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Ex-Vivo Perfusion of Donor Hearts: The Feasibility of Banked Blood for Normothermic Machine Perfusion

Hong Chee Chew [1, 2], Ling Gao [1], Jeanette E. Villaneuva [1], Aoife Doyle [1], Mark Hicks [1], Andrew Jabbour [1, 2], Kumud K. Dhital [1, 2], Peter S. Macdonald [1, 2, *]

1. Transplantation Laboratory, Victor Chang Cardiac Research Institute, Darlinghurst, Australia; E-Mails: chew.damac@gmail.com; l.gao@victorchang.edu.au; j.villaneuva@victorchang.edu.au; A.doyle@victorchang.edu.au; mark.hicks@svha.org.au; andrew.jabbour@svha.org.au; kdhital@me.com; peter.macdonald@svha.org.au
2. Heart Transplant Clinic, St Vincent's Hospital, Darlinghurst, Australia

* **Correspondence:** Peter S. Macdonald; E-Mail: peter.macdonald@svha.org.au

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Abstract

***Background*:** Normothermic machine perfusion (NMP) utilises 1.2-1.5L of donor blood (DB) with a target perfusate haematocrit of 25% to reanimate the donor heart. Limitations to current practise include small donor size and donor anaemia which can impact on the recovery of these hearts. Furthermore, in donation after circulatory death (DCD), blood collection may delay delivery of preservation solutions resulting in longer warm ischaemic times for the heart and other organs. Banked blood (BB) is a potential alternative to donor blood. We investigated the effect of banked blood for the reanimation and perfusion of donor hearts in a DCD porcine model.

***Methods*:** Series 1: Landrace pigs (n=12) underwent DCD withdrawal with subsequent procurement of the heart; 6 were reperfused with autologous blood; 6 were reperfused with BB (collected 48 hours prior). Hearts were maintained on NMP for 5 hours with continuous pressure and flow measurement. Serial venous and arterial lactate measurements were

performed as a marker of organ health. Series 2: Ten further studies in the BB group with nitric oxide (NO) pre-treatment (n=5), or sodium nitroprusside (SNP) infusion (n=5).

***Results*:** BB demonstrated severe metabolic and electrolyte disturbances requiring correction before organ perfusion. Both groups were successful in cardiac reanimation and demonstrated favourable lactate trends during perfusion. Perfusion with BB resulted in higher coronary perfusion pressure (CPP) which continued to rise overtime with resultant deterioration in cardiac function. The administration of NO delayed but did not prevent the rise in CPP. SNP infusion prevented the rise in CPP but resulted in severe hyperkalaemia.

***Conclusions*:** Reperfusion of DCD hearts with BB during NMP results in significantly higher perfusion pressures leading to progressive deterioration of cardiac recovery. Treatment with nitro vasodilators produced short-lived benefits.

Keywords

Banked blood; normothermic machine perfusion; donation after circulatory death heart transplant

1. Introduction

Machine perfusion is now utilised in the resuscitation of multiple donor organs including heart, lung, liver and kidneys, in place of or as an adjunct to cold storage for clinical transplantation [1-8]. Early to mid-term results have shown favourable outcomes despite the increased utilisation of 'marginal' organs which were previously discarded [6, 7, 9, 10]. Notwithstanding the continued debate around perfusion methods (i.e. hypothermic vs. normothermic) and the merits of individual device technology, the overall positive role of machine perfusion in donor organ management is now widely established.

An important component of machine perfusion is the composition of the solution with which the organ is perfused whilst on the device. Perfusates can range from enriched whole blood to specialised crystalloid solutions and vary depending on the perfusion device used. In heart transplantation, the only clinically approved device is the Transmedics Organ Care System (OCS Heart) which uses normothermic perfusion of the donor heart [11-13]. The perfusate uses a combination of donor blood, which is collected at the time of organ procurement, mixed with a proprietary crystalloid solution. In contrast, other hypothermic cardiac perfusion devices (in animal trials) have used non-blood based perfusates [14-17].

Whilst a blood-based perfusate appears to be the logical choice for ex-situ normothermic perfusion of the donor heart, time and volume constraints become major logistical factors potentially impeding the satisfactory resuscitation of the donor heart. Limitations to collecting the necessary volume required for machine perfusion can be due to lower blood volume in a smaller adult or paediatric donor, blood loss following events leading to death or low haematocrit secondary to resuscitation efforts. Prolonged blood collection time can be due to low blood volume, to donor size or volume loss, and under resuscitation during the organ retrieval process with resultant hyperviscosity in the absence of antemortem heparin. Although these issues may be infrequent, it remains a major concern as donor hearts are scarce. In addition, prolonged blood

collection time may delay recovery of other organs from the multi-organ donor. In contrast, non-blood based perfusate is not affected by these considerations, however, prolonged non-blood-based reperfusion in normothermic conditions has been associated with the development of significant tissue oedema, myocardial injury and impaired myocardial functional recovery [18].

A strategy to use banked blood could replenish and potentially replace the need to salvage donor blood to prime the perfusion circuit and consequently mitigate against the disadvantages of using donor blood. The use of banked blood on organs including the lungs, liver and kidneys has not shown any unfavourable results. However, in the setting of normothermic perfusion (NMP) of the donor heart, usage of banked blood as perfusate is not recommended despite there being no published evidence of detrimental outcomes using banked blood reperfusion in the heart to date. The aim of this pre-clinical study was to investigate the efficacy and safety of banked blood as a perfusate during normothermic machine perfusion of the DCD heart.

2. Materials and Methods

2.1 Animals

Juvenile Landrace pigs (n=12) between 55-65kg were used. All animal care and handling were performed in accordance to the standards outlined in the Australian Code for the Care and Use of Animals for Scientific Purposes (8th edition) (2013) and experimental protocols were approved by the institutional animal ethics committee (ARA #13/10).

2.2 Materials

Carotid and jugular cannulation with Arrow 5FG and 8FG percutaneous vascular sheath introducer systems (Teleflex, Mascot, Australia) were used to monitor arterial blood pressure (MAP) and central venous pressure (CVP). Side ports were also used to provide high volume infusions if necessary.

One litre Celsior Solution (Genzyme, Naarden, The Netherlands) was used to flush the heart after blood collection was completed. The flush solution was supplemented with recombinant human erythropoietin (Eprex 5000IU, JANSSEN, North Ryde, Australia), glyceryl trinitrate (GTN 100mg, Hospira, Mulgrave, Australia) and zoniporide (Pfizer Inc, Groton, CT) as described previously [19].

Banked blood was collected and supplied by the Faculty of Veterinary Science, Camden Campus, University of Sydney. Blood collection was performed 48 hours prior to experiments, from donor animals by Faculty Veterinarians under sterile conditions. Blood was collected in 450mL double blood pack containing citrate phosphate dextrose and adsol preservation solutions (CPD/Adsol, Baxter Healthcare Corp, Old Toongabbie, Australia) to a total volume of 1.5L and stored at 4^{o}C according to blood bank practices.

2.2.1 Normothermic Perfusion Device

A similar setup like the Organ Care System was used as the normothermic perfusion device. The perfusion circuit was based on the Langendorff model, with retrograde aortic flow and antegrade coronary perfusion. A single centrifugal pump was used to fill the aorta. Aortic flow was measured

using a flowmeter and adjusted based on coronary flow and aortic root pressure. Coronary flow was estimated by measuring pulmonary artery outflow using a standard flowmeter.

Perfusate solution: In addition to banked blood or donor blood, the circuit was primed with 500mL of Gelofusine, 500mg of methylprednisolone, 1 ampoule of Centrum multivitamin, 20mM of sodium bicarbonate and 1g of cephazolin. The perfusate was oxygenated with 5% carbogen (BOC Gases) delivered at 2Litres per hour by an oxygenator connected to the perfusion circuit.

2.3 Anaesthesia

Animals were pre-medicated with intramuscular ketamine (10mg/kg), midazolam (1mg/kg) and atropine (50mcg/kg) on arrival. Once sedated, further anaesthesia with inhaled isoflurane (2-4%) was given while vascular access was obtained. Tracheal intubation was performed with 7-7.5mm endotracheal tube and anaesthesia maintained with inhaled isoflurane and IV fentanyl (100-200mcg boluses).

Ventilation was maintained with a tidal volume of 10ml/kg, positive end expiratory pressure (PEEP) of 5 mmHg. Continuous monitoring of arterial pressure, central venous pressure, ECG and pulse oximetry was performed up until donor heart explantation. Baseline arterial blood gas analysis was performed using a handheld iSTAT point of care system (Abbott Point of Care Inc, Princeton, NJ).

2.4 Experimental Protocol

Baseline haemodynamic parameters were established through carotid and jugular cannulation as described above. Median sternotomy was performed, and the pericardium opened. The animal was given a bolus dose of midazolam and fentanyl prior to withdrawal of ventilatory support. Documentation of haemodynamic parameters was performed following cessation of ventilator support to mimic the clinical donation after circulatory death (DCD) scenario [20]. The time of circulatory arrest was taken as the time of equalisation of central venous pressure and systemic arterial pressure. A further stand off period of 5 minutes was introduced to comply with the jurisdictional legislation for DCD organ retrievals in the clinical setting.

2.4.1 Series 1

Donor Blood Group (DB). Upon completion of 'stand-off' time, the right atrial appendage was incised and a large bore venous cannula was introduced. Blood was collected into a standard 2 Litre collection bag with 20000 Units Heparin. Upon collection of 1-1.2 L of blood, the venous cannula was removed and collected blood was siphoned into the primed device (see materials: priming solution) using a leukocyte filter (Pall medical). The primed blood solution was sampled for blood gas and biochemistry. Any electrolyte disturbance and pH derangement were corrected by addition of $NaHCO_3$, calcium gluconate, magnesium sulphate and insulin.

A purse string suture was placed around the aortic root, and a 12G aortic root cannula was introduced and secured. The preservation solution, previously primed and prepared, was connected to the root cannula. The ascending aorta was cross clamped and the inferior vena cava transected. One litre of preservation flush was administered commencing at an infusion pressure of 150mmHg. Cold saline and ice were used to topically cool the chest cavity. Once the flush was

completed, the root cannula was removed, and purse string tied off. The heart was excised, and the aorta and pulmonary artery were cannulated and connected to machine perfusion. A left ventricular vent was introduced and secured through the open left atrium.

The aortic root was connected after adequate deairing, and aortic flow (AF) was initiated at 0.9 L/min. Coronary flow (CF) was maintained between 650-750 ml/min and mean arterial pressure (MAP) between 60-70 mmHg. The heart was ventricular-paced at 90 bpm in all experiments. Serial arterial and venous samples were taken for lactate profile, biochemistry and blood gases. The heart was maintained on the device for 4-5 hours during which AF, CF and MAP were recorded regularly to document any changes.

Banked Blood Group (BB). Banked blood was siphoned through a leukocyte filter (Pall) into the circuit reservoir containing 20000 Units of heparin along with the priming solution. The primed blood was sampled for blood gas and biochemistry, and any electrolyte and pH derangement were corrected.

The animals in the BB group underwent DCD withdrawal as described in series 1, however, donor blood collection was not undertaken. The heart once excised and cannulated was installed on the perfusion device with the same settings as previously mentioned. Blood sampling and flow monitoring were performed as for the DB group, and the heart was maintained for 4-5hours.

Outcomes Measured. Haemodynamic changes and biochemical alterations were assessed at baseline and during NMP at regular intervals. Blood analysis was performed in all experiments at 3 set time points: pre-withdrawal, post-priming on the device and immediately after initiation of NMP. In addition, banked blood was analysed prior to mixing with priming solution. Further sampling during NMP was based on recovery of the donor heart. Serial arterial and venous lactate measurements were performed hourly except during the early phases of organ reperfusion, or when flow or pressure adjustments were made

Biochemical Changes. Blood samples were analysed using point-of-care iSTAT handheld analyser at regular intervals as described. Arterial samples were analysed using CG-8 cartridges (Abbott Inc, Princeton, NJ), providing basic blood gas measurements including O_2 and CO_2 partial pressure, [HCO_3^-], [K^+], [Ca^{2+}], blood glucose and haematocrit level.

Haemodynamic Changes. Target haemodynamic parameters during NMP were based on clinical NMP recommendations by Transmedics OCS Heart™. CF was maintained between 0.65-0.75L/min by changing pump flow in the presence of permissible perfusion pressure (MAP 60-70mmHg). Pharmacological intervention with Transmedics maintenance solution containing adenosine as a coronary vasodilator or adrenaline to reduce CF through vasoconstriction was undertaken if haemodynamic parameters fell outside of the above targets [21, 22]. AF, CF and MAP were measured continuously during the 4-5 hour period of NMP. Haemodynamic data were recorded during each blood sampling timepoint.

Assessment for Viability. In clinical practice, we and others have used lactate level and arterial venous lactate differentials to determine if the donor heart is suitable for transplantation [23]. In this study, blood perfusate was sampled from both arterial and venous outlets for lactate measurement using point-of-care iSTAT CG-4 cartridges. Sampling was performed every hour until termination of the study.

Study Endpoint. The experiment was terminated in the following situations:

- Completion of study at 5hours of NMP
- Arterial and venous lactate <0.3mM (limitation of CG-4 measurement)

- Asystolic arrest

2.4.2 Series 2

An additional 10 experiments were performed based on outcomes of series 1. Banked blood was treated with nitric oxide gas (NO) and sodium nitroprusside (SNP) infusion to mitigate measured changes in MAP in the BB group.

NO group (N=5) – Primed bank blood was treated with NO gas at 20ppm for an hour prior to initiation of NMP. Blood sampling was measured after completion of treatment for any biochemical and acid/base alterations. All other aspects of the experiment were conducted as described in series 1 above.

SNP group (N=5) – SNP (50mg/5ml, DBL/Globex Pharmaceuticals Ltd.) was diluted to a concentration of 0.5 mcg/mL using 0.9% NaCl. SNP infusion was commenced at 1ml/hr using a syringe driver and was titrated to maintain a MAP between 60-70 mmHg during NMP. All other aspects of the experiment were conducted as described in series 1 above.

3. Results

3.1 Series 1

3.1.1 Experimental Variables

Pig weights from DB and BB groups were 63 ± 7kg vs. 64 ± 5kg (P = 0.4). Warm ischaemic times for the groups were DB 21 ± 4mins vs. BB 18 ± 5mins (P = 0.1) and instrumentation time until NMP were DB 28 ± 5mins vs. BB 25 ± 8mins (P=0.25).

All hearts in the DB group were perfused for the full duration of the study, with the exception of one heart terminating prematurely at 285 mins due to cardiac arrest. Three hearts, including the abruptly arrested heart, achieved arterial and venous lactate of <0.3mM at 4hours of NMP.

All hearts in the BB group were perfused for the full duration of the study, except for one heart which demonstrated severe right atrioventricular distension secondary to irreversible high MAP of 129 mmHg after 240 mins.

3.1.2 Biochemical Outcomes

When comparing biochemical baseline properties between DB vs. BB, there were significant differences in a number of parameters including potassium concentration (DB 3.9±0.2mM vs. BB 8.7±0.6mM; P<0.05), pH (DB 7.55±0.05 vs. BB 6.52±0.03; P<0.05) bicarbonate concentration (DB 34.5±3.7mM vs. BB undetectable), and calcium concentration (DB 1.3±0.04mM vs. BB <0.25mM). There was also a trend for increased haemoconcentration in BB (Hct 0.35±0.15) when compared to DB (0.24±0.03) (P=0.06).

The BB group required extensive acid/base and electrolyte correction: this was performed through correction of severe acidosis with $NaHCO_3$ (27.5 ± 4.2 mM) and correction of hypocalcaemia with calcium gluconate (6.6 ±1.6 mM). Post correction electrolytes are presented in Table 1.

Table 1 Biochemical and acid base measurements post-priming and post-correction.

		DB	BB	P
K^+	Post-Priming	6.5±1.9	7.7±1.2	P=0.11
	Post-Correction	5.3±2.6	6.8±1.7	P=0.14
pH	Post-Priming	7.33±0.04	6.81±0.09	P<0.05
	Post-Correction	7.27±0.06	7.11±0.16	P=0.02
HCO_3^-	Post-Priming	25.2±2.6	14.8±3	P<0.05
	Post-Correction	21.2±1.7	20.1±8.8	P=0.38
Ca^{2+}	Post-Priming	0.9±0.05	0.25	P<0.05
	Post-Correction	0.75±0.3	0.32±0.07	P<0.05
Hct	Post-Priming	0.15±0.05	0.2±0.03	P=0.03
	Post-Correction	0.14±0.02	0.16±0.02	P=0.07

Banked blood demonstrated significantly lower pH and hypocalcaemia requiring correction prior to reanimation of donor heart. Despite aggressive replacement with calcium gluconate, we were unable to achieve similar levels of [Ca^{2+}] as the DB group. Despite this, there were no apparent impact on contractility during reperfusion when comparing DB and BB group.

Haematocrit (Hct) was significantly higher post-priming, but did not exhibit any significant difference after correction due to the higher volumes of corrective fluids required.

Despite intensive correction of the underlying electrolyte and acid base imbalance, BB group had greater acid/base and biochemical disturbance at the time of cardiac perfusion. The BB group maintained a higher Hct at the time of cardiac perfusion.

3.1.3 Functional Outcomes

Haemodynamic outcomes were measured at baseline and hourly until termination of the study. Whilst CF and AF were maintained during NMP, there were increasing differences in MAP between the DB and BB groups over time (P<0.05). At commencement of NMP, AF (DB 0.81±0.05L/min vs. BB 0.84±0.12L/min; P=0.31) and MAP (DB 71±13mmHg vs. BB 76±22mmHg; P=0.36) were required to maintain CF. Whilst DB parameters remained stable throughout perfusion, there was a progressive and statistically significant increase in MAP in the BB group which was unresponsive to adenosine infusion. MAP measured prior to termination of the study was 64±8 mmHg in hearts perfused with DB compared to 99±36 mmHg in hearts perfused with BB (p<0.01).

AF and MAP measurements are presented in Table 2 and trends during ex-situ reperfusion are presented in Figure 1.

Table 2 AF and MAP during NMP.

Time (mins)	AF (L/min)			MAP (mmHg)		
	DB	BB	P	DB	BB	P
60	0.81±0.04	0.8±0.09	0.45	62±12	87±21	0.02
120	0.78±0.04	0.82±0.08	0.16	65±12	86±17	0.03
180	0.80±0.05	0.87±0.13	0.13	64±8	82±19	0.05
240	0.79±0.07	0.81±0.05	0.27	65±9	90±25	0.04
300	0.85±0.04	0.83±0.07	0.34	64±8	99±36	0.1

Aortic Flow (AF) were similar in both groups, but there was significantly higher perfusion pressure (MAP) in the BB group. Whilst perfusion pressure remains stable throughout the experiment in the DB group, there is progressively rising pressure in the BB group after 4 hours of machine perfusion.

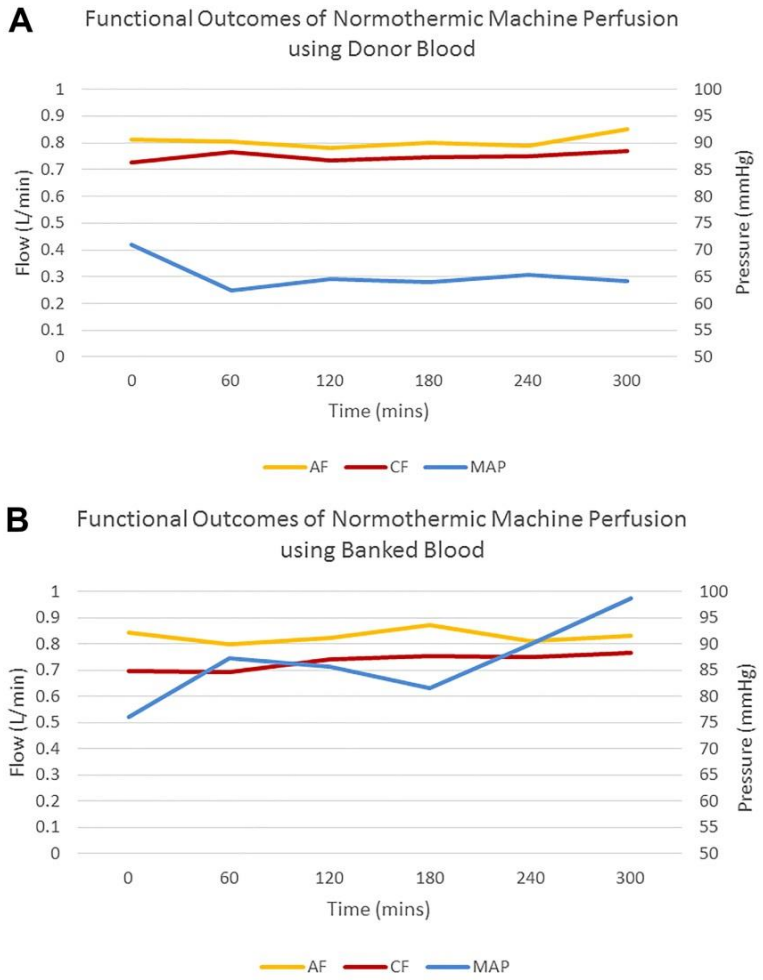

Figure 1 (A) AF, CF and MAP during NMP using donor blood; (B) AF, CF and MAP during NMP using banked blood. Comparing DB to BB, the starting MAP was higher in the BB group which progressively increases overtime. CF and AF are similar in both groups, and remain stable throughout the experiment.

3.1.4 Lactate Measurements

Serial lactate measurements were performed hourly from arterial and venous outlets. Whilst both DB and BB showed trends of diminishing lactate over time with evidence of myocardial consumption, the baseline lactate in the BB group was higher compared to the baseline in the DB group (BB 6.3±1.5mM vs DB 2.5±0.6mM; P<0.01). Lactate levels measured prior to termination were BB (arterial 1.7±1.3mM vs. venous 1.6±1.3mM) and DB (arterial 0.50±0.33mM vs. venous 0.51±0.35mM); Arterial and venous lactate levels in the BB group were significantly higher than in the DB group at the termination of study (P=0.03). Arterial and venous lactate trends for both groups are presented in Figure 2.

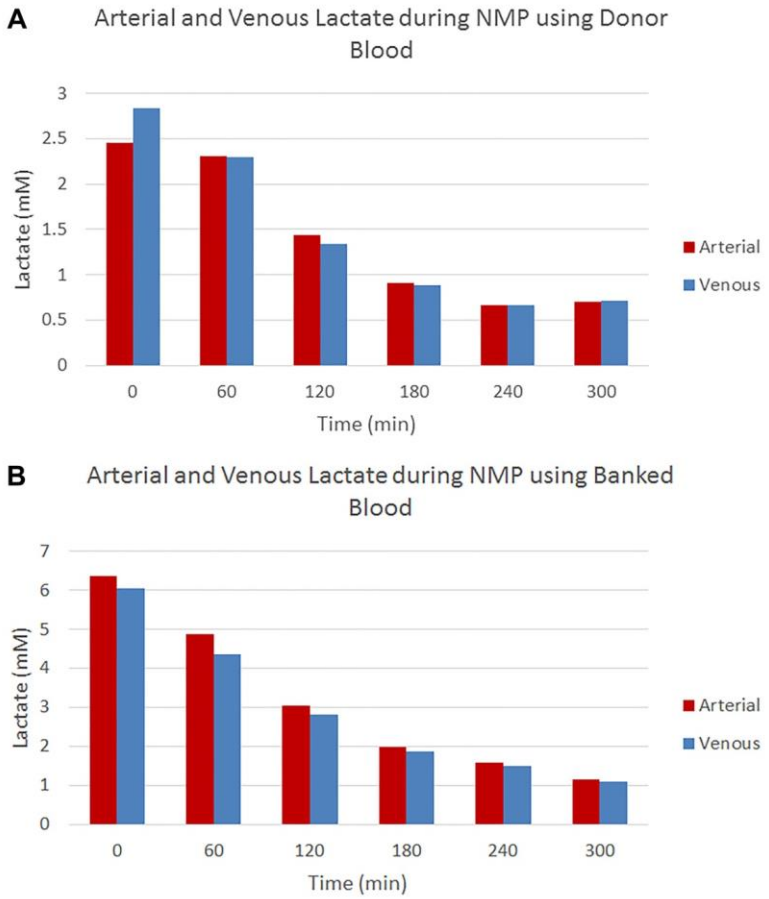

Figure 2 (A) Arterial and Venous Lactate in Donor Blood group; (B) Arterial and Venous Lactate in Banked Blood group. Overall lactate trend in DB and BB group are similar except BB group had higher starting lactates. Both groups demonstrated lactate extraction when comparing arterial to venous sampling.

3.1.5 Visual Assessment

Visual assessment of the right ventricle, during the early stages of reperfusion, demonstrated satisfactory contraction in both groups, with no evidence of right atrial distension after reanimation and establishing of a stable rhythm through ventricular pacing at 90 bpm. Over time, right atrial and ventricular distension was observed in the BB group in association with a rising MAP.

3.2 Series 2

3.2.1 Outcome

Following the first series of studies which demonstrated a progressive rise of MAP in the BB group, attempts to mitigate the pressure rise using alternative pharmacological interventions to adenosine were attempted. We hypothesised that the rise in MAP was due to depletion of S-nitrosohaemoglobin and disruption of nitric oxide bioavailability within banked blood [24]. Efforts to replenish nitric oxide bioavailability in bank blood using NO gas and SNP were performed.

Whilst NO-treated BB extended the duration of effective control of MAP, this effect was lost after 3-4 hours of reperfusion. This was followed by a rapid rise in MAP with deterioration of donor heart metabolism reflected by the rise in lactate (n= 4/5). Further treatment of banked blood with NO gas did not result in any improvement of MAP. This is presented in Figure 3.

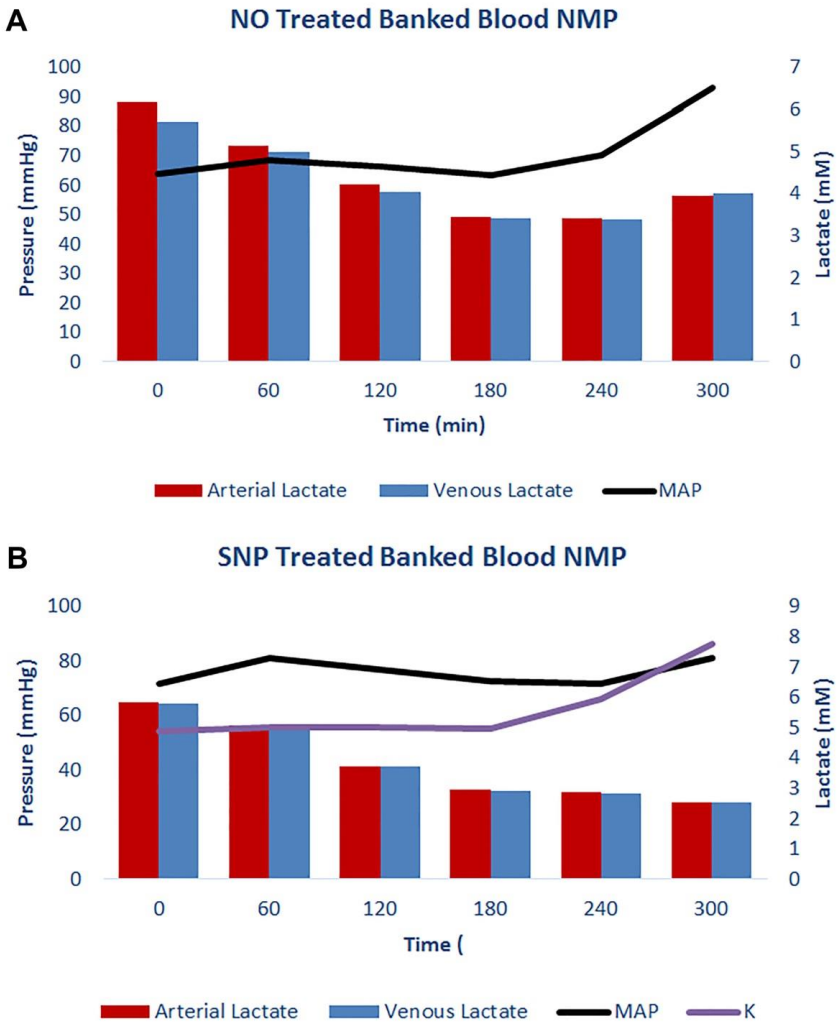

Figure 3 (A) NO treated Banked Blood outcomes; (B) SNP treated Banked Blood outcomes. NO and SNP treated banked blood demonstrated lower starting MAP, however the effect of NO appears to diminish at 3 to 4 hours of machine perfusion with gradual increase of MAP. SNP had sustained effect on MAP but resulted in abrupt hyperkalaemia after 4 to 5 hours of treatment.

In comparison, SNP infusion provided effective and sustained control of MAP. However, we observed an abrupt rise in [K^+] by 5-6 hours which resulted in sudden cardiac stand-still in four hearts. The observed hyperkalaemia occurred in isolation, with no gross changes in the other electrolytes measured or the perfusate pH. This is presented in Table 3.

Table 3 Perfusate blood biochemistry results for SNP-BB Group (n=5).

Time Point (min)	[K^+] (mM)	[Ca^{2+}] (mM)	[HCO_3^-] (mM)	pH	Glucose mg/dL
0	4.8±1.6	0.32±0.06	18.4±1.8	7.24±0.05	16.4±13.5
60	5±1.7	0.33±0.05	21.4±2.7	7.30±0.05	16.2±3.7
120	5±0.8	0.33±0.05	22.7±2.8	7.33±0.03	17.8±7.5
180	5±0.8	0.33±0.05	23.4±3.6	7.34±0.03	18.5±12.1
240	6±1.5	0.34±0.06	22.7±3.6	7.29±0.07	17.3±13
300	7.8±2.0	0.32±0.04	22.5±4.8	7.25±0.09	14±1.5.4

Abrupt rise in [K^+] was noted in the BB group treated with SNP after 4 hours of machine perfusion. Despite aggressive treatment with sodium bicarbonate and insulin infusion, the hyperkalaemia was irreversible and eventuated in the asystolic arrest of the donor heart on device.

4. Discussion

Our current experience in clinical DCD heart procurement has shown that early institution of cardiac protective flush facilitates organ recovery post-implantation. The process of blood collection, can potentially delay the institution of preservation flush by two minutes. Furthermore, the collection of adequate blood volume can be challenging in the clinical scenario and can be affected by the donor pre-mortem haemoglobin, donor size and fluid balance status. This is further confounded by some donor hospitals which do not permit ante-mortem interventions such as heparin administration and blood transfusions. Low Hct during NMP may lead to earlier onset of oedema, and carries the potential risk of decreased tissue oxygen perfusion. Current OCS recommends a target Hct >0.25. Hence the ability to use banked blood is highly attractive.

Unlike DBD hearts, DCD hearts, at the time of NMP, usually demonstrates high coronary flows with low perfusion pressure. This can be explained by the loss of coronary vasomotor tone which requires higher doses of adrenaline to improve perfusion pressure. In our study, there were no demonstrable differences in the early periods of reperfusion of both DB and BB, suggesting similar baseline pathophysiology of the donor heart.

Although the lactate concentration in the perfusate fell over time, with evidence of lactate extraction in both groups, the lactate concentrations in the BB group were higher at all time points. Despite the favourable lactate trend, coronary perfusion pressure rose steadily in the BB group consistent with increasing coronary vascular resistance over the time course of the experiment. After 4 hours of NMP hearts perfused with BB displayed increasing right atrial and ventricular distension and visual deterioration of myocardial contraction.

In contrast, despite lower Hct in the DB group, perfusion pressure remained stable throughout the entire course of the experiment; and the right ventricular function did not appear to be compromised on visual inspection. These hearts showed no evidence of right atrioventricular distension throughout the 5 hours of NMP.

The results of our porcine study indicate that perfusion of DCD hearts with BB results in progressive coronary vasoconstriction which is refractory to infusion of adenosine. We postulate that the higher pressures seen in the BB group was due to depletion of S-nitrosohaemoglobin and disruption of endogenous nitric oxide signalling by banked blood [25-27]. The resultant vasoconstriction in the coronary vessels lead to higher perfusion pressures. The introduction of an NO donor successfully prevented the increase in arterial pressure, but this effect was short-lived in the case of supplementation of BB with NO gas and associated with potential detrimental electrolyte and arrhythmic effects as seen in the SNP group.

Banked blood contains citrate which is normally metabolised rapidly by the liver when packed red cells are transfused into a donor. The absence of a clearance mechanism for citrate during NMP is the most likely explanation for the marked hypocalcaemia observed in the perfusate after the addition of BB. Hypocalcaemia was only partly corrected with the addition of calcium gluconate. It is unclear whether the hypocalcaemia contributed to the haemodynamic deterioration in the BB perfused hearts or whether more aggressive supplementation may have fully corrected this electrolyte abnormality. These issues will need to be addressed in future experiments.

Limitations: There are a number of limitations to our study. We did not prospectively cross match the blood donors with the heart donor. This decision was based on the advice provided by the herd veterinarian and our experience with heart transplantation between members of the same herd where we have not observed any hyperacute rejection responses. We believe that the progressive fall in the lactate level in the BB perfused hearts, the reversal of the coronary vasoconstrictor response at least temporarily with NO gas or SNP and the absence of any haemolysis in the perfusate make it unlikely that the coronary vasoconstriction observed in the BB perfused hearts was due to immunological incompatible blood transfusion. Another limitation was that the hearts were not transplanted subsequently for full functional recovery assessment.

5. Conclusions

Current experiments comparing DB to BB as the perfusate for donor porcine hearts during NMP have shown favourable lactate profiles with both perfusates. BB demonstrated significantly more deranged biochemical and acid base profiles at baseline. With partial correction of these abnormalities, DCD hearts were able to be reanimated and demonstrated favourable lactate profiles during the initial 4 hours on machine perfusion. However, BB reperfusion of donor heart is associated with higher coronary perfusion pressure which worsens overtime and is associated with progressive distension of the right sided chambers.

Attempts to prevent the progressive increase in MAP during BB perfusion with NO gas supplementation or infused SNP showed short term success which attenuates after 4-5 hours. With NO gas supplemented BB, MAP rises rapidly after 4 hours with rapid deterioration in cardiac function. In contrast, SNP infusion during BB perfusion exerted a more sustained effect on MAP

but led to severe hyperkalaemia. Based on our findings, we conclude that without further modification, banked blood is unsuitable for normothermic perfusion of the donor heart.

6. Future Directions

Collection of blood volume in the DCD setting can be challenging in smaller patients or patients with anaemia. Based on current experience, we would not recommend blood transfusion directly into the NMP circuit; rather pre-operative transfusion of the donor appears to be a safer option and has not been shown to have any immediate effect on CF and MAP during NMP.

The role of 'blood-washing', by reprocessing banked blood through a cell-saver system may attenuate the effects witnessed in the current study. Furthermore, artificial oxygen carriers or blood analogues are potentially alternatives to replace blood volume requirements for NMP but have been associated with increased tissue oedema during NMP [18]. Stored blood rejuvenation therapy has been shown to increase red blood cell (RBC) ATP and 2,3-DPG level, this effect however is dependent on the RBC storage duration and losses its effect with prolonged storage time [28].

Current reperfusion devices use a closed circuit which may lead to build up of toxic metabolites which can be injurious to the recovering heart. The introduction of a hemofiltration or dialysis system as an adjunct, especially when NMP time exceeds 4 hours may prevent the build-up of toxic substances and reduce tissue oedema through haemoconcentration.

In addition, the current Transmedics NMP device does not allow full assessment of left ventricular function. In our current experiments, we observed a favourable lactate profile in BB perfused hearts despite progressive increase in coronary vascular resistance and visual evidence of progressive distension of the right heart chambers. This highlights the potential shortcomings of current NMP system and the requirement for a more holistic assessment method for assessing donor heart viability during NMP.

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Faculty of Veterinary Science, Camden Campus, University of Sydney.

Author Contributions

Hong Chee Chew: Research design and performance of research, data analysis and writing of paper; *Ling Gao:* Performance of research; *Jeanette Villanueva:* Performance of research; *Aoife Doyle:* Performance of research; *Mark Hicks:* Performance of research; *Andrew Jabbour:* Writing of paper; *Kumud Dhital:* Research design and writing of paper; *Peter S Macdonald:* Supervisor involved in research design, performance and writing of paper.

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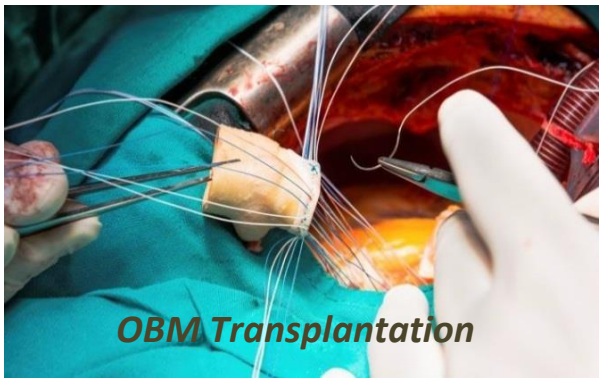
Competing Interests

The authors have declared that no competing interests exist.

References

1. Tenderich G, Tsui S, El-Banayosy A, Dhital K, Schulte-Eistrup S, Schulz U, et al. The 1-year follow-up results of the PROTECT patient population using the organ care system. J Heart Lung Transplant. 2008; 27: S166-S166.
2. Dhital KK, Iyer A, Connellan M, Chew HC, Gao L, Doyle A, et al. Adult heart transplantation with distant procurement and ex-vivo preservation of donor hearts after circulatory death: A case series. Lancet. 2015; 385: 2585-2591.
3. Cypel M, Yeung JC, Machuca T, Chen M, Singer LG, Yasufuku K, et al. Experience with the first 50 ex vivo lung perfusions in clinical transplantation. J Thorac Cardiov Surg. 2012; 144: 1200-1207.
4. Esmailian F, Kobashigawa JA, Naka Y, Mancini D, Soltesz E, Hsich E, et al. The PROCEED II international heart transplant trial with the organ care system technology (OCS). J Heart Lung Transplant. 2013; 32: S95-S96.
5. Hameed AM, Pleass HC, Wong G, Hawthorne WJ. Maximizing kidneys for transplantation using machine perfusion: From the past to the future: A comprehensive systematic review and meta-analysis. Medicine. 2016; 95: e5083.
6. Sandal S, Luo X, Massie AB, Paraskevas S, Cantarovich M, Segev DL. Machine perfusion and long-term kidney transplant recipient outcomes across allograft risk strata. Nephrol Dial Transplant. 2018; 33: 1251-1259.
7. Angelico R, Perera MT, Ravikumar R, Holroyd D, Coussios C, Mergental H, et al. Normothermic machine perfusion of deceased donor liver grafts is associated with improved postreperfusion hemodynamics. Transplant Direct. 2016; 2: e97.
8. Jayant K, Reccia I, Virdis F, Shapiro AMJ. The role of normothermic perfusion in liver transplantation (TRaNsIT Study): A systematic review of preliminary studies. HPB Surg. 2018; 2018: 14.
9. DiMaio JM, Morse M, Teeter WA, Cobert ML, West LM, Jessen ME, et al. Donor hearts not offered or rejected for transplantation - A lost opportunity? J Heart Lung Transplant. 2013; 32: S156.
10. Wierup P, Haraldsson Å, Nilsson F, Pierre L, Scherstén H, Silverborn M, et al. Ex vivo evaluation of nonacceptable donor lungs. Ann Thorac Surg. 2006; 81: 460-466.
11. Macdonald PS, Chew HC, Connellan M, Dhital K. Extracorporeal heart perfusion before heart transplantation: The heart in a box. Curr Opin Organ Transplant. 2016; 21: 336-342.
12. Messer SJ, Axell RG, Colah S, White PA, Ryan M, Page AA, et al. Functional assessment and transplantation of the donor heart after circulatory death. J Heart Lung Transplant. 2016; 35: 1443-1452.
13. Vela MM, Sáez DG, Simon AR. Current approaches in retrieval and heart preservation. Ann Cardiothorac Surg. 2018; 7: 67-74.

14. Van Caenegem O, Beauloye C, Vercruysse J, Horman S, Bertrand L, Bethuyne N, et al. Hypothermic continuous machine perfusion improves metabolic preservation and functional recovery in heart grafts. Transplant Int. 2015; 28: 224-231.
15. Cobert ML, Merritt ME, West LM, Ayers C, Jessen ME, Peltz M. Metabolic characteristics of human hearts preserved for 12 hours by static storage, antegrade perfusion or retrograde coronary sinus perfusion. J Thorac Cardiov Surg. 2014; 148: 2310-2315.e1.
16. Michel SG, La Muraglia GM, 2nd, Madariaga ML, Titus JS, Selig MK, Farkash EA, et al. Twelve-hour hypothermic machine perfusion for donor heart preservation leads to improved ultrastructural characteristics compared to conventional cold storage. Ann Transplant. 2015; 20: 461-468.
17. Steen S, Paskevicius A, Liao Q, Sjöberg T. Safe orthotopic transplantation of hearts harvested 24 hours after brain death and preserved for 24 hours. Scand Cardiovasc J. 2016; 50: 193-200.
18. White CW, Hasanally D, Mundt P, Li Y, Xiang B, Klein J, et al. A whole blood–based perfusate provides superior preservation of myocardial function during ex vivo heart perfusion. J Heart Lung Transplant. 2015; 34: 113-121.
19. Watson AJ, Gao L, Sun L, Tsun J, Doyle A, Faddy SC, et al. Enhanced preservation of pig cardiac allografts by combining erythropoietin with glyceryl trinitrate and zoniporide. Am J Transplant. 2013; 13: 1676-1687.
20. Iyer A, Chew HC, Gao L, Villanueva J, Hicks M, Doyle A, et al. Pathophysiological trends during withdrawal of life support: Implications for organ donation after circulatory death. Transplantation. 2016; 100: 2621-2629.
21. Dhital K, Simon A, Macdonald P. Ex-vivo donor heart perfusion: Testing the limits of cardiac resilience. Heart Lung Circ. 2015; 24: 1191-1192.
22. García Sáez D, Zych B, Sabashnikov A, Bowles CT, De Robertis F, Mohite PN, et al. Evaluation of the organ care system in heart transplantation with an adverse donor/recipient profile. Ann Thorac Surg. 2014; 98: 2099-2106.
23. Ardehali A, Esmailian F, Deng M, Soltesz E, Hsich E, Naka Y, et al. Ex-vivo perfusion of donor hearts for human heart transplantation (PROCEED II): A prospective, open-label, multicentre, randomised non-inferiority trial. Lancet. 2015; 385: 2577-2584.
24. Kanias T, Gladwin MT. Nitric oxide, hemolysis, and the red blood cell storage lesion: Interactions between transfusion, donor, and recipient. Transfusion. 2012; 52: 1388-1392.
25. Reynolds JD, Bennett KM, Cina AJ, Diesen DL, Henderson MB, Matto F, et al. S-nitrosylation therapy to improve oxygen delivery of banked blood. Proc Natl Acad Sci. 2013; 110: 11529-11534.
26. Riccio DA, Zhu H, Foster MW, Huang B, Hofmann CL, Palmer GM, et al. Renitrosylation of banked human red blood cells improves deformability and reduces adhesivity. Transfusion. 2015; 55: 2452-2463.
27. Risbano MG, Kanias T, Triulzi D, Donadee C, Barge S, Badlam J, et al. Effects of aged stored autologous red blood cells on human endothelial function. Am J Resp Crit Care Med. 2015; 192: 1223-1233.
28. Barshtein G, Gural A, Manny N, Zelig O, Yedgar S, Arbell D. Storage-induced damage to red blood cell mechanical properties can be only partially reversed by rejuvenation. Transfus Med Hemother. 2014; 41: 197-204.



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