figure 1). The liver was suspended in a net and placed in a container filled with balanced crystalloid solution such that it could float and not be subject to pressure injury. At 180 minutes after death in

Table 1. Liver biochemical measurements during normothermic extracorporeal liver perfusion (NELP)

	Time after NELP started (hours)				
	0	6–12	12–24	24–36	36–48
Liver 1					
Bilirubin, total, μmol/L	2	2	7	NA	NA
ALP, U/L	23	21	87	NA	NA
ALT, U/L	490	511	1256	NA	NA
AST, U/L	937	1073	2957	NA	NA
Ammonia, μmol/L	28	35	79	NA	NA
Lactate, mmol/L	4.9	4.1	2.2	NA	NA
Glucose, mmol/L	11.1	15.6	21.8	NA	NA
Liver 2					
Bilirubin, total, μmol/L	5	na	6	na	na
ALP, U/L	74	na	83	na	na
ALT, U/L	3362	na	4013	na	na
AST, U/L	na	na	na	na	na
Ammonia, μmol/L	169	na	152	na	na
Lactate, mmol/L	9.9	2.3	1.9	na	1.4
Glucose, mmol/L	12.3	15.7	17.7	na	6.9

NA = not applicable. ALP = alkaline phosphatase. ALT = alanine aminotransferase. AST = aspartate aminotransferase. na = not available.

Patient 1, and at 215 minutes after death in Patient 2, NELP was initiated and the livers became uniformly perfused (Figure 2).

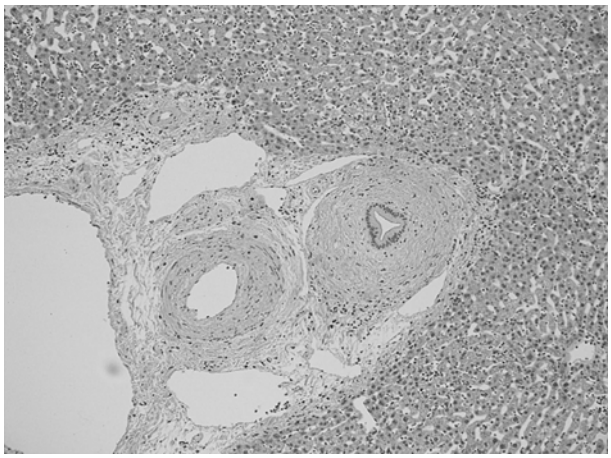
During the experiment, the portal flow was maintained at 700–1000 mL/min at a portal pressure of 5–15 mmHg and the hepatic artery flow was maintained at 100–300 mL/min with a perfusion pressure between 40 mmHg and 60 mmHg. After 10 minutes of NELP, epoprostenol (prostacyclin) (Flolan, GlaxoSmithKline) was added to the reservoir. Oxygen flow was adjusted to maintain Pa_{O_2} at 60–120 mmHg and Pa_{CO_2} at 20–60 mmHg. Both livers had continued bleeding from vessels in the porta hepatis and around the aortic stump into the container of the supporting bath. Hence, a sump circuit was set up to pump the serosanguinous ooze back into the circuit at pump speeds varying from 10 to 60 mL/min (Figure 2).

Table 2. Paracetamol removal during normothermic extracorporeal liver perfusion (NELP)

	Paracetamol level, μmol/L		
Time after NELP initiated	5–10 min*	60 min*	120 min*
Liver 1			
2 hours	444	11	< 8
22 hours	231	24	< 8
Liver 2			
2 hours	161	48	< 8
22 hours	277	113	65
40 hours	250	117	80

* Time from paracetamol injection to measurement.

Figure 3. Histological appearance of bile ducts after 24 hours of perfusion (appearance is normal)



Assessment of hepatic function during NELP

After NELP was in place and stable, paracetamol (200 mg by IV push) was injected into the reservoir to investigate the liver's ability to metabolise it. This process was repeated twice after about 20 then 40 hours, with sampling for paracetamol levels at 1 hour and 2 hours after each injection. Perfusion was continued unchanged for the observation period. At the time of paracetamol administration, blood was taken for measurement of liver enzymes, bilirubin and ammonia levels.

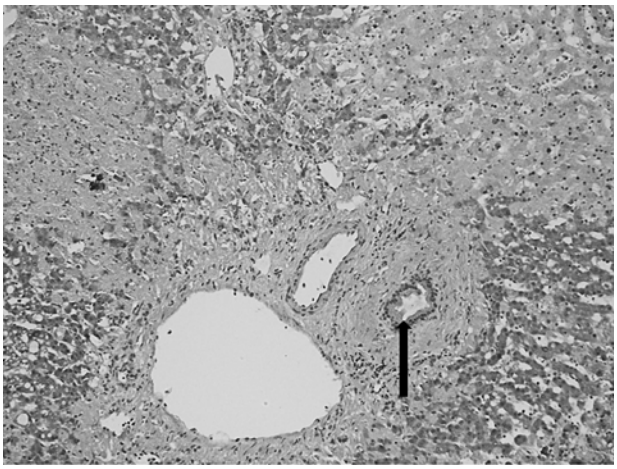
Results

Throughout the experiment both livers had normal macroscopic appearance, were soft to touch and appeared initially uniformly well perfused. The second liver, however, began to show visible peripheral patches of likely ischaemia after > 30 hours of perfusion. NELP was delivered for 24 hours to the first liver and for 43 hours to the second liver.

Functional assessment

Bile production was visibly maintained throughout the experiment for both livers. The blood lactate concentration decreased from a high baseline level to normal levels in both experiments, and glucose levels remained high despite

Figure 4. Histological appearance of liver parenchyma after 43 hours of perfusion. A normal bile duct (arrow) can be seen surrounded by areas of normal parenchyma and patchy infarction (pale areas, upper left and right)



the addition of another 100 units of insulin to the reservoir during the experiments. The available changes in biochemical measurements during the two perfusion experiments are shown in Table 1. Paracetamol was effectively removed during both experiments within 120 minutes of injection, although the effectiveness of such removal decreased with time (Table 2).

Histological assessment

The liver perfused for 24 hours showed patchy areas of ischaemic hepatocyte injury focally involving Zones 2 and 3, and well preserved biliary ducts (Figure 3).

The liver perfused for 43 hours showed larger and more frequent patches of ischaemia in centrilobular areas, extending focally up to Zone 1. Portal tract changes included bile ductular proliferation, periductal oedema, and mild mixed inflammation along with canalicular and bile ductile cholestasis. Bile ducts (small ducts in portal tracts and larger ducts) all appeared normal or, at most, showed mildly irregular epithelium. All these changes were considered to be similar in nature to those seen in early liver transplant biopsies, and were interpreted as ischaemia–reperfusion injury.

Discussion

Key findings

We conducted a proof-of-concept experiment of extended NELP of two human livers after DCD to assess the feasibility, efficacy and sustained liver functional capacity with such treatment over a 24- and 43-hour perfusion period. By using NELP with separate hepatic artery and portal vein cannulation, parenteral nutrition and physiological perfusion pressures and gas tensions, we achieved successful restoration and maintenance of function for an extended period. During this period, the liver was able to normalise lactate levels, metabolise paracetamol, produce bile and, in the case of the first liver, broadly contain ammonia levels. Moreover, histological assessment showed only limited evidence of ischaemic injury to liver cells or the biliary tract at 24 hours.

In the liver that was perfused for 43 hours, however, paracetamol removal deteriorated over time and histological analysis revealed several large patches of hepatocyte ischaemia even though significant bile duct injury was not seen (Figure 4).

Relationship to previous findings

One significant advantage of NELP is that it confers the ability to assess real-time liver viability during preservation. Butler and colleagues reported successful normothermic extracorporeal porcine liver perfusion for 72 hours.[9] During this period, the isolated livers maintained acid–base balance, electrolytes, protein synthesis and bile production. Normothermic perfusion has also been shown to be superior to conventional cold storage in terms of such viability analyses.[6,7] We have previously reported successful NELP of postDCD porcine livers[12] and the successful short-term (several hours) normothermic perfusion of a human liver obtained after DCD.[13] We have now achieved human liver perfusion after DCD and have maintained it in two livers for a period of 24 and 43 hours, respectively. To our knowledge, no other groups have reported such extended maintenance of NELP.

Significance of findings

The results of this experiment provide further support for the notion that NELP is a reproducible technique. To our knowledge, this is the first successful perfusion and functional preservation of a human liver after DCD using NELP for such an extended period. This technique, once refined, might open the door to the possible use of DCD livers for extracorporeal support of patients with fulminant liver failure.

Conclusions

Our experiments show the ability to maintain liver perfusion and function in a human DCD liver for > 40 hours, using NELP. This promising technique offers a means of protecting DCD livers and enabling their preservation for periods sufficient to potentially allow several options for use from possible transplantation to enabling extracorporeal liver support, or both.

Competing interests

None declared.

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