Normothermic extracorporeal perfusion of isolated porcine liver after warm ischaemia: a preliminary report

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ABSTRACT

- Liver transplantation is a major life-saving procedure, and donation after cardiac death (DCD) has increased the pool of potential liver donors.
- However, DCD livers 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 proof-of-concept experiments using a DCD model in the pig to assess the short-term (4 hours) feasibility and functional efficacy of NELP. Using extracorporeal membrane oxygenation, parenteral nutrition, separate hepatic artery and portal vein perfusion, and physiological perfusion pressures, we achieved NELP and evidence of function (bile production, paracetamol removal, maintenance of normal ammonia and lactate levels) for 4 hours in pig livers subjected to 15 and 30 minutes of cardiac arrest before explantation.
- Our experiments justify further investigations of the feasibility and efficacy of human DCD liver preservation by ex-vivo perfusion.

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Liver transplantation is the best treatment for patients with end-stage liver disease and fulminant liver failure. However, demand for liver transplantation continues to exceed organ supply.[1,2] The shortage of organs has led to increasing consideration of donation after cardiac death (DCD). Unfortunately, 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 primary graft failure.[4] Thus, 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 hypothermic machine perfusion[5] and normothermic machine perfusion with oxygenated blood.[6-10] To further explore the short-term feasibility and functional efficacy of normothermic extracorporeal liver perfusion (NELP) of a DCD liver, we have conducted proof-of-concept studies using DCD pig livers.

Methods

Animals

Ethics approval for the study was obtained from the Animal Research Ethics Committee of the School of Veterinary Science at the University of Melbourne. Large White cross Landrace pigs (40–50 kg) were procured for the experiment. The pigs were sedated with an intramuscular injection of ketamine hydrochloride (10 mg/kg; Parnell Laboratories, Alexandria, NSW, Australia) and xylazine hydrochloride (0.2 mg/kg; Ilium Xylazil-20, Troy Laboratories, Smithfield, NSW, Australia). An ear vein was cannulated and anaesthesia was induced using intravenous thiopentone sodium (10 mg/kg; Thiobarb powder, Jurox, Rutherford, NSW, Australia), followed by endotracheal intubation. General anaesthesia was maintained using isoflurane (Isorrane, Baxter Healthcare, Old Toongabbie, NSW, Australia) in oxygen and the pig's heart rate, SpO_2 and end-tidal CO_2 were monitored continuously.

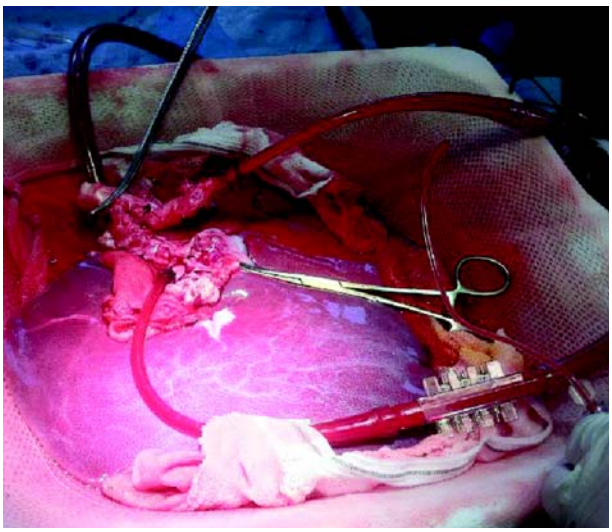
Perfusion circuit

An extracorporeal perfusion circuit was constructed using a hollow-fibre oxygenator (Affinity CB511, Medtronic Australasia, North Ryde, NSW, Australia), a centrifugal pump (CBBP-80 Plus, Medtronic), an 800 mL soft reservoir (MVR800, Medtronic), tubing (Medtronic, 3/8 inch internal diameter, polyvinyl chloride), a gate clamp, pressure transducers (ITL Healthcare, Chelsea Heights, Vic, Australia) and flow probes (DP-38, Medtronic).

After the centrifugal pump, a roller pump (Cobe Cardiovascular Australia, Dandenong, Vic, Australia) was also added to the circuit to perfuse the hepatic artery. Thus, these 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 animal before liver procurement and 500 mL of Hartmann's solution. 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 animal during NELP by an infusion of 100 mL of parenteral nutrition solution (Kabiven G19%, Fresenius Kabi, Bad Homburg, Germany) in which 100 units of insulin were added throughout the period of liver perfusion. The infusion was delivered at 10 mL/h.

Figure 1. Liver perfused by normothermic extracorporeal circuit with oxygenated blood entering the hepatic artery and portal vein and exiting deoxygenated via the inferior vena cava cannula in Pig 1



Before surgery, 25 000 units of heparin were administered to the pig intravenously. After a midline abdominal incision, the iliac vein 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.

Thirty minutes after the incision, cardiac arrest was induced by intravenous injection of pentobarbitone sodium (200 mg/kg) (Lethabarb, Virbac (Australia), Milperra, NSW, Australia). Fifteen minutes after the cardiac arrest in the first animal and 30 minutes after cardiac arrest in the second animal, liver in-situ perfusion by gravity through the portal vein with cold (4°C) Euro-Collins solution (Baxter) commenced. The liver was then procured and transferred to the back table in a container filled with ice-cold solution for preparation before perfusion (ligation of possible bleeding vessels, identification of biliary duct, trimming of vessels for subsequent perfusion).

The preservation solution was then flushed with Hartmann's solution. The aortic stump (Sarns 20 gauge cannula, Terumo, Macquarie Park, NSW, Australia), inferior vena cava stump (32 gauge bullet-tipped cannula, Medtronic) and portal vein (22 gauge cannula, Medtronic) were cannulated. The liver was suspended in a net and placed in a container filled with haemofiltration replacement solution (Accusol, Baxter) so that it could float and was not subject to pressure areas. After 60 minutes of preparation of the liver and 90 minutes after cardiac arrest, normothermic extracorporeal membrane oxygenation (ECMO) perfusion was started and immediately the liver became perfused (Figure 1).

The portal vein flow was maintained at 750–1000 mL/min at a pressure of around 5–15 mmHg and hepatic artery flow maintained at 100–150 mL/min at a perfusion pressure of 40–50 mmHg. After 10 minutes of full perfusion, 1.5 mg of prostacyclin (Flolan, Glaxo Wellcome, United Kingdom) was added to the reservoir for endothelial protection and nutrition also started. Perfusate samples were taken during the perfusion every 60 minutes and oxygen flow and air flow to the oxygenator were adjusted to maintain PaO_2 between 80 and 100 mmHg and $PaCO_2$ between 30 and 50 mmHg.

Because the liver had trivial but continued bleeding from the branches of the vessels derived from the ligated mid-aorta used to ensure hepatic artery perfusion during NELP, extra Hartmann's solution was given into the reservoir to maintain ECMO flow in Pig 1, but not in Pig 2. Liver perfusion was discontinued electively at 240 minutes and tissue samples were taken for histological investigation.

Results

Biochemical and bile generation assessment

In both animals, lactate concentration decreased from elevated levels at the start to normal levels after 4 hours of perfusion. Glucose concentration fell from 22.2 mmol/L to 7.1 mmol/L in 120 minutes and was maintained within the reference range in the first animal but remained elevated despite additional insulin and decreased parenteral nutrition

Table1. Key arterial blood gas-derived indices during normothermic extracorporeal liver perfusion*

	Time after extracorporeal membrane oxygenation started (min)				
Variable	0	60	120	180	240
pH	7.51/7.15	7.23/7.28	7.39/7.31	7.34/7.41	7.55
PCO_2, mmHg	11/32	39/41	41/42	43/33	27.0
PO_2, mmHg	186/118	85/85	93/79	63/104	131
Glucose, mmol/L	22.2/37.0	13.7/44.0	7.1/36.0	5.2/23.2	4.9
Lactate, mmol/L	3.2/4.1	1.4/4.8	2.1/3.0	2.4/2.5	1.9

* Values for Pig 1 separated from values for Pig 2 by "/" symbol. No arterial blood gases measured in Pig 2 at 240 minutes.

Table 2. Liver biochemistry during normothermic extracorporeal liver perfusion (NELP)*

	Time after NELP started (min)				
Biochemistry	0	60	120	180	240
Bilirubin, total, μmol/L†	< 2	< 2	< 2	< 2	< 2
ALP, U/L	69/8	58/51	47/52	32/57	22/57
ALT, U/L	59/13	55/34	42/36	33/37	28/42
GGT, U/L	25/2	20/41	14/33	9/30	5/28
Ammonia, μmol/L	62/12	44/30	25/22	37/22	29/28

ALP = alkaline phosphatase. ALT = alanine aminotransferase. GGT = γ-glutamyltranspeptidase.
* Values are presented for each animal and separated by the / symbol; values for Pig 1 were influenced by dilution with Hartmann's solution due to the need to replace oozing from small vessels near the aortic stump. † Same value for both animals.

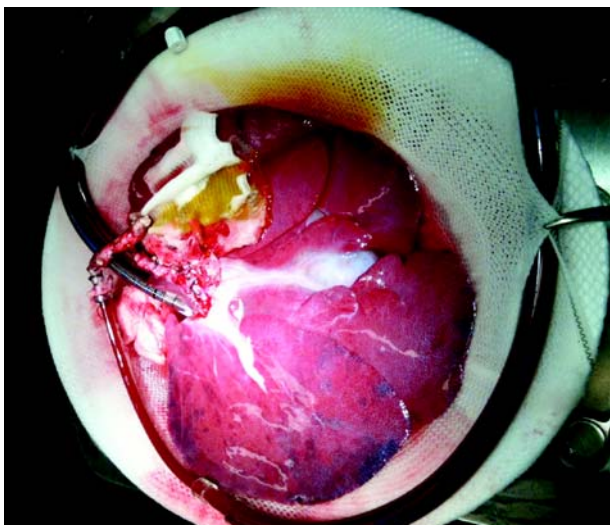
Table 3. Changes in paracetamol levels during normothermic extracorporeal liver perfusion (NELP), by experimental animal*

	Time after NELP started (min)				
	0	60	120	180	240
Pig 1	< 8 μmol/L	391 μmol/L	20 μmol/L	< 8 μmol/L	< 8 μmol/L
Pig 2	< 8 μmol/L	< 8 μmol/L	137 μmol/L	< 8 μmol/L	< 8 μmol/L

* Highest levels were immediately after administration.

in the second (Table 1). Liver enzymes remained in the normal or near normal range. Bilirubin levels remained low throughout the experiment. Ammonia was also maintained at essentially normal levels (Table 2). Total bile flow rate was about 8–12 mL/h (Figure 2).

Figure 2. Liver perfused by normothermic extracorporeal circuit with bile production



Some peripheral areas of the liver do not appear optimally perfused.

Drug clearance assessment

After 60 or 120 minutes of ECMO, paracetamol was administered as a bolus into the reservoir at a dose of 200 mg, to investigate the liver's metabolic capacity. Paracetamol concentration dropped to nearly undetectable or undetectable levels within 60 minutes (Table 3).

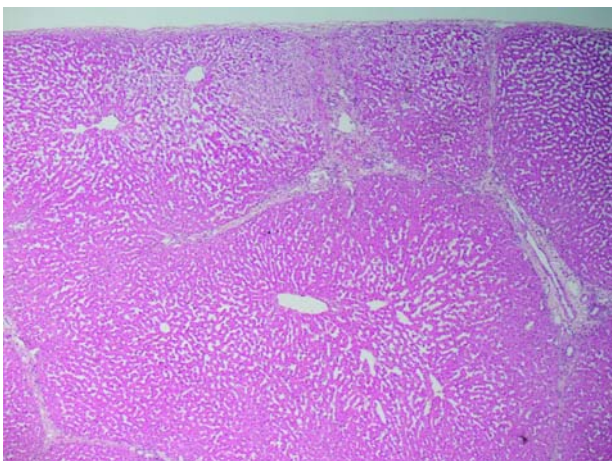
Histological assessment

Histological analysis found preservation of hepatic architecture with generalised sinusoidal dilatation. Both showed mild patchy ischaemic necrosis, predominantly subcapsular and involving zone 3 hepatocytes in Pig 2, and forming a 18 × 5 mm linear zone in Pig 1. These ischaemic foci were characterised by hepatocyte shrinkage, cytoplasmic pallor and/or vacuolation and nuclear pyknosis, and were estimated to involve about 5% (in Pig 1) and 15% (in Pig 2) of the sampled parenchyma (Figure 3). Hepatic infarction was not seen. There was no lobular inflammation, haemorrhage or cholestasis. The findings were consistent with preservation injury.

Discussion

We conducted proof-of-concept experiments using NELP after DCD in two pigs, to assess the short-term feasibility and efficacy of such treatment over a 4-hour perfusion period. We found that using NELP, parenteral nutrition,

Figure 3. Histological section of peripheral liver from Pig 2



There is an area of subcapsular ischaemia that corresponded to a macroscopically visible area of poor perfusion. The vast majority of the visible parenchyma is intact.

separate hepatic artery and portal vein perfusion and physiological perfusion pressures, we achieved successful restoration and maintenance of function for 4 hours. During this period, the liver was able to normalise lactate levels, metabolise paracetamol, produce bile and maintain normal liver enzyme, bilirubin and ammonia levels. Moreover, histological assessment showed no evidence of ischaemic infarction and most of the parenchyma appeared normal.

One significant advantage of normothermic machine perfusion is that it confers the ability to assess real-time liver viability during preservation. Butler et al reported the successful 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. Moreover, normothermic perfusion preservation has 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 that they achieved restoration of metabolic and functional parameters after 4 hours.[11] Fondevila et al studied normothermic perfusion and oxygenation in pig livers but only for a limited period of time before transplantation, only after donor treatment with extracorporeal membrane oxygenation, and with no functional assessment.[12] Gravante et al perfused livers after minimal ischaemic time and after cold protection and transportation from the abattoir to the laboratory. They also performed no functional tests.[13] Thus, to our knowledge, we are only the second group to have delivered NELP immediately after warm ischaemia and to have maintained it for several hours with functional and pharmacological assessment. Further confirmation of the reproducibility of such technology and technique is important to the evolution of liver preservation methods.

The results of the current experiment provide further support for NELP as a reproducible technique. To our knowledge, this is the first experimental successful DCD liver preservation using normothermic extracorporeal perfusion in Australia and New Zealand. This technique, once refined, might help expand the donor pool to marginal livers in the future. In addition, it may be used in conjunction with conventional blood purification techniques for patients with fulminant hepatic failure.

Conclusion

Our experiments demonstrate the ability to maintain good liver function in DCD livers for 4 hours, using NELP delivered with parenteral nutrition, separate hepatic artery and portal vein perfusion and physiological perfusion pressures. This technique offers a means of protecting DCD livers and enabling their extended assessment and possible use for transplantation.

Competing interests

None declared.

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References

1 Zamperetti N, Bellomo R, Piccinni P, et al. Reflections on transplantation waiting lists. *Lancet* 2011; 378: 632-5.
2 Organ Procurement and Transplantation Network; Scientific Registry of Transplant Recipients. 2010 OPTN/SRTR annual report: transplant data 2000–2009. http://www.srtr.org/annual_reports/2010/ (accessed Aug 2012).
3 Chan EY, Olson LC, Kisthard JA, et al. Ischemic cholangiopathy following liver transplantation from donation after cardiac death donors. *Liver Transpl* 2008; 14: 604-10.
4 de Vera ME, Lopez-Solis R, Dvorchik I, et al. Liver transplantation using donation after cardiac death donors: long-term follow-up from a single center. *Am J Transplant* 2009; 9: 773-81.
5 de Rougemont O, Breitenstein S, Leskosek B, et al. One hour hypothermic oxygenated perfusion (HOPE) protects nonviable liver allografts donated after cardiac death. *Ann Surg* 2009; 250: 674-83.
6 St Peter SD, Imber CJ, Lopez I, et al. Extended preservation of non-heart-beating donor livers with normothermic machine perfusion. *Br J Surg* 2002; 89: 609-16.
7 Imber CJ, St Peter SD, Lopez de Cenarruzabeitia I, et al. Advantages of normothermic perfusion over cold storage in liver preservation. *Transplantation* 2002; 73: 701-9.
8 Schon MR, Kollmar O, Wolf S, et al. Liver transplantation after organ preservation with normothermic extracorporeal perfusion. *Ann Surg* 2001; 233: 114-23.
9 Butler AJ, Rees MA, Wight DG, et al. Successful extracorporeal porcine liver perfusion for 72 hr. *Transplantation* 2002; 73: 1212-8.
10 Brockmann J, Reddy S, Coussios C, et al. Normothermic perfusion: a new paradigm for organ preservation. *Ann Surg* 2009; 250: 1-6.
11 Xu H, Berendsen T, Kim K, et al. Extracorporeal normothermic machine perfusion resuscitates pig DCD livers with extended warm ischemia. *J Surg Res* 2012; 173: e83-8.
12 Fondevila C, Hessheimer AJ, Maathuis M-H J, et al. Superior preservation of DCD livers with continuous normothermic perfusion. *Ann Surg* 2011; 254: 1000-7.
13 Gravante G, Ong SL, Metcalfe MS, et al. Cytokine response of electrolytic ablation in an ex vivo perfused liver model. *ANZ J Surg* 2010; 80: 537-41. ❑