CARDIAC ALLOGRAFTS FROM DONATION AFTER CIRCULATORY DEATH DONORS – ARE THESE HEARTS VIABLE FOR USE IN CARDIAC TRANSPLANTATION?

A thesis in fulfilment of the requirements for the degree of Doctor of Philosophy

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Cardiac Transplantation remains the definitive treatment of End-Stage Heart Failure beyond medical therapy, with excellent recipient survival and quality of life. However, its major limitation remains in the finite number of cardiac allografts available for transplantation. Whilst all hearts have previously been procured solely from brain dead donors, the search for additional sources of organs have led investigators to Donation after Circulatory Death (DCD) donors.

The body of work enclosed in this thesis investigates the viability of hearts from these DCD donors for use in cardiac transplantation. These hearts have been avoided, having been exposed to varying periods of warm ischaemia - referring to hypoxic and hypoperfusion insults at normothermic conditions. However, there is inherent tolerance to these insults prior to irreversible damage. The initial series of experiments utilised a large animal model to investigate the warm ischaemic time (WIT) limit prior to irrecoverable damage at a biochemical, metabolic & functional level. These studies demonstrated irrecoverable damage beyond 20 minutes WIT. However, utilising post-conditioning strategies aimed at activating cellular protective pathways, tolerance could be increased to 30 minutes. The next series of experiments were aimed at demonstrating clinical relevance. Utilising a large animal orthotopic transplant model, post-conditioned hearts exposed to 30 minutes WIT were transplanted into recipient animals following 4 hours of storage. Storage of the allografts were either through the current standard of care (cold storage) or with ex-vivo perfusion (EVP) preservation (Transmedics OCS). Superiority and viability of cardiac allografts post-transplantation were only demonstrated when preserved with EVP.

Based on these results and a series of human pre-clinical studies, the first human DCD cardiac transplant of the modern era was performed in July 2014. The series of the first 3 of these heart transplants was published in the LANCET journal as a major medical breakthrough.

Since 2014, a total of 14 clinical DCD heart transplants have been performed with all recipients discharged home. These results demonstrate successful clinical translation of the animal studies. It also aptly responds to the question posed in the title of this thesis – that these hearts are truly viable for use in cardiac transplantation.

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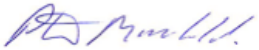

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PRESENTATIONS

2015

Australia and New Zealand Society of Cardiothoracic Surgery (ANZSCTS) ASM, 15–18 November 2015; Adelaide, AUSTRALIA

2013

12th Congress of the International Society of Organ Donation and Procurement, 21–24 November 2013; Sydney, AUSTRALIA

Transplantation Society of Australia & New Zealand 31st ASM 24–27 June 2013; Canberra, AUSTRALIA

Royal Australasian College of Surgeons ASC, Auckland, New Zealand 6–10 May 2013; Auckland, NEW ZEALAND

International Society of Heart & Lung Transplantation (ISHLT) 33rd Annual Meeting 24–27 April 2012; Montreal, CANADA

2012

Australia and New Zealand Society of Cardiothoracic Surgery (ANZSCTS) ASM, 11–14 November 2012; Hobart, AUSTRALIA

24th International Congress of The Transplantation Society (TTS), 15–19 July 2012; Berlin, GERMANY

Transplantation Society of Australia & New Zealand 30th ASM 27–29 June 2012; Canberra, AUSTRALIA

International Society of Heart & Lung Transplantation (ISHLT) 32nd Annual Meeting 17–21 April 2012; Prague, CZECH REPUBLIC

2011

Transplantation Society of Australia & New Zealand 29th ASM 29 June – 1 July 2011; Canberra, AUSTRALIA

Alfred Hospital Symposium – Can Hearts from Donations after cardiac death be used in Transplantation? 18 November 2011; Melbourne, AUSTRALIA

PRIZES

2015

Australia and New Zealand Society for Cardiothoracic Surgery ASM: ***Poster prize***

2013

Transplantation Society of Australia & New Zealand: ***President's Prize, Young Investigator's Award***

Royal Australasian College of Surgeons ASC, Auckland: ***Trainee Research Prize***

2012

Australia and New Zealand Society for Cardiothoracic Surgery ASM: ***Young Achiever's Award***

Transplantation Society of Australia & New Zealand: ***Young Investigator's Award***

2011

Transplantation Society of Australia & New Zealand: ***Young Investigator's Award***

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1.1 End-stage heart failure and transplantation in Australia

An ageing population and more survivors of myocardial infarctions contribute to the growing pandemic in Australia that is Chronic Heart Failure (CHF). CHF affects two to three per cent of the population and rises to over 23% in those over the age of 65 (1). An estimated 30,000 Australians receive the diagnosis of CHF each year (2). The burden on the Australian health system is significant: more than 45,000 hospital admissions in 2009–2010 were a result of CHF (3), at an estimated cost of $1 billion per year (4).

For the subset of patients who have end stage heart failure (ESHF), the prognosis is grim. Poor quality of life, recurrent hospitalisations and a mortality of up to 50% at one year is what confronts this critically ill population (5). For ESHF patients who are resistant to medical management, cardiac transplantation has remained the treatment of choice over the last 40 years.

Heart transplantation is the gold standard treatment of ESHF, providing a nearly 90% one-year survival, a 75% seven-year survival and significant improvement in quality of life (6,7). Since the first heart transplant in South Africa in 1967 (8), the numbers of transplants have grown with their outcomes markedly improved. Today, over 5000 transplants a year are conducted in over 300 countries worldwide (9). In Australia, since the commencement of a heart transplant program in 1984, over 2000 heart transplants have been performed.

1.2 Challenges in cardiac transplantation today

The most significant problem that faces cardiac transplantation today is the significant disparity between supply and demand.

Improved outcomes post transplantation has been a result of continued progress in donor management, organ preservation, intensive care, and immunosuppressive therapy. For the growing ESHF population, who face a 50% mortality at one year, transplantation provides a one-year survival of ~ 90% (6). The expanding ESHF population, in addition to improvements in outcomes with and increased use of mechanical circulatory support (ventricular assist devices – VADs) to bridge patients to transplantation, ensures more patients require and survive to transplantation. This, along with expanding recipient selections, results in an ever-growing demand for transplants.

In contrast, the numbers of donors and donor hearts suitable for transplantation has declined. Over the last two decades, the numbers of cardiac transplants performed has steadily declined as a result of limitations in organ availability (Figure 1). This trend has improved in the last few years, but still remains below transplant rates in the 1990s.

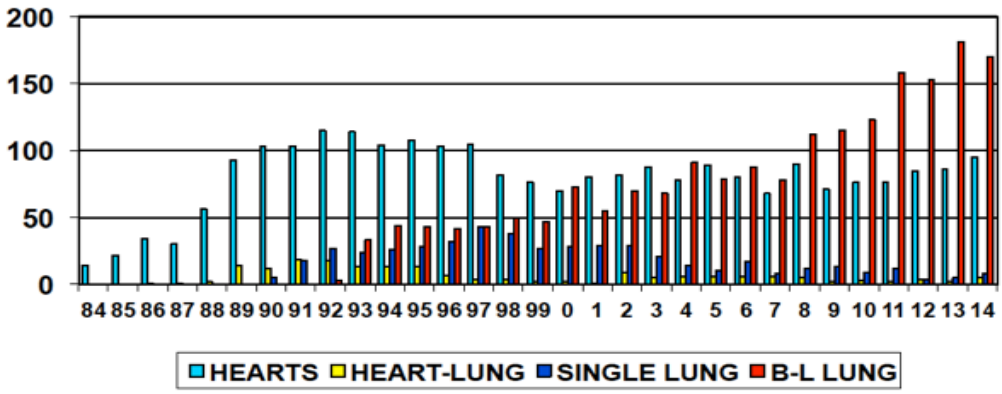

***Figure 1**- Heart transplant numbers in Australia and New Zealand 1984-2014. Source: Australia and New Zealand Cardiothoracic Organ Transplant Registry 2014 report(10)*

Whilst improvements in road safety has played a role in declining numbers of donors in the last two decades, overall donor numbers in Australia are lower than other parts of the developed world. Figure 2(a) highlights the difference, with 16.9 organ donors per million population in Australia, compared to 26 in the USA and 35 in Spain. While donor numbers have increased in Australia in the last few years (Figure 2(b)), it still remains lower than is required to meet the growing need. However, despite an increase in donor numbers, the proportion of suitable cardiac allografts remains low.

This disparity between number of donor hearts available and the increasing demand amongst the ESHF population has forced researchers to look at expanding the donor pool and evaluating alternative sources.

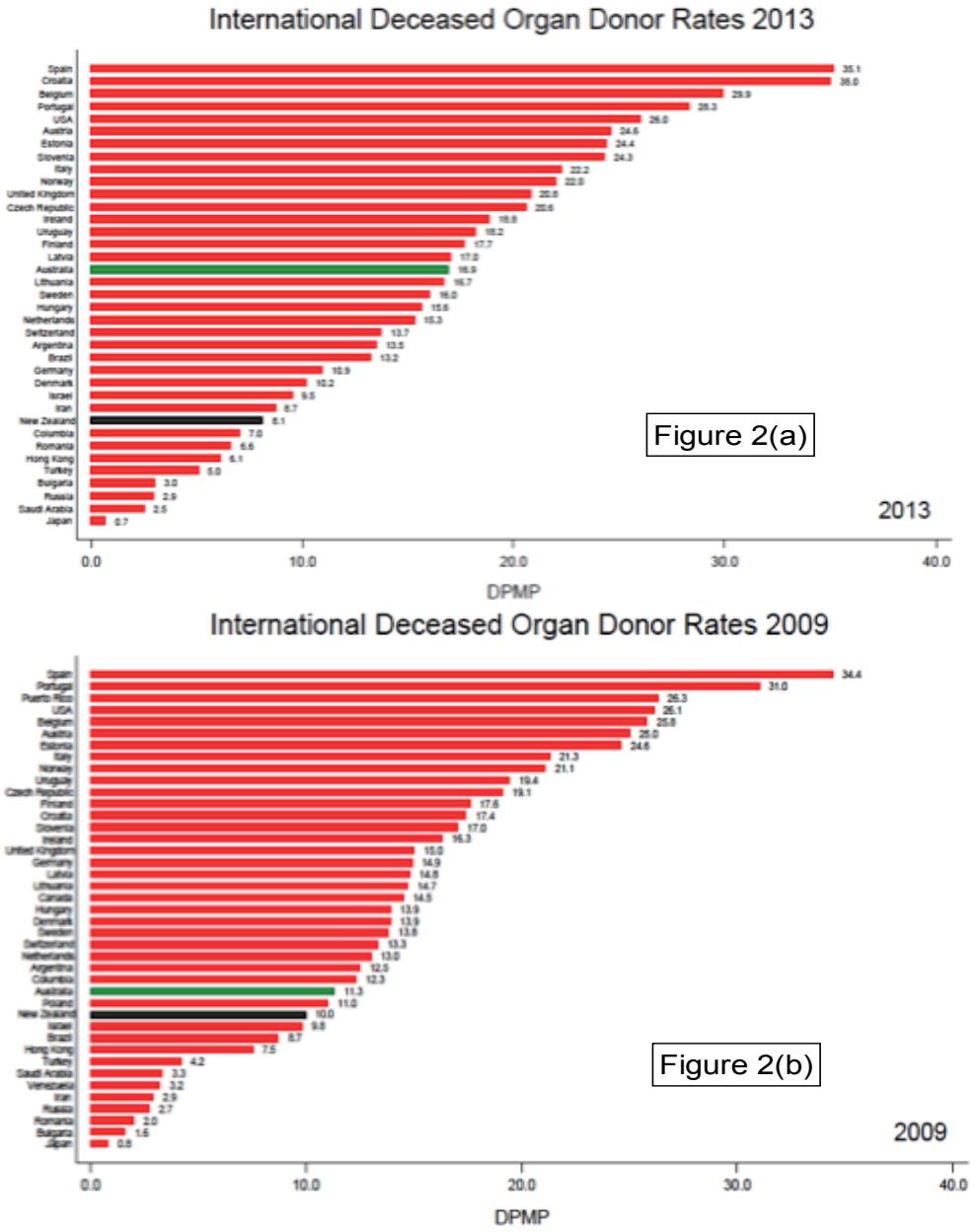

Figure 2 *– Organ donation rates in Austrlaia compared with the rest of the world in 2013 (a) and 2009 (b).*
Source: Australia and New Zealand Organ Donation Registry, 2014 Annual Report (11).

1.3 Expanding the donor pool

The use of 'marginal donors' has been one avenue that has been pursued in a bid to increase transplant numbers—these are donors with features and risk profiles that lie outside the ideal donor characteristics, but are still thought to be suitable for cardiac allograft donation. In Australia, the combination of a smaller population and a low organ donation rate has resulted in increased utilisation of hearts from older 'marginal' donors (12,13,14) and sub-optimal organs from younger donors. The Australian geography also forces greater procurement distances to retrieve donor hearts, contributing to longer ischaemic times—mean ischaemic times in Australia and New Zealand in 2014 was 234 minutes. It has been established that longer ischaemic times are detrimental for organ recovery, with International Society of Heart Lung Transplantation (ISHLT) data highlighting the increase in one-year mortality with ischaemic times of over three hours (Figure 3). The combination of these two factors, advanced donor age and prolonged ischaemic time, markedly increases the mortality and morbidity after heart transplantation (15).

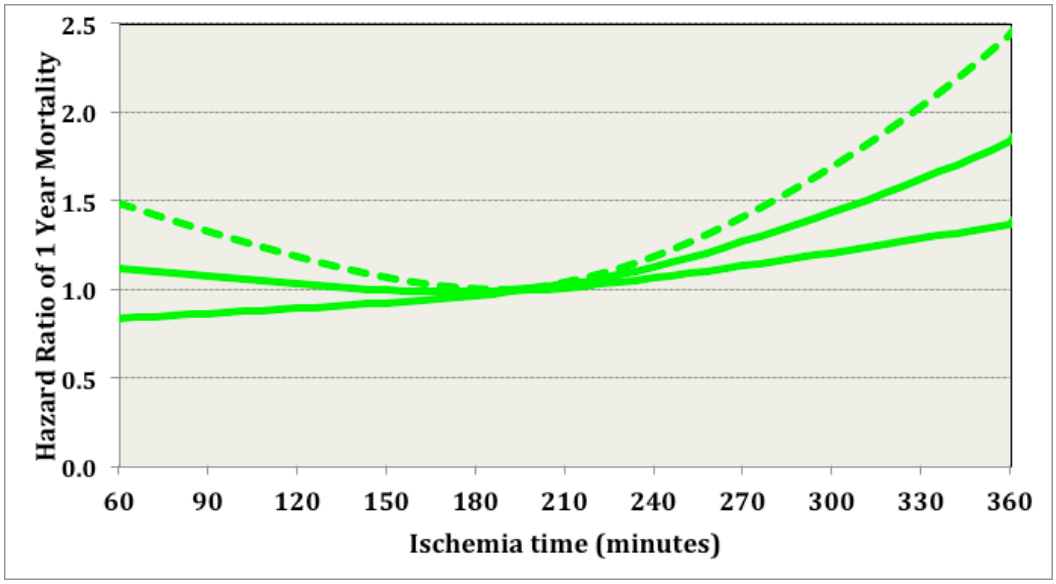

Figure 3 *– Ischemic Time and 1 year mortality; p<0.0001*
Source: The International Society of Heart and Lung Transplantation Registry 2014 Adult Heart Transplantation Slides (15)

One of the costs of this strategy is an increased incidence of Primary Graft Failure (PGF), a devastating complication in the immediate post-transplant period. It manifests as severe ventricular dysfunction of the donor graft and carries significant mortality and morbidity. In the last decade advances in pharmacological treatment and mechanical circulatory support have improved the outlook for heart transplant recipients who develop this complication. Despite these advances, PGF is still the leading cause of death in the first 30 days post transplantation (15).

As transplant centres test the boundaries of organ viability in an attempt to maximise organ usage, PGF is becoming an increasingly relevant complication. With the use of increasing numbers of marginal donors, and as we embark on the utilisation of hearts exposed to significant ischaemic insults (DCD hearts), the need for better understanding of PGF is paramount. A published review of this complication forms the first published paper of this thesis.

1.4 Alternate sources of donor organs – DCD donors

While brain death donors have remained the sole source of cardiac allografts for transplantation in the modern era, this has not always been the case. The concept of brain death (BD) was officially recognised in 1968 with the development of BD criteria by the Harvard ad-hoc committee (16). Prior to this milestone, in the absence of BD recognition, a small number of cardiac transplants were conducted using hearts from donors who had undergone circulatory arrest, referred to as donation after circulatory death (DCD).

One of these was the first clinical heart transplant ever performed in 1967 (8). Performed by Christian Barnard, the heart was procured from a 25-year-old female DCD donor who had been involved in a motor vehicle accident. With donor and recipient in adjacent theatres, ventilator support was withdrawn from the donor. Following cessation of circulation, the donor heart was explanted and subsequently transplanted. While the transplant was successful and the heart successfully supported the recipient circulation, the patient succumbed to infection in the setting of immunosuppression 18 days post-op. The first cardiac transplant in Australia,

performed in 1968 by Harry Windsor at St Vincent's Hospital (Sydney), was also from a DCD donor (17).

Following this initial experience with DCD allografts, the recognition and legalisation of BD in the late 1960s ensured a means to avoid the obligatory warm ischaemia of DCD donors. Since this point, BD donors have remained the sole source of hearts for transplantation over the last 40 years. Over this period, significant progress in all facets of cardiac transplantation has occurred and dramatically improved the short- and long-term outcomes with organ transplantation. A result of this has been the growing demand for transplantation. It is this same progress, as well as the need to address the organ shortage problem, that has re-opened interest in DCD organs.

The concept of cardiac transplantation from DCD donors has been actively investigated over the last decade, but without any successful clinical translation. In order to understand the limitations and challenges of DCD organs in cardiac transplantation, a thorough understanding of the DCD donor and organ donation process is paramount.

1.5 Who/what are DCD donors?

In Australian legislation, there are two recognised methods of declaring death – specifically defined as 'irreversible cessation of all functions of the brain: BRAIN DEATH' or irreversible cessation of circulation of blood in the body: CIRCULATORY DEATH (DCD) (e.g. the *NSW Human Tissue Act s33 1983*).

1.5.1 Brain death

BD is a recognised entity in intensive care, where two different specialists are required to identify a set of diagnostic signs prior to declaration of death. These donors are assessed for organ donation and if deemed suitable, family consent is discussed following which the donors are taken to theatre for organ procurement. Hearts from BD donors are exposed to no warm ischaemia during the retrieval process. With death having already been declared using neurological criteria, the heart can be arrested using special preservation solutions by the retrieving team. The cold ischaemic time

begins following arrest and includes hypothermic storage in transport during which low-level metabolic activity minimises ischaemic damage.

1.5.2 Circulatory death

The other recognised mode of death is through cessation of circulation. This is the mode of death in the majority of circumstances both in the community and in hospitals. It is usually the result of cardiorespiratory arrest, resulting in pump (heart) failure and therefore cessation of circulation. There are numerous circumstances and categories of circulatory arrest (Maastricht classification: see below), at present a small subset of this potential pool, where controlled and planned withdrawal can occur, is being utilised for organ donation. The donor withdrawal process and steps leading to circulatory cessation are all of critical importance and are outlined in Figure 4(a). The typical DCD donor is in the intensive care unit (ICU) and ventilated; these patients have sustained catastrophic irreversible cardiorespiratory or neurological injury and are considered for withdrawal of life-sustaining treatment by intensive care specialists. In this setting, following discussion with family, withdrawal of care is agreed upon. An important aspect is the identification of patients who are likely to have rapid progression to death following withdrawal, with this likely to happen in less than 90 minutes. The other criteria of selection are similar to BD donors. If deemed suitable for organ donation and consent acquired, the process of organ donation begins.

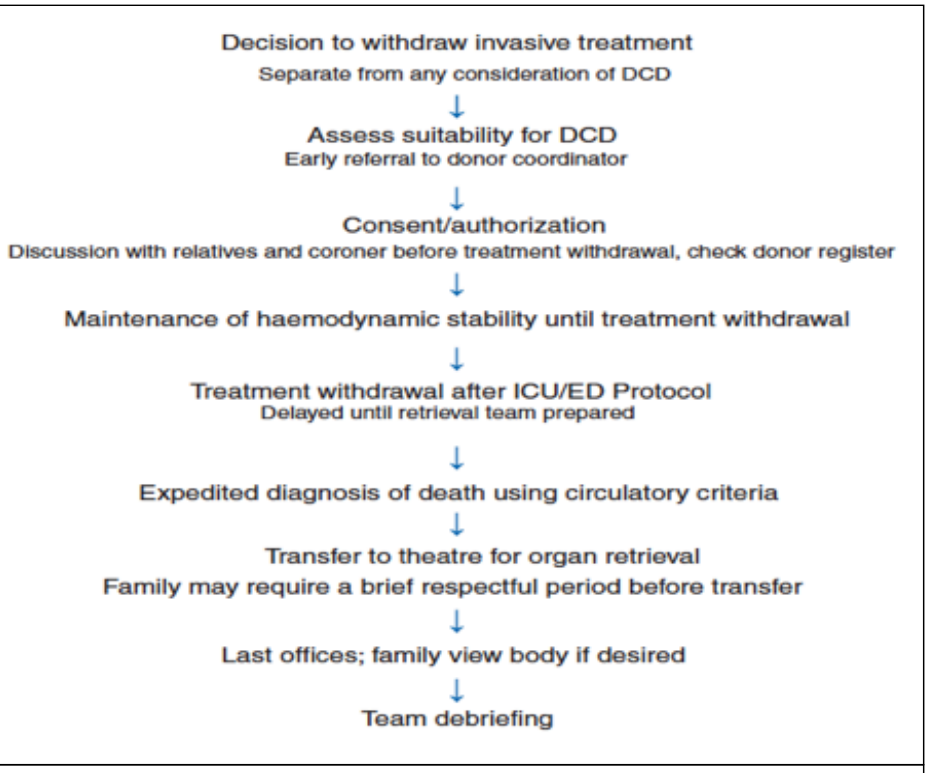

Figure 4 (a) – The DCD withdrawal process
Source: Modified from the publication: Organ and Tissue Donation after Death, for Transplantation. NHMRC 2007 (20)

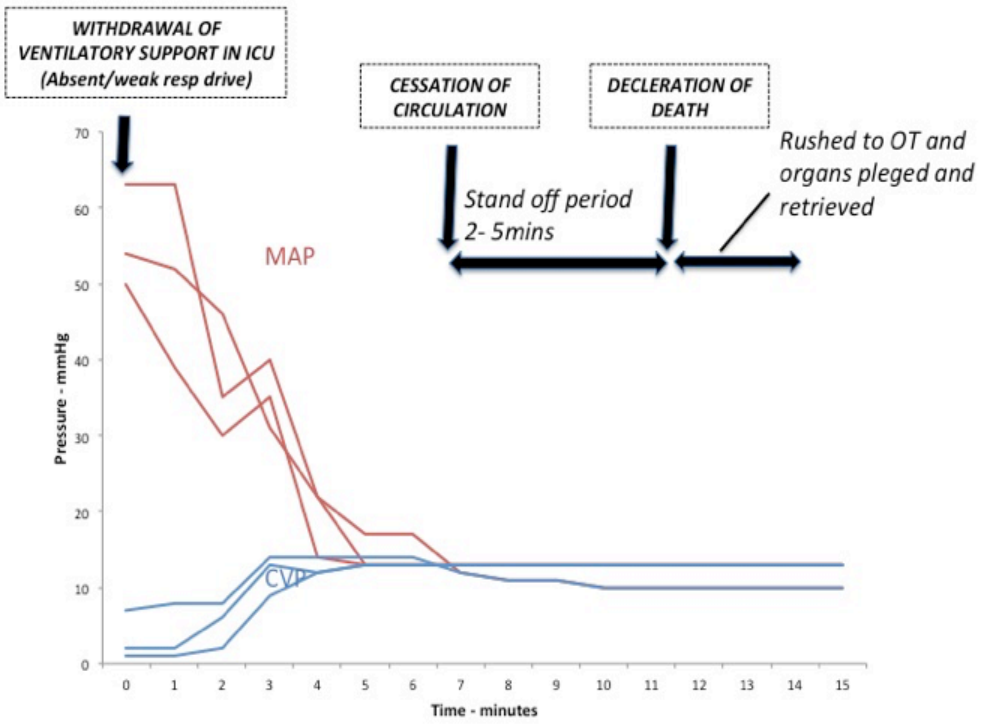

Figure 4(b) *– The DCD withdrawal process and haemodynamic changes leading to circulatory arrest*

The withdrawal process can occur in the ICU or the operating theatre (OT), however usually occurs in the ICU to allow family to be in attendance during this period. All life sustaining treatment is withdrawn, and monitoring, including ECG and intra-arterial line (IAL), remain in-situ. No treatment for the benefit of the donation process (heparin etc.) can be given prior to death in most jurisdictions, but drugs to ensure the patient's comfort can be administered. A sample chart of the haemodynamic changes that occur following withdrawal are shown in Figure 4(b). Death is declared with the use of the invasive monitors to ascertain irreversible cessation of circulation, and is done so by a member outside the organ retrieval or transplant team. Following circulatory cessation, a two- to five-minute stand off period is employed to ensure no reanimation or auto-resuscitation of the heart in vivo. There have been sporadic cases reported where there is re-establishment of circulation within the few minutes after declaration of circulatory arrest and death (18). Known as the Lazarus phenomenon, these cases have been noted following failed CPR rather than in the controlled DCD donor (19).

Despite this, given the theoretical risk of this phenomenon, the standoff period is employed and if no further activity is evident during this period, death is declared. The donor is subsequently rushed to theatre where organs are rapidly flushed with cold preservation solution and procured.

The critical difference with DCD donors is the inherent warm ischaemic time (WIT) during the withdrawal process. This refers to the period between withdrawal of care and organ flush with preservation solution, and is also referred to as the agonal phase. It is during this time that the organs are subject to numerous insults. Rapid hypoxia develops following withdrawal of ventilator support, and from this point all organs are subject to ischaemia in a normothermic environment. As a result of the hypoxia, cardiac pump failure ensues and there is a resulting drop in arterial pressures and a rise in venous pressures—the resulting hypoperfusion further contributes to ischaemic damage. Eventual equalisation of pressures and circulatory arrest results in donor death. These processes in the lead up to circulatory arrest, as well as the obligatory stand off period, are detrimental to all organs and are collectively known as warm ischaemic damage.

It is this warm ischaemic damage that has largely limited the use of cardiac allografts from DCD donors. The widespread knowledge of the detrimental impact of ischaemic damage secondary to acute coronary artery occlusion and its rapid irreversibility has had clinicians question the viability of DCD hearts following sustained periods of warm ischaemia.

1.6 Maastricht criteria

As previously mentioned, the event of circulatory arrest occurs in various settings and there is potential for organ retrieval in all of these events. In an attempt to categorise these into clinically relevant scenarios that are universally accepted, ‘Maastricht’ categories were established. These have been refined and are now broken down into controlled and uncontrolled donors situations, referring to the circumstances of cardiac arrest (Figure 5).

Category	Description	Type of DCD
I	Dead on arrival	Uncontrolled
II	Unsuccessful resuscitation	Uncontrolled
III	Anticipated cardiac arrest	Controlled
IV	Cardiac arrest in a brain-dead donor	Controlled
V	Unexpected arrest in ICU patient	Uncontrolled

Figure 5 *– Modified Masstricht criteria (22)*

Until now, the focus of DCD organs in Australia and the world has remained on category III donors (and category IV, although uncommon), where a controlled setting offers a limited warm ischaemic time and allowance for the logistics of organ procurement and recipient preparation. However, some groups around the world have ventured into the category I and II groups (22).

Whilst the inclusion of category I and II donors add numbers to the donors pool for several organs, it is likely that these uncontrolled situations may be well beyond the point of reversible ischaemic damage to the cardiac allograft. In addition, the use of category I and II donors requires a significant increase in logistical organisation that is currently not established in Australia. For now, the controlled setting of category III donors offers the best setting to limit warm ischaemia and therefore to answer the question of DCD cardiac allograft viability for transplantation.

1.7 DCD donors in clinical transplantation today

While the damaging forces of warm ischaemia have caused apprehension and avoidance of DCD cardiac allografts until now, numerous other DCD organs are currently being utilised clinically with promising results. An understanding of the issues and complications encountered with these organs, as well as their warm ischaemic tolerance, is useful as we begin to research cardiac viability for transplantation.

DCD organs are now being routinely used in clinical kidney, liver, pancreas, and lung transplantation. Results with kidney and lung transplantation using these organs have been at least as good as organs acquired from the BD donor. However, DCD liver

transplantation results have been inferior to BD liver transplantation, with increased rates of ischaemic biliary complications.

1.7.1 Kidney transplantation

The results of kidney transplantation have been comparable between BD and DCD donors, with similar long-term survival. While there have been reports of higher rates of early graft dysfunction, this does not appear to affect longer-term rates of graft failure, survival and glomerular filtration rate decline (23,24).

It has also been reported that dialysis patients who are on the waiting list will have increased life expectancy after a DCD kidney transplant, when compared to continuation of dialysis treatment and later receiving a conventional donation after brain death (DBD) kidney (25).

The impact of DCD kidneys on the transplantation scene has been marked, and currently account for up to one-third of kidney transplants in the UK, and 11% of kidney transplants in the US (26,27,28).

1.7.2 Liver transplantation

The outcomes with DCD liver transplantation have been sub-optimal. Most local, national and international registry data concur that DCD recipients experience inferior graft survival in comparison to DBD donors (30-33). This is chiefly related to the increased rates of ischaemic biliary strictures (16% in DCD donors vs. 3% in DBD donors in one report) (34). In addition, the increased rates of hepatic artery thrombosis in the absence of ante-mortem heparinisation may also play a role.

However, while DCD livers are associated with worse graft and patient survival when compared with DBD livers, it does offer superior survival when compared with remaining on the waiting list (34).

1.7.3 Lung transplantation

Very promising results have been reported with DCD lung transplantation (35,36). The Australian experience with DCD lungs commenced in 2006 across several units throughout the country. Excellent results have been achieved with superior medium-term outcomes when compared with BD lung transplantation during the same period, Figure 6 (37). It is postulated that the absence of the BD catecholamine storm in DCD donors may potentially explain the lower rates of primary graft dysfunction (PGD) and Bronchiolitis Obliterans Syndrome (BOS) in lung transplantation.

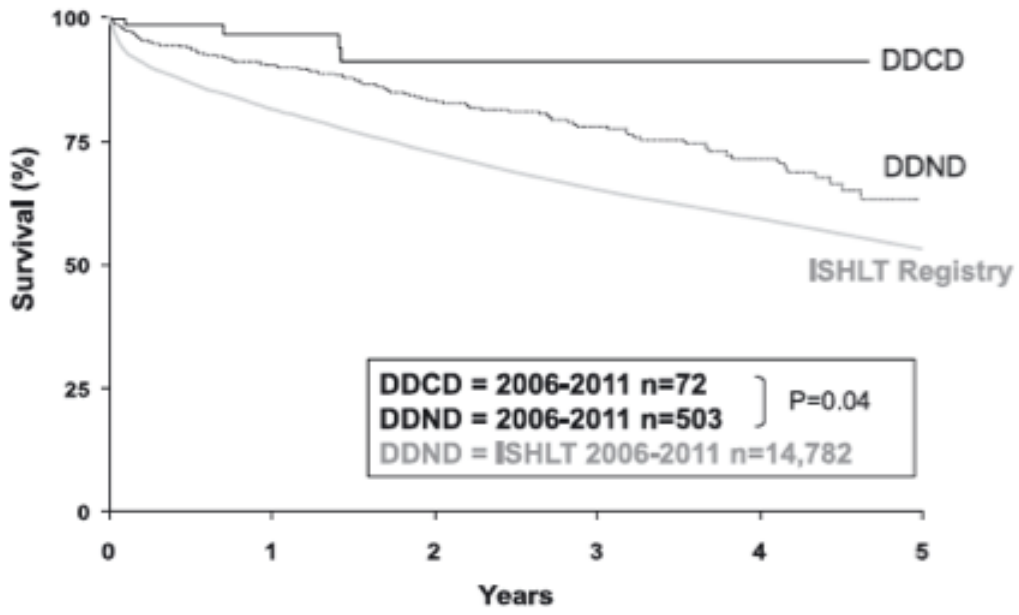

Figure 6 *- Actuarial survival of DDCD donor LTx recipients (p = 0.04) versus DDND LTx recipients versus International Society for Heart and Lung Transplantation Registry survival: 2006–2011. Source: Levvey BJ et al. Excellent clinical outcomes from a national donation-after-determination-of-cardiac-death lung transplant collaborative. Am J Transplant 2012 – Fig 3.*

These promising results with kidney and lung transplantation has established their role in transplantation today, and their use will increase worldwide as more units partake in DCD organ transplantation. The impact of the introduction of DCD lung transplantation is evident in Australia's experience, with transplant numbers increasing by 28% (37).

With the success of kidney and lung transplantation the goal would be to translate this progress to cardiac transplantation. Whilst the lung is more resilient to warm ischaemia due to its capacity for oxygen extraction from both vascular perfusion as well as alveolar oxygen exposure, the same cannot be said for the heart. Hearts are highly dependent on aerobic metabolism, with oxygen delivery via coronary perfusion alone. It has been shown that the effect of hypoventilation has a greater impact than hypoperfusion in the DCD setting for lung viability post-transplant (38). Hearts on the other hand, reliant on perfusion alone, face a rapid onset of ischaemia that results in far earlier transition to irreversible damage and non-viability than other organs. The timing of this transition from reversible ischaemia to irreversible damage is unclear, and forms part of the assessment undertaken in one of the publications included.

To date, several pre-clinical studies and a handful of clinical case-reports (non-transplanted hearts) have been published about the utilisation of DCD hearts for transplantation. Whilst results have been promising, there has been no clear consensus about the viability of these hearts for transplantation and there remains concern about their use in transplantation. In addition, no studies have been attempted in a model that completely mimics the clinical setting and readily allows easy clinical transplantation. A review of the DCD heart literature is provided in the following section.

1.8 DCD hearts: pre-clinical studies

As mentioned, the earliest DCD cardiac transplantation work dates back to the first human heart transplant done by Christian Barnard in 1967 (8). With warm and cold ischaemic times being very short, the transplant was a success. However patient survival was limited by infection in the setting of the immunosuppression used at the time. Within 12 months of this, the official recognition of BD paved the way for DBD heart transplantation, which has since remained the only source of adult cardiac allografts.

However, the scarcity of organs from such donors has led investigators to re-evaluate alternate sources. The reality of modern transplantation relies on distant

procurements, with donor and recipient location only very rarely in the same institution. As investigators look at DCD hearts once again, the setting is different to what it was over 50 years ago. Researchers need to assess the viability of these hearts exposed to longer WITs to ensure clinical relevance, as well as their tolerance of longer procurement distances. Research in overcoming these challenges has been underway over the past two decades.

Pre-clinical work into DCD hearts have relied on numerous animal models, including primates, dogs, pigs and rats. Models have ranged from non-transplant ex vivo perfusion assessments to clinically relevant orthotopic transplantation.

The DCD insult has been recreated using exsanguination or an asphyxia model, and warm ischaemic times have ranged from under 20 minutes to 60 minutes. Whilst some are more clinically applicable than others, they all provide insight into the nature of the DCD insult and degree of allograft recovery.

One of the earlier works by Gundry et al (39) showed significant promise of DCD graft viability. In five juvenile baboons weighing only 3.6 kg, a clinically relevant asphyxia model was employed and DCD hearts exposed to WIT of between 15 and 31 minutes underwent orthotopic transplantation. These hearts were successfully weaned off CPB and kept alive for up to 34 days. The young age and small size of these animals may be of more relevance to pediatric transplantation. In addition, there was pre-treatment of the donor with agents to limit ischaemia reperfusion injury (IRI)—such an intervention that would not be permitted in the clinical setting. Despite these, this study provided much early promise for DCD cardiac allografts.

Studies exposed the hearts to varying periods of warm ischaemia ranging from 15 minutes (39) to 60 minutes (40). In previous studies, researchers elected a set WIT to expose the heart to, however, to date no group have compared the effect of differing WIT on recovery; thus, there appears no clear consensus on the limit of WIT. In addition, the use of differing donor cardioplegia agents and post-mortem treatments causes variation in WIT tolerance and cardiac recovery, further confounding the process of defining a WIT limit for DCD cardiac allografts. Whilst hearts exposed to a

WIT of less than 30 minutes appear to have recovery suitable for transplantation (41), hearts exposed to 30 minutes or greater appear to have impaired functional outcomes post-reanimation. Following a WIT of 30 minutes and despite continuous cardioplegia perfusion preservation, Repse et al showed a significantly worse recovery when compared to control/normal hearts in a canine model (42). Osaki et al reported that despite utilising controlled initial reperfusion to limit reperfusion injury, there was at best only a 60% recovery (c/w donor baseline) of cardiac output following a total WIT of ~40 minutes (43). With longer WITs, the recovery deteriorates further. Hirota et al reported that in dogs, following a WIT of 60 minutes, there was only a 50% recovery of cardiac index in an ex vivo working model perfusion setup (40). On the other side of 30 minutes, there appears to be more promising results reported. Scheule et al reported that following mean WITs of 25 minutes, albeit in an exsanguination model in abattoir pigs, there was no difference in functional parameters on an isolated working heart setup between DCD and control (no warm ischaemia) hearts (44). Ali et al showed in a clinically relevant model that DCD hearts exposed to a WIT of 15 minutes (mean) had recovery comparable with DBD hearts—all animals were weaned off CPB and displayed no differences in load independent biventricular contractility (41).

Despite varying interventions and strategies of reperfusion, there appears to be a point at which cardiac recovery is impaired. While it is difficult to compare studies with varying models and strategies, the evidence thus far suggests deterioration of cardiac recovery at WITs of beyond 25 to 30 minutes.

1.8.1 DCD model

Several confounding features exist in the literature that warrants attention. The most relevant DCD model is animal asphyxia, which most closely resembles the clinical setting. Several studies that have shown promise of DCD allografts for transplantation have used an exsanguination model, which employs rapid exsanguination of donor animal blood at the commencement of withdrawal. This obviates one of the insults that the heart is exposed to during WIT: distension. Osaki et al published an important paper that defines the difference in these models; comparing cardiac arrest via

asphyxia or exsanguination, they reported an increase in left ventricular end-diastolic volume (LVEDV) (to 132% of baseline post-arrest) and impaired left ventricle (LV) function post-transplant of asphyxiated donors (45). Ali et al reported a significant decrease in right ventricle (RV) ejection fraction (despite a normal RV ESPVR) but complete recovery of LV function following resuscitation of DCD hearts in an asphyxia model (41). The RV in particular appears to be at risk of injury from the impact of increased venous pressures and circulatory load. In addition, exsanguination removes the progressive biochemical and catecholamine changes that the heart is exposed to during the agonal phase. It is postulated that the combination of distension, as well as the catecholamine-mediated injury of the RV seen in DBD donors (46), contribute to marginality of the RV in DCD donors (41).

Hence, the promising results advocating DCD allograft viability and suitability for transplantation by several investigators (44,47,48) must be assessed in light of these organs being sheltered from the above insults.

1.8.2 Non-transplant model

Several of the papers published to date report results in a non-transplant model—using an ex vivo working heart setup, donor hearts are explanted and cannulated for reperfusion and reanimation. Depending on the nature of the perfusate, such setups allow the hearts to be continuously perfused in an arrested state (40,42,44), or in an unloaded beating state (40). With the use of an oxygenated perfusate such as blood, they can be transitioned into a loaded 'working' state through filling of the left atrium at various preloads (40,42,44,48). Whilst this is an alternate and potentially superior method of preservation (compared with cold storage preservation), the lack of orthotopic transplantation makes this model less relevant. There is an inherent additional cold and warm ischaemic time that is added during transplantation that is not accounted for. The DCD heart's ability to withstand further periods of ischaemia beyond the initial insult is unclear and no firm conclusions can be drawn about organ viability until assessed post-transplantation. Some investigators have avoided a transplant model but have mimicked the transplant process insult with the addition of

40 minutes warm ischaemia post preservation (42). However, the relevance of the transplant model is also in its ability to assess the heart's capacity to support left- and right-sided circulation in vivo. Whilst there are reports of four-chamber (left and right heart) working heart setups (49), this is technically more challenging and has not been translated to DBD or DCD cardiac allografts. With questions raised about the ability of the right heart to recover from the DCD insult, the transplant model appears at present to be the most accurate way to assess its recovery.

1.9 Ischaemia reperfusion injury

1.9.1 Donor pre-treatment

In an attempt to pre-condition the heart and mitigate IRI, investigators have infused donors with agents to aid in minimising IRI (39). The evidence for this is covered in the next few paragraphs, but the diminished relevance of pre-mortem intervention is important to note. Clinically the 'dead donor rule' ensures no allowance for pre-mortem intervention in the donor that assists in the organ donation process (50). While there are some jurisdictions where pre-withdrawal administration of heparin is allowed (37), this is not universal at this point in time. While donor pre-treatment is unlawful, any intervention post declaration of death to decrease IRI is acceptable and likely of great benefit to DCD allografts.

Myocardial injury occurs during several phases and includes the ischaemic period, the acute reperfusion phase, and a more delayed reperfusion phase. In the acute coronary syndrome setting, attempts to achieve early reperfusion by reopening acutely occluded vessels have made large strides in limiting the ischaemic period. With improved pre-hospital therapy and more rapid transit from presentation to the catheterisation laboratory, there have been very significant reductions in mortality in patients with myocardial infarctions.

As much as the ischaemic period is a source of considerable damage to the myocardium, the time of reperfusion itself plays an equally large role in myocardial injury. This period continues to be overlooked clinically, with only a short window of

opportunity to provide intervention and ongoing research in elucidating the mechanisms involved. The ability to intervene to limit myocardial reperfusion injury was first outlined by Murry et al nearly three decades ago (51). These authors described the phenomenon of ischaemic pre-conditioning, whereby episodes of sub-lethal intermittent ischaemia and reperfusion conferred resistance against a subsequent lethal episode of myocardial ischaemia. Benefits of pre-conditioning in reducing infarct size, preserving vascular endothelial function and reducing apoptosis have all been reported since then (51-54). The clinical relevance of pre-conditioning the heart prior to an unanticipated ischaemic episode is absent in ACS, but has been studied and shown to be of benefit in the transplant (55) and cardiac surgery setting (56) where onset of ischaemia is anticipated. Since Murry et al, there has been significant progress in delineating the extracellular signaling and intracellular targets at play following the pre-conditioning stimulus. The role of mitochondria—and specifically mitochondrial permeability transition pores—various survival kinases pathways, and calcium homeostasis have been identified as key players in IRI (57).

While ischaemic pre-conditioning is an important strategy in limiting IRI, of more practical application is the ability to intervene at the time of reperfusion following an ischaemic insult. Referred to as ischaemic *post*-conditioning, this more recently discovered phenomenon has greater clinical relevance. It has been shown that intermittent bursts of ischaemia and reperfusion (similar to pre-conditioning) *after* an ischaemic insult provide an equally protective effect on myocardial injury (58). Furthermore, the mechanisms activated in this process are similar to pre-conditioning. The importance of ischaemic post-conditioning is its relevance to DCD cardiac allografts. While DBD donors can be pre-conditioned prior to the preservation cold ischaemia, no intervention can be undertaken in the DCD donor until after the period of warm ischaemia. Whilst intervention to prevent ischaemic damage in DCD donors is limited to decreasing WIT, the scope to limit reperfusion injury is large and warranted.

Since the study by Zhao et al it has been shown that post-conditioning confers protection from reperfusion injury in a manner similar to pre-conditioning via recruitment of signal transduction pathways (59). These pathways include the

reperfusion injury salvage kinase (RISK) pathway (60), the survival activating factor enhancement (SAFE) pathway (61) and the nitric oxide synthase pathway (62). All these pathways converge at the mitochondrion, where the outcome appears to determine myocyte survival. The mitochondrion plays a large and significant role in IRI (63,64). Its detrimental role is not only a result of impaired ATP synthesis under hypoxic ischaemic conditions, but also because it represents a 'switchboard' for the above signaling pathways controlling cell death (57). These signals impact on the mitochondrial permeability transition pores (mPTP), which normally tightly control the ion barrier between the matrix and the cytosol. A critical determinant of cell death in IRI is the opening of this mPTP, which occurs in the first few minutes of reperfusion.

Opening of the mPTP, located in the inner mitochondrial membrane, renders the otherwise impermeable inner membrane freely permeable to solutes up to 1500 Da in size (65,66). The result is swelling of the mitochondrial matrix, rupture of the outer mitochondrial membrane, and release of pro-apoptotic factors into the cytosol that leads to cell apoptosis (67). In addition, mPTP opening uncouples mitochondrial oxidative phosphorylation and loss of the mitochondrial membrane potential, resulting in cell necrosis if the pores remain open and ATP depletion occurs. Conditions that promote its opening at time of reperfusion include a high mitochondrial calcium and inorganic phosphate load, ATP depletion, oxidant stress and a corrected matrix pH (68-70).

Cytosolic calcium overload results from the switch to anaerobic metabolism during the ischaemic period. As a result of decreased pH from lactate production, the Na-H+ exchanger is activated by extruding the H+ from the cell in exchange for intracellular Na+ overload. This results in activation of the Na-Ca exchanger ending in cytosolic Ca overload. Furthermore, the lack of ATP during ischaemia inactivates the Na-K ATPase further increasing cytosolic Na and therefore Ca overload. During this acidic environment, the mPTP remains closed (71).

At reperfusion, several cellular changes occur: pH normalises as a result of lactic acid washout, mitochondrial calcium overload results from activation of the mitochondrial

calcium uniporter, and reactivation of the electron transport chain releases reactive oxidative species (ROS). This creates a milieu for mPTP opening and resulting reperfusion injury as outlined above.

It is this opening of the mPTP, occurring at the time of reperfusion, that has been the target of researchers and interventions in the fight against IRI. It is of particular importance in DCD hearts, where intervention at the time of reperfusion is permitted and limiting the effect of IRI is paramount for organ viability. Therefore, various strategies have been investigated in inhibiting mPTP opening at reperfusion. This search has resulted in the identification of the RISK and SAFE pathway as integral parts of the post-conditioning protective benefit (72,73). Whilst the activation of these signaling cascades was initially attempted using mechanical approaches (Zhao et al), further elucidation of molecular pathways has allowed researchers to identify pharmacological agents that activate similar mechanisms.

The RISK signaling cascade involves the signaling elements of Akt (74-77) and ERK1/2 (74,78,79), activated via cardio-protective stimuli. The nature of these stimuli and the exact mechanism of protection in ischaemic post-conditioning are still not clearly defined. Despite this, there have been studies that strongly suggest the role of Akt and ERK1/2 in inhibiting mPTP opening (80-84). Agents that have been postulated to activate both Akt and ERK1/2 include adenosine (85,86), erythropoietin (87-89) and NHE inhibition agents (90).

The other signaling cascade of interest is the SAFE pathway, which involves the activation of transcription factors JAK2-STAT3 in mediating IRI (91,92). Mitochondrial STAT3 activation has been shown to have a significant cardio-protective effect through post-conditioning pig hearts with regional myocardial ischaemia and reperfusion (93). Post-conditioning the heart causes phosphorylation of STAT3 in the mitochondria resulting in better preservation of complex 1 respiration and inhibition of the mPTP. (93,94). Apart from mechanical stimuli to activate JAK-STAT, leptin (95), erythropoietin (74,79,89) and NHE inhibition agents (90) all work in activating JAK-STAT.

As mentioned above, nitric oxide (NO) homeostasis also has a role in IRI, and is impaired in ischaemia and reperfusion. As a result of ischaemia and resulting intracellular Ca influx, endothelial nitric oxide synthase (eNOS) is activated. Following an initial burst of NO release, NO levels decline from decreased levels of L-arginine (substrate) and tetrahydrobiopterin (cofactor) (96-98). Upon reperfusion, the ongoing activated eNOS undergoes 'uncoupling' and releases ROS instead (99,100). NO levels are low, and its replacement exogenously has been shown to be of benefit in limiting IRI. While the well-known effects of NO include cGMP mediated vasodilation and decreased platelet aggregation, it also has effects in opening mitochondrial ATP sensitive K+ channels and exerts a negative feedback effect on eNOS, thereby limiting the release of ROS (the link between ROS and opening of the mPTP is known) (101). The benefit of GTN as a source of exogenous NO has been shown in cardiac allograft preservation in small and large animal studies (55,102).

Erythropoietin has been investigated in both small and large animals, being evaluated in ACS (74,88,89) and more recently transplant models (55). It is best known for its role in erythropoiesis, acting to direct erythroblasts away from apoptosis and instead differentiate and mature into erythrocytes. The mechanism of this effect is via activation of intracellular PI3K-Akt (RISK) and JAK-STAT (SAFE) signaling cascades, the same proposed pathways as in ischaemic post-conditioning. Activation of these cascades by EPO to provide cardio-protection has been demonstrated (74-77,79). Its benefit has also been shown in a large animal DBD transplant model, supporting its role in ischaemic conditioning and limiting IRI in a clinically relevant setting (55).

Another area of active interest is the sodium-hydrogen exchanger (NHE) as a target for intervention. As detailed above, this transporter is activated in ischaemia as a result of intracellular acidosis. The effects of intracellular sodium (Na) and calcium overload result in mitochondrial calcium overload upon reperfusion and subsequent mPTP opening. The end result of this is cell necrosis/apoptosis. Whilst interventions to inhibit mPTP have been investigated as outlined above, numerous investigators have evaluated the NHE as a target to prevent calcium overload. NHE inhibition using agents such as amiloride, cariporide and zoniporide has been shown in numerous small and

large animal studies to limit myocardial IRI (103-106). In addition to their effects in limiting intracellular calcium overload, it has also been shown that zoniporide supplementation of Celsior preservation solution causes activation of ERK and STAT3 in isolated rat hearts (90).

The combination of these agents in GTN, Na-H+ exchange inhibitor and EPO, with their varying roles in pre-conditioning the heart through the signaling cascades of RISK, SAFE and NOS, have been studied extensively and shown to be of significant benefit in mitigating IRI in small and large animal cardiac allograft preservation and transplant models (55,101,107,108). With the knowledge that the mechanisms of ischaemic pre- and post-conditioning are very similar, the use of these pharmacological agents in mitigating IRI in the DCD setting is of particular relevance. The DCD allograft, given its exposure to a significant ischaemic period, is certain to have further damaging reperfusion injury, the impact of which has been established. While little can be done about the inherent warm ischaemia, interventions to mitigate IRI are critical in demonstrating any viability of DCD cardiac allografts in transplantation.

1.10 Warm ischaemic time definition

The definition of warm ischaemia has varied between investigators and for different organs. While the definition of the agonal phase in the DCD setting most commonly refers to the period from withdrawal of ventilator support to circulatory arrest, the definition of warm ischaemia has been inconsistent. Proposed definitions include: time from withdrawal of ventilator support to organ flush with preservation solution, time from BP systolic of under 50 mmHg to organ flush, time from oxygen saturations of <70% to organ flush or time from circulatory arrest to organ flush. Levvey et al proposed the definition of BP(s) of <50 mmHg as the most practical approach to defining WIT for DCD lung transplantation (109). This arbitrary figure has been proposed based on practical application and data recordings during the withdrawal process, however there is no evidence in pre-clinical or clinical reports that a systolic BP of 50 mmHg has any relevance to onset of significant organ ischaemia. In addition,

the impact of hypoxia has been demonstrated as being of more relevance than hypoperfusion (BP <50 mmHg) in DCD lung viability (38).

The most commonly used timeframe for WIT is the interval between withdrawal and organ preservation flush. Until more data is collected from DCD donors and subsequent transplant outcomes, this definition provides the simplest and most comprehensive measure of WIT. At this early stage of assessing DCD cardiac viability, it would appear safer to assume WIT from time of withdrawal to include all hypotensive and hypoxic insults regardless of the degree.

1.11 DCD hearts: clinical studies

As outlined previously, the first heart transplant ever performed and the first heart transplant in Australia were from DCD donors. Not soon thereafter the recognition and legalisation of BD ensured the transition to DBD cardiac transplantation. With the absence of any warm ischaemia with DBD donors, there was simply no reason to re-consider DCD cardiac transplantation. For the decades that followed focus remained on immunosuppression, fine tuning surgical techniques and improving organ preservation during the cold ischaemic time.

But in the last two decades, with a drop in donor numbers and increased demand for transplantation, attention has once again returned to DCD cardiac allografts. Although numerous pre-clinical studies have been conducted and a few sporadic clinical reports have been published, until now **no** adult DCD heart transplants have been performed. A large part of this has been the fear of ischaemic damage and suspected non-viability. Since the initial few original transplants in the 1960s, there exists only two reports of adult human DCD non-transplanted hearts and one case series of three successful paediatric DCD heart transplants.

In 2009, Ali et al reported a case of in vivo cardiac reanimation in a DCD donor (110). The donor was a 57-year-old female with a catastrophic intracranial bleed; she had no significant cardiac history or any inotropic requirement pre-withdrawal. Cardiac asystole was rapid, occurring one-minute post withdrawal. A total WIT from

withdrawal to re-establishment of circulation was 24 minutes, and comprised a five-minute stand off, transport to the OT and surgical entry into the chest followed by cannulation for cardiopulmonary bypass (CPB). Head vessels were clamped to prevent cerebral circulation and conductance catheters were inserted into the LV and RV to assess contractile function. The donor was weaned off CPB and cardiac function was assessed on 5 mcg/kg/min of dopamine. LV function was promising with PV loop analysis comparable to normal hearts; however, RV function appeared to take on an abnormal ischaemic appearance on PV loop analysis. Despite this the heart was able to once again support the donor circulation with normal central venous and PA pressures. While these results were promising for DCD cardiac allograft viability, there were several questions raised as a result of this report. Concerns have been raised about the ethics of re-animating the heart in vivo and particularly of re-establishing circulation in vivo. The declaration of death in DCD donors is based on the irreversible cessation of circulation; therefore re-establishing circulation in the donor understandably raises moral and ethical questions. Furthermore, the clamping of head vessels raises further moral doubts that will likely face strong opposition when establishing a clinical program. The assessment of organ viability following warm ischaemia is addressed; however, it does not take into account the cold ischaemic time/preservation period that exposes these hearts to further insults, recovery following which remains unanswered. Finally, this report raises further questions about the RV's ability to withstand the DCD insults of distension and warm ischaemia. Despite the above concerns this report provides promise for DCD cardiac allograft viability, and rightfully points to the need for further research in this field.

More recently in 2014, Osaki et al reported the attempted resuscitation of five human DCD hearts (111). Comparison was made between five DBD hearts declined for transplant (based on donor coronary disease, age and high risk social history) with five DCD hearts with a WIT of between 26 and 174 minutes. Following preservation flush with UW solution and subsequent explantation, cold storage preservation was employed for both donor types with mean cold ischaemic times of 211 minutes (DBD) and 177 minutes (DCD). Assessment of functional recovery was in an ex vivo working heart perfusion system, where LV ESPVR was calculated. Of the five DCD hearts, one

heart had a WIT of 174 minutes and as expected was unable to be resuscitated. The remainder had WITs ranging between 26 and 40 minutes. While all of these could be resuscitated to sustain a loaded state on the ex vivo system, they displayed a non-significant trend towards inferior LV ESPVR when compared to DBD hearts. It is difficult to determine the significance of these findings. DCD hearts subjected to WITs of beyond 30 minutes in pre-clinical studies have appeared to display inferior recovery (42), and it is likely that the human hearts in this report with longer WITs may have also sustained more severe ischaemic damage and resulting impaired functional recovery. The control hearts in this report are also not ideal, as these are marginal DBD hearts that have been rejected for transplantation and hence would be expected to have impaired recovery. So whilst there appears only a trend to impaired ESPVR of DCD hearts to DBD hearts (6.9+/- 0.7 vs. 5.6 +/-1.5 mmHg/ml), the control does not necessarily indicate viability for transplantation. Also, the impact on the RV was not assessed in this report. The authors did successfully reanimate these human DCD hearts ex vivo—this is of significance as it appears that for DCD cardiac transplantation to become a reality there needs to be a method of ex vivo assessment post warm ischaemia to help determine organ viability.

The only DCD cardiac transplants that have been done in the modern era have been in the paediatric population (112). In 2008, Boucek et al from Denver USA reported in NEJM three successful DCD paediatric transplants. Mean donor age was 3.7 days and mean WIT (from withdrawal to declaration of death/preservation flush) was 18.3 minutes. Preservation flush was administered immediately following declaration of death via femoral arterial balloon catheters placed in the ascending aorta. Recipient mean age was 2.2 months, and total cold ischaemic time was 106 minutes. One recipient needed ECMO post-transplant (recipient was on ECMO pre-transplant), but the remainder had no requirement for mechanical support or high dose inotropes. Mean length of stay post-transplant was 20 days, which did not differ from DBD cardiac transplant controls. All three recipients were alive at six months with normal ventricular function on echocardiogram. There was also no difference in rejection rates, and no late deaths at a 3.5-year follow up.

This paper also raised several questions and ethical concerns, illustrated by the editorials that followed the publication (113). The most significant of these was the duration of the stand off period prior to declaration of death—whilst the first donor had a three-minute stand off, the remaining two had only a 1.25-minute stand off. Concerns were raised about the ethicality of the shorter stand off, and no further DCD heart transplants have been reported from this institution. While the duration has varied across jurisdictions, the shortest stand off period accepted is two minutes. As outlined earlier, the fear of spontaneous cardiac reanimation or the Lazarus phenomenon post arrest is the basis for this. Although there are no cases of this phenomenon being reported in DCD donors but rather following CPR cessation, the concern remains: the backlash from the medical profession to the shortened stand off period is testament to the sensitivity of the medical community to alteration of accepted policy for declaration of death in DCD donors. It also stresses the importance of maintaining awareness of both ethicality and morality in DCD donors, and not compromising on these principles in an attempt to limit warm ischaemia. Other aspects that should be highlighted in this case series are the pre-withdrawal administration of heparin and insertion of femoral arterial sheaths for preservation solution infusions. Such interventions would not be permitted in Australian jurisdictions, as they would not abide by the 'dead donor rule'. Despite all these ethical dilemmas, this case series is a cardinal paper in highlighting the successful transplantation of DCD cardiac allografts exposed to WIT of up to 27.5 minutes. At least in a pediatric population it demonstrates the potential promise of DCD allografts whilst also emphasising the importance of ethical considerations and community awareness.

All of the above human DCD reports have shown promise of these cardiac allografts for transplantation. Both the pre-clinical and clinical reports are still yet to determine the WIT limit of cardiac viability, but it appears that the limit of WIT tolerance lies below 30 minutes. Further work also needs to be done in identifying a mode of cardiac assessment post warm ischaemia; although Boucek et al reported success using cold storage, it is unlikely that adult cardiac surgeons will be convinced to implant a heart exposed to such a significant insult without assessing recovery pre-transplant. Ex vivo

perfusion, as employed by Osaki et al may be necessary for this purpose (111). Of further promise is that there appears to be DCD cardiac viability without the use of additional interventions to limit IRI; it is yet to be seen if such intervention improves the left heart recovery reported by Osaki et al (111) and the right heart reported by Ali et al (110).

1.12 Donor heart preservation studies

In the absence of a preservation strategy between organ procurement and recipient implantation, cells rapidly shift from aerobic to anaerobic metabolism. With inefficient and limited ATP synthesis, anaerobic metabolism results in rapid depletion of energy stores and accumulation of toxic metabolites. In addition, paralysis of the Na+/K+ ATPase occurs contributing to cellular oedema. If ischaemia is not acutely reversed, cell death ensues.

The main goal of organ preservation is to ensure the maintenance of organ viability until recipient implantation. The current standard of care in cardiac allograft preservation is cold preservation solution flush of the organ followed by cold storage– deceleration of metabolic activity with hypothermia forms the basis of organ protection with this method, albeit for a limited period of time (114). Although hypothermia alone limits metabolic activity it is unable to cease all damaging cellular processes. Over time the small degree of anaerobic metabolism causes ATP depletion, Na+/K+ ATPase alterations, impaired Ca2+ homeostasis, mitochondrial derangements, and increased release of reactive oxygen species (ROS) all contributing to deleterious effects on cellular viability (115).

The use of additional preservation solutions aims to provide further cellular protection beyond hypothermia alone. The preservation solution is utilised in two time periods: firstly to arrest the DBD hearts through a flush down the coronary arteries, and secondly as the hypothermic solution the heart is stored in during cold storage transport.

Whilst its major purpose is to cause diastolic arrest in the DBD donors, it also serves to provide energy substrates, prevent myocardial oedema, and mitigate the injurious effects that occur at time of reperfusion (IRI).

The many commercial and in-house cardioplegic/preservation solutions in clinical use emphasise the complexity of the molecular and cellular mechanisms that underlie ischaemic and reperfusion related injury. It also highlights the lack of consensus about the optimal strategy for organ preservation (116). Cardioplegic/storage solutions, such as St Thomas' Solution No. 2 (Plegisol), Bretschneider (Custodiol), UW solution and Celsior, have been in widespread clinical use since the early 1990s (117-119) and appear to provide adequate protection of 'standard criteria' donor hearts subjected to ischaemic times of less than four hours (120). However, the cardioprotective capacities of such formulations may be suboptimal for the increasing numbers of "marginal" donor hearts seen in current clinical practice, particularly those subjected to longer ischaemic times. Whilst the solution that is used in our institution is Celsior no clear consensus has been reached about the superiority of one agent over another with varying results reported in the literature (121-123). It has been suggested that Celsior appears to have a greater protection for higher risk grafts (124), but the lack of convincing evidence is a consequence of the literature containing no adequately powered randomised control trial to answer this question.

The composition of Celsior includes mannitol to counter oedema, histidine to act as a buffer and offset acidosis, and glutathione to provide antioxidant activity against ROS. Of significance is that Celsior has been studied extensively in our laboratory (55,101,107,108) and appears compatible with agents added to limit IRI, an intervention that is felt to be critical in resuscitating DCD hearts and demonstrating any potential viability.

Following organ explant, the method of transport from donor hospital to the recipient has remained the use of an ice-filled esky and applying the principles of cold hypothermic storage. As mentioned, this provides a limited period of protection for standard donors, but its safety diminishes beyond this. Despite hypothermia and

preservation solution additives, exposure to a prolonged anaerobic environment results in depletion of metabolic substrate, progressive cellular acidosis, gradual Ca overload and hyperkalaemia induced endothelial injury, all contributing to intracellular and extracellular swelling and culminating in a priming of the cell for potentially lethal IRI upon reperfusion (57).

The suboptimal protection of marginal hearts has forced investigators to look at alternate methods of organ preservation. The use of non-hyperkalaemic strategies such as adenosine-lignocaine cardioplegia has been recently investigated – maintenance of the membrane potential and avoidance of hyperkalaemia have been demonstrated to be associated with less myocyte and microvascular damage, resulting in improved functional recovery when compared with standard hyperkalaemic cardioplegic solutions (125). The use of lignocaine in hyperkalaemic solutions such as Del Nido has already been demonstrated to deliver longer safe cardioplegic arrest times in paediatric and adult settings (126).

In an attempt to allow oxygen provision during preservation beyond cardioplegic solutions, mechanical perfusion systems, utilising various oxygenated blood and non-blood based solutions to continuously perfuse allografts ex vivo have been investigated for the last two decades with promising results.

1.13 Machine perfusion preservation of donor hearts

It is interesting to note that despite donor and recipient being present in adjoining theatres, the first human heart transplant utilised a DCD heart that was continuously perfused with donor blood during the interval (8). Since then, with the predominant use of DBD organs, cold storage has provided a simple and safe method of organ perfusion adequate for standard donors. In the setting of marginal donors with longer ischaemic times, static storage demonstrates higher rates of primary graft failure and higher mortality at one year (120,127).

The benefits of continuous perfusion preservation have been demonstrated in several pre-clinical studies. The provision to reinstate aerobic metabolism allows preservation

of ATP stores (128,129,130), as well as maintenance of ionic homeostasis through better membrane function and reduced oxidative stress in the myocardium (131). Improved myocardial oxygen utilisation (128,132) and greater fatty acid turnover during machine perfusion (133) also appears to translate to superior oxygen utilisation even post reperfusion in the recipient (134). Clinically, these benefits allow the tolerance of longer transport times and the potential for longer retrieval periods. The avoidance of a cold ischaemic time may in turn also reduce the degree of reperfusion injury.

It is clear that the build-up of harmful metabolites is damaging to these allografts—lactate accumulation has been shown to contribute to impaired ventricular recovery (135,136), and adenosine predisposes to increased ROS generation upon reperfusion and resultant IRI (137,138). Washout of these metabolites therefore forms an important part of the benefit of continuous perfusion (139).

Whilst it has been assumed that hypothermia down to four degrees is paramount for cold storage and limitation of metabolic activity, hypothermia is not without its adverse effects. Hypothermia causes cellular disturbances in ion homeostasis that threaten cellular survival, but this effect is postulated to occur only at temperatures less than 25 degrees. However mild hypothermia at 32 to 35 degrees avoids this and provides protection due to activation of cellular pathways rather than limiting energy utilisation. It has been suggested that this temperature range also contributes to ischaemic conditioning thereby providing further protection from IRI (140). While the principle of cold storage preservation cannot afford such mild hypothermic conditions, machine perfusion and an oxygenated perfusate certainly can. Machine perfusion allows for more precise control of myocardial temperatures, a more homogenous cooling effect and avoids the thermal damage that can arise from very low temperatures in cold storage (141,142).

The benefits of machine perfusion have been demonstrated in numerous pre-clinical studies. At a molecular level, lower rates of structural changes in the myocyte (129), DNA damage (131) and cellular apoptosis following reperfusion (128) have all been

reported. At a functional level in large animal models, better post-reperfusion ventricular performance at standard and prolonged storage intervals (129,143,144, 145), lower inotropic requirements post-transplantation (146) and improved survival post extended perfusion periods (147,148) have been demonstrated.

Machine perfusion offers benefit for the increasing proportion of recipients undergoing redo-transplantation or who are bridged to transplant with ventricular assist devices (VADs). In this population, it allows ongoing oxygenated perfusion of the allograft while recipient explant is completed negating the pressure burden of minimising cold ischaemic time in such circumstances. In addition, it is postulated that with these allografts sustaining less ischaemic injury and endothelial damage during the transplant process this may be associated with lower rates of chronic rejection and vasculopathy long term (149).

The benefits of machine perfusion have been reported with other organs. Clinical kidney transplants using machine perfusion preservation have reported lower rates of delayed graft failure (150-152) and allowed expansion of the donor pool (153). An important study in liver transplantation demonstrated a survival benefit for liver grafts after normothermic perfusion when compared to cold storage (154). A landmark paper in lung transplantation has been the use of ex vivo lung perfusion to resuscitate and utilise marginal lungs for lung transplantation with good results (155). Furthermore, benefits of machine perfusion extend beyond better preservation and assessment by providing a platform for organ intervention and optimisation—this has the potential to influence longer-term outcomes through immunomodulation and gene therapy strategies (156,157). The above interventions can take place with better effectiveness in a normothermic environment (158) without concern of systemic toxicity of a gene vector system in an isolated organ system (159). While such efforts are far from clinical applicability, the potential for such endeavours exists with this technology.

The use of machine perfusion in heart preservation is still in its infancy with no established clinical program with large numbers. With only one clinically approved device and several emerging approaches at this point in time (160-162), there is still

much work to be done in identifying the optimal perfusion strategy and perfusate. While lung preservation strategies have utilised devices for organ resuscitation and assessment following retrieval to the recipient hospital (155), hearts are more susceptible to cold ischaemic time and warrant a portable device to transport the device. Although this adds a degree of complexity, it is likely to be critical in minimising any further ischaemia (cold) for these marginal DCD allografts. One of the published papers of this thesis seeks to answer this question.

The perfusates that have been investigated include blood at varying temperatures (144,163), hypothermic crystalloid cardioplegia (164,165), blood cardioplegia at 26 degrees (166) and blood substitutes such as bovine haemoglobin (167). Part of the benefit of machine perfusion is its ability to assess myocardial viability following retrieval and prior to decision for transplantation, although this is not feasible with all forms of machine perfusion. An approach that has been utilised by some researchers is hypothermic cardioplegic machine perfusion of an arrested heart, with lower oxygen consumption and less complexity of operation, which has shown to be effective in organ preservation (168,169). However it fails to allow a platform for organ assessment. Some researchers have tried to overcome this limitation by utilising two separate methods: by having a period of continuous perfusion with a cardioplegic solution with the heart arrested followed by a period of normothermic perfusion in a separate machine to enable assessment of the working heart. While this is suitable for research applications, it lacks clinical applicability due to the duplication of cost and complexity of operation, and the risk of allograft damage from the added challenge of connecting and perfusing on two separate circuits. It has also been shown that DCD liver allografts, organs that appear to be particularly sensitive to warm ischaemia, fare worse when exposed to prolonged cold ischaemia prior to machine perfusion (170,171). Whilst an advantage of this hypothermic cardioplegic continuous perfusion strategy lies in its maintenance of a low oxygen requirement of the heart, it must be noted that oxygen demand is markedly reduced, even when the allograft is preserved in an empty beating state—empty beating normothermic hearts require 75% to 90% less oxygen than a loaded working heart (114).

Ex vivo organ assessment is particularly relevant to DCD allografts and several methods have been evaluated. Working heart assessment of developed pressure or cardiac output via adjustment of preload and afterload (49,169) offers a mode of functional assessment while the use of biochemical markers, such as lactate extraction provides metabolic profiling. The simplicity of arterial-venous lactate differential measures is part of the reason why this forms the mode of organ viability assessment in the only clinically approved ex vivo cardiac perfusion device: TransMedics organ care system (OCS) (172). While some research teams have utilised arrested heart machine perfusion preservation and assessment via in vivo orthotopic transplantation, this lacks clinical translation for any marginal heart—surgeons will demand confirmation of viability prior to implantation. A mode of ex vivo assessment potentially allows both expansion of the donor pool of marginal organs, and decreases the rate of primary graft failure by discarding organs that fail viability testing. Given this, it would seem sensible to utilise one device for machine perfusion that allows both oxygenated perfusate preservation and allograft assessment.

The use of tepid or normothermic blood allows preservation in a beating state and also allows the option for functional or metabolic assessment. Donor blood as perfusate is utilised in the clinical device (TransMedics OCS), with initial trials of this device confirming non-inferiority to cold storage in standard donors (172). The true test lies in marginal donors, initial results of which have been promising in keeping with the potential of machine perfusion to increase the donor pool (173).

Despite all of these benefits, machine perfusion has its challenges. When compared to cold storage, increased cost and complexity, the need for additional personnel and training are required. Another concern with machine perfusion has been the development of myocardial oedema. Associated with perfusion and pump parameters, hydrostatic pressure and perfusate oncotic pressure, the development of oedema is associated with diastolic dysfunction and has raised concern (174). The development of oedema has been reported by some investigators (168,175) but not by others (132,143) and appears to be dependent on several key factors. Peltz et al demonstrated the association of myocardial oedema with higher flow rates,

highlighting the importance of perfusion parameters (165). In addition, the composition of the perfusate and the use of oncotic agents have been shown to correlate with the degree of myocardial oedema (176).

While intracellular oedema is thought to be more irreversible and harmful for cell and organ viability than interstitial oedema, it is still unknown if machine perfusion contributes to one preferentially, and this warrants further investigation. It has been noted that despite the development of oedema there does not always appear to be any impact on functional recovery (164,177,178). Contributing factors to the development of organ oedema in ex vivo continuous perfusion include the nature of flow (continuous versus diastolic pulsatile), the absence of lymphatic drainage of the heart (179), and the choice of perfusate and preservations strategy (arrested heart hypothermic cardioplegic perfusion versus beating heart preservation). The benefits of beating heart preservation in limiting oedema have been suggested in cardiac surgery and in allograft preservation (164,162,180). Limiting the development and impact of oedema is an area of ongoing research in identifying optimal perfusates and perfusion strategies.

As experience in using machine perfusion grows, it is likely that the impact of cost and complexity will diminish. Although further work in minimising myocardial oedema and identifying a suitable perfusate is required, the potential benefits of machine perfusion in minimising ischaemic damage and reperfusion injury appears to outweigh the few limitations.

Machine perfusion may be of particular relevance for DCD cardiac allografts and their potential use in transplantation. Given the already significant period of damaging warm ischaemia, these hearts have a pertinent need to minimise any further cold ischaemic insult. In addition, there is an unquestionable need to assess viability of these grafts following their ischaemic insult. Finally, the platform for further intervention to resuscitate and optimise these organs is invaluable and may mark the difference between suitability for transplantation and non-viability. In these ways, machine perfusion offers avenues to address the challenges put forth by DCD cardiac

allografts and may well be critical in demonstrating any promise for DCD cardiac transplantation.

1.14 Ethical challenges in DCD heart transplantation

Organ donation rests upon appropriate management and decision making by the medical fraternity to ensure advocacy of donor wishes whilst maintaining ethicality, and is thus an area of active discussion (181). The 'dead donor rule' of transplantation refers to the act of procuring life-sustaining organs, such as the heart, only following the death of the donor. Procurement earlier than this is considered illegal and immoral. The two legally recognised modes of death are BD and circulatory death. Circulatory death implies irreversible cessation of blood circulation in the body requiring the cessation of pump or cardiac function in the donor.

DCD donors stem from the latter category of donors succumbing to circulatory death. The term 'DCD' has stood for either donation after cardiac death, donation after circulatory death or cardio-circulatory death. For declaration of death in DCD donors, reliance is on irreversible cessation of circulation, however this is determined by cessation of cardiac pump function. Therefore the focus in such donors has previously been on the heart, hence the widespread use of the term 'cardiac death'. The use of such a definition implies *irreversible* loss of function of the heart; however, the successful reanimation of the heart from such donors clearly demonstrates reversibility of cardiac function. While the cardiac and circulatory function are closely intertwined, the term 'cardiac death' is inaccurate and appears inappropriate and not in keeping with the legal definition of death. Proponents of change highlight that the circulation remains the defining feature. When patients are placed on the heart-lung machine, it is the circulation that is maintained while the heart is stopped. Thus, it remains critical that the irreversible cessation of circulation is the defining feature of death, and not the state of the heart. This, along with the recent push for DCD heart transplantation, has forced many jurisdictions to address the misleading nomenclature. In Australia, the term DCD has been changed from donation after

‘cardiac death’ to donation after ‘circulatory death’ to reflect more accurately the cause of death (182).

The focus on a two to five minute window post asystole/cessation of circulation is to ensure no ‘auto-resuscitation’, referring to the spontaneous reanimation of the heart in the donor—this would restore circulation and would imply return to life. As outlined previously, the basis for this is from numerous clinical reports of spontaneous cardiac reanimation and subsequent return of circulation following cessation of cardiopulmonary resuscitation. Although there have been no reports of this Lazarus phenomenon in the DCD setting, it would be prudent to maintain an at least two minute stand off period. Anything shorter has been shown previously to trigger a medical community backlash (183).

Another point to emphasise is that the donor’s family and ICU team have no intention to re-start the heart in vivo, as the focus has been to withdraw care given the poor prognosis. Therefore, once cessation of cardiac activity and circulation has occurred in the donor, death is declared. With the focus being on circulation, reanimation of the heart in the recipient has no impact on what has occurred in the donor.

The DCD patient has, in the majority of cases, severe neurological injury. The extent of the injury does not meet the criteria for brain death. Based on the grim prognosis, palliation is decided upon following discussion between the family and intensive care specialists. It is likely that in the setting of pre-existing brain injury, that a period of impaired or absent circulation of over 10 to 15 minutes will likely result in BD (184-187). It is this argument that is used by some investigators to allow re-institution of circulation and re-animation of the heart in the donor (188). Furthermore, other clinicians have suggested the inclusion of loss of higher brain function in addition to circulatory arrest in the definition of DCD—the argument posed by Tibballs et al is that cardiac donation from DCD donors negates the irreversibility of donor circulation; by adding the loss of higher brain function as a part of death declaration in the donor, this ensures that circulation is not the only basis for DCD death (189). Others have argued for a change of the definition of BD from ‘whole brain’ (currently used) to higher brain

function, which focuses on the irreversible loss of consciousness. This may potentially allow some DCD donors to have death declared by neurological criteria.

As we progress towards the potential use of hearts from DCD donors, there is a pertinent need to ensure that these ethical debates and issues are resolved with consultation from both the general community and the medical fraternity. It is also important to highlight that the most vital part of the donation process is the informed consent and discussion with the donor family. The entire donation process must safeguard the donor and have ongoing awareness of the ethical, moral and legal boundaries of organ donation.

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CHAPTER 2–5: PUBLICATIONS

Introduction

Despite several decades of research into DCD hearts, questions over their viability remain. In part, the reason for this is the ongoing uncertainty regarding the severity of the ischaemic insult and the presence of irreversible damage.

A series of experiments were planned and conducted in a large animal model and facility. Landrace pigs have been used previously in this laboratory, and therefore provided an established large animal model. While pre-clinical work to date has been undertaken in testing DCD hearts in ex vivo setups and in transplant models, there has been no clear consensus on the question of WIT tolerance of these hearts. Questions about the limit of WIT and irreversible damage remain unanswered. Using a withdrawal model that mimics the clinical setting, the paper entitled ***Increasing the tolerance of DCD hearts to warm ischemia by pharmacological post-conditioning*** aimed to answer this question. Exposed to varying periods of warm ischaemia from 20 to 40 minutes, the recovery of hearts on a blood perfused ex vivo working heart setup was analysed. In addition, lessons learnt from a decade of work on ischaemic conditioning in this laboratory were utilised. The findings of this work demonstrating the benefit of several pharmacological agents in activating ischaemic pre-conditioning and post-conditioning were tested in the DCD setting. Specifically, the hypothesis that pharmacological post-conditioning enhanced the tolerability of the DCD heart to warm ischaemia was then tested.

Following the identification of a WIT limit and the benefit of pharmacological post-conditioning, the next stage of experiments was to assess these findings in an orthotopic transplant model. In addition, an important question about the ideal preservation strategy for these hearts was assessed. With experience in ex vivo perfusion preservation gained in the first series of experiments, the next stage involved the use of the only clinically approved EVP device on the market. Hence this second stage of pre-clinical experiments, outlined in the paper entitled ***Normothermic***

ex vivo perfusion provides superior organ preservation and enables viability assessment of hearts from DCD donors, tested the viability of these DCD hearts pushed to the limit of WIT tolerance utilising post-conditioning strategies. Two specific hypotheses were addressed in this paper: firstly that normothermic ex vivo perfusion provides superior preservation of the DCD heart than static cold storage, and secondly that NEVP allows viability assessment of the DCD heart to be undertaken before committing the recipient to transplantation. This work was conducted in a manner that mimicked the clinical setting as closely as possible, thereby allowing maximal clinical relevance of any findings.

After a period of clinical non-transplant trials involving retrieval of human DCD hearts to the laboratory, it was felt that there was enough evidence for the clinical translation. Based on the results of the above pre-clinical work, the world's first human DCD adult orthotopic heart transplant of the modern era was conducted at St Vincent's Hospital Sydney in July 2014. The results of the first four retrievals and three transplants are outlined in the paper entitled ***Adult heart transplantation with distant procurement and ex-vivo preservation of donor hearts after circulatory death: a case series*** and published in the Lancet.

With clinical translation and an established program at our institution, the next stage involved refinement of understanding of the DCD process. The withdrawal period involves several haemodynamic, metabolic and biochemical changes that impact both the organ and the use of donor blood as a perfusate. With a goal of defining these trends to allow a better understanding of the DCD process and insults, the paper entitled ***Pathophysiological trends during withdrawal of life support: implications for organ donation after circulatory death (DCD)*** was written and is currently under review by the American Journal of Transplantation.

In the climate of donor shortages and failure to meet the demand for cardiac allografts, transplant centres around the world are forced to utilise donors that stretch the limits of acceptability for transplantation. These marginal donors, who include DCD donors, are at higher risk of early PGF. A better understanding of this complication and

its consequences are paramount as we approach the routine use of DCD cardiac allografts in transplantation; a review of this topic titled ***Primary graft failure after heart transplantation*** was therefore written and published.

CHAPTER 2 – PUBLICATION 1

TITLE: Primary Graft Failure After Heart Transplantation

JOURNAL: Journal of Transplantation; Article ID 175768

YEAR: 2011

Declaration

I certify that this publication was a direct result of my research towards this PhD, and that reproduction in this thesis does not breach copyright relations.

Foreword

Primary Graft Failure (PGF) represents a serious and life-threatening complication following heart transplantation. It is at particular risk of occurring in the setting of marginal donor organ use. Marginality refers to donors who are selected outside the ideal characteristics, but felt to be of adequate organ viability for transplantation, albeit with a higher risk of PGF.

Several factors make PGF relevant in the investigation of DCD cardiac allografts. The warm ischaemic insults of DCD donors make these cardiac allografts innately marginal. Whilst the hypothesis is that with shorter warm ischaemic times there is only reversible damage and maintenance of organ viability, there is little doubt that these hearts are more vulnerable to PGF in the post-transplant period. Furthermore, there has been suggestion that the use of ex-vivo perfusion better preserves hearts, and therefore lowers the risk of PGF. This forms another hypothesis in these studies. With PGF a significant endpoint in several facets of the investigation of DCD cardiac allografts, it appears prudent to review the current literature and outcomes with PGF in cardiac transplantation today.

This publication was utilised at the 33rd Annual International Society of Heart and Lung Transplant (ISHLT) meeting during which a consensus conference on PGF took place on 23 April, 2013. The subsequent report from the consensus conference published in the Journal of Heart and Lung Transplantation in 2014 also references this work and includes an expert opinion piece from Professor Peter Macdonald (1).

1. Kobashigawa J, Zuckermann A, Macdonald P et al. Report from a consensus conference on primary graft dysfunction after cardiac transplantation. J Heart Lung Transplant. 2014;33(4):327-340.

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Review Article

Primary Graft Failure after Heart Transplantation

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Primary graft failure (PGF) is a devastating complication that occurs in the immediate postoperative period following heart transplantation. It manifests as severe ventricular dysfunction of the donor graft and carries significant mortality and morbidity. In the last decade, advances in pharmacological treatment and mechanical circulatory support have improved the outlook for heart transplant recipients who develop this complication. Despite these advances in treatment, PGF is still the leading cause of death in the first 30 days after transplantation. In today's climate of significant organ shortages and growing waiting lists, transplant units worldwide have increasingly utilised "marginal donors" to try and bridge the gap between "supply and demand." One of the costs of this strategy has been an increased incidence of PGF. As the threat of PGF increases, the challenges of predicting and preventing its occurrence, as well as the identification of more effective treatment modalities, are vital areas of active research and development.

1. Introduction

Heart transplantation is an effective method of treatment for end-stage heart failure, with more than 5,000 transplants being conducted each year in over 300 countries [1]. The survival rate after heart transplantation has improved steadily over the last two decades with virtually all of the improvement being in survival during the first few months [1]. Despite this improvement in early post-transplant survival, there is little if any evidence that deaths due to primary graft failure (PGF) have decreased over this period. In a large retrospective study of 7,259 heart transplant recipients during the decade from 1990 to 2000, Young and colleagues reported that the one month mortality after heart transplantation was 6.9% with 43% of these deaths due to PGF [2]. This compares with the most recent audit of the International Society of Heart and Lung Transplantation Registry which reported a one month mortality after transplantation of 8% with 39% of these deaths resulting from PGF [1]. It is clear from these data that PGF continues to be the single most common cause of death within the first month after heart transplantation [1]. In addition, the high morbidity associated with PGF and its treatment is likely to be a major contributor to deaths that are attributed to other causes such as infection and rejection over subsequent months.

2. Incidence

The reported incidence of PGF after heart transplantation varies widely between studies with estimates ranging between 2.3 and 26% [3–11]. Most of the variability can be attributed to inconsistent definitions of PGF used by different authors. In a large retrospective review of the UNOS Registry, Russo and colleagues defined PGF as death or retransplantation within the first 90 days of transplantation and reported an incidence of only 2.5% [5]; however, as argued by others, the use of such a definition based on "hard endpoints" is likely to underestimate the true incidence of the clinical syndrome as it only detects those with the worst clinical outcomes [12, 13]. In contrast, when PGF has been defined as the need for high-dose inotropes or mechanical assist devices in the immediate post-transplant period, most investigators have reported incidence rates of 10–20% or higher [3, 4, 7–9].

Table 1: Suggested diagnostic criteria for primary graft failure [7, 9].

Presence of	Evidenced by
Ventricular systolic dysfunction—left, right, or biventricular dysfunction	Echocardiographic evidence of dysfunction
Cardiogenic shock lasting more than one hour	Low systolic blood pressure < 90 mmHg and/or low cardiac output—<2 L/minute/m^2 Despite adequate intracardiac filling pressures—CVP > 15 mmHg and/or PAWP > 20 mmHg
Circulatory support	Use of ≥2 inotropic agents/vasopressors including high-dose epinephrine or norepinephrine and/or use of a mechanical assist device—IABP, ECMO, VAD
Appropriate time frame	Onset < 24 hours after transplantation
Exclusion of secondary causes of PGF	For example, cardiac tamponade and hyperacute rejection

The changing demographics of donors and recipients observed in cardiac transplantation over the last two decades appear to be contributing to an increase in the incidence of PGF [9, 11, 14]. Transplant centres face significant donor shortages and growing waiting lists. This is no more evident than in Australia, where the combination of a relatively small population and low organ donation rate has resulted in increased utilisation of hearts from older "marginal" donors [11, 14, 15] and suboptimal organs from younger donors. In addition to this, greater procurement distances to retrieve donor hearts in Australia contribute to prolonged ischaemic times. The combination of these two factors, advanced donor age and prolonged ischaemic time, markedly increases the risk of PGF and death after heart transplantation [5, 16].

3. Definition and Diagnostic Criteria

PGF is a syndrome in which the transplanted heart fails to meet the circulatory requirements of the recipient in the immediate post-transplant period as a consequence of either single or biventricular dysfunction. It is manifested as hypotension and low cardiac output in the presence of adequate filling pressures [17]. In most instances, it is likely to result from a multifactorial process with contributing elements from the donor, recipient, and the transplant process.

A universally accepted clinical definition for PGF has been lacking and is urgently needed. Several authors have suggested minimal diagnostic criteria [7, 9], which are summarised in Table 1. The primary diagnostic criterion for PGF is evidence of ventricular dysfunction which may involve the left, right, or both ventricles occurring within the first 24 hours of heart transplantation. The major clinical manifestation of this dysfunction is severe haemodynamic instability with cardiogenic shock. A diagnosis of PGF should only be made when other causes of acute graft failure such as cardiac tamponade and hyperacute rejection have been excluded.

The severity of PGF can be graded according to the level of support needed to restore haemodynamic stability. In less severe cases, intravenous inotropic support with two or more agents may be sufficient to achieve this, whereas in more severe cases mechanical circulatory assistance (including intra-aortic balloon pump, extracorporeal membrane oxygenator (ECMO), or any ventricular assist device) is required. A three-level grading system based on the severity of primary graft dysfunction has been developed for lung transplantation and shown to be strongly predictive of one-month mortality [18, 19]. It seems likely therefore that the severity of cardiac PGF has an equally significant prognostic value after transplantation. In view of this, a standardised clinical definition of PGF incorporating a severity grading system is urgently needed.

4. Aetiology and Pathogenesis

Acute ischaemia-reperfusion injury with myocardial stunning has been postulated as a predominant factor in the development of PGF. The donor heart is subjected to a series of insults during the transplant process including brain death and its sequelae, hypothermic storage, warm ischaemia, and finally reperfusion. Donor hearts vary in their ability to withstand these insults. It is clear, for example, that the hearts from older donors have an increased susceptibility to PGF [5, 16] which may be explained by the observation that aged myocytes have a reduced ability to withstand ischaemia-reperfusion injury [20].

Brain death in the donor is associated with a series of events that result in impaired myocardial contractility. These events include the rapid release of catecholamines immediately after brain death contributing to myocardial ischaemia, calcium overload, calpain activation, and changes in the calcium sensitivity of contractile proteins [21, 22]. The surge in endogenous catecholamine release immediately after brain death followed by the administration of exogenous catecholamines during donor resuscitation may contribute to desensitization of the myocardial beta-receptor signal transduction system after brain death and to the activation of multiple proinflammatory mediators [23–26]. In addition, decreased serum levels of various hormones including triiodothyronine, cortisol (after a transient increase), and insulin have been reported and likely contribute to the depression of myocardial contractility [27].

Most donor hearts are stored in a cold preservation solution and transported on ice. Hypothermic storage slows but does not completely arrest cellular metabolism.

Table 2: Risk factors for primary graft failure.

Donor factors	Recipient factors	Procedural factors
Age [2, 5, 7, 9, 16, 33]	Age [3, 33]	Ischaemic time [2, 3, 7, 9, 11, 33]
Cardiac dysfunction on echo [2, 3, 11]	Ventilator support [2]	Donor recipient weight mismatching [2]
High-dose inotropic support [3, 6, 34]	Intravenous inotropic support [9], Mechanical support [3, 5]	Female donor to male recipient [2, 5, 35]
Cause of brain death [3, 36]	Pulmonary hypertension [17, 29–31]	Concomitant lung retrieval [5]
Primary graft dysfunction of other organs [8]	Overweight [37], Diabetes mellitus [9]	

Consequently, progressive ischaemic injury is an inevitable consequence of prolonged static storage. In addition, loss of normal aerobic metabolism paralyses the transmembrane Na+/K+ ATPase pump leading to cellular swelling and the switch to anaerobic metabolism during cold storage results in a rapid decline in high-energy phosphates and the development of lactic acidosis [28]. Finally, reperfusion injury results in further calcium overload and oxidative stress both of which can contribute to the mechanism of stunning [21, 28]. Thus, at every stage of the transplant process, the heart is exposed to cellular stresses that may adversely impact on myocardial function and ultimately lead to the syndrome of PGF.

Primary graft failure may also occur in circumstances where the donor heart has not been subjected to substantial ischaemia-reperfusion injury. Under these circumstances, recipient factors are the principal cause of PGF. There are two clinical scenarios where this is likely to occur. The first is the presence of a fixed high pulmonary vascular resistance in the recipient [29–31]. In this circumstance, the right ventricle of the donor heart is unable to overcome the afterload imposed by the elevated pulmonary vascular resistance, and selective or predominant right ventricular failure ensues. In one series of 911 patients, 28 of 130 deaths were due to acute graft failure with 43% of this early mortality (12 of 28 patients), attributed to severe preoperative pulmonary hypertension causing right-sided circulatory failure, low cardiac output and eventually biventricular failure [32]. The second scenario is when the recipient is critically ill on ventilatory and/or acute mechanical circulatory support often with evidence of multisystem failure and sepsis [2, 3, 5]. In this circumstance, the "hostile environment" of the recipient results in PGF. The pathophysiology of PGF in this setting is poorly understood but probably involves the concerted action of multiple proinflammatory cytokines on the transplanted heart.

In most instances, it is likely that the combination of donor, procedural and recipient factors leads to the syndrome of PGF. For example, an older donor heart that has been subjected to a prolonged ischaemic time may fail in a recipient with an elevated pulmonary vascular resistance whereas a younger donor heart may not. On the other hand, the same older donor heart may function adequately in a haemodynamically stable recipient with low pulmonary vascular resistance. Hence, matching donors to recipients with regard to risk factors for PGF are critical to minimising the risk of this life-threatening complication.

5. Risk Factors for PGF

Given the significant contribution of PGF to early mortality after cardiac transplantation, identification of predictive factors is important. Multiple risk factors for PGF have been identified by different authors. They can be divided into those that are donor related, those that are recipient related and those related to the transplant procedure.

As shown in Table 2, multiple donor and recipient factors have been associated with an increased risk of PGF. Principal among these are increasing donor and recipient age, both of which have also been identified as major risk factors for one-year mortality after transplantation [1]. The review of the Australian & New Zealand Cardiothoracic Transplant Registry reveals that there has been a steady rise in mean donor and recipient age over the last 2 decades [15] with the mean donor age exceeding 40 years of age for the first time in 2010 (personal communication with Mr. Ross Pettersson).

Another potent risk factor for PGF identified in multiple studies is donor heart ischaemic time, referring to the period from the arrest of the donor heart to time of graft reperfusion in the recipient. It is apparent from the ISHLT Registry that one-year mortality risk after heart transplantation increases steadily with every minute of ischaemic time in excess of 3 hours [1]. Marasco et al. estimated that the risk of PGF increased by 43% for every hour of extra ischaemic time beyond 4 hours [7]. As with donor age, there has been a significant increase in donor heart ischaemic time for heart transplants performed in Australia and New Zealand from a mean of less than 3 hours prior to 1990 to a mean in excess of 4 hours for most of the last decade [15]. In our own recently reported experience of ECMO support for PGF, donor heart ischaemic times of 5 hours or longer were associated with a fivefold increase in the risk of PGF [11].

These data indicate that the current techniques used to preserve the donor heart during procurement and transport have limited efficacy. Unfortunately, prolonged ischaemic times in heart transplantation are sometimes logistically unavoidable. There is a clear need to develop more effective preservation strategies—either by bolstering the cardioprotective efficacy of the storage solution or through use of oxygenated ex vivo perfusion systems. Counter-intuitively, Russo et al. reported an increased risk of PGF with ischaemic time of less than 1 hour, citing the potential limited cooling period being insufficient to achieve the benefits of cellular protection with global hypothermia [5].

Several authors have reported that donor heart dysfunction as evidenced by a low left ventricular ejection fraction on echocardiography, unstable donor haemodynamics, or the need for high doses of catecholamines is a potent risk factor for PGF [2, 3, 6, 11]. Historically, donor hearts that displayed these characteristics would have been regarded as unsuitable for transplantation; however, increased demand for transplantation has led to many Transplant Units including our own making use of these "marginal" hearts [3, 6, 11, 14]. The expectation is that the myocardial dysfunction evident in the donor is a result of stunning and is recoverable over time despite the current lack of a useful clinical measure that can reliably distinguish reversible from irreversible myocardial dysfunction in the brain dead donor. Of all the clinical information available regarding a potential heart donor, a young donor age (<30 years) and the absence of any known history of heart disease are probably the two pieces of clinical information that our group most relies on when deciding to use a donor heart with overt myocardial dysfunction prior to procurement [11].

Donor-recipient size mismatch has also been identified as a significant contributing factor in the development of PGF. In one study, the combination of a donor-recipient weight ratio of less than 0.8 with pulmonary hypertension in the recipient (>4 wood units) was associated with PGF [5]. Several studies have found that the transplantation of a female donor heart into a male recipient was associated with increased PGF, with size mismatch being the likely connection. A possible link to immunological processes and increased rejection episodes have also been described [2, 35].

The concurrent donation of other organs may also have a role in PGF, specifically the donation of lungs [5]. The proposed aetiologies include additional flush volume that may contribute to RV distension and dysfunction, and release of pulmonary vascular cytokines at time of arrest which can result in ventricular dysfunction [5]. The association of PGF in multiple organs retrieved from the same multi-organ donor has also been reported, highlighting the potential for significant donor influences in the development of PGF [8]. This also allows predictability of PGF through monitoring of other organs transplanted from that specific donor [8].

The presence of ventilator or ECMO support in the recipient prior to and at the time of transplantation has been shown to be a significant risk factor for PGF [2, 5]. These patients are usually critically ill with evidence of multi-organ dysfunction and often sepsis. Conversion of these patients to long-term mechanical support with a left ventricular assist device or total artificial heart is associated with significant mortality [38], but if successful enables resolution of any acute multi-organ dysfunction with subsequently safer transplantation when the patient's condition has stabilised. Although a trend to increased PGF has been reported in patients who are bridged to transplantation with long-term implanted VADs [5], post-transplant survival of these patients does not appear to be compromised [15, 39].

Risk factors do not act in isolation, and it is likely that the interaction between donor, recipient, and procedural factors is a major determinant of the risk of PGF. A clear example of this is the interaction between donor age and ischaemic time reported by Russo et al. [16]. In that study, there was no detectable adverse effect of ischaemic time on survival after heart transplantation when the donor was less than 20 years of age. In contrast, when the donor age increased above 20 years, a prolonged ischaemic time had a significant negative impact on survival [17]. This effect became even more marked when the donor age exceeded 35 years. The association of increasing donor age with PGF is likely related to the decreased ability of the aging heart to tolerate ischaemic insults as well as the increased incidence of intrinsic cardiac pathology with age [20].

5.1. PGF Predictive Tool. Given that multiple factors in the donor, the transplantation process and the recipient contribute to the risk of PGF, the development of a predictive tool, and scoring system that combines known risk factors has been reported [9, 40]. Suggested variables have included donor and recipient age, donor inotropic dependence, recipient right atrial pressure, and ischaemic time [9]. With further understanding of the aetiology of PGF, as well as identification and confirmation of risk factors, an accurate predictive scoring tool is imminent in the near future. The utility of any predictive tool remains to be determined, but it does serve to emphasise the importance of careful donor-recipient matching in the prevention of this life-threatening complication.

6. Management

The treatment of PGF remains extremely challenging—a substantial 30-day mortality rate is seen despite intensive pharmacological as well as mechanical circulatory support (IABP, ECMO, VAD) used in this critical period [1, 2]. In milder cases of primary allograft dysfunction, high-dose inotropic agents may be sufficient to restore myocardial contractility and haemodynamic stability. A variety of inotropic agents have been used to treat PGF include catecholamines, phosphodiesterase inhibitors, and more recently levosimendan [41–43].

With more severe cases of graft failure, mechanical circulatory support with intra-aortic counterpulsation or VA extracorporeal mechanical support (ECMO) may be needed to maintain haemodynamic support and perfusion of vital organs. In our institution, the decision to institute ECMO has been made early, that is, in the operating room when there has been difficulty with separating from cardiopulmonary bypass despite a trial of inotropic/vasopressor support [11]. We believe that early institution of ECMO not only allows the heart more time to recover from the multiple stresses to which it has been exposed but also prevents development of multisystem organ failure which would otherwise occur if there is a period of uncorrected cardiogenic shock. Recent advances in ECMO circuit design have resulted in a significantly improved survival rates and fewer complications compared with practice not longer than a decade ago, when paracorporeal ventricular assist devices were used for left ventricular support and centrifugal pumps for right ventricle support [7, 11, 44, 45].

Heart transplant recipients with PGF remain supported on ECMO until graft function improves. In our experience,

Table 3: Pharmacological activation of prosurvival kinases in a model of donor heart preservation.

Agent (s)[1]	Storage time (h)	Poststorage CO recov[2]	Prosurvival kinase phosphorylation[3]			Other salient findings	Ref.
			Akt	ERK	STAT3		
GTN (0.1 mg/mL)	6	2.5	0	+	nd[4]	↓ cleaved Casp 3	[61]
Carip (10 μM)	6	3.5	0	++	nd[4]	↓ cleaved Casp 3	[61]
INO 1153 (1 μM)	6	2.5	+ + +	+	nd[4]	Recovery of function abolished by Akt inhib	[62]
Zonip (1 μM)	6	14	0	+ + +	+ + +	Zonip abolished LDH release; ↓ cleaved Casp 3; Inhib of STAT3 phos abolished recovery of f'n.	[63]
Neureg (14 nM)	6	13	++	+ + +	+ + +	Recovery of function abolished by Akt inhib	[64]
EPO (5 units/mL)	6	16	0	0	+ + +	Inhib of STAT3 phos abolished recovery of f'n.	[65]
Neureg + GTN + Carip	10	13	++	0	+	Triple supplement ↓ contraction band necrosis	[64]

[1]Agent(s) added to Celsior arresting and storage solution. Abbreviations/drug classes are as follows: GTN—glyceryl trinitrate (nitric oxide donor); Carip—cariporide, Zonip—zoniporide, (both sodium/hydrogen exchange inhibitors); INO 1153—poly(ADPribose) polymerase inhibitor; Neureg—recombinant human Neuregulin-1 peptide; EPO—erythropoietin. [2]Recovery of cardiac output expressed as fold increase over Celsior-stored hearts ($P \leq 0.05$); [3]increase in survival kinase phosphorylation over Celsior-stored hearts; + + +: intense; ++: moderate; +: weak; [4]nd-not determined;

this has generally been within 72 hours; however, heart recovery has been observed as early as 1 day and as late as 7 days after transplant [11]. Assessment of the timing of cardiac recovery is usually judged by daily bedside echocardiography with brief reduction in ECMO flow during echocardiographic examination. The majority of the patients in our series have had peripheral femoral venous and arterial cannulas placed for ECMO support, and in most cases, it has been possible to remove these cannulae in the intensive care unit without the need to return to the operating theatre.

In cases with pre-existing recipient pulmonary hypertension, PGF is usually manifested as right ventricular dysfunction in the immediate post-transplant period. Treatment includes administration of specific pulmonary vasodilators such as inhaled nitric oxide to lower pulmonary vascular resistance [46] however, mechanical circulatory support may be needed [45]. Long-term administration of selective pulmonary vasodilators (prostacyclin, sildenafil) or in some cases implantation of left ventricular assist devices in potential heart transplant recipient with fixed pulmonary hypertension has been reported to produce sustained lowering of pulmonary vascular resistance allowing orthotopic heart transplantation to be performed without any increase in perioperative graft failure or mortality [47, 48].

7. Prognosis

PGF is the leading cause of death in the first month after heart transplantation. Although registry studies indicate that the number of early deaths due to PGF has not changed over the last two decades [1, 2], this is in the setting of an increasing incidence of PGF reported in the literature [3, 4, 11, 14]. This suggests that the prognosis for patients diagnosed with PGF is improving, most likely as a result of the improved efficacy and safety of pharmacological and ECMO support in these critically ill patients [3, 11, 14]. In our own experience of 17 patients supported on ECMO for PGF, one month survival was 82% [11].

The impact of PGF beyond the first month after transplantation is less clear, but also likely to be significant. Severe ischaemia-reperfusion injury has been shown experimentally to upregulate multiple proinflammatory mediators which may prime the graft for acute rejection [25, 26] and also predispose the graft to allograft vasculopathy [49], both of which could contribute to graft failure at later time points.

8. Prevention and Areas for Future Improvement

Given the cumulative impact of the multiple risk factors that contribute to the development of PGF, careful matching between donor and recipient is critical to minimising the risk of PGF. Unfortunately, the logistics of transplantation sometimes dictate that unfavourable risk factor interactions cannot be avoided. While some risk factors (e.g., donor and recipient age) are not modifiable, other risk factors (e.g., donor heart ischaemia-reperfusion injury sustained following brain death or during organ procurement and preservation) may be amenable to therapeutic intervention.

The period between brain death and heart retrieval is one in which heart function can deteriorate rapidly. Optimal management of the brain dead donor during this

period remains a contentious issue. More than 90% of brain dead donors receive one or more inotropic or vasopressor infusions most commonly noradrenaline [50]. While low-dose infusions of catecholamines appear to be safe, high-dose infusions increase the risk of PGF and should be avoided [3, 6, 34]. There has been a longstanding interest in the administration of pituitary-dependent hormones in the optimisation of donor organ quality after brain death. Vasopressin is an effective alternative to noradrenaline for maintaining blood pressure, and its use may prevent the need for escalating doses of noradrenaline [51, 52]. On the other hand, the value of thyroid hormone and corticosteroids in this setting is still controversial. While large-scale retrospective analyses support a role for these drugs [53, 54], prospective randomised controlled trials to date have failed to demonstrate any improvement in cardiac function or outcome after transplantation [55, 56].

The period of heart storage and transport is the second period that offers an opportunity to intervene. Currently most hearts are stored and transported in cold cardioplegic/preservation solutions. The many commercial and in-house cardioplegic/preservation solutions in routine clinical use not only emphasise the complexity of the molecular and cellular mechanisms that underlie ischaemia-reperfusion injury but also the lack of consensus as to the optimal strategy for organ preservation [57]. Cardioplegic/storage solutions such as St Thomas' Solution No. 2 (Plegisol), Bretschneider (Custodiol), and Celsior, have been in widespread clinical use since the early 1990's [58–60] and appear to provide adequate protection of "standard criteria" of donor hearts subjected to ischaemic times of less than 4 hours [1]. The cardioprotective capacities of such formulations may be suboptimal for the increasing numbers of "marginal" donor hearts seen in current clinical practice, particularly those subjected to prolonged ischaemic times.

Elucidation of the mechanisms of ischaemia-reperfusion injury over this same period of time has suggested novel strategies to enhance the cardioprotective capacities of existing preservation solutions. The search for an overarching protective strategy against cardiac reperfusion injury has been advanced by the realisation that ischemic pre- and postconditioning as well as a number of pharmacological agents that mimic these physiological strategies can activate prosurvival signalling pathways such as PI3K/Akt, ERK 1/2 and STAT3 at reperfusion (for review see Hausenloy et al., [66]). Consistent with this mechanism, we have recently demonstrated that rat hearts arrested and stored for 6 or 10 hours in Celsior solution supplemented with the conditioning agents glyceryl trinitrate (GTN), a nitric oxide donor, and cariporide, a sodium hydrogen exchange inhibitor significantly improved poststorage cardiac function that could be abolished by inhibition of the mitochondrial K_{ATP} channel, a key target of prosurvival signalling pathways [67]. These findings have recently been further verified in a translational porcine orthotopic heart transplant model incorporating donor brain death. Here, donor hearts arrested 6 hours after brain death and stored in Celsior supplemented with GTN and cariporide could be successfully weaned from cardiopulmonary bypass after 14-hour hypothermic storage [68]. In addition, we have demonstrated that appropriate pharmacological supplementation of the arresting and storage solution can activate survival signalling after reperfusion in a model of a normal donor heart exposed to storage times that would class them as "marginal" (6 hr storage) or unsuitable for transplant (10 hour storage) (Table 3).

An alternative to cold static storage is ex vivo perfusion. There is limited experience with this approach in heart transplantation [69]; however, a recent large randomised controlled trial in deceased kidney transplantation revealed a significant reduction in primary graft dysfunction and improved graft survival at one year after transplant in machine-preserved kidneys [70]. These benefits were particularly marked in kidneys obtained from marginal donors [71].

In summary, the increasing reliance on "marginal" donors to meet the ever-increasing demand for heart transplantation means that PGF is likely to remain a frequent complication. Although there have been significant improvements in the treatment of established PGF, it still carries a high morbidity and mortality. While it is possible that some cases of PGF may be prevented by careful matching of donors and recipients, complete prevention of PGF will require the development of more effective donor management and donor heart preservation strategies. These remain high-priority areas for ongoing basic and clinical research.

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CHAPTER 3 – PUBLICATION 2

TITLE: Increasing The Tolerance of DCD Hearts to Warm Ischemia by Pharmacological Post-conditioning

JOURNAL: American Journal of Transplantation 14(8):1744-52.

YEAR: 2014

Declaration

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Foreword

There is little doubt that prolonged periods of warm ischaemia cause damage to the myocardium with little hope of recovery. With shorter periods of warm ischaemia however, there appears a limited timeframe during which only reversible myocardial ischaemia occurs, raising the possibility of organ viability for transplantation.

One of the questions that remain unanswered in the literature is the limit of warm ischaemic tolerance of cardiac allografts. Until this publication, there has been no comparison of differing warm ischaemic times and their impact on functional and metabolic recovery. Utilising a model that closely mimics the clinical scenario, this question was investigated in porcine hearts.

In addition, there has been decades of work looking at ischaemic conditioning strategies to minimise ischaemia-reperfusion injury. As outlined in section 1.9 of the literature review, our lab has shown over the last decade that there are pharmacological agents that can be utilised in stimulating pre- and post-conditioning strategies and improving outcomes with BD donor heart transplantation. In the setting of DCD hearts, as the ischaemic insult has already taken place prior to any intervention permitted by the 'dead donor rule', ischaemic **post**-conditioning strategies are required. The second aspect of this paper evaluates the benefit of these pharmacological agents in post-conditioning these hearts and assesses for any improvement in subsequent recovery.

The results of this study forms the basis for the 30 minute WIT cut-off that is currently being used in the clinical DCD heart transplantation program recently established at St Vincent's Hospital Sydney.

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Increasing the Tolerance of DCD Hearts to Warm Ischemia by Pharmacological Postconditioning

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Donation after circulatory death (DCD) offers a potential additional source of cardiac allografts. We used a porcine asphyxia model to evaluate viability of DCD hearts subjected to warm ischemic times (WIT) of 20–40 min prior to flushing with Celsior (C) solution. We then assessed potential benefits of supplementing C with erythropoietin, glyceryl trinitrate and zoniporide (Cs), a combination that we have shown previously to activate ischemic postconditioning pathways. Hearts flushed with C/Cs were assessed for functional, biochemical and metabolic recovery on an *ex vivo* working heart apparatus. Hearts exposed to 20-min WIT showed full recovery of functional and metabolic profiles compared with control hearts (no WIT). Hearts subjected to 30- or 40-min WIT prior to C solution showed partial and no recovery, respectively. Hearts exposed to 30-min WIT and Cs solution displayed complete recovery, while hearts exposed to 40-min WIT and Cs solution demonstrated partial recovery. We conclude that DCD hearts flushed with C solution demonstrate complete recovery up to 20-min WIT after which there is rapid loss of viability. Cs extends the limit of WIT tolerability to 30 min. DCD hearts with ≤30-min WIT may be suitable for transplantation and warrant assessment in a transplant model.

Keywords: Donation after circulatory death (DCD), *ex vivo* perfusion, ischemia reperfusion injury (IRI), ischemic postconditioning, warm ischemic time (WIT)

Abbreviations: AF, aortic flow; C, Celsior preservation solution; Cs, supplemented Celsior solution; DCD, donation after circulatory death; EVP, *ex vivo* perfusion; IRI, ischemia reperfusion injury; LAP, left atrial pressure; NDD, neurological determination of death; OCS, Organ Care System; WIT, warm ischemic time; WM, working mode

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Introduction

Heart transplantation remains the most effective treatment for refractory end-stage heart failure. Currently, with rare exceptions, donors after neurological determination of death (NDD) are the only source of allografts for heart transplantation. The paucity of available NDD donor hearts has limited the number of transplants that can be performed, resulting in substantial recipient waiting list mortality (1). Several groups have proposed the use of donation after circulatory death (DCD) hearts as an additional source of cardiac allografts for transplantation (2–4); however, the possibility of irreversible myocardial damage during the unavoidable warm ischemic period has limited clinical translation.

Over the last few years, the positive impact of DCD organs in lung transplantation has become evident. The advent of DCD lung donation has resulted in a 28% increase in transplant numbers in Australia with outcomes comparable to those from NDD donors (5). Livers, however, appear more sensitive to the effects of warm ischemia, with increasing rates of ischemic biliary strictures and worse survival following DCD liver transplantation (6,7). Similar concerns regarding the impact of warm ischemia and irreversible ischemic injury have thus far precluded the use of DCD hearts as cardiac allografts. Contributing to these concerns is the lack of any reliable method to assess the extent of ischemic injury in hearts that are subjected to static storage in hypothermic solutions.

Clinically, the obligatory warm ischemic time (WIT) refers to the period between withdrawal of ventilator support and the administration of cold preservation solution. During this time, the heart is subjected to hypoxia, hypoperfusion and distension. Despite these insults, several preclinical studies in porcine, canine and primate models have suggested the potential use of DCD hearts for cardiac transplantation. These studies employed varying WITs and differing models of cardiocirculatory death (2–4). However, to our knowledge, no study has compared the recovery of DCD hearts following various WITs, to ascertain the limit of warm ischemia prior to irreversible myocardial damage. Here, we evaluated the impact of predetermined periods of warm ischemia on the functional and metabolic recovery of hearts retrieved from porcine DCD donors with the aim of determining the duration of myocardial ischemia before the onset of irreversible myocardial injury.

The concept of cardioprotection by ischemic conditioning has been extensively investigated since Murry et al (8) demonstrated the phenomenon of ischemic preconditioning, where repeated short periods of ischemia protected the heart from a subsequent longer ischemic insult. More recently, a similar benefit of ischemic postconditioning (intervention after the onset of ischemia) has been shown (9,10). Similar cellular and mitochondrial protective mechanisms are activated in ischemic preconditioning and postconditioning, with actions initiated through either mechanical or biochemical stimuli and acting to minimize ischemia reperfusion injury (IRI) (11–13). Our group has previously reported that glyceryl trinitrate, erythropoietin and zoniporide, when added as single or combined supplements to Celsior (C) solution, activate the intracellular kinases which mediate ischemic preconditioning and postconditioning (14–17). We have further shown that supplementing C solution with these three agents reduces IRI in an isolated working rat heart model (16) and in an NDD porcine transplant model (18). In the DCD setting, interventions to enhance donor organ viability can only be administered after the onset of the ischemic insult and death of the donor. Given these considerations, we also tested the hypothesis that supplementing the preservation solution with these pharmacological agents increases tolerance of DCD hearts to warm ischemia.

Materials and Methods

Juvenile *Landrace* species pigs were used, with experimental protocols approved by the Garvan/St. Vincent's Animal Ethics Committee (AEC). All animals were cared for according to the standards outlined in the Australian Code for the Care and Use of Animals for Scientific Purposes, 8th edition (2013).

Animals and anesthesia

Thirty pigs weighing between 55 and 65 kg were used. Animals were premedicated with an intramuscular injection of ketamine (10 mg/kg), midazolam (1 mg/kg) and atropine (50 μg/kg). Animals were intubated (7–7.5 mm cuffed endotracheal tube) and ventilated with a tidal volume of 10 mL/kg and positive end-expiratory pressure of 5–7 mmHg. Anesthesia was maintained using inhalational isoflurane (2–5%) and regular IV fentanyl (100–200 μg).

Mean arterial and central venous pressures, pulse oximetry, end-tidal CO_2, core temperature and electrocardiogram were monitored continuously. Arterial blood gas samples were analyzed at presurgical and prewithdrawal time points.

Surgical procedure

Right internal carotid artery and internal jugular vein were cannulated for arterial and central venous pressure monitoring, respectively.

After administration of lignocaine (1 mg/kg) to prevent arrhythmias, a median sternotomy was performed and pericardium opened. Heparin (300 IU/kg) was administered and the heart inspected for gross abnormalities. Baseline biochemistry and metabolic profiles were assessed.

Withdrawal

We used an asphyxia model to closely mimic the clinical setting. After bolus doses of midazolam and fentanyl to ensure adequate sedation were administered and prewithdrawal blood samples taken, ventilation was ceased and the endotracheal tube disconnected. With the pleura opened, the lungs were inspected to confirm absence of spontaneous breathing. Times to circulatory arrest and zero oxygen saturation were recorded; however, hearts were left untouched for strict predetermined periods of warm ischemia irrespective of circulatory cessation times. Circulatory arrest time was defined as time when mean arterial pressure matched central venous pressure. Warm ischemia was defined as the period between withdrawal of ventilator support and administration of preservation solution. Control hearts were not exposed to any WIT. Table 1 outlines the various groups.

Immediately prior to the end of the predetermined WIT, cannulas were inserted into the right atrium (for blood collection) and aortic root (for preservation solution flush). Blood (~1.2 L) was drained from the animal just prior to aortic cross-clamp application and C preservation solution (Genzyme, Cambridge, MA; 1 L precooled to 4°C)—administered with the heart vented. The chest cavity was subsequently filled with cold saline slush. In the "supplemented" groups (Cs), pharmacological agents known to activate ischemic conditioning pathways (14–16) were added to the C solution. These supplements and their doses are listed in Table 1. Post-C flush, the hearts were excised and submerged in cold saline while being cannulated for *ex vivo* perfusion (EVP).

Ex vivo *perfusion circuit*

Perfusate volume consisting 1.2 L of donor animal blood, 500 mL of Gelofusine (4% bovine-derived gelatin; B. Braun, Melsungen, Germany),

Table 1: Treatment groups and pharmacological supplements added to Celsior solution

Group	WIT	n	Preservation solution
Control	0	6	Celsior
20-C	20	6	Celsior
30-C	30	6	Celsior
30-Cs	30	6	Supplemented Celsior
40-C	40	3	Celsior
40-Cs	40	3	Supplemented Celsior

WIT, warm ischemic time. Supplemented Celsior—1000 mL Celsior with addition of 5 U/mL erythropoietin (Eprex; Janssen, North Ryde, NSW, Australia); 1 nM zoniporide (Pfizer, West Ryde, NSW, Australia); 100 mg/L glyceryl trinitrate (Hospira, Melbourne, VIC, Australia).

250 mL of standard Krebs solution and heparin (10 000 IU). The circuit comprised the Capiox® SX18R oxygenator (Terumo Corporation, Ann Arbor, MI) and a roller pump. Hearts were initially perfused in resting/Langendorff mode, where retrograde aortic flow (AF) perfused the coronaries at set pressures and allowed an empty beating state. Pulmonary artery (PA) ejection was used to gauge coronary flow. Hearts were switched to working mode (WM) by filling the left atrium at set pressures and then measuring cardiac output, calculated by the sum of aortic and PA ejection (coronary flow). The two modes of perfusion are outlined in Figure 1.

Ex vivo *perfusion protocol*

Hearts were attached to the perfusion circuit as shown in Figure 1 and perfused in Langendorff mode for 60 min at a pressure of 45–50 mmHg. Temperature was gradually increased from 28 to 34°C. Hearts in ventricular fibrillation/tachycardia were cardioverted once temperature rose to 34°C. At the end of this hour, the temperature was increased to 37°C and dobutamine commenced at 5 μg/kg/min. Hearts were then switched to WM function for 180 min, during which functional, metabolic and biochemical profiles were assessed. As a measure of contractility, a left atrial pressure (LAP) versus AF challenge was conducted to generate Starling curves—LAP was controlled using the height of the preload chamber (Figure 1).

Control group

Hearts (n = 6) in the control group were not exposed to a withdrawal period or any warm ischemia. Hearts were flushed with unsupplemented C solution (1 L) and subsequently explanted and cannulated for EVP.

Outcome measures

The assessments of functional recovery, biochemical parameters and myocardial edema were used to gauge the impact of increasing periods of warm ischemia, and any benefit of ischemic postconditioning strategies.

Troponin and lactate levels

In vivo baseline measurements of troponin were recorded to ensure no underlying pathology. On the EVP circuit, coronary inflow and coronary effluent were sampled hourly for spectrophotometric determination of lactate at 660 nm (Roche Modular P instrument; Roche Diagnostics, Basel, Switzerland). Troponin T levels were determined using chemiluminescence immunoassay (Roche E170 immunoassay) on coronary effluent samples.

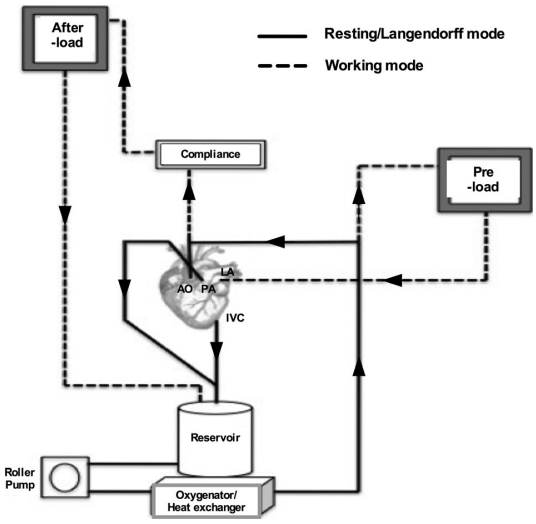


Figure 1: *Ex vivo* perfusion circuit. The beating heart *ex vivo* rig allowed assessment of functional, biochemical and metabolic parameters in Langendorff (resting) and working modes.

Myocardial oxygen consumption

Myocardial oxygen consumption (MvO_2) was measured in the setting of standardized preload and afterloads and inotropic support, and at similar heart rates. MvO_2 was calculated using the equation, $MvO_2 = CBF \times (CaO_2 - CvO_2)$, where CBF—coronary blood flow; CaO_2 and CvO_2—coronary inflow and coronary effluent oxygen content. CBF was measured directly on the *ex vivo* circuit. Oxygen content was determined using the measured coronary inflow and effluent oxygen saturations (SaO_2), hemoglobin levels (Hb, g/dL) and the partial pressure of oxygen (PaO_2), and calculated using the equation $Ca/vO_2 = Hb(g/dL) \times 1.34 \times (SaO_2/100) + (PaO_2 \times 0.0031)$ (19).

Myocardial edema

At the conclusion of the experiment, left ventricular myocardium was sampled for edema assessment. Samples were immediately wrapped in preweighed foil and weighed (wet weight). Following storage at 80°C for 72 h, they were re-weighed for dry weight. Fluid content was calculated by measuring wet weight/dry weight ratios using the following equation: water content % = ([wet weight − dry weight]/wet weight) × 100, as has been described previously (20,21). ''Normal'' water content was determined from myocardial sample obtained from a control heart that was not exposed to any WIT or EVP.

Functional parameters and statistical methods

Functional parameters evaluated included cardiac output, AF versus LAP curves and MvO_2, which were normalized to heart weight (g) to standardize between animals. Statistical analyses were performed using Prism 6.0b (GraphPad Software, Inc., La Jolla, CA). Data are expressed as mean ± SE. Differences between groups were determined using one- or two-way analysis of variance (ANOVA) depending on the number of factors assessed, followed by *post hoc* analysis using Tukey's multiple comparisons test. p-value < 0.05 was considered significant.

Results

There was no difference between groups with regard to time to circulatory arrest and to zero oxygen saturations (Table 2). Male:female ratios across the groups are also recorded.

Functional recovery

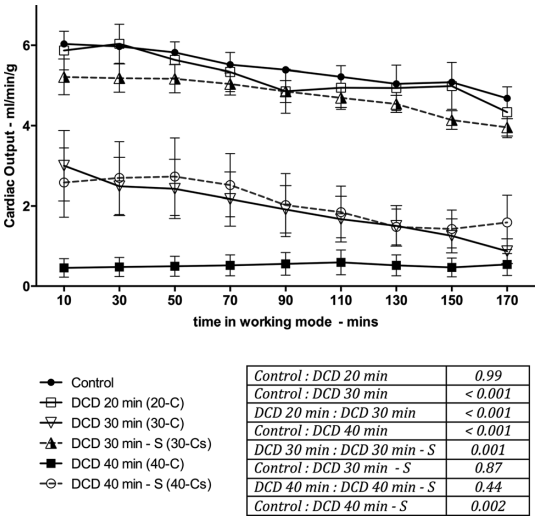
Cardiac output recovery after flush with C solution: Cardiac output during the 180 min of left heart WM was measured at a fixed LAP of 15 mmHg (Figure 2). Cardiac output of control hearts recovered to a maximum of 6.03 ± 0.48 mL/min/g at 10 min of WM. Thereafter, CO gradually declined reaching 4.68 ± 0.45 mL/min/g at the end of the protocol. Compared with controls, the recovery of cardiac output was unchanged in 20-C hearts ($p = 0.99$ vs. control hearts). In contrast, only partial recovery of cardiac output was observed in 30-C hearts ($p < 0.001$ vs. control hearts), while 40-C hearts had minimal recovery ($p < 0.001$ vs. control hearts).

Table 2: Gender ratios, time to circulatory arrest and time to zero arterial oxygen saturation (SaO_2) for the different treatment groups

Group	WIT	n	Male:female ratio	Time to circulatory arrest (min)	Time to $SaO_2 = 0$ (min)
Control	0	6	1:1	n/a	n/a
20-C	20	6	1:1	7.50 ± 0.34	3.8 ± 0.8
30-C	30	6	1:1	7.33 ± 0.56	2.5 ± 0.5
30-Cs	30	6	2:1	6.50 ± 0.43	3.0 ± 0.3
40-C	40	3	2:1	8.33 ± 1.86	3.0 ± 0.6
40-Cs	40	3	2:1	6.67 ± 0.88	3.0 ± 0.6
				$p = 0.48$	$p = 0.69$

WIT, warm ischemic time.

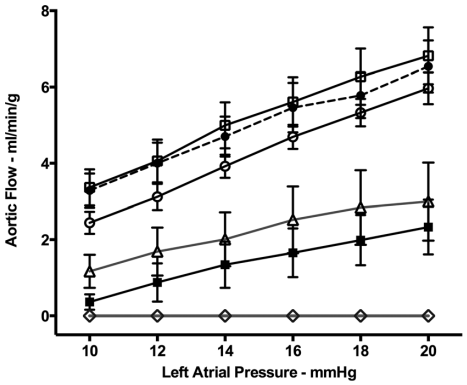
Cardiac output recovery after flush with supplemented C solution: Hearts in the 30-Cs group showed enhanced cardiac output recovery compared with 30-C hearts (30-Cs vs. 30-C; $p = 0.001$), with improvements in CO to levels comparable to control hearts (control vs. 30-Cs; $p = 0.87$; Figure 2). There was a nonsignificant benefit of C supplementation for hearts exposed to 40-min WIT (40-Cs vs. 40-C; $p = 0.44$); however, overall recovery remained inferior to control hearts (control vs. 40-Cs; $p = 0.002$, Figure 2).



Control : DCD 20 min	*0.99*
Control : DCD 30 min	*< 0.001*
DCD 20 min : DCD 30 min	*< 0.001*
Control : DCD 40 min	*< 0.001*
DCD 30 min : DCD 30 min - S	*0.001*
Control : DCD 30 min - S	*0.87*
DCD 40 min : DCD 40 min - S	*0.44*
Control : DCD 40 min - S	*0.002*

Figure 2: Cardiac output recovery of hearts exposed to WIT of 0–40 min on the working heart circuit. Following 60 min of resting mode perfusion, hearts were switched to and maintained in working mode for a further 180 min, during which cardiac output was calculated as the sum of aortic flow and coronary flow. Cardiac output is reported as mL/min and calculated per g of heart weight. Cardiac output was measured 10 min after conversion to working mode and repeated every 20 min. Hearts exposed to 30- and 40-min WIT were divided into two groups to assess for benefit of supplemented Celsior (Cs—addition of pharmacological agents to Celsior to activate ischemic conditioning pathways)—30-C versus 30-Cs and 40-C versus 40-Cs. WIT, warm ischemic time.

Left atrial pressure versus aortic flow: AF was measured at varying LAPs to gauge contractility by generation of Starling curves (Figure 3). Control hearts had an increase in AFs from 3.28 ± 0.45 mL/min/g at 10 mmHg LAP to 6.54 ± 0.68 mL/min/g at 20 mmHg. Hearts in the 20-C group had comparable profiles to control ($p = 0.98$), while hearts in the 30-C group had significant deterioration in AF ($p < 0.001$ vs. control). In contrast, 30-Cs hearts had superior AFs (30-Cs vs. 30-C; $p < 0.001$) that were comparable to controls ($p = 0.26$). Hearts in the 40-C group were unable to generate AF at any LAP during this challenge, indicating severe myocardial damage. Hearts in the 40-Cs group were able to generate aortic ejection (40-C vs. 40-Cs; $p = 0.03$); however, contractility remained significantly inferior to controls ($p < 0.001$).



Control : DCD 20 min	*0.98*
Control : DCD 30 min	*<0.001*
Control : DCD 30 min - S	*0.26*
DCD 30 min : DCD 30 mins - S	*<0.001*
DCD 40 min : DCD 40 min - S	*0.03*
Control : DCD 40 min - S	*<0.001*

Figure 3: Aortic flow versus left atrial pressure. Aortic flow was measured during an increase in left atrial pressure from 10 to 20 mmHg to generate Starling curves. The challenge was conducted at 120 min postreperfusion (60 min postchange to working mode). Aortic flow was measured as mL/min/g heart weight.

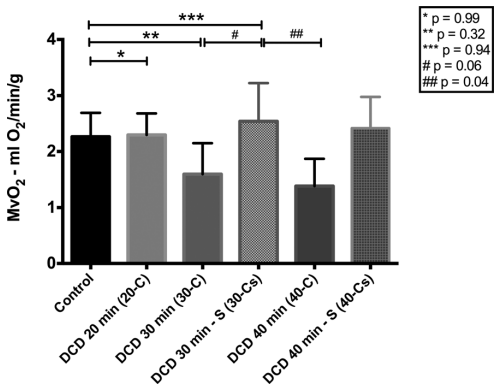


Figure 4: Myocardial oxygen consumption at 60 min post-reperfusion. Calculations based on measurements of SaO_2, PaO_2, PvO_2, hemoglobin and heart weight (g).

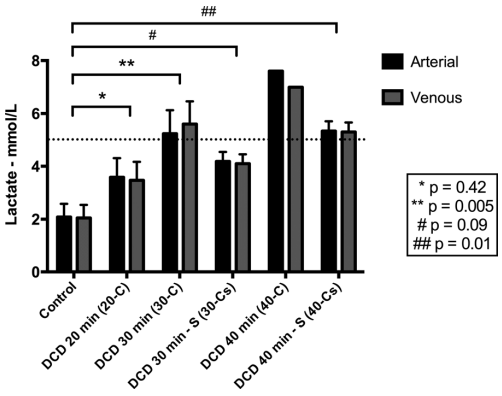


Figure 5: Arterial (coronary inflow) and venous (coronary effluent) lactate levels at 180 min postreperfusion. Dashed line represents the lactate level of 5 mmol/L. Levels below 5 mmol/L during normothermic *ex vivo* perfusion have been reported to be associated with cardiac viability for transplantation (22).

Myocardial oxygen consumption: MvO_2 was measured hourly during the *ex vivo* period, with similar trends throughout. Figure 4 shows MvO_2 at the end of 60 min of resting mode, with control hearts consuming 2.27 ± 0.18 mL O_2/min/g. While 20-C hearts had MvO_2 similar to control ($p = 0.92$). The 30-C hearts had a trend toward lower consumption ($p = 0.32$). In contrast, 30-Cs hearts demonstrated a trend toward higher oxygen consumption than 30-C hearts ($p = 0.06$), which was comparable to that of control hearts ($p = 0.94$). Hearts in the 30-Cs group also had significantly higher consumption than hearts in the 40-C group ($p = 0.04$).

Cardiac rhythm: The rhythm recovery of the heart on the *ex vivo* circuit also varied. While control hearts and 20-C hearts recovered to a sinus rhythm with synchronous atrial and ventricular contractions, hearts in the 30-C or 40-C groups all had atrial dysrhythmia. While a ventricular rhythm was noted with these hearts, the atria generated no synchronized contractions during the EVP period (Video S1). In contrast, all hearts in the Cs groups recovered to a normal sinus rhythm (Video S2), demonstrating a significant benefit of postconditioning strategies to rhythm recovery.

Metabolic profiles

Measurement of metabolic parameters during EVP of the human heart from NDD donors has been used to gauge myocardial viability prior to transplantation (22). Metabolic markers were therefore assessed.

Lactate: Lactate profiles at 180 min post-EVP are shown in Figure 5. The majority of hearts continued to extract lactate throughout the EVP period. At 180 min postreperfusion, hearts in the control and 20-C groups had coronary venous lactate levels below 5 mmol/L, while hearts in the 30-C group had levels above 5 mmol/L (30-C vs. control, $p = 0.005$). In contrast, at the same time point, hearts in the 30-Cs group had coronary venous lactate levels <5 mmol/L (30-Cs vs. control, $p = 0.09$). Hearts in the 40-C and 40-Cs groups had poor profiles with coronary venous lactate levels remaining above 5 mmol/L.

Troponin: Troponin concentrations in the perfusate during EVP increased progressively over time in all groups, related in part to the absence of any clearance mechanism for troponin (Figure 6). Measurement of troponin at 60 min postreperfusion allowed an assessment of the impact of WIT (as opposed to the impact of EVP) and the presence of any benefit of pharmacological postconditioning (Figure 6A). At this time point, control hearts had troponin levels of 1052 ± 195 ng/L. Hearts in the 20-C group had similar levels ($p = 0.93$). Pharmacological postconditioning significantly decreased troponin release from 30-min WIT hearts (30-C vs. 30-Cs: $p = 0.002$), to levels comparable with control hearts ($p = 0.99$). Hearts in the 40-Cs had a nonsignificant trend toward lower troponin release compared with hearts in the 40-C group, and remained significantly greater than control levels (40-C vs. control: $p = 0.007$). Troponin release during the remainder of the EVP time displayed similar trends to that observed at 60 min (Figure 6B). There was significantly higher troponin release following 30-min WIT (30-C vs. 20-C: $p < 0.01$); however, hearts in the 30-Cs group had significantly lower troponin levels that were comparable with control hearts (30-C vs. 30-Cs: $p < 0.01$; 30-Cs vs. control: $p > 0.25$).

Myocardial edema

No difference in water content was noted between control hearts (no WIT, no EVP) and experimental control hearts (no

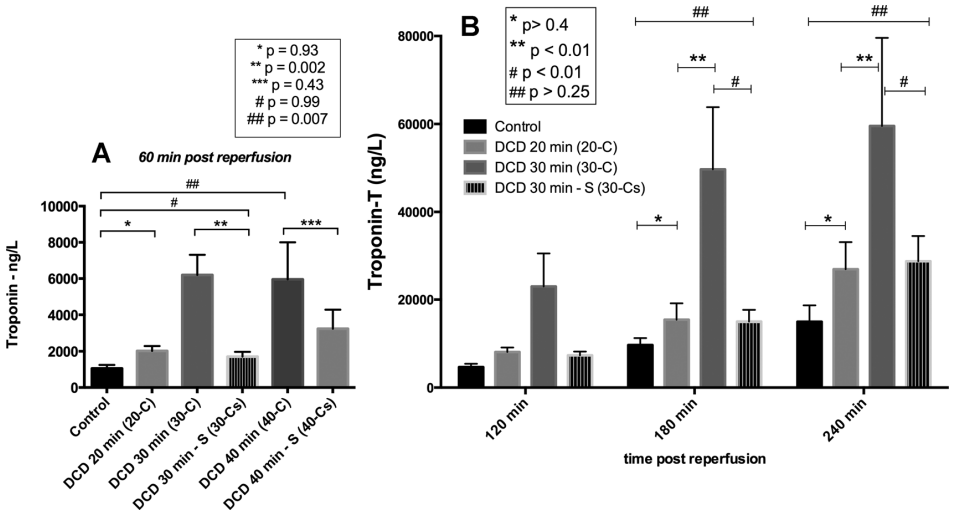


Figure 6: Troponin levels at 60 min postreperfusion for all treatment groups (A) and during the remainder of *ex vivo* perfusion period (120–240 min) for control, 20-C, 30-C and 30-Cs groups (B).

WIT, 4 h EVP), with water content of 81.61 ± 0.18% versus 80.97 ± 0.04%, respectively ($p = 0.97$). Figure 7 shows the water content for the various WIT groups. Hearts in the 20-C group had a small nonsignificant increase in myocardial water content. Hearts exposed to ≥30-min WIT developed significant myocardial edema (30-C and 40-C: both $p < 0.001$ vs. control). Hearts in the 30-Cs and 40-Cs groups demonstrated trends toward less edema than hearts in the 30-C and 40-C groups, but still had higher water content than control ($p = 0.02$).

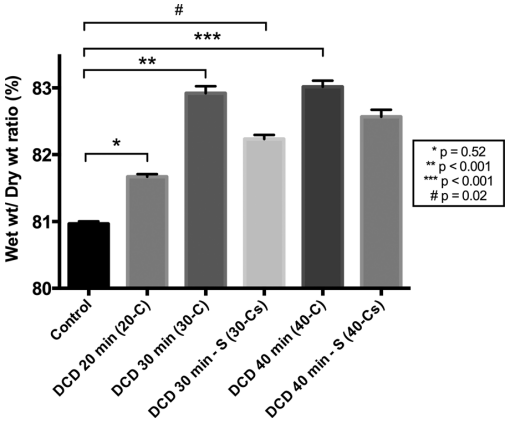


Figure 7: Myocardial edema – water content (wet weight/dry weight ratio %); left ventricular myocardial samples were used to assess water content.

Discussion

Uncertainties regarding the extent and reversibility of the myocardial injury that occurs to the heart during withdrawal of life support have largely prevented the development of human heart transplantation from DCD donors. In this study, we employed a large animal asphyxia model of controlled donation after cardiac death (23) to investigate the tolerability of the heart to normothermic ischemia. In contrast to the exsanguination model which avoids cardiac distension, the asphyxia model more closely mimics the human DCD pathway as it exposes the heart to triple insult of hypoxia, hypoperfusion and distension (24). In the present study, we observed complete functional and metabolic recovery of hearts subjected to 20 min ischemia, partial recovery after 30 min ischemia and no recovery after 40 min ischemia when hearts were flushed with C solution. Troponin release from 20-min WIT hearts was comparable to that of nonischemic control hearts following reperfusion, whereas troponin release from 30- and 40-min WIT hearts was dramatically increased. These observations suggest that the onset of irreversible myocardial injury commences after 20 min of normothermic ischemia and are consistent with early studies of experimental myocardial infarction by Jennings and Ganote (25). These authors reported that canine hearts subjected to normothermic ischemia developed irreversible myocardial injury when the duration of ischemia exceeded 20 min (25).

Previous studies of myocardial recovery in large animal DCD models have exposed the heart to WITs ranging from 15 to 30 min, with varying results (2,3,26). Ali et al (3) in a porcine model demonstrated good biventricular functional recovery

postorthotopic transplantation following 20.9 ± 2.8-min WIT, while Osaki et al (26) reported partial recovery of hearts subjected to 30-min WIT in a porcine transplant model using controlled initial reperfusion. Similarly, Repse et al (2) also showed inferior cardiac power recovery of canine hearts subjected to 30-min WIT compared with normal control hearts. The limited human experience to date has been with DCD hearts subjected to relatively short WITs. Boucek et al (27) reported three successful pediatric heart transplants from DCD donors with WITs of 11.5–27 min (mean of 18.3 min). Ali et al (28) reported the reanimation of a human adult heart *in vivo* following a total WIT of 23 min; postresuscitation, the heart was able to support the donor's circulation.

Having established the tolerable normothermic ischemic time for DCD hearts flushed with C preservation solution we then tested the hypothesis that supplementation of C solution with agents known to activate postconditioning pathways would extend the tolerable normothermic ischemic time. Our group has previously reported that glyceryl trinitrate, erythropoietin and zoniporide, when added as single or combined supplements to C solution, activate the same intracellular kinases that mediate ischemic preconditioning and postconditioning (14–17). We have further shown that supplementing C solution with these agents reduces IRI in an isolated working rat heart model (16) and in an NDD porcine transplant model (18). In the present study, flushing DCD hearts with C solution supplemented with these three agents significantly extends the tolerable normothermic ischemic time by approximately 10 min allowing complete functional recovery of 30-min WIT hearts and partial recovery of 40-min WIT hearts. Importantly in terms of clinical translation, our cardioprotective strategy was effective when administered postmortem. Postconditioning is a phenomenon that has been demonstrated in multiple other organs including kidney, liver and lungs (29–32). Hence, our findings may also have implications for other organs retrieved from DCD donors.

We also examined lactate concentrations in the perfusate and across the coronary circulation of the DCD hearts during EVP. These values have been used to assess the viability of human hearts retrieved from NDD donors during EVP on the Transmedics Organ Care System (OCS) (22). The lactate profiles of 20-min WIT hearts were similar to those of control hearts and met the viability criteria that have been established for the Transmedics OCS: an overall lactate concentration in the perfusate of less than 5 mmol/L and evidence of lactate extraction across the heart (coronary sinus [lactate] < coronary arterial [lactate]) (22). Thirty-minute WIT hearts flushed with supplemented C solution also had lactate profiles which met Transmedics OCS criteria for viability whereas 30-min WIT hearts flushed with unsupplemented C solution did not. High lactate levels during EVP were also observed in the 40-min WIT hearts which demonstrated poor functional recovery and high troponin release. These findings have major implications for clinical translation in that measurement of lactate concentrations during *ex vivo* normothermic perfusion of reanimated hearts from DCD donors may allow assessment of the heart's viability and suitability for transplantation before commencement of the recipient procedure.

Study Limitations

Two potentially important differences between our model and the clinical DCD setting exist. The first is the *ante-mortem* administration of heparin. Given the concern regarding intra-coronary or intra-cardiac thrombus formation following circulatory arrest, heparin was administered *ante-mortem* for these experiments. *Ante-mortem* administration of heparin is permitted in some jurisdictions but not in others. In a recent clinical review of DCD lung transplant outcomes, early function of the transplanted lung was not adversely affected by the absence of *ante-mortem* heparin (33). Whether this finding applies to the heart is unknown and further studies assessing DCD hearts in the absence of *ante-mortem* heparin are warranted. The second difference is the duration of the stand-off period following circulatory arrest. We acknowledge that the use of fixed ''stand-off'' times after cessation of the circulation is a point of difference from the clinical scenario of DCD organ donation. The intent here was to explore the limit of the DCD heart to warm ischemia before the onset of ''irreversible'' ischemic injury when the heart is flushed with C preservation solution. In these series of experiments, the time to circulatory cessation was similar in all the groups at approximately 7 min. While the clinical stand-off time is 5 min, we extended this stand-off period (by up to 35 min in some animals) to expose the heart to predetermined total WITs ranging from 20 to 40 min. Hence, the ischemic insult that these hearts were exposed to is likely to be greater than the clinical equivalent, given the longer stand-off period during which there is complete circulatory arrest.

Conclusion

Impaired functional, biochemical and metabolic recovery is seen following resuscitation of DCD hearts exposed to WITs of greater than 20 min. Pharmacological postconditioning extends the tolerance of DCD hearts to warm ischemia by approximately 10 min, allowing complete functional recovery of hearts exposed to WITs of up to 30 min. Using a WIT cut-off of 30 min, the potential to increase the numbers of available allografts for cardiac transplantation from DCD donors is substantial (34). Assessment of the feasibility of heart transplantation from DCD donors using a combined approach of pharmacological postconditioning and EVP to enhance organ preservation is warranted.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Video S1: Hearts exposed to a WIT of $\geq$30 min had an atrial dysrhythmia, lacking synchronized atrial contractions.

Video S2: Hearts exposed to a WIT of 20 min, and hearts exposed to postconditioning strategies following WITs of $\geq$30 min, all recovered to sinus rhythm.

CHAPTER 4 – PUBLICATION 3

TITLE: Normothermic ex vivo perfusion provides superior organ preservation and enables viability assessment of hearts from DCD donors

JOURNAL: American Journal of Transplantation 15(2):371-80

YEAR: 2015

Declaration

I certify that this publication was a direct result of my research towards this PhD, and that reproduction in this thesis does not breach copyright relations.

Foreword

Whilst the last paper (publication 2) was used to display WIT tolerance and organ viability, these next sets of experiments were conducted with a goal of demonstrating clinical relevance. Chapter 3 demonstrated that utilising ischaemic post-conditioning strategies, a WIT of up to 30 minutes conferred full functional and metabolic recovery on an ex-vivo working heart platform. While the 40-minute WIT group had inferior functional recovery, there was no difference in oxidative metabolism parameters, suggesting potential for recovery. Despite this, the demonstrated complete recovery of 30 minute WIT hearts formed the basis for the next series of experiments.

A DCD asphyxia model similar to the clinical setting was utilised and, apart from pre-heparinisation, no ante-mortem interventions were conducted. Time from donor explant to recipient implantation was timed to approximately four hours, in keeping with the mean interval time at St Vincent's Hospital Sydney. The ex vivo perfusion (EVP) device utilised was the TransMedics Organ Care System, a device approved for clinical use and recently purchased by St Vincent's Hospital. Using an orthotopic transplantation model, transplantation times, surgical techniques and cardiopulmonary bypass weaning protocols were all kept in line with usual clinical practice. By maintaining all the above facets in the conduct of these experiments, maximal clinical relevance was ensured and therefore if viability were demonstrated, clinical translation would be an appropriate next step.

In addition to this, the direct comparison of a clinically approved EVP device and current practice of cold storage preservation has never been undertaken with DCD cardiac allografts. As outlined in section 1.13 of the literature review, there have been numerous pre-clinical studies advocating for EVP and its superior organ preservation. With no greater need for optimal preservation and minimal further damage than in DCD hearts, this hypothesis was tested in a clinically applicable manner in this series of experiments.

Results of this set of experiments paved the way for clinical translation.

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Normothermic *Ex Vivo* Perfusion Provides Superior Organ Preservation and Enables Viability Assessment of Hearts From DCD Donors

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The shortage of donors in cardiac transplantation may be alleviated by the use of allografts from donation after circulatory death (DCD) donors. We have previously shown that hearts exposed to 30 min warm ischemic time and then flushed with Celsior supplemented with agents that activate ischemic postconditioning pathways, show complete recovery on a blood-perfused *ex vivo* working heart apparatus. In this study, these findings were assessed in a porcine orthotopic heart transplant model. DCD hearts were preserved with either normothermic *ex vivo* perfusion (NEVP) using a clinically approved device, or with standard cold storage (CS) for 4 h. Orthotopic transplantation into recipient animals was subsequently undertaken. Five of six hearts preserved with NEVP demonstrated favorable lactate profiles during NEVP and all five could be weaned off cardiopulmonary bypass posttransplant, compared with 0 of 3 hearts preserved with CS ($p < 0.05$, Fisher's exact test). In conclusion, DCD hearts flushed with supplemented Celsior solution and preserved with NEVP display viability before and after transplantation. Viability studies of human DCD hearts using NEVP are warranted.

Abbreviations: CPB, cardiopulmonary bypass; CS, cold storage; DCD, donation after circulatory death; IPC, ischemic postconditioning; IRI, ischemia reperfusion injury; NEVP, normothermic *ex vivo* perfusion; WIT, warm ischemic time

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Introduction

Heart transplantation is limited by the shortage of suitable donor organs. This has led some investigators to evaluate the potential viability of hearts from donation after circulatory death (DCD) donors (1–5). Organ donation via the DCD pathway is associated with variable periods of warm ischemia. The period between withdrawal of life support and administration of organ preservation solution, referred to as the warm ischemic time (WIT), exposes the heart to the triple insults of hypoxia, hypo-perfusion and cardiac distension (6–8). Beyond the WIT, the donor heart is subjected to further insults—during storage and again at the time of reperfusion. Concerns regarding the extent of myocardial injury sustained during the WIT and subsequent storage, combined with the inability to assess myocardial viability prior to transplantation, have been major barriers to the clinical development of heart transplantation using DCD donor hearts.

DCD hearts, having sustained an unavoidable warm ischemic injury, ideally require a preservation modality that minimizes further ischemic injury, offers a platform for organ resuscitation, and provides a portal for assessment of graft viability prior to transplantation. Cold storage (CS) fails to address these needs, however normothermic *ex vivo* perfusion (NEVP) may provide these benefits and thereby allow superior recovery and assessment of DCD hearts.

Recently, using a porcine DCD model, we reported that the tolerable WIT could be extended from 20 to 30 min by

modification of the organ preservation solution used to flush the heart (6). DCD hearts subjected to 30 min WIT and then flushed with Celsior solution supplemented with erythropoietin, glyceryl trinitrate and zoniporide (a combination that we have shown activates myocardial ''post-conditioning'' pathways (9)), demonstrated full functional recovery on a blood-based NEVP system, whereas DCD hearts subjected to 30 min WIT and then flushed with standard Celsior solution demonstrated poor recovery.

The aim of this study was to validate these findings in a clinically relevant model. Utilizing the same DCD asphyxia model that incorporated 30 min of warm ischemia followed by flushing of the donor heart with supplemented Celsior solution, we hypothesized that NEVP would provide superior preservation of the donor heart than CS and that it would also enable viability assessment of DCD hearts to be undertaken prior to transplantation.

Methods

A porcine model of orthotopic heart transplantation was utilized as previously described (10,11). Juvenile *Landrace* pigs were used, with experimental protocols approved by the Garvan Institute/St Vincent's Hospital Animal Ethics Committee. All animals were cared for according to the standards outlined in the Australian Code for the Care and Use of Animals for Scientific Purposes 8th edition (2013).

Experimental groups

Ten orthotopic heart transplants were conducted in two groups with differing preservation strategies. Group A hearts (n = 8) were preserved using a blood perfused NEVP system (TransMedics^{R} Organ Care System [OCS]; TransMedics, Inc., Andover, MA) and seven hearts were transplanted, while Group B hearts (n = 3) were preserved using CS prior to transplantation (Figure 1).

All donor hearts were exposed to 30 min of warm ischemia in a DCD asphyxia model, prior to being flushed with cold Celsior solution supplemented with erythropoietin (5000 U/L), glyceryl trinitrate (100 mg/L) and zoniporide (1 μmol/L), as described previously (6,11).

Animals and anesthesia

Ten orthotopic transplants were conducted using 20 pigs weighing between 55 and 65 kg. For each donor/recipient pair, the larger animal was used as the donor. One intended donor heart was reperfused on the OCS but not transplanted due to poor metabolic and functional recovery. Animals were premedicated with an intramuscular injection of ketamine (10 mg/kg), midazolam (1 mg/kg) and atropine (50 μg/kg). Animals were intubated (7–7.5 mm cuffed endotracheal tube) and ventilated with a tidal volume of 10 mL/kg and PEEP of 5–7 mmHg. Anesthesia was maintained using inhalational isoflurane (2–5%) and regular IV fentanyl (100–200 μg bolus). There was continuous physiological monitoring of cardiac rhythm, mean arterial pressure (MAP), central venous pressure (CVP), pulse oximetry, end-tidal CO_2 and core temperature. Arterial blood gas samples were analyzed at regular intervals.

Surgical procedure—donor/recipient animals

The left internal carotid artery and internal jugular vein were cannulated via a neck incision for arterial and CVP monitoring, respectively. After

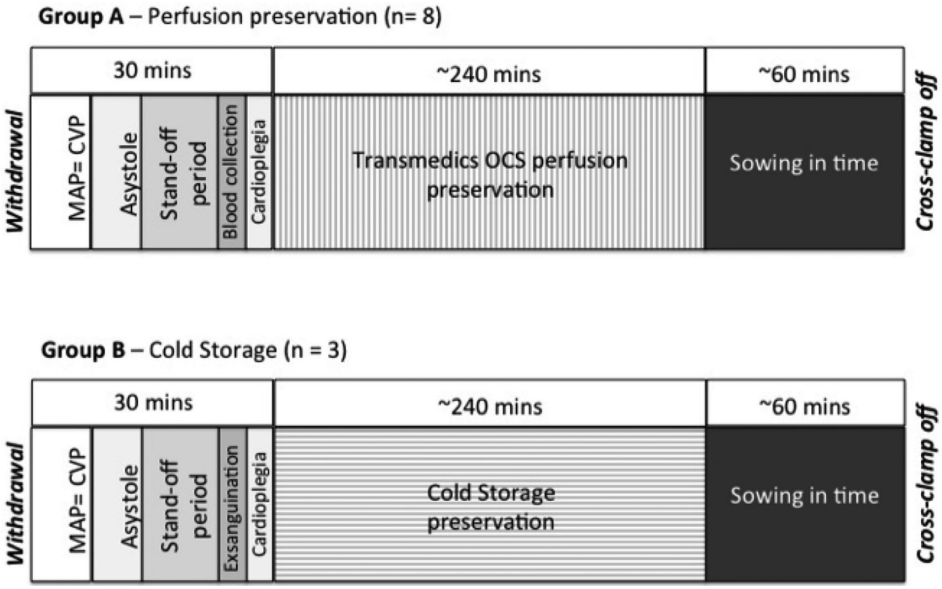

Figure 1: Experimental timeline for Group A (TransMedics^{R} OCS perfusion preservation) and Group B (Cold Storage preservation) studies. Identical withdrawal processes, total WIT and cardioplegia were used for both groups. Blood was collected from Group B animals at the same time as Group A animals who had blood collected to prime the *ex vivo* perfusion circuit. This was performed in order to expose all hearts to similar periods of cardiac distension.

administration of lignocaine (1 mg/kg) to prevent arrhythmias, a median sternotomy was performed and the pericardium opened. Heparin (300 IU/kg) was administered and the heart inspected for gross abnormalities. Blood was taken for baseline hemoglobin and biochemistry. Purse-string sutures were placed in the apex of the left and right ventricle and Millar Pressure-Volume (PV) Loop catheters (Millar, Inc., Houston TX) were inserted through needle stab incisions. Contractility and diastolic functional data were obtained during temporary vena caval occlusion using the implanted catheters and the MPVS ultra system (Millar, Inc.). Catheters were then removed and the incisions closed.

Donor animal—withdrawal

We used an asphyxia model to mimic the clinical setting, as described previously (6). Animals were preoxygenated with 100% oxygen prior to withdrawal. After a bolus dose of midazolam and fentanyl to ensure adequate sedation, ventilation was ceased and the endotracheal tube disconnected. With the pleura opened, the lungs were inspected to confirm absence of spontaneous breathing. Varying times to circulatory arrest and cessation of electrical activity were noted, however, hearts were left untouched for 30 min of warm ischemia irrespective of electrical activity/circulatory cessation times. Warm ischemia was defined as the period between withdrawal of ventilator support and administration of preservation solution.

Donor Group A—TransMedics^R OCS preservation

Immediately prior to the end of the 30 min WIT, cannulae were inserted into the right atrium (for blood collection) and aortic root (for preservation solution flush). Blood (approximately 1.2 L) was drained from the animal over 1–2 min (all donor animal hematocrits were $\geq$25%). Immediately following blood collection, the aorta was cross-clamped and supplemented Celsior preservation solution (Genzyme, Cambridge, MA; 1L precooled to 4°C) was administered with the heart vented via incisions in the left atrial appendage (LAA) and inferior vena cava (IVC). The chest cavity was subsequently filled with cold saline slush. After Celsior flush, the hearts were excised and submerged in cold saline while being cannulated for NEVP on the TransMedics^R OCS. The OCS was prepared as per the manufacturer's instructions with 500 mL of TransMedics^R priming solution and 1000 mL of TransMedics^R maintenance solution (running at 0–30 mL/h). Collected blood was passed through a leukocyte filter (Pall LeukoGuard BC2; Pall Corporation, Port Washington, NY) prior to being added to the device reservoir. Hearts were perfused in resting mode for4 h maintaining aortic pressures of 60–80 mmHg and coronary flows of 550–800 mL/min (Figure S1) as recommended by the manufacturer for human heart preservation (12). If an increase in aortic pressure was required, epinephrine (0–0.08 μg/min) infusion was utilized. Bradycardic (heart rate $<$ 60) hearts were paced (VVI) using epicardial ventricular pacing wires.

Donor Group B—CS

Immediately prior to the end of the 30 min WIT, blood was collected as per Group A and the aortic root cannula was inserted. Hearts were perfused with 900 mL of supplemented Celsior solution at 4°C, while being vented via incisions in the LAA and IVC. The chest cavity was subsequently filled with cold saline slush. After excision, hearts were stored in an organ bag with 100 mL of supplemented Celsior solution at 4°C, surrounded by cold saline slush and placed in a sealed plastic container of ice to maintain hypothermic storage for a similar 4-h period.

Recipient procedure—Group A and Group B

The recipient animals were anesthetized in a similar manner to the donor animal. Following insertion of lines for hemodynamic monitoring, a median sternotomy was performed. Heparin (300 IU/kg) was administered and the pigs placed on aorto-bicaval cardiopulmonary bypass (CPB), with active cooling to 32°C. Both preservation groups had similar mean ''storage'' times to match the average clinical cold ischemic times faced at our institution. After excision of the native heart, the donor heart was transplanted orthotopically using the bi-atrial anastomosis technique of Lower and Shumway (13). During the anastomosis time of approximately 60 min, intermittent cold blood cardioplegia (1:4 St Thomas Solution/autologous whole blood) was given (14). Following completion, the PV loop catheters were re-inserted through the previous incisions. The animals were rewarmed and the hearts reperfused. The hearts were defibrillated as required and paced using ventricular pacing wires (VVI at 110 bpm). At 50 min postreperfusion, a dobutamine infusion (5–10 μg/kg/min) was commenced if MAP on CPB was greater than 40 mmHg. If MAP was less than 40 mmHg, epinephrine (0.05 μg/kg/min) was commenced. A norepinephrine infusion was added if required to maintain a MAP $>$ 40 mmHg.

After 60 min of reperfusion, the first attempt was made to wean the recipient animal off CPB with a goal of maintaining a MAP of $\geq$40 mmHg. If this attempt was unsuccessful, the animal was placed back on CPB, and two further attempts at weaning were made at 2 and 3 h postreperfusion. These attempts were undertaken at higher doses or varying combinations of inotropic support (dobutamine up to 20 μg/kg/min or epinephrine up to 0.1 μg/kg/min). If these were also unsuccessful, the hearts were deemed nonviable. If the animal was successfully weaned off CPB, it was monitored for a further 3 h, during which hemodynamic and metabolic data were collected. Repeat contractility and diastolic functional data were re-recorded during temporary IVC occlusion immediately prior to termination of the experiment.

Outcome measures

Ex vivo *perfusion preservation:* Metabolic profiles of cardiac allografts preserved on the OCS were assessed using transmyocardial lactate extraction (15). Simultaneous sampling from the coronary inflow and coronary effluent ports on the OCS perfusion circuit was undertaken at hourly intervals. Lactate concentrations in the perfusate were measured with an automated iSTAT analyzer (Abbott, Inc., Princeton, NJ) according to the manufacturer's instructions. A total concentration of lactate $<$5 mmol/L in the perfusate combined with myocardial lactate extraction (coronary inflow lactate $>$coronary effluent lactate) was considered evidence of myocardial viability (15).

Orthotopic transplantation: NEVP and CS preservation methods of DCD hearts were compared. The primary outcome was the ability to wean off CPB posttransplantation, and to maintain hemodynamic stability for 3 h postweaning. Heart rate, arterial and CVPs were recorded continuously. Metabolic status was also monitored (acid-base status, lactate) to assess adequacy of tissue perfusion.

Statistics

Statistical analyses were performed using Prism 6.0b (GraphPad Software, Inc., La Jolla, CA). Data are expressed as mean ± SE. One-way analysis of variance was used to determine differences between groups pre- and posttransplant. Categorical variables were compared using Fisher's exact test. A p-value $<$0.05 was considered significant.

Results

Baseline donor characteristics

There was no difference in donor and recipient animal weights (Table 1) or in the WITs between the two groups (Table 2).

Table 1: Donor and recipient animal weights between the two groups

	TransMedics^R OCS preservation	Cold storage preservation	p-Value
Donor weight (kg)	64.9 ± 1.9	62.3 ± 2.0	0.70
Recipient weight (kg)	61.9 ± 1.7	60.7 ± 2.3	0.99
Donor/recipient ratio	1.05 ± 0.01	1.03 ± 0.01	0.99

DCD heart viability during NEVP preservation

Hearts from eight DCD donor animals were assessed during NEVP. Of the eight hearts evaluated, seven had declining lactate levels in the perfusate during NEVP. All seven hearts showed lactate extraction throughout their time on NEVP and by the third hour, the perfusate lactate concentration was below 5 mmol/L (Figure 2). In one heart, perfusate lactate concentrations remained above 5 mmol/L throughout NEVP and the heart was producing lactate by the third hour (Figure 2). This heart required epicardial pacing and was the only heart that also required epinephrine infusion to maintain perfusion pressure. In addition to the poor metabolic profile, this heart had gross impairment of contractile function and impaired recovery of rhythm on NEVP. Based on these characteristics, the heart was judged to be nonviable and therefore not transplanted into a recipient animal. The seven hearts with favorable lactate profiles were transplanted. Of these seven hearts, five did not require epicardial pacing during the NEVP period.

Posttransplant recovery of NEVP versus CS hearts

Of the seven orthotopic transplants performed with NEVP hearts, two recipient animals were excluded due to procedural complications unrelated to donor allograft recovery. One recipient animal developed malignant hyperthermia immediately following anesthetic induction, while the other transplant was aborted due to a major malfunction of the CPB prior to completion of the transplant procedure.

Excluding these two transplants, the remaining five hearts were successfully transplanted and all were weaned off CPB. These hearts were able to support the recipient animal's circulation with the use of inotropic support (dobutamine/epinephrine), vasopressors (norepinephrine) and ventricular pacing. All five hearts were monitored for 3 h postweaning, and were able to maintain stable hemodynamics without any further escalation in inotropic support (Table 3). MAP remained above 40 mmHg in all animals throughout the 3 h after weaning from CPB. Serial measurements of systolic blood pressure and mean CVP are shown in Figure 3.

Table 2: Withdrawal (W/D), preservation, and anastomosis times of the two groups with differing preservation strategies

Period	TransMedics^R OCS preservation	Cold storage preservation	p-Value
W/D to MAP = CVP	7.1 ± 0.6	7.3 ± 0.3	n.s.
W/D to electrical asystole	12.6 ± 2.9	16.3 ± 4.4	n.s.
W/D to blood collection	25	25	n.s.
W/D to cardioplegia	30	30	n.s.
Preservation time	233 ± 3	231 ± 17	n.s.
Anastomosis time	62.4 ± 1.6	60.0 ± 3.1	n.s.

Values are in minutes and reported as mean ± standard error of the mean. Cessation of circulation was determined by equalization of mean arterial pressure (MAP) and central venous pressure (CVP), when MAP = CVP.

None of the three hearts in the CS group could be weaned from CPB, despite escalation of inotrope doses, ventricular pacing and repeated attempts at weaning (Figure 3). Furthermore, it was not possible to establish a stable cardiac rhythm in any of the CS hearts, all of which demonstrated poor contractility prior to attempted weaning. Excluding the two technical failures in the NEVP group, recovery of the six DCD allografts preserved with NEVP was superior to that of the three allografts preserved using CS ($p < 0.05$ Fisher's exact test, CS vs. NEVP).

Representative systolic and diastolic functional assessment of an NEVP heart (TM07) is displayed in Figure 4. Due to technical reasons, we were unable to obtain contractility measurements in all transplanted hearts. However, data from TM07 pre- and posttransplant provide indicative trends: left ventricular (LV) dP/dt max, cardiac output, and the slope of the preload recruitable stroke work (PRSW) and end-systolic pressure-volume relationship slope were similar pre- and posttransplant, and are consistent with viable recovery of contractile function (with inotropic support). The less negative LV dP/dt min, lower LV and right ventricular (RV) end-diastolic volumes and the increased slope of RV end-diastolic pressure-volume relationship (EDPVR) are consistent with impaired relaxation of both ventricles with more marked impairment of the right ventricle.

Metabolic recovery

Serum lactate levels and arterial pH were used to assess metabolic recovery and tissue perfusion. Serum lactate levels increased during CPB in all recipient animals. However there was no further increase in serum lactate levels after weaning from CPB (Figure 5). Blood pH was high pre-CPB and fell during and after CPB but remained within the normal range throughout the observation period (Figure 5).

Discussion

In our previously published research utilizing a porcine DCD asphyxia model, we demonstrated that the tolerable WIT of hearts retrieved from DCD donors could be extended by approximately 10 min using a pharmacological postconditioning strategy (6). In the current study, we have extended these findings to a clinically relevant porcine orthotopic heart transplant model, demonstrating that DCD hearts

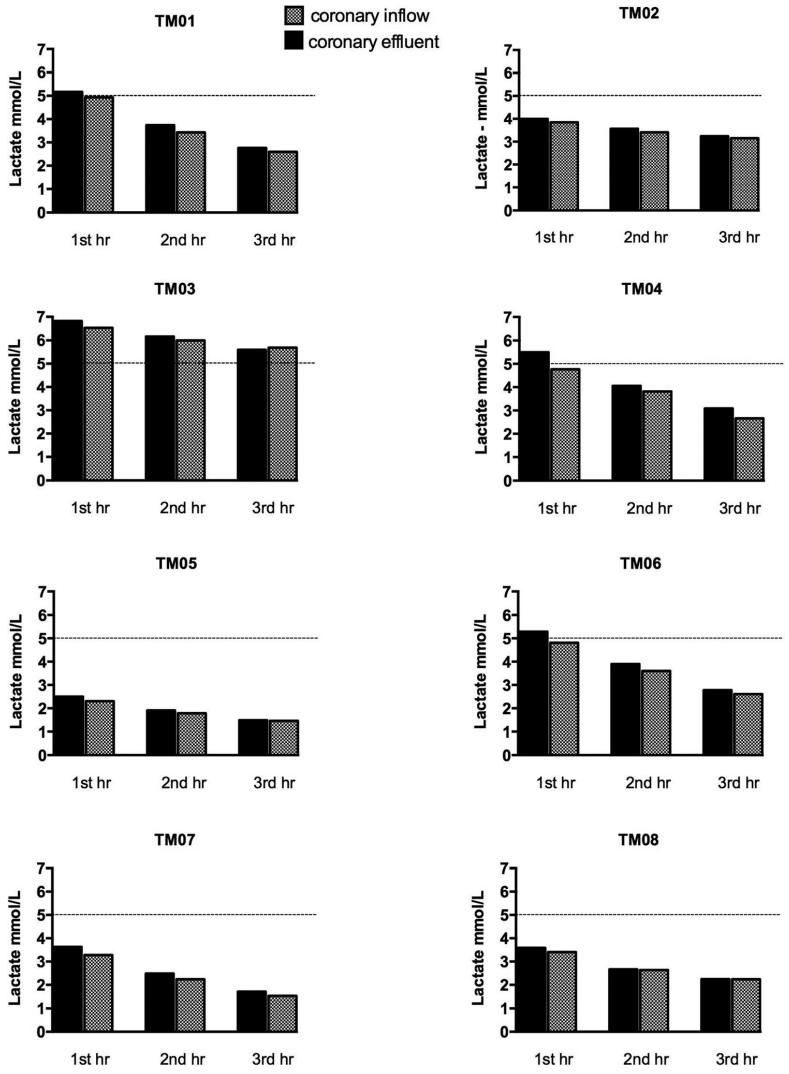

Figure 2: Lactate profiles of eight hearts preserved with the TransMedics^{R} OCS system over the first 3 h of preservation. Results for each animal are shown.

exposed to 30 min WIT can be successfully transplanted using a pharmacological postconditioning strategy, followed by 4 h preservation using NEVP. In contrast, DCD hearts undergoing the same pharmacological postconditioning strategy, but then stored for an equivalent time period under cold static storage conditions, demonstrated immediate primary graft failure.

CS versus ex vivo *perfusion*

CS has been used as the standard preservation modality for cardiac allografts for the last 40 years. Limitations to CS stem from the presence of ongoing low levels of anaerobic metabolic activity (16), which is reflected in the increased risk of primary graft failure in the setting of prolonged ischemic times (17–19). The development of *ex vivo*

Table 3: Inotropic support and pacing at time of weaning from CPB for the five DCD hearts preserved with *ex vivo* perfusion and subsequently successfully transplanted

	Adrenaline	Dobutamine	Noradrenaline	Pacing mode/rate
TM04	Nil	10 μg/kg/min	0.08 μg/kg/min	VVI 110
TM05	Nil	5 μg/kg/min	0.03 μg/kg/min	VVI 110
TM06	0.05 μg/kg/min	10 μg/kg/min	Nil	VVI 110
TM07	0.04 μg/kg/min	5 μg/kg/min	0.08 μg/kg/min	VVI 110
TM08	Nil	5 μg/kg/min	0.03 μg/kg/min	VVI 110

No further increase in doses of inotropes/vasopressors than those displayed below were required.

perfusion technology offers an alternative and potentially superior method of organ preservation. A number of preclinical studies have suggested its superiority over CS for myocardial viability and functional recovery (20–22). The benefit of EVP is likely greatest in the setting of extended criteria donors, who demonstrate increased susceptibility to the detrimental effects of anaerobic metabolism during CS (19,20,23). Similarly, DCD hearts, having sustained a period of obligatory warm ischemia, are high-risk allografts that require optimal preservation. While hypothermic nonblood EVP has been shown to be superior to cold static storage in experimental DCD heart transplant studies, this mode of preservation does not allow any assessment of viability to be undertaken prior to transplantation (3,5,21,22). In contrast, blood-based NEVP allows preservation in a beating state and thereby a platform for both resuscitation and assessment. The impact of the obligatory warm ischemia on DCD hearts is likely to vary between hearts depending on donor characteristics and the time to circulatory arrest. Therefore, we believe that a method for assessing viability of the DCD heart prior to implantation is an essential step in the translation of this procedure from the research laboratory to the clinic.

NEVP for preservation of DCD hearts

Recently, White et al (24) reported the immediate posttransplant outcomes of two separate protocols involving NEVP in a porcine DCD heart transplant model. In protocol one, they administered cold hyperkalemic cardioplegic solution in the donor prior to NEVP, and again at the time of implantation. In protocol two, they administered tepid normokalemic adenosine/lignocaine (AL) in Steen solution as the initial cardioplegia. They reported better donor heart recovery with the second strategy and concluded that this was due to enhanced cardioprotection through avoidance of hyperkalemia and profound hypothermia. It is noteworthy however that the time to reperfusion on the NEVP device was on average 10 min longer with protocol one, a potentially critical time difference given the susceptibility of the porcine DCD heart to ischemia following withdrawal of life support (6). Nonetheless, their findings with AL solution, together with our previously published findings using a pharmacological postconditioning solution, highlight the importance of the composition of the initial flush solution for DCD hearts and the potential for limiting myocardial injury after death. It remains to be seen whether further modifications to the cardioplegic flush

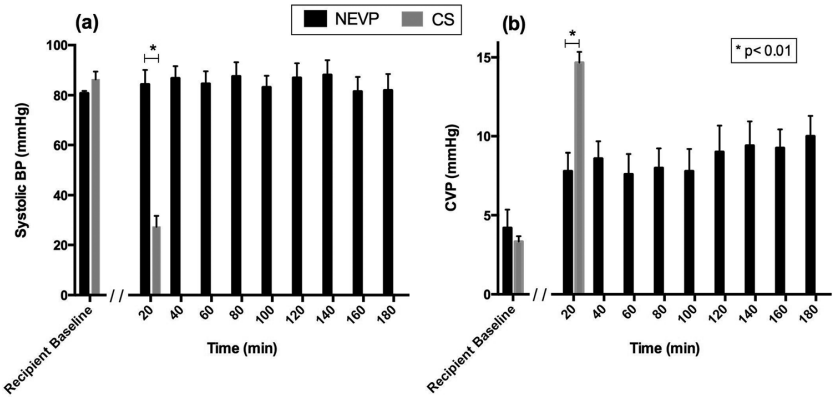

Figure 3: Hemodynamic profile of hearts after weaning from CPB. (a) Systolic blood pressure (BP) and (b) central venous pressure (CVP) were recorded in the recipient animals prior to institution of cardiopulmonary bypass (CPB). Subsequent values were recorded after weaning from CPB—for 3 h postweaning in NEVP hearts (black bars) and for 20 min after the final attempt at weaning in CS hearts (gray bars). Data shown are the mean ± 1 SEM.

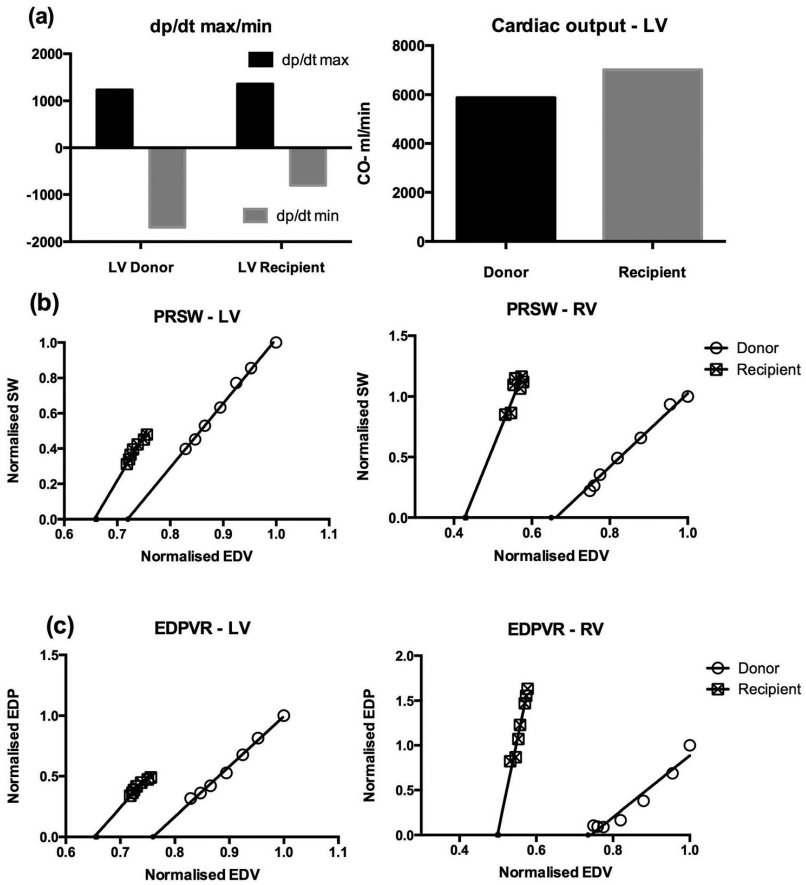

Figure 4: Functional assessment of TM07: measures of cardiac contractility and diastolic function for both ventricles were obtained in the donor following initial instrumentation (pretransplant) and repeated in the recipient (posttransplant) after weaning from CPB and immediately prior to termination of the experiment. (a) Upper left panel shows LV dp/dt maximum and minimum values pre- and posttransplant. Upper right panel shows cardiac output at the same time points. (b) and (c) Middle and lower panels show preload recruitable stroke work (PRSW) and end-diastolic pressure-volume relationships (EDPVR) for both ventricles pre- and posttransplant. PRSW and EDPVR were derived from pressure-volume loops recorded during temporary IVC occlusion. To reduce potential confounding from modeling errors, the stroke work, end-diastolic pressure and end-diastolic volume data were normalized to the pretransplant steady state value for each ventricle as described previously (32).

solution can further prolong the tolerable WIT for the DCD heart. This is an important area for further research.

The TransMedics^{R} OCS is the only NEVP device that is approved for clinical use for heart transplantation. Hearts are preserved in an unloaded beating state using continuous warm blood-based perfusion and viability assessed by evaluating lactates profiles in the perfusate (15). To date, over 200 human hearts have been preserved in Europe, North America and Australia with this device (25,26). While these studies have demonstrated noninferiority to CS with preservation of standard hearts from neurological determination of death donors, the greatest benefit of this and other *ex vivo* perfusion devices is likely to be in the setting of extended criteria donors (20,25), studies of which have been limited. In our experiments, seven of eight DCD hearts that were exposed to 30 min of warm ischemia and subsequently preserved on the OCS displayed lactate profiles that met prespecified criteria for allograft viability (15). The subsequent successful transplantation of these hearts validates the use of the lactate profile during NEVP as a marker of DCD cardiac

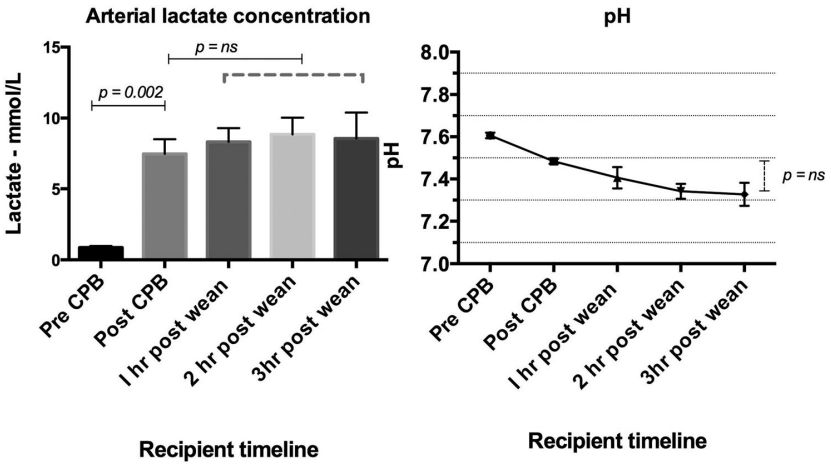

Figure 5: Arterial lactate and pH levels pre- and postweaning from CPB of the 5 transplanted DCD hearts.

allograft viability. Whether lactate measures alone are adequate for assessing viability, or whether additional forms of assessment are required for these high risk allografts remains to be determined.

Mechanistically, the benefit of NEVP over CS is most likely related to reduced myocardial ischemia/reperfusion injury in NEVP-preserved hearts. Timely institution of NEVP with oxygenated blood re-establishes aerobic metabolism, provides cellular nutrients that allow restoration of high energy substrates and removes metabolic waste products (27). Collectively these actions prevent ongoing ischemic injury and the reperfusion injury that follows more prolonged ischemia. In contrast, the immediate transfer of a heart that has sustained a severe warm ischemic injury into a cold ischemic environment slows the rate of ischemic injury but does nothing to reverse it. Exhaustion of cellular energy stores during CS ultimately results in activation of apoptotic and necrotic cell death upon reperfusion (28). This reperfusion injury is manifested as primary graft failure (17).

While the DCD hearts were able to support the recipient animal's circulation, there is evidence that these hearts had sustained significant injury as evidenced by the level of inotropic support required in the immediate posttransplant period. In addition, in the recipient animal in which we were able to obtain more detailed functional assessments, there was evidence of biventricular diastolic dysfunction particularly involving the right ventricle. This is not unexpected as marked RV distension has been reported during withdrawal of life support and may exacerbate myocardial injury (8). This finding has implications for clinical translation and suggests that DCD hearts may not be suitable for recipients with an elevated pulmonary vascular resistance (29,30).

Assessment of viability of DCD hearts prior to transplantation

Another major finding of our study is the value of NEVP in identifying severely damaged DCD hearts prior to transplantation—the one heart that demonstrated an abnormal lactate profile also demonstrated an erratic rhythm and visibly poor contractility during NEVP. In comparison with the ''viable'' donor hearts, there was no major difference in donor characteristics, hemodynamics or features of the warm ischemic period. This observation highlights the importance of a reliable method of viability assessment to prevent the catastrophic consequences of transplanting a nonviable graft into a recipient.

Limitations

The model we have utilized to display viability of DCD hearts still differs from clinical reality. Hearts were left untouched in the donor for a 30-min WIT regardless of time to circulatory cessation. In most clinical jurisdictions, a stand-off period of only 5 min is required after death, which is determined by cessation of circulation. DCD hearts in this study, however, were exposed to a longer and more severe insult than would occur clinically due to the longer stand-off period.

Because of technical equipment issues, we were unable to assess contractility recovery of all DCD hearts post-weaning from CPB. Therefore, we assessed hemodynamic state and tissue perfusion based on gross hemodynamic data, inotropic, and vasopressor support details and metabolic status. Although all animals developed elevated lactic acid levels during CPB, this is a well-recognized complication of hypothermic CPB in pigs and not

necessarily indicative of inadequate myocardial or tissue perfusion (31). Importantly, there was no further increase in arterial lactate levels after weaning from CPB. The duration of observation of the transplanted heart after weaning from CPB was limited to 3 h. It is possible that the hearts may have failed if observed for a longer period. However, all animals remained stable both in terms of hemodynamic status and inotropic support during this high risk period for development of primary graft failure (29,30).

Conclusions

Using a pharmacological postconditioning strategy and NEVP, DCD hearts exposed to 30 min WIT can be resuscitated and successfully transplanted. This offers a feasible time frame for recovery of human hearts from DCD donors, and may significantly increase the number of cardiac allografts available for transplantation. In addition, NEVP enables viability assessment of the DCD heart to be undertaken prior to donor heart implantation. Our studies suggest that DCD hearts, despite having sustained a significant ischemic insult, can be resuscitated to a state of viability for transplantation. Viability studies of human DCD hearts are warranted and are under way.

Acknowledgments

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1: Coronary flow and perfusion pressure of the eight hearts during the NEVP period.

CHAPTER 5 – PUBLICATION 4

*(*2nd author)*

TITLE: Adult heart transplantation with distant procurement and ex-vivo preservation of donor hearts after circulatory death: a case series

JOURNAL: Lancet 385(9987):2585-91

YEAR: 2015

Declaration

I certify that this publication was a direct result of my research towards this PhD, and that reproduction in this thesis does not breach copyright relations.

Foreword

Following the demonstration of organ viability in a clinically relevant model, the next stage involved clinical translation. Protocols for DCD donor selection, cardiac allograft and retrieval process, as well as criteria for organ viability/suitability for transplantation were all established. Ethical approval for a clinical DCD heart transplantation program was acquired, and discussions and presentations to the ICUs of all institutions partaking in this study were undertaken.

With regards to the retrieval process, specific roles of the surgeon and assistant were outlined to minimise the time from donor arrival in the operating room to blood collection and organ preservation flush. In addition, discussions were held with the abdominal surgeons about the conduct of proceedings during multi-organ procurements. This was done in order to avoid any limitation of the abdominal organ procurement process, and to allow smooth collaboration between the various surgical teams. With this in mind, a speedy entry into the thoracic cavity for blood exsanguination was prudent to avoid delaying the abdominal surgeons need to clamp the aorta and vent the IVC.

With establishment of protocols and adequate discussions and collaborations with intensive care specialists, concurrent surgeons, donor coordinators, and theatre staff, the program was readied for clinical translation.

The first distant procurement DCD heart transplant in the world was conducted at St Vincent's Hospital Sydney in July 2014. Following its success, further DCD heart transplants were successfully performed at our institution—a case series of the first three of these were published in the Lancet and forms Chapter 5. In total six DCD hearts transplants have been performed to date, with all recipients discharged home with normal cardiac function on echocardiography.

With six additional heart transplants performed in the first 12 months of the program, this represents an approximate 25% increase in transplant numbers. Forming a major milestone in the history of transplantation, the advent of DCD cardiac transplantation

has been recognised as a significant medical breakthrough (1). It also goes a long way in addressing the mortality that exists on cardiac transplant waiting lists. Furthermore, following the success of St Vincent's Hospital clinical program, two other units have followed the lead and have conducted successful DCD cardiac transplants: Papworth Hospital and Harefield Hospital in the United Kingdom. To date, 15 DCD heart transplants have been performed at St Vincent's Hospital Sydney, with 100% survival.

The successful establishment the DCD heart transplant program at our institution, followed by success around the world, unequivocally demonstrates that DCD hearts are viable for use in cardiac transplantation. However, several features regarding the clinical outcomes must be noted. Two of the six DCD hearts used so far have required mechanical assistance to be weaned off cardio-pulmonary bypass, in keeping with the diagnosis of PGF. While both these hearts recovered completely to demonstrate normal biventricular function within a week post-transplant, it nevertheless highlights the presence of a temporary period of 'stunned' myocardium following the warm ischaemic insult. In addition, with only recent establishment of a clinical program, only short-term outcomes are apparent and no comment on the longevity of these allografts can be made. With time, the medium- to long-term outcomes of these hearts will become available to analyse.

To note, the preservation solution utilised was the Modified St Thomas' solution, rather than the Celsior solution used in the pre-clinical studies. Whilst the St Thomas' solution was supplemented with agents to stimulate ischaemic post-conditioning, this solution was used instead of Celsior due to Transmedics Inc preference. The difference in outcome cannot be commented on given the absence of a direct comparison, however it must be noted that there are significant dfferences in electrolyte compositon with lower potassium and calcium concentrations in Celsior solution.

Nevertheless, the series of pre-clinical experiments outlined in Chapters 3 and 4, and the successful clinical translation demonstrated in the following publication clearly answers the question posed in the title of this dissertation: that DCD cardiac allografts are viable for use in cardiac transplantation.

1. Patterson R. World-first dead heart transplant at Sydney's St Vincent's Hospital a game changer. The Australian (2014), 24th October.

Adult heart transplantation with distant procurement and ex-vivo preservation of donor hearts after circulatory death: a case series

Kumud K Dhital, Arjun Iyer, Mark Connellan, Hong C Chew, Ling Gao, Aoife Doyle, Mark Hicks, Gayathri Kumarasinghe, Claude Soto, Andrew Dinale, Bruce Cartwright, Priya Nair, Emily Granger, Paul Jansz, Andrew Jabbour, Eugene Kotlyar, Anne Keogh, Christopher Hayward, Robert Graham, Phillip Spratt, Peter Macdonald

Summary

Background **Orthotopic heart transplantation is the gold-standard long-term treatment for medically refractive end-stage heart failure. However, suitable cardiac donors are scarce. Although donation after circulatory death has been used for kidney, liver, and lung transplantation, it is not used for heart transplantation. We report a case series of heart transplantations from donors after circulatory death.**

Methods **The recipients were patients at St Vincent's Hospital, Sydney, Australia. They received Maastricht category III controlled hearts donated after circulatory death from people younger than 40 years and with a maximum warm ischaemic time of 30 min. We retrieved four hearts through initial myocardial protection with supplemented cardioplegia and transferred to an Organ Care System (Transmedics) for preservation, resuscitation, and transportation to the recipient hospital.**

Findings **Three recipients (two men, one woman; mean age 52 years) with low transpulmonary gradients (<8 mm Hg) and without previous cardiac surgery received the transplants. Donor heart warm ischaemic times were 28 min, 25 min, and 22 min, with ex-vivo Organ Care System perfusion times of 257 min, 260 min, and 245 min. Arteriovenous lactate values at the start of perfusion were 8·3–8·1 mmol/L for patient 1, 6·79–6·48 mmol/L for patient 2, and 7·6–7·4 mmol/L for patient 3. End of perfusion lactate values were 3·6–3·6 mmol/L, 2·8–2·3 mmol/L, and 2·69–2·54 mmol/L, respectively, showing favourable lactate uptake. Two patients needed temporary mechanical support. All three recipients had normal cardiac function within a week of transplantation and are making a good recovery at 176, 91, and 77 days after transplantation.**

Interpretation **Strict limitations on donor eligibility, optimised myocardial protection, and use of a portable ex-vivo organ perfusion platform can enable successful, distantly procured orthotopic transplantation of hearts donated after circulatory death.**

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Introduction

The first successful clinical heart transplantation was done with a heart donated after circulatory death in 1967 by Christiaan Barnard and the South African Groote Schuur Hospital team.[1] In that era, before the establishment of brain-stem death criteria, numerous heart transplantations were carried out around the world with the donor and recipient located in adjacent operating rooms.[2] The introduction of brain-death legislation and the adoption of cardioplegic arrest and static cold preservation, enabled distant procurement and avoided the necessity of transferring donors to the recipient hospital.

Unlike hearts from brain-dead donors who still have a beating heart, for which cardiac structure and function can be assessed after death, hearts donated after circulatory death have unknown functional status, risk of occult pathology, and substantial warm ischaemic insult. The difficulties of assessing the suitability of hearts donated after circulatory death and of co-locating multiorgan donors and recipients has meant that heart transplantation has had to rely solely on donation after brain death.

New policies related to donation after circulatory death have aimed to narrow the gap between the number of patients awaiting a new heart and the number of suitable organs available.[3] Use of organs donated after circulatory death has improved the number and outcomes of kidney and lung transplantation,[4,5] and to a lesser extent, liver transplantation.[6]

A series of three successful paediatric heart transplantations from colocated neonatal donors after circulatory death was described in 2008,[7] in whom in-situ cooling following pre-withdrawal heparinisation and insertion of femoral cannulae were done. In 2009, in-situ resuscitation of an adult human heart donated after circulatory death with subsequent weaning from cardiopulmonary support was reported.[8] The major hurdles to the transplantation of hearts from human donors after circulatory death are the ability to mitigate warm

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ischaemia during withdrawal of life-support, the need to preserve the heart during transportation from the donor to the recipient hospital, and the need to assess the viability of the heart before transplantation. In preclinical studies, we have shown that the tolerance of a heart donated after circulatory death to warm ischaemia can be enhanced by modification of the initial flush solution[9] and that normothermic ex-vivo perfusion preserves the heart better than hypothermic storage and enables the heart's viability to be assessed.[10] These findings, combined with those of other investigators,[9–15] have led to a renewed effort to explore the potential for clinical heart transplantation from donors after circulatory death.[16]

The transportable Organ Care System (TransMedics; Andover, MA, USA) enables both standard and marginal-criteria ex-vivo donor hearts to be preserved,[17] and enables detection of occult pathology during normothermic ex-vivo perfusion. The heart Organ Care System has been used for 246 orthotopic heart transplantations worldwide. We report the first three successful human heart transplants after distant procurement of orthotopic hearts donated after circulatory death.

Methods

Recipients

The patients were included in the Marginal Heart Study at St Vincent's Hospital (Sydney, Australia), which involves a protocol for the use of extended-criteria donor hearts including those donated after circulatory death. This single-centre study defined extended-criteria for both hearts donated after brain death and hearts donated after circulatory death. We considered all Maastricht category III controlled hearts from donors after circulatory death aged younger than 40 years with less than 30 min from withdrawal of support to delivery of cardioplegia. We limited donor age to 40 years to minimise the risk of retrieving hearts with pre-existing pathology and because of concern about the tolerability to ischaemic injury of hearts from older donors.[18] The 30-min warm ischaemic time was chosen on the basis of preclinical studies.[9] The recipient and donor characteristics are shown in table 1.

Patient 1 was a 57-year-old woman with end-stage familial dilated cardiomyopathy admitted for orthotopic heart transplantation 6 weeks after placement on the waiting list for rapid deterioration of her symptoms and with less than 30 days out of hospital in the preceding 4 months.

Patient 2 was a 43-year-old man with cardiomyopathy presumed secondary to viral myocarditis 4 years previously. Over the past 12 months, he had substantial deterioration with recurrent hospital admissions for decompensated heart failure requiring treatment with levosimendan. He was admitted for cardiac transplantation 4 days after placement on the waiting list

Patient 3 was a 57-year-old man with arrhythmogenic right ventricular dysplasia who had been waiting 321 days on the transplantation list. He had had several storms of ventricular tachycardia and multiple shocks from an internal defibrillator.

The study was approved by the St Vincent's Hospital Research Ethics Committee, and endorsed by the New South Wales Ministry of Health and the New South Wales DonateLife Organ & Tissue Service. All recipients provided written informed consent.

Donors and donation procedures

Potential donors were referred by DonateLife agencies for consideration for heart and lung transplantation. Donors had medical history recorded and routine investigations done including venous and arterial blood tests, microbiological cultures, electrocardiography, haemodynamic assessment, and chest radiography. An echocardiogram, if done before referral, was also assessed, as well as the requirement for any vasopressor or inotropic drugs. The process of organ donation and subsequent withdrawal of life-support was done by the intensive care team, who were separate from the thoracic and abdominal organ retrieval teams. The observation period after cessation of circulation varies in Australia and is legally defined by each state. It is at least 2 min in New South Wales and 5 min in other jurisdictions.

At the end of the observation period the donors were declared deceased and quickly transferred to an operating room. The location of withdrawal of support varied from an adjacent operating room, an anaesthetic bay, or an intensive care unit. The thoracic and abdominal retrieval teams were ready before life-support was withdrawn. The donors were only intubated and prepared for surgery on arrival at the retrieval operating room. A median sternotomy and laparotomy were done simultaneously with a large venous cannula placed directly into the grossly distended right atrium to enable rapid collection of 1·5 L of blood to prime the ex-vivo perfusion apparatus.

	Recipient 1	Recipient 2	Recipient 3	Donor 1	Donor 2	Donor 3
Age (years)	57	43	57	26	26	27
Sex	Male	Female	Male	Male	Male	Male
Diagnosis	Familial DCM	Viral DCM	ARVD*	Hypoxia	Trauma	Trauma
Blood group	A	A	O	A	A	O
Height (cm)	163	176	170	183	173	182
Bodyweight (kg)	71	70	79	92	70	79
Ejection fraction (%)	20	18	19	75	50	NA
LVEDD (mm)	84	61	67	..	..	..
TPG (mm Hg)	7	5	8	..	..	..
Creatinine concentration (μmol/L)	99	135	149	..	..	..
eGFR (mL/min BSAc)	44	65	42	..	..	..
Total bilirubin concentration (μmol/L)	30	60	42	..	..	..

DCM=dilated cardiomyopathy. ARVD=arrhythmogenic right ventricular dysplasia. LVEDD=left ventricular end-diastolic dimension. eGFR=estimated glomerular filtration rate. NA=not available. TPG=transpulmonary gradient.

Table 1: **Recipient and donor characteristics**

Heparin was only added to the blood collection bag and not administered to the donor, as per New South Wales state regulations on donation after circulatory death. A clamp was placed on the ascending aorta and 1 L of cold crystalloid St Thomas' cardioplegia supplemented with erythropoietin (5000 units per L) and glyceryl trinitrate (100 mg/L) was delivered via the aortic root at a pressure of 150 mm Hg. The heart was vented by cutting across the left atrial appendage and the inferior vena cava at the pericardial reflection.

After delivery of both cardioplegia and pneumoplegia, the heart was immediately explanted with transection at the mid-aortic arch, across the pulmonary artery at its bifurcation, across the superior vena cava at its confluence with the innominate vein, and leaving behind sufficient left atrial tissue with the pulmonary veins as required for bilateral lung transplantation. In all three patients, the liver, both lungs, and kidneys were also retrieved for transplantation.

Ex-vivo preservation

The donor hearts were attached to the Organ Care System after cannulation of the aorta and pulmonary artery. The Organ Care System circuit prime was made up by mixing 1·5 L of donor blood that had been passed through a leucocyte filter (Pall LeukoGuard BC2; Pall Corporation, Port Washington, NY, USA) with 500 mL of TransmedicsR priming solution containing buffered electrolytes, mannitol, vitamins, and steroids. A TransmedicsR proprietary maintenance solution (1 L) containing isotonic electrolytes, aminoacids, dextrose-insulin, and low-dose adenosine was infused at a rate of 0–30 mL/h during ex-vivo perfusion to keep coronary flow within an acceptable range of 650–900 mL/min. Two of the three hearts needed 5 J direct current cardioversion for initial ventricular fibrillation on reperfusion. The rhythm subsequently converted to sinus bradycardia requiring pacing with biventricular epicardial pacing wires for two patients. The third heart started beating spontaneously in sinus rhythm and did not require pacing.

A vent was placed in all hearts via the left atrium to decompress the left ventricle, and then the superior vena cava and inferior vena cava were both closed. The heart was positioned so that oxygenated blood directly entered the ascending aorta in a retrograde manner and then necessarily down the coronary arteries. Blood then returns to the right side of the heart and is diverted up a cannula placed in the pulmonary artery before draining into the circuit reservoir. The apparatus principally uses aortic pressure, coronary flow, and arteriovenous lactate concentrations to assess cardiac function, with a lower venous concentration indicating lactate uptake and therefore satisfactory myocardial function. An infusion of low-dose adenosine, another infusion containing adrenaline, and adjustable circuit pump flow were used to control coronary vascular resistance and heart rate to keep parameters within the following ranges: aortic pressure 65–90 mm Hg; coronary flow 650–900 mL/min; heart rate 65–100 beats per min. Simultaneous sampling from the coronary inflow and coronary effluent ports on the perfusion circuit was done at regular intervals to measure myocardial lactate extraction. Lactate concentrations in the perfusate were measured with an automated iSTAT analyser (Abbott; Princeton, NJ, USA) according to the manufacturer's instructions. A total concentration of lactate of less than 5 mmol/L in the perfusate combined with myocardial lactate extraction (coronary inflow lactate>coronary effluent lactate) was considered evidence of myocardial viability.[19]

Role of the funding source

None of the funders had any role in data collection, analysis, or interpretation, writing of the report, or in the decision to submit for publication. KKD, AI, MC, HCC, CS, AD, EG, PJ, PS, and PM had access to all the data. KKD, AI, MC, HCC, EG, PJ, AJ, AK, CH, RG, PS, and PM were responsible for decision to submit for publication.

Results

Cessation of circulation occurred in less than 20 min in all three patients and the start of cardioplegia delivery took another 3–6 minutes (table 2). Attachment of the heart to the Organ Care System took an additional 23–28 min, as a result of the additional time needed to deliver pneumoplegia for procurement of lungs.

For patient 1, closure of both the inferior vena cava and the superior vena cava led to an immediate distension of the heart, particularly the right side. The

	Donor 1	Donor 2	Donor 3
Withdrawal parameters			
Location of withdrawal	Operating theatre	Intensive care unit	Anaesthetic bay
Withdrawal to systolic blood pressure <50 mm Hg (min)	7	5	11
Withdrawal to SaO_2 <50% (min)	8	2	1
Withdrawal to cessation of circulation (min)	16	10	11
Observation period (min)	2	2	5
Warm ischaemic time (min)*	28	25	22
OCS parameters			
Pacing	Yes	Yes	No
Adrenaline infusion (μg/h)	5	5	5–7
Adenosine infusion (mg/h)	0–21	0–21	0–21
Total OCS perfusion time (min)	257	260	245
Total ischaemic time (min)†	90	96	107
A-V lactate at start of perfusion (mmol/L)	8·30–8·10	6·79–6·48	7·60–7·40
A-V lactate at end of perfusion (mmol/L)	3·60–3·60	2·80–2·30	2·69–2·54

OCS=Organ Care System. A-V=arteriovenous. *Time from withdrawal of support to cardioplegia delivery. †Composite of the time from cessation of circulation to instrumentation on the OCS apparatus plus the time from cardioplegia delivery at the end of OCS perfusion to post-transplant reperfusion.

Table 2: **Donor heart management**

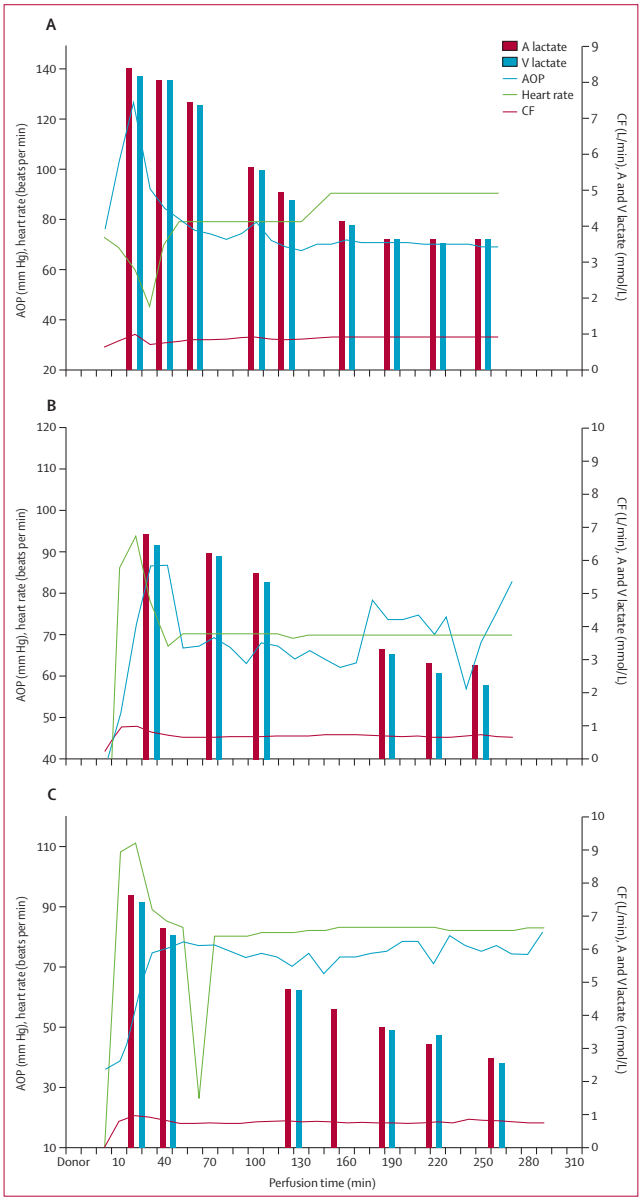

Figure 1: **Aortic pressue, heart rate, coronary flow, and lactate concentrations during ex-vivo perfusion**
(A) Donor 1, (B) donor 2, (C) donor 3. A lactate=arterial lactate. V lactate=venous lactate. AOP=aortic pressure. CF=coronary flow.

superior vena cava tie was immediately removed, allowing right ventricle decompression and the heart paced through direct epicardial pacing leads. Figure 1 shows aortic pressure, coronary blood flow, heart rate, and lactate values for both arterial and venous samples for the three patients. Despite the favourable downward trend in serum lactate concentrations, the right ventricle continued to show substantial dyskinesia for the first 2 h. During this time, the heart was transported by road to the recipient hospital. Thereafter, right ventricle function improved greatly and both coronary blood flow and mean aortic pressure remained constant and in the prescribed range. The difference in arteriovenous lactate improved further and remained stable at less than 5 mmol/L. An analysis of the data logged during machine perfusion of the first heart suggested that there was an acute and inadvertent rise in pump flow during the initial phase of machine perfusion that was likely to be iatrogenic. We were cautious during manipulation of pump flow in the subsequent two transplantations and we did not see similar right ventricle dysfunction after initial attachment to the Organ Care System.

The second and third hearts were retrieved at a greater distance by air and had excellent perfusion parameters and absorbing lactate values. The decision to proceed with transplantation was made only once the perfusion and lactate profiles met Organ Care System parameters. Only then were the recipients anaesthetised and placed on cardiopulmonary bypass. Ex-vivo perfusion was turned off, supplemented cold St Thomas' cardioplegia delivered to the donor heart with prompt electromechanical arrest, and the heart taken off the Organ Care System apparatus for implantation.

A fourth donor was a 35-year-old woman who had been in a motor vehicle accident. The time from withdrawal of ventilator to cessation of circulation was 35 min and the total warm ischaemic time was 49 min, which exceeded our limit. The heart was therefore excluded from the heart transplant pathway and implemented on the Organ Care System apparatus for a research protocol for which there was prior consent. The initial arteriovenous lactate concentrations were 9·6–9·8 mmol/L and eventually increased to 11·0–11·0 mmol/L with substantial dyskinesia and poor cardiac contractility.

After completion of left atrium anastomosis, cold blood cardioplegia was administered via the aortic root. The pulmonary artery and aortic anastomoses were then completed and followed by warm blood hyperkalaemic reperfusion before removal of the recipient cross-clamp and start of cardiac reperfusion on cardiopulmonary bypass. The inferior vena cava and superior vena cava anastomoses were done with the heart beating in two patients with bi-caval connections. The third case involved a bi-atrial anastomosis. In this case, the right atrial anastomosis was also done on the beating heart. The total ischaemic times for the three patients were

90 min, 96 min, and 107 min and consisted of the time from cessation of circulation in the donor to attachment to the Organ Care System, plus time from cardioplegic arrest of the donor heart on the Organ Care System to cardiac reperfusion in the recipient.

The hearts were then reperfused on cardiopulmonary bypass for 20 min for each hour of ischaemia. After weaning from cardiopulmonary bypass, both visually and on trans-oesophageal echocardiography, the right ventricle of the first recipient showed only mild impairment whereas the left ventricle was severely impaired. The recipient was then placed on venoarterial femoro-femoral extra-corporeal membrane oxygenation. An intra-aortic balloon was also placed percutaneously. Thereafter, biventricular function improved daily with removal of intra-aortic balloon 24 h later and decannulation of extra-corporeal membrane oxygenation on day 4. The third patient had a similar Takotsubo-type cardiomyopathy affecting the left ventricle and required an intra-aortic balloon for weaning off cardiopulmonary bypass, which was subsequently removed on day 2. Right ventricle function was good throughout. The second heart recipient was weaned off cardiopulmonary bypass with ease and needed only small doses of inotropic support. Peri-operative trans-oesophageal echocardiogram showed excellent biventricular function.

Patient 1 was discharged on day 26 and remained well with no evidence of ischaemic injury in any endomyocardial biopsy (figure 2). 105 days after surgery, she had mildly diminished left ventricle contractility coinciding with moderate cellular rejection. She was briefly admitted for steroid-pulse treatment and then returned home where she remains well, with normal left ventricular function at 176 days after transplantation.

The second recipient has had normal biventricular function on all echocardiograms. He also developed moderate cellular rejection on endomyocardial biopsy at day 20 but this was not associated with any reduction in left ventricle function. The biopsies showed no evidence of ischaemic injury on histological examination. He was discharged 28 days after surgery and remains well at 91 days after transplantation.

The third recipient has had hyperdynamic biventricular function since 2 days after surgery, when the intra-aortic balloon was removed. His endomyocardial biopsies have also been negative for ischaemic injury and, to date, there has been no evidence of cellular rejection. His planned discharge on day 13 was postponed because of a moderate pericardial effusion, which was drained without complication. He was discharged on day 21 and remains well 77 days after transplantation.

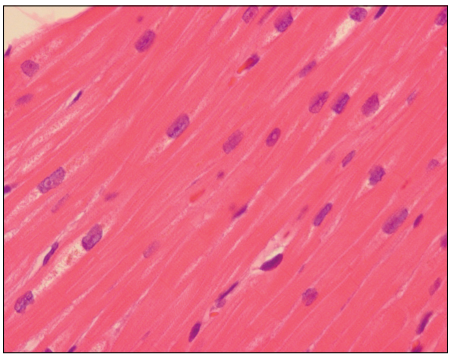

Figure 2: **Endomyocardial biopsy sample from patient 1 on day 8**
Shows normal myocardial architecture and no evidence of ischaemia reperfusion injury or rejection. Haematoxylin and eosin stain. Magnification ×400.

Discussion

To our knowledge, this report describes the first successful clinical heart transplantations after circulatory death with donor organs procured at a distance necessitating reanimation, resuscitation, and transportation with use of an ex-vivo cardiac perfusion device (panel). Of the strategies to slow the growing discrepancy between the number of patients awaiting transplantation and the scarcity of suitable donors, the use of organs donated after circulatory death has been successful for lung and intra-abdominal organ transplants. Strong endorsements of protocols for such transplants by national and international regulatory bodies have led to wider adoption of this strategy, with organs donated after circulatory death contributing an increasing percentage of the total number of donors, especially in Australia, Belgium, Netherlands, Spain, UK, and USA.[3]

The results of kidney transplantation are much the same for kidneys donated after circulatory death and those from brain-dead donors, with similar long-term survival despite a higher incidence of delayed graft function in patients given kidneys from donors after circulatory death.[4] Outcomes of liver transplantation from donors after circulatory death have been poorer, with more frequent biliary strictures and primary graft failures ascribed to a greater sensitivity to warm ischaemia.[6] Nevertheless, other studies[20] report similar outcomes for livers donated after circulatory death and those donated after brain death.

Promising results have been reported for lung transplantation after circulatory death. The International Society of Heart & Lung Transplantation DCD Registry has shown similar outcomes at 1 year between lung transplants donated after brain death and those donated after circulatory death.[21] Data from a multicentre Australian study[5] showed survival of 97% at 1 year and 90% at 5 years for patients given lungs donated after circulatory death compared with 90% at 1 year and 61% at 5 years for patients given lungs donated after brain death.

Several large studies of animals[9–13] and a series of studies of ex-vivo human heart resuscitation,[8,14,16,22] have shown the feasibility of using hearts donated after

Panel: **Research in context**

Systematic review

We searched PubMed with the terms "donation after circulatory death", "donation after cardiac death", "non heart beating donation", "ex vivo perfusion", and "heart transplantation" to find relevant articles language in any language published up to Aug 14, 2014. We searched for both animal and human studies of donor hearts from a non-brain-dead but deceased donors. Several studies describe satisfactory ex-vivo reanimation of animal[9–13] and human[14,15] hearts donated after circulatory death. Heart transplantations done in the late 1960s[1] and a report[7] of three neonatal heart transplantations after circulatory death in 2008, both refer to colocated donors and recipients. There were no reports of clinical heart transplantation with distantly procured hearts from donors after circulatory death.

Interpretation

To our knowledge, this report describes the first successful clinical heart transplantations after circulatory death with donor organs procured at a distance necessitating reanimation, resuscitation, and transportation with use of an ex-vivo cardiac perfusion device. Our findings confirm that human hearts donated after circulatory death can be adequately preserved and their function assessed in a physiological ex-vivo platform before safe clinical transplantation with excellent outcome. A broader adoption of this strategy would lead to a significant increase in the number of heart transplantations and limit the loss of patients on transplantation waiting lists as well as potentially reducing the number of patients requiring urgent palliative bridging strategies.

circulatory death for clinical transplantation. In a study of donation after circulatory death in pigs,[9,23] we have shown that pharmacological post-transplantation conditioning, achieved by supplementing cardioplegia with erythropoietin, glyceryl trinitrate, and zoniporide, increased the tolerance of the heart to warm ischaemia. Moreover, the commercially available portable ex-vivo heart Organ Care System has made it possible to maintain physiological perfusion of a donor organ during distant organ procurement.[10,24] The device has been used for both resuscitation and assessment of marginal hearts donated after circulatory death for transplantation[17] as well as in research assessing functional recovery of unused human hearts donated after circulatory death.[15]

Although several groups are developing more robust means to assess ex-vivo myocardial function, in this study we had the ideal donor–recipient match, in which the donor hearts' metabolisms improved sufficiently with ex-vivo perfusion to warrant a clinical transplantation. The delayed graft function in our first patient might be analogous to that reported for kidneys transplanted from donors after circulatory death.[25] In this regard, a strategy of prophylactic extracorporeal membrane oxygenation support, which is safe and effective,[26] might enable hearts donated after circulatory death to recover. Roughly 17% more transplantations could be done by use of hearts donated after circulatory death.[27–29]

Our findings could fuel further ethics debates, particularly with respect to the definition of death; the conflict between death of a donor and death of individual organs; the acceptable length of observation from cessation of circulation to declaration of death; and the variable acceptance for a range of pre-mortem interventions aimed at safeguarding organ function at the cost of inconvenience to the donor. Donation of organs after circulatory death is a well-established practice. In this respect, we believe that the heart is no different to the lungs, liver, or kidneys. All these organs remain viable for a short time after permanent cessation of circulation. In developing a heart transplantation programme from donors after circulatory death, we adhered to established jurisdictional criteria and processes for determination of circulatory death and subsequent removal of organs for transplantation. In addition to the necessity of further refining strategies to counteract the effects of warm ischaemia, and improving the technical aspects of ex-vivo heart preservation and assessment, we believe it is time to move this debate towards development and implementation of broader international consensus guidelines.

Expansion of heart transplantation from donors after circulatory death would help to reverse the trend of fewer organs per donor after circulatory death compared with brain-dead donors. It would also enable doctors to better honour the wishes of donors and their relatives to maximise opportunities for organ transplantation, and clinicians' professional responsibility to reduce the time spent on transplantation waiting lists caused by the shortage of suitable donor hearts.

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CHAPTER 6 – PUBLICATION 5

TITLE: Pathophysiological trends during withdrawal of life support: implications for organ donation after circulatory death (DCD)

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Declaration

I certify that this publication was a direct result of my research towards this PhD, and that reproduction in this thesis does not breach copyright relations.

Foreword

The establishment of a clinical DCD heart program confirms the viability of these hearts and their place in cardiac transplantation. The next step is focused on improving organ resuscitation and enhancing ex vivo perfusion care. This can only be done by better understanding the pathophysiological changes that occur during the warm ischaemic period and therefore a better assessment of the insult that these hearts are exposed to.

The haemodynamic and electrical changes during the agonal phase allows insight into the death process and thereby its diagnosis. The rapidity of the development of metabolic derangements are noteworthy and may offer clues to any permitted ante-mortem interventions that may blunt these dramatic changes. Biochemical changes in vivo are relevant to ex vivo donor blood perfusion preservation—changes such as a profound hyperkalaemia may expose the allograft to further insults during EVP.

Catecholamine surges have been typical of brain death donors with their impact well studied. However there has been little demonstrated about the catecholamine release in DCD donors. In addition, there is little knowledge of the source of any catecholamine surge in the DCD patient. Whilst the argument has been that the surge is of little significance in the setting of a deteriorating circulation, very little is known about their levels in the heart. This is investigated in these studies with systemic and coronary sinus measures of catecholamine levels.

It is felt that the study of these haemodynamic, metabolic, biochemical and hormonal changes during the agonal period provides a better understanding of the insults that the heart is exposed to and offers clues to better resuscitation. Furthermore, defining these derangements encourages debate about the ideal perfusate for EVP, whether donor blood or otherwise.

Original Basic Science—General

Pathophysiological Trends During Withdrawal of Life Support: Implications for Organ Donation After Circulatory Death

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Background. Donation after circulatory death (DCD) provides an alternative pathway to deceased organ transplantation. Although clinical DCD lung, liver, and kidney transplantation are well established, transplantation of hearts retrieved from DCD donors has reached clinical translation only recently. Progress has been limited by concern regarding the viability of DCD hearts. The aim of this study was to document the pathophysiological changes that occur in the heart and circulation during withdrawal of life (WLS) support. **Methods.** In a porcine asphyxia model, we characterized the hemodynamic, volumetric, metabolic, biochemical, and endocrine changes after WLS for up to 40 minutes. Times to circulatory arrest and electrical asystole were recorded. **Results.** After WLS, there was rapid onset of profound hypoxemia resulting in acute pulmonary hypertension and right ventricular distension. Concurrently, progressive systemic hypotension occurred with a fall in left atrial pressure and little change in left ventricular volume. Mean times to circulatory arrest and electrical asystole were 8 ± 1 and 16 ± 2 minutes, respectively. Hemodynamic changes were accompanied by a rapid fall in pH, and rise in blood lactate, troponin-T, and potassium. Plasma noradrenaline and adrenaline levels rose rapidly with dramatic increases in coronary sinus levels indicative of myocardial release. **Conclusions.** These findings provide insight into the nature and tempo of the damaging events that occur in the heart and in particular the right ventricle during WLS, and give an indication of the limited timeframe for the implementation of potential postmortem interventions that could be applied to improve organ viability.

(*Transplantation* 2016;100: 2621–2629)

A significant disparity exists between the demand for heart transplantation and the availability of suitable donor organs, resulting in substantial mortality of patients on waiting lists around the world.[1] Although the utilization of lungs from donation after circulatory death (DCD) donors has changed the landscape of lung transplantation in Australia since 2006,[2] the utilization of hearts from such donors has rarely been attempted.[3,4] Concerns regarding myocardial damage during the obligatory warm ischemic time (WIT) that accompanies withdrawal of life support (WLS)

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A.I. conducted all experiments in series 1, conducted initial analysis, and wrote initial draft. H.C.C. conducted all experiments in series 2, conducted initial analysis of these experiments and wrote revised draft. L.G. provided anesthesia for all experiments, provided critical review of analyses and article. J.V. assisted in conduct of experiments in series 2, provided critical review of analyses and article. M.H. assisted in study design, conduct of experiments, provided critical review of analyses and article. A.D. assisted in conduct of series 1 experiments, provided critical review of analyses and experiments. G.K. assisted in study design, conduct of experiments, provided critical review of analyses and article. A.J. assisted in study design, conduct of experiments, provided critical review of analyses and article. P.C.J. assisted in study design and provided critical review of analyses and article. M.P.F. assisted in study design and provided critical review of analyses and article. R.P.H. assisted in study design and provided critical review of analyses and article. R.M.G. assisted in study design and provided critical review of analyses and article. K.K.D. assisted in study design and provided critical review of analyses and article. P.S.M. developed the study protocol, oversaw the conduct of the experiments and wrote the final draft.

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and the inability to assess viability of cold-stored hearts has limited clinical translation.

Several preclinical studies and recent clinical reports have reported viability and successful orthotopic transplantation of DCD hearts[4-8]; however, questions remain regarding the maximal tolerable WIT and the ideal preservation strategy. Cessation of ventilatory support at withdrawal in the setting of poor respiratory drive results in profound hypoxemia. Although continued exposure to alveolar air limits hypoxic injury to DCD lungs,[9,10] organs reliant on oxygenated blood perfusion, such as the heart, lack this advantage. In addition, high aerobic dependence of the heart renders it particularly vulnerable to ischemic injury.[11]

Another potential cardiac insult after WLS is ventricular distension. With decreasing ejection and increasing end-diastolic volumes, the heart is exposed to progressively higher intraventricular pressures. Detrimental effects of ventricular distension have been reported in cardiac surgery[12] and during the DCD withdrawal period.[13,14]

Now that heart transplantation from DCD donors is a clinical reality, an important goal of ongoing research is to improve the tolerability of the DCD heart to warm ischemia. With this goal in mind, the aim of the present study was to provide a detailed description of the pathophysiological changes after WLS in a porcine DCD model. To this end, central hemodynamic and ventricular volumetric changes, blood gas derangements, as well as myocardial catecholamine release were assessed over a range of WITs.

MATERIALS AND METHODS

Details of the animals used and study protocol have been reported previously.[7] Juvenile *Landrace* pigs were used, with experimental protocols approved by the Garvan/St Vincent's Animal Ethics Committee. All animals were cared for according to the standards outlined in the Australian Code for the Care and Use of Animals for Scientific Purposes 8th edition (2013) and overseen by the Garvan/St. Vincent's Hospital Animal Ethics Committee.

Animals and Anesthesia

Forty-two pigs weighing 55 to 65 kg were used. Animals were premedicated with intramuscular ketamine (10 mg/kg), midazolam (1 mg/kg), and atropine (50 μg/kg). Animals were intubated using cuffed 7 to 7.5 mm endotracheal tubes, with anaesthesia induced/maintained using inhalational isoflurane (2-5%) and intravenous fentanyl (100-200 μg bolus).

Ventilation was maintained with a tidal volume of 10 mL/kg, positive end-expiratory pressure of 5-7 mm Hg.

Mean arterial pressure (MAP), central venous pressure (CVP), pulse oximetry and electrocardiogram were monitored. Arterial blood gases were analyzed regularly, including presurgical baselines.

Surgical Procedure and DCD Protocol

The right internal carotid artery (RICA; 5Fr) and right internal jugular vein (RIJV; 8Fr) were cannulated for arterial and CVP monitoring. A median sternotomy was performed, and the pericardium opened.

Series 1

An asphyxia model was used to mimic the clinical setting. Heparin (300 mg/kg) was administered intravenously before WLS. After bolus dose of midazolam (10 mg) and fentanyl (100 μg) to ensure sedation and analgesia, ventilation was ceased and endotracheal tube disconnected. Absence of spontaneous breathing was confirmed. Times to circulatory arrest and electrical asystole were recorded; however, hearts were left untouched for predetermined periods of warm ischemia (between 20 and 40 minutes), irrespective of electrical activity/circulatory cessation times.

Warm ischemia was defined as the period between withdrawal of ventilator support and administration of preservation solution. Circulatory arrest was defined as the time when MAP matched CVP. The groups were assigned as outlined in Table 1.

Immediately before the end of predetermined WIT, cannulae were inserted into right atrium (for blood collection) and aortic root (for administration of preservation solution). Blood (~1.2 L) was drained just before aortic cross-clamping and administration of Celsior preservation solution, (Genzyme, Cambridge, MA; 1 L precooled to 4°C), with the heart vented via incisions in the left atrial appendage and inferior vena cava, and the chest cavity filled with cold slush.

These experiments were performed to assess recovery of DCD hearts exposed to varying WITs on an ex vivo perfusion (EVP) circuit as reported previously.[7] Donor animal blood was used as perfusate, with 2 separate methods of blood collection assessed. Using 40Fr single stage venous cannula in the right atrium, blood was drained into a collection bag with roller pump assistance in 1 group and by simple gravity drainage in the second group—gravity-assisted blood collection was performed in a separate group of 6 animals exposed to 30-minute WIT (in addition to the 12 × 30 minutes WIT animals reported in Table 1).

Outcome Measures

Hemodynamic changes, biochemical alterations, and hormonal responses were assessed at baseline and during WLS. Blood analysis was performed in all animals at 3 set timepoints: prewithdrawal, 5 min postwithdrawal, and immediately postelectrical asystole. Further sampling times were based on the predetermined WIT, and not all parameters were measured at all timepoints. The number of observations at each timepoint varied according to the time to electrical asystole and the subsequent WIT.

Hemodynamic Changes

Mean arterial pressure and CVP and oxygen saturations (left ear pulse oximeter) were measured at presurgical, prewithdrawal, and at set timepoints during the withdrawal period (n = 24; saturation measures are reported in 22/24, because 2 experiments had technical issues with the probe).

TABLE 1.

Groups exposed to varying predetermined warm ischaemic times, irrespective of time to circulatory arrest

Warm ischemic time, min	Number	Male-female
20	6	3:3
30	12	7:5
40	6	4:2

Animals of both sexes were used with numbers listed above.

Blood Gas Changes

Blood gases were analysed using the iSTAT "point of care" handheld analyzer with EG-6 cartridges (Abbott Inc, Princeton, NJ). This provided additional measures of the partial pressures of O_2 and CO_2, oxygen saturations, and pH (n = 6-24).

Biochemical Changes

Troponin-T was measured in arterial blood at presurgical, prewithdrawal, and regular postwithdrawal timepoints (n = 5-20) using chemiluminescence immunoassay (Roche E170 immunoassay). Potassium, bicarbonate, and lactate concentrations were measured in arterial blood at corresponding timepoints using the iSTAT analyzer (n = 5-24). In addition, potassium levels in withdrawn blood were compared between the 2 blood collection methods (n = 6-18).

Catecholamine Levels

Whole blood samples were taken simultaneously from the RICA and a nonoccluding coronary sinus catheter for measurement and comparison of systemic and local myocardial adrenaline and noradrenaline release (n = 5). Catecholamine levels were measured using a Shimadzu HPLC-ECD Antec-Leydon detector.

Series 2

An additional 12 experiments were performed to more closely investigate the central hemodynamic and ventricular volumetric changes during the first 10 minutes of the withdrawal period. In this set of experiments, no antemortem heparin was administered. In addition to the previously mentioned invasive monitoring methods, a Swan-Ganz catheter was introduced into the RIJV for pulmonary artery pressure (PAP) measurement. After sternotomy, the left atrium was cannulated to allow continuous measurement of left atrial pressure (LAP). Sonometric crystals were used to measure volumetric changes in the ventricles during withdrawal (left ventricle n = 6; right ventricle n = 6). Five crystals were attached to the epicardium of a single ventricle ((1) apical, (2) basal, (3) anterior wall, (4) posterior wall, (5) septal wall), and a Millar pressure catheter was inserted into the corresponding ventricle.

Hemodynamic and Volume Changes

Mean arterial pressure, CVP, PAP, LAP, left or right ventricular volume, and intracavitary pressure were recorded at baseline and from the beginning of WLS until asystole. We used SonoSOFT 3.1.3 Software (Sonometrics Corp., London, ON) to acquire and analyze the data files as described previously.[15]

Statistical Methods

Statistical analyses were performed using Prism 6.0b (GraphPad Software, Inc, La Jolla, CA). Normally, distributed data are expressed as mean ± SE. Nonparametric data are expressed as median values. Differences between groups were determined using 1- or 2-way analysis of variance depending on the number of factors assessed; followed by post hoc analysis using Tukey test to correct for multiple comparisons. Nonparametric data were compared using the Mann-Whitney test. A *P* value less than 0.05 was considered significant.

RESULTS

Series 1

Haemodynamic changes

After WLS, there was an immediate and progressive fall in systemic arterial pressure. Central venous pressure rose within the first 5 minutes after withdrawal. Mean time from withdrawal to circulatory cessation (MAP = CVP) was 8 ± 1 min (Figure 1A). During the first 2 to 3 minutes after WLS, there was a small increase in heart rate. Thereafter, heart rate fell progressively (Figure 1B). Time to electrical asystole was significantly longer than time to cessation of circulation occurring at a mean of 16 ± 2 minutes post-withdrawal with 6 animals demonstrating electrical activity beyond 25 minutes (Figure 1C).

Blood Gas and pH Changes

Oxygen saturation was measured invasively (SO_2-RICA) and noninvasively (SaO_2-ear oximetry). SaO_2 fell rapidly from 100% to below 30% by 2 minutes postwithdrawal (Figure 2A). SO_2 confirmed similar findings, with SO_2 falling to 12% ± 1% at 5 minutes postwithdrawal. The partial pressure of oxygen fell rapidly to 14 ± 1 mm Hg by 5 minutes post-withdrawal, whereas carbon dioxide increased to 84 ± 3 mm Hg

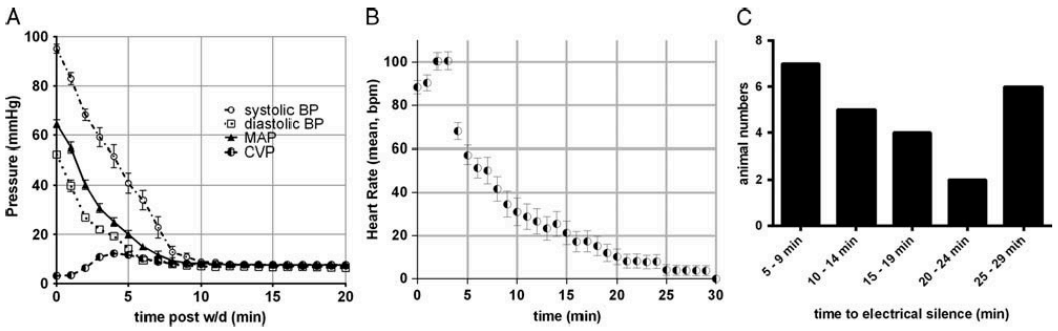

FIGURE 1. A, Systolic and diastolic BP, MAP, and CVP during withdrawal period (n = 24) expressed as mean ± SE. B, Mean heart rate during 30 minutes postwithdrawal, displayed as bpm (n = 24) expressed as mean ± SE; (c) of the 24 hearts evaluated, time to electrical silence (asystole) reported as number of animals during each time interval. bpm, beats per minute.

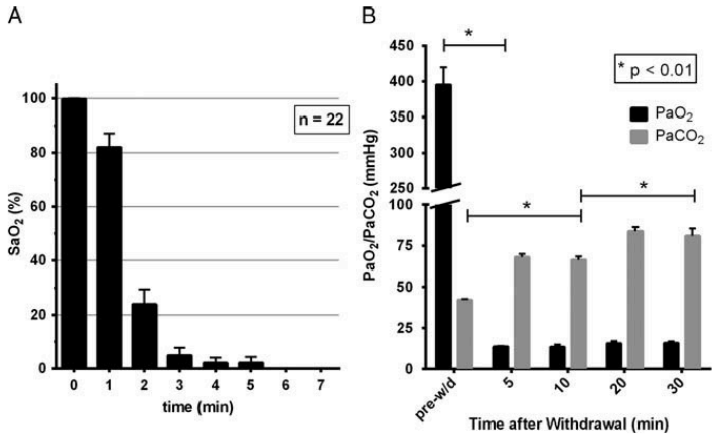

FIGURE 2. A, Noninvasive oxygen saturations—SaO_2 readings of ear probe; n = 22 (of total n = 24, 2 experiments encountered technical issues with the saturation probe); graph demonstrates the rapid onset of hypoxia in the presence of asphyxia; expressed as mean ± SE. B, PaO_2 and $PaCO_2$ was measured over the 30 min WIT period (n = 6-24) expressed as mean ± SE. PaO_2, partial pressure of oxygen; $PaCO_2$, carbon dioxide.

(Figure 2B). Profound acidosis developed during the WIT-pH (Figure 3A) decreased to 7.17 ± 0.01. A progressive lactic acidosis was evident with increasing WIT-blood lactate levels peaked at 13 ± 2 mmol/L at 40 minutes postwithdrawal (Figure 3B).

Biochemical Changes

Blood troponin-T levels rose progressively during the WIT (Figure 4), peaking at 202 ± 26 ng/L at 40 minutes postwithdrawal. Blood potassium concentrations [K+] increased from a baseline level of 3.9 ± 0.3 mmol/L to 5.6 ± 0.1 mmol/L by 5 minutes postwithdrawal. Thereafter, no further significant increases were noted during in situ sampling (Figure 5A).

[K+] of samples from blood collected (for EVP) using a roller pump are displayed in Figure 5B. [K+] of collected blood rose dramatically with increasing WIT before blood collection (Figure 5B). When the same quantity of blood was collected using gravity drainage after 30 minutes WIT, there was only a small nonsignificant increase in the [K+] in the collected blood compared with the in situ [K+] after 30 minutes WIT (in situ [K+] after 30 minutes WIT 5.9 ± 0.2 mmol/L versus [K+] in blood collected with gravity assistance 6.3 ± 0.5 mmol/L, p = ns). In contrast, [K+] in blood collected with roller pump assistance after 30 minutes WIT was markedly elevated reaching 10.5 ± 0.7 mmol/L ($P < 0.001$ vs in situ [K+] or [K+] in blood collected by gravity drainage; Figure 5C).

Catecholamine Levels

Simultaneous measures of adrenaline and noradrenaline from carotid artery and coronary sinus catheters revealed elevations of both catecholamines postwithdrawal (Figure 6; n = 5). There was a significant increase in both amines at both locations at 4 minutes postwithdrawal compared with baseline. At 20 minutes, there was further increase of both amines in blood sampled from the coronary sinus but not from the carotid artery.

Series 2

Baseline pressure measurements in all animals were MAP, 65 ± 10 mm Hg; PAP, 17 ± 3 mm Hg; CVP, 6 ± 2 mm Hg; LAP, 8 ± 1 mm Hg. After cessation of ventilation, a progressive decrease in MAP was noted as in series 1. In contrast to

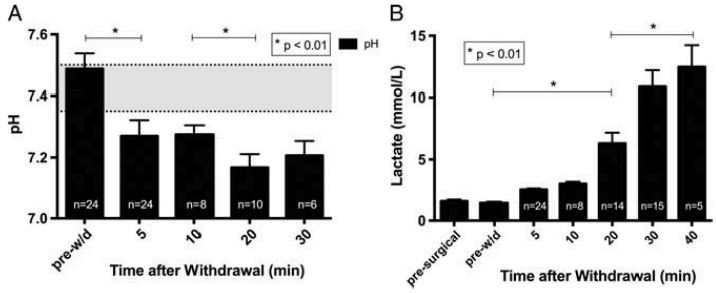

FIGURE 3. A, pH levels during the withdrawal period. The bar on the left indicates normal pH before withdrawal, and the shaded territory indicates the normal pH range of 7.35 to 7.5 (n = 6-24) expressed as mean ± SE. B, Mean lactate levels at various time-points prior to and after withdrawal. Lactate levels in mmol/L (n = 5-24) expressed as mean ± SE.

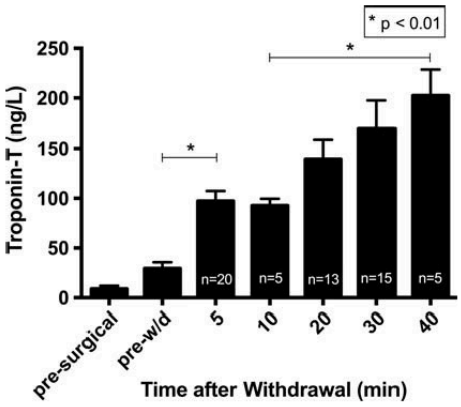

FIGURE 4. Troponin T levels at timepoints before and after withdrawal. Troponin measured as ng/L (n = 5-20) expressed as mean ± SE.

the progressive decline in MAP, there was an initial rise in CVP and PAP (to 12 ± 3 mm Hg; and 21 ± 3 mm Hg, respectively, both $P < 0.05$ compared with baseline). Simultaneously with the rise in CVP and PAP, LAP fell reaching a nadir of 6 ± 3 mm Hg ($P < 0.05$ vs baseline). With progression to mechanical asystole, all pressures eventually equalized (PAP, 8 ± 1 mm Hg; CVP, 8 ± 1 mm Hg; LAP, 8 ± 2 mm Hg) (Figure 7).

Volume Changes

Representative pressure volume loops for the right and left ventricles during the first 5 minutes after WLS are shown in Figure 8. In the right ventricle, the end-diastolic pressure volume point was displaced upward and to the right, whereas the end-diastolic pressure volume point for the left ventricle was displaced downward and to the left. Mean baseline right and left ventricular volumes were 162 ± 30 mL and 159 ± 52 mL, respectively. Measurements were calculated at minute intervals until equalization of central hemodynamic pressures. After WLS, right ventricle end-diastolic volume increased by 23% to 199 ± 57 mL ($P = 0.083$ vs baseline, 1-sided P value) and left ventricle end-diastolic volume increased by 4% to 169 ± 49 mL (Figure 9).

DISCUSSION

This study demonstrates in a porcine asphyxia model that the absence of respiratory drive after WLS results in rapid onset of profound hypoxemia with progression to circulatory arrest within 10 minutes in most animals. Significantly longer times to electrical asystole when compared with circulatory arrest times were noted. No cases of autoresuscitation were observed after circulatory arrest. The presence of ongoing electrical activity in the absence of cardiac output has also been reported in human DCD donors.[16,17] Importantly, from a clinical standpoint, death is determined at the time of cessation of the circulation and not electrical asystole which can persist for many minutes after circulatory arrest.

A definition of functional warm ischemia as the interval between systemic arterial systolic pressure of less than 50 mm Hg and the administration of organ flush solution has been proposed for the lungs, liver, and kidneys of DCD donors.[10,18,19] A number of observations in our model suggest that the onset of myocardial ischemia in the DCD donor may occur earlier than the onset of ischemia for other organs. As shown in Figure 1, systolic blood pressure fell below 50 mm Hg within the first 5 minutes, at which time diastolic blood pressure was only 20 mm Hg. Given that the majority of coronary blood flow occurs during diastole, it is likely that the onset of coronary hypoperfusion and myocardial ischemia occurs well before systolic blood pressure reaches 50 mm Hg. Lung tissue, even in the absence of adequate perfusion, can maintain tissue ATP levels through alveolar oxygen exposure.[9] This differs from other organs including the heart where coronary perfusion remains the sole avenue of oxygen delivery. In our model, profound hypoxemia developed rapidly with the arterial oxygen saturation falling from 100% prewithdrawal to 30% within 2 minutes of cessation of ventilation, when systolic blood pressure was still above 50 mm Hg (Figures 1 and 3).

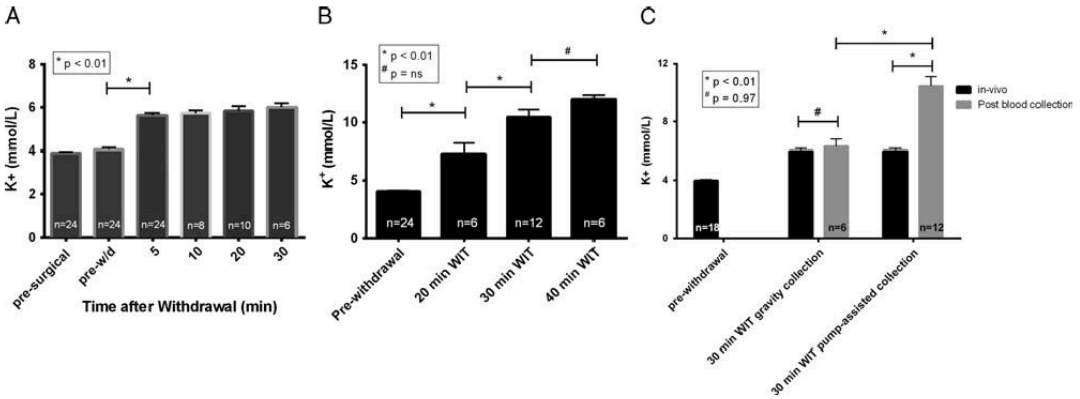

FIGURE 5. A, In vivo measurement of potassium levels before and after withdrawal of ventilator support. Potassium (K+) as mmol/L (n = 7-24) expressed as mean ± SE. B, Ex vivo measurement of potassium, sampled from pump assisted blood collected after varying WIT periods (20-40 min; n = 6-24. C, Ex vivo measurement of potassium, sampled from either gravity assisted (n = 6) or pump-assisted methods (n = 12) after the same 30 minutes WIT period. Both graphs display in vivo prewithdrawal potassium levels as control. Potassium (K+) as mmol/L; expressed as mean ± SE.

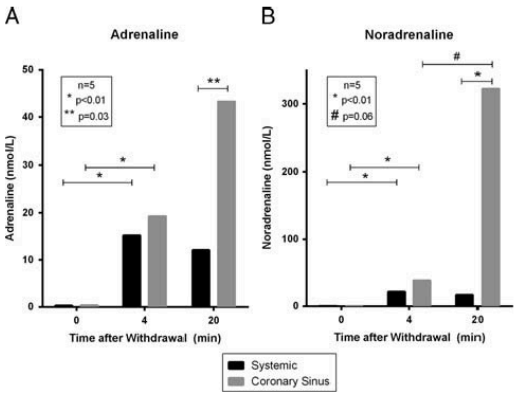

FIGURE 6. Median adrenaline (A) and noradrenaline (B) levels during warm ischaemia period postwithdrawal. Measured in nmol/L (n = 5). Differences between sampling sites and time points analyzed using nonparametric Mann-Whitney *U* test.

Along with the rapid desaturation, occurring within 2 minutes after withdrawal, we observed a significant increase in PAP and CVP and a significant fall in LAP. These changes are most likely due to hypoxemia-induced acute pulmonary vasoconstriction, resulting in increased right ventricular afterload and decreased left ventricular preload. Acute hypercapnia and acidosis or elevation of endothelin-1 may also contribute to the pulmonary vasoconstrictor response.[20,21] Although both ventricles are subjected to profound normothermic ischemia, these observations suggest that there is an additional insult to the right ventricle, resulting from the abrupt increase in pulmonary vascular impedance. The differential effect of WLS on the diastolic filling pressures in the right and left ventricles (as reflected by the CVP and LAP, respectively) also has implications for the rate of decline in coronary blood flow to each ventricle. As coronary perfusion pressure is largely determined by the difference between aortic diastolic perfusion pressure and intraventricular diastolic pressure, the more rapid loss of the perfusion gradient between aortic diastolic pressure and the right ventricular pressure, as approximated by the CVP (as compared with the pressure gradient between aortic diastolic pressure and the left ventricular pressure, as approximated by the LAP) suggests that right ventricular myocardial perfusion is compromised more rapidly than left ventricular perfusion.

Our findings complement those reported recently by White et al[14] in a similar porcine model of DCD donation. In that study, the authors inferred a pulmonary vasoconstrictor response based on changes in the left and right ventricular pressures after WLS, but they did not directly measure PAP or LAP. These authors also reported a 20% increase in the right ventricular end-diastolic volume during WLS with a small fall in left ventricular volume. Although the change in right ventricular volume observed in our study during WLS did not reach statistical significance, the magnitude of the change was identical to that reported by White et al.[14] Although hypoxia and hypoperfusion are obligatory insults in all DCD organs, the additional insult of distension is likely to impact negatively on recovery[13,14]—ventricular distension contributes to decreased diastolic coronary flow, subendocardial ischemia, overstretch of cardiac muscle, and damage to the endocardium.[22] Regardless of the mechanism, our observations suggest that the right ventricle suffers a more severe insult than the left ventricle during WLS in the DCD setting. Although the clinical implications of this finding remains uncertain, the additional insult to the right ventricle suggests that caution should be exercised in the selection of recipients for hearts obtained from DCD donors, avoiding recipients with fixed elevation of pulmonary vascular resistance. The greater right ventricular injury apparently mediated by pulmonary vasoconstriction raises the potential role of prophylactic pulmonary vasodilator therapy in management of WLS before recovery of DCD hearts. There is some experimental evidence for this with Kato et al[23] demonstrating in

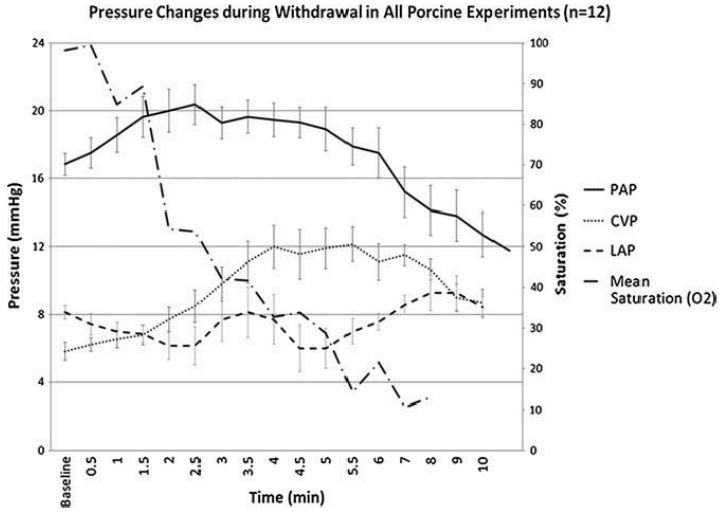

FIGURE 7. Central haemodynamic changes during withdrawal of life support (n = 12).

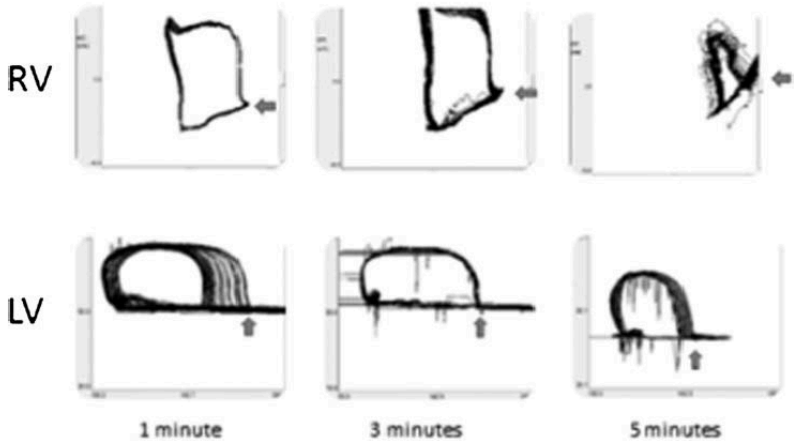

FIGURE 8. Representative pressure volume loops obtained from RV and LV during withdrawal of life support. RV, right ventricle; LV, left ventricle.

a canine model of DCD heart donation that intravenous administration of the endothelin antagonist, FR139317, 10 minutes before WLS, blunted the rise in PAP during WLS and was associated with better recovery of the donor heart after transplantation. As with other antemortem interventions, the administration of pulmonary vasodilator therapy before WLS would need careful ethical consideration and jurisdictional approval.

Cardiac myocyte death is an inevitable consequence of progressive hypoxia and acidosis.[24-26] Withdrawal of life leads to the rapid development of both these conditions with progressive lactic acidemia (>10 mmol/L) and hypercarbia (>80 mmHg) contributing to severe acidosis. The correlation between WIT and troponin rise observed in our study is in keeping with progressive myocardial damage with longer periods of exposure to the above insults. Acidosis during myocardial ischemia activates the sodium hydrogen exchanger, NHE1, which may contribute to myocardial dysfunction both during ischemia and upon reperfusion.[26] Administration of selective inhibitors of the NHE1 after the onset of ischemia but before the onset of reperfusion has been shown to reduce myocardial injury in experimental models of normothermic ischemia reperfusion injury including sustained cardiac arrest,[27] and for this reason, inhibition of NHE1 is an attractive therapeutic target for myocardial protection in DCD donors.[7,28]

Significant changes in potassium levels were also evident during WIT. Acidosis is the most likely cause for this rapid development. It is uncertain whether other factors, such as myocyte necrosis or red cell hemolysis, contribute to this early rise; however, it is noteworthy that no further increase in potassium was observed in vivo after circulatory arrest. Subsequent collection of donor blood from the right atrium using a large bore cannula with gravity assistance was associated with little change in the potassium concentration of collected blood. In contrast, the progressive increase in

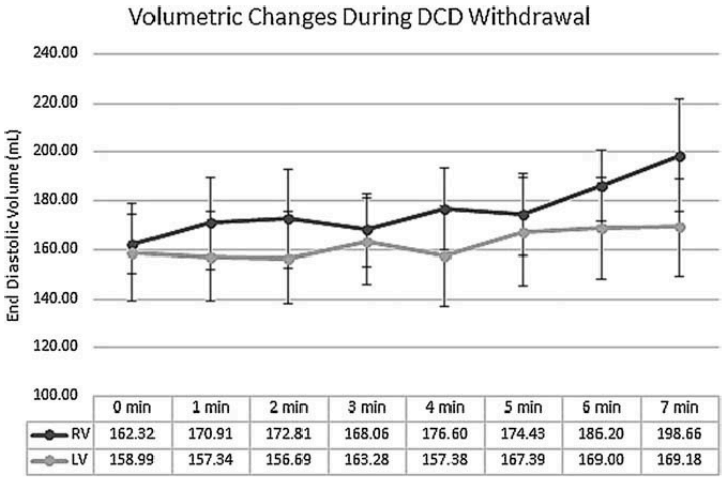

FIGURE 9. Changes in LV and RV volumes during withdrawal of life support (n = 12).

potassium concentration of blood collected using a roller pump suggests increased fragility of red cells with increasing WIT. Although plasma free hemoglobin was not measured, the dramatic further increase in blood potassium observed with roller pump-assisted blood collection is most likely explained by mechanical disruption of fragile red cells by the pump.

The catecholamine storm of brain dead donors has been associated with detrimental donor organ function.[15,29,30] It has been suggested that the absence of this in DCD donors may contribute to potentially superior outcomes in lung transplant recipients.[2,31] This hypothesis has been challenged however by findings from Ali et al[5] in a porcine model in which they observed similar or greater catecholamine release in DCD donors compared with brain stem death donors. The increase in heart rate observed within the first few minutes of WLS in our study and also reported by others may be explained by activation of the sympathetic nervous system in response to falling systemic blood pressure and hypoxemia as recently suggested by White et al.[14] Our observation of a higher coronary sinus level of noradrenaline and adrenaline (compared with systemic measures) at 4 and 20 minutes is consistent with dramatic release of myocardial catecholamines during and after WLS. These data suggest that the presence of a catecholamine surge in DCD donors, similar to that observed in brain dead donors, however the extent of catecholamine-induced injury to the heart and exposure of other organs to these damaging catecholamines in the setting of a rapidly deteriorating circulation is unclear.

If DCD hearts are to become a viable option for cardiac transplantation, EVP technology will likely play an important role in resuscitation of these organs. This is an active area of research with ongoing debate about the ideal perfusate.[5,6,8,32] A normothermic blood-based perfusate, allowing provision for oxygenation and therefore beating heart assessment, seems to be the leading contender.[6,32] Although donor blood is an easily accessible resource, the time required for its collection (up to 90 seconds in clinical cases reported to date),[4] introduces a further delay in the recovery of other organs. In addition, as shown in the present study, blood collected from DCD donors has marked abnormalities in its composition—it is markedly acidotic and hyperkalemic, with further potassium rise encountered when blood collection is undertaken using roller pump-assisted instead of gravity-assisted collection. Furthermore, increased levels of catecholamines in collected blood could also adversely affect cardiomyocyte survival and myocardial function.[5] These findings highlight the need for further research aimed at optimizing the composition of the perfusate used for EVP of DCD hearts to limit further organ damage.

Limitations

Several limitations must be noted. First, the donors were healthy adolescent animals that were anesthetized before WLS. In the clinical setting, most potential DCD donors have sustained critical neurological injuries and have been on life support for days before a decision to WLS. Moreover, the time to circulatory arrest after WLS is difficult to predict with only a minority of DCD donors progressing as rapidly as the porcine model used in our experiments.[33] Although the time to circulatory arrest reported in our study was similar to that reported by others using similar open-chested porcine models,[13,14,23] longer times to circulatory arrest have been reported by authors examining recovery of abdominal organs in porcine DCD models where the chest cavity has been left intact.[34,35] Second, the standoff period in series 1 was greater than would be allocated clinically. This was done to expose the donor hearts to set warm ischemic times, irrespective of circulatory cessation time. In addition, the use of isoflurane anaesthesia in our model and similar large animal DCD models reported by others[13,14,23,34,35] is another point of difference between the experimental laboratory and the clinical setting. A potential preconditioning effect of isoflurane anaesthesia in these animals before WLS cannot be excluded[36]; however, we think this is unlikely to have influenced the tempo of hemodynamic and metabolic changes that occurred during the normothermic ischemia because we have previously demonstrated a potent postconditioning effect of supplemented Celsior solution in DCD hearts recovered from isoflurane-anesthetized animals.[7] Finally, the antemortem administration of heparin in series 1 is not permitted in some jurisdictions.

CONCLUSIONS

The need for an additional source of cardiac allografts in transplantation is great, and DCD hearts offer a potential source of organs. A better understanding of the detrimental processes that occur during the warm ischemic period is needed and may provide insights into optimal resuscitation of these hearts. In these series of experiments, we have demonstrated the profound hemodynamic, biochemical, and hormonal derangements that occur during the warm ischemic period with a disproportionate injury to the right ventricle caused by acute pulmonary vasoconstriction. Although many of these insults cannot be avoided, there is scope for (ethically acceptable) antemortem and postmortem interventions to limit the profound hemodynamic and metabolic derangements and progress to be made in identifying and optimizing a suitable perfusate for EVP.

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CHAPTER 7 DISCUSSION AND CONCLUSION

Heart transplantation remains the gold standard therapy for medical treatment-resistant ESHF. With improving outcomes and a 90% one-year survival, the demand for this surgical treatment continues to overwhelm cardiac allograft supply. The result of this has been the extension of suitable donor criteria and the acceptance of marginal donors. This has resulted in higher rates of PGF, an early complication associated with higher morbidity and mortality.

A source of donors that has previously been largely overlooked in the clinical setting has been DCD donors. The fear of warm ischemic damage to the heart, an organ that is particularly sensitive to its effects, has limited the use of hearts from such donors. The last two decades has seen many investigators assess viability of these DCD hearts for transplantation. But despite these efforts, there has been no consensus that has supported translation to adult humans. There has been a lack of pre-clinical work that has complete clinical relevance and translatability, and several questions have remained unanswered.

Our efforts were to address the deficits in the knowledge of DCD hearts, to ascertain the limit of warm ischemic tolerance, to identify the best available preservation strategy, to find methods of limiting IRI, and to assess parameters of confirming viability. These were all performed in a large animal model set-up to mimic the clinical setting as closely as possible. We identified a 30-minute WIT cut-off and demonstrated the benefits of pharmacological post-conditioning in limiting IRI buying an additional 10 minutes of tolerated warm ischaemia. We proceeded to display the superiority of EVP over cold storage, both as a superior modality of preservation and in allowing measurement of metabolic parameters of viability, and demonstrated the above in a clinically relevant orthotropic transplant model. With convincing results in pre-clinical work, we proceeded to clinical translation, with the team conducting the world first distant procurement DCD adult heart transplant in July 2014. This was followed by a further five DCD heart transplants to date, demonstrating the viability of these organs

for cardiac transplantation. Our clinical breakthrough was followed by the performance of two successful DCD cardiac transplants at Papworth Hospital in the UK.

The pre-clinical work and subsequent clinical translation confirm the viability of these DCD allografts in cardiac transplantation. The performance of an additional six transplants in the first 12 months of the DCD heart program at St Vincent's Hospital amounts to a ~25% increase in the number of cardiac transplants now performed. In 2013, approximately 14.5% and 15.5% of recipients on the waiting list in the US and Europe died whilst awaiting a cardiac allograft (1,2). Similar mortalities were seen in Australia despite a large campaign to increase organ donation rates. Given this figure, a 25% increase in transplants is very relevant and exceeds expectations. The University of Wisconsin evaluated their DCD donor pool and based on DCD cardiac allograft suitability identified an additional 17% hearts from DCD donors (3). Singhal et al reported an assessment of the Gift for Life program and identified a four to six per cent increase in the number of heart transplants (4). However, approximately 17% of DCD donors were eliminated due to incomplete data. Noterdaeme et al estimated a similar 15% increase in heart transplant activity from DCD allografts after interrogating the local Belgium donor database (5). We conducted a similar evaluation of our DCD donor pool between 2007 and 2013 and identified an additional 16% of cardiac allografts from DCD donors (6). The additional 25% must be considered in light of a conservative approach to DCD donors with utilisation of cardiac allografts from donors with only minimal risk factors. An increase of this proportion is significant and marks the start of a changing paradigm in the heart transplantation scene.

While success clinically has been achieved, it must be noted that of the six DCD heart transplants done so far at St Vincent's Hospital, two of them required temporary mechanical support for PGF post-operatively. While both these hearts made a rapid recovery and displayed normal ventricular function on echocardiogram within a week post-transplant, these DCD hearts are vulnerable organs that have just sustained a significant warm ischaemic insult. The utility of mechanical support for a portion of these DCD hearts highlights the susceptibility of these allografts and the need for

ongoing work in resuscitating these organs. There is little that is permitted ethically to limit the ischaemic insult, but there is still plenty of scope for intervention to limit IRI.

Our pre-clinical work confirmed that pharmacological ischaemic post-conditioning is effective in limiting reperfusion injury and improving recovery post-warm ischaemia. The agents that have been evaluated and shown to improve outcomes in DCD hearts in our publications have included GTN, EPO and zoniporide. We have also previously shown that the addition of sodium-hydrogen exchange inhibitors (cariporide and zoniporide) to the other agents is associated with better myocardial recovery (7,8). However, this class of agent is not available for clinical use due to adverse findings of a recent clinical trial of a drug of the same class. Cariporide, a sodium-hydrogen exchange inhibitor (NHE-1), was studied in the EXPEDITION trial in patients undergoing coronary artery bypass grafting (9). Whilst there was a significant decrease in myocardial ischaemia in the cariporide group (compared with placebo), there was a higher rate of fatal cerebrovascular events (CVE) associated with this agent. Postulated mechanisms for this include a pro-coagulant state with abrupt withdrawal of cariporide, and the limitation of cellular regulation of acidosis. Whilst it has also been suggested that the increased rates of CVE may be specific to the molecular structure of cariporide and not a class effect of NHE-1 inhibitors, and despite numerous other studies demonstrating the cerebro-protective effects of NHE inhibition (10,11,12), there has been withdrawal of all NHE inhibitors from the market in response to the EXPEDITION trial. Hence, the recent clinical DCD heart transplants were conducted using only two of these agents (EPO and GTN).

There are several important points that should be considered when considering the clinical moratorium on the use of NHE inhibitors. The dose of cariporide that was used in the EXPEDITION trial (180 mg loading followed by 20–40 mg/hour for 48 hours) was much greater than has been shown to be of benefit in pre-clinical studies of organ preservation (2.8 mg in 1L Celsior solution) (7). The cerebrotoxicity of cariporide in EXPEDITION would be postulated to be of minimal significance at such a dose. In addition, the use of this agent in organ preservation in the donor setting where the donor is deceased either as a result of brain death or circulatory arrest. Hence any

degree of neurotoxicity, which is likely insignificant at the dose proposed, is also irrelevant in a deceased donor. Finally, there have also been transplant studies that have been conducted using an alternate NHE inhibitor in the form of zoniporide (13). With a different chemical structure, but similar proven myocardial protection, zoniporide may offer the myocardial benefit without the neurotoxicity. However, this is yet to be shown.

The benefit of NHE inhibitors in improving allograft function has been demonstrated. Whilst their use in the CABG setting is prohibited their use for organ post-conditioning should not be—their use in a deceased donor negates the neurotoxicity concern. In addition, the use of an alternate NHE inhibitor at markedly lower doses than that utilised in the EXPEDITION trial adds to their case. In the meantime, alternate agents are also being actively investigated in our laboratory. Cyclosporin A, acting to inhibit the opening of the mitochondrial permeability transition pore, is an important determinant of cell death in the setting of acute ischaemia-reperfusion injury (IRI). Studies have demonstrated its use in limiting IRI (14). Dantrolene, a ryanodine receptor anagonist, acting to inhibit organelle release of calcium into the cytosol, has also been demonstrated to limit IRI through its effects on calcium homeostasis (15). Both these agents are thus under active investigation as post-conditioning agents in heart transplantation.

The other facet that has allowed DCD heart transplantation to become a clinical reality is the use of ex vivo perfusion preservation. It has been demonstrated over the last few decades that EVP offers superior preservation to cold storage (16-18). Clinical translation of this has only been recent due to the cost and increased complexity of operation. It now appears that these devices have an established role in determining viability and providing superior preservation, specifically for marginal donors (19). With regards to DCD allografts, methods of proving organ viability remain an area of active investigation. While metabolic parameters are the cornerstone of the TransMedics device at present, there continues debate as to its adequacy. Notably, two allografts that demonstrated ‘viable’ lactate profiles required short-term mechanical support post-transplant. Many researchers, including our initial studies,

have utilised working mode ex vivo assessment to determine suitability. This adds a level of complexity to managing these organs on EVP devices. Despite this, it appears that a form of safe and reproducible functional assessment in a loaded or stressed heart is the next step in the development of EVP science. In addition to this, the utility of coronary angiograms offers a way to weed out unsuitable organs (20). Finally, the challenge of myocardial oedema with EVP needs to be addressed. Despite additives to limit oedema, the use of flow controlled perfusion results in endothelial changes and significant water gain (21). This also likely contributes to myocardial stunning post-transplant and therefore increased risk of mechanical support. The science of EVP, with optimal pressure and flow targets, as well as additives to maintain the integrity of the endothelial glycocalyx, requires ongoing work.

One of the revelations in DCD transplantation has been the result with DCD lung transplantation. Not only has there been evidence of non-inferiority when compared to DBD lung transplantation (23), there are reports of better outcomes with DCD lung transplantation (24). Although the explanation for this remains unclear, it has been postulated that the lack of BD and the absence of organ exposure to the ensuing autonomic storm might be the difference. In addition, the resilience of the lung to ischaemia is greater than other organs. It has been shown both in our work and others that a catecholamine surge also exists in DCD donors. However, while DBD donor organs are exposed to this for hours to days, DCD organs have only minimal exposure in the setting of deteriorating circulation. The impact on DCD cardiac allografts is unclear, bearing in mind the greater susceptibility to warm ischaemia. Another longer term outcome that may be affected in DCD heart transplantation is the incidence of allograft vasculopathy. The etiology of this process is strongly linked to innate immune mechanisms and a strong risk factor is the early period of ischaemia reperfusion injury (25). The correlation between arguably a greater degree of IRI in DCD hearts and development of allograft vasculopathy long term will need to be monitored. As worldwide acceptance of DCD heart transplantation grows following the breakthrough in 2014, such longer-term outcomes will become evident with time.

The ethical acceptability of DCD cardiac transplantation is a topic of ongoing debate and discussion. One important step in addressing the ethics was to change the nomenclature from donation after 'cardiac' death to donation after 'circulatory' death. This change in Australia allows more consistency with the legal definition of death in DCD requiring cessation of circulation. Donation after cardiac death, on the other hand, implies irreversibility of cardiac function that is clearly not the case and not legally consistent. However many authors argue that the use of hearts in transplantation renders the circulatory irreversibility void and therefore poses ethical and legal challenges in DCD cardiac transplantation (26). Whilst irreversible cessation of circulation in the donor is used for declaration of death universally, there are some jurisdictions where circulation is re-established in the donor following declaration of death. Large et al from Papworth Hospital used cardiopulmonary bypass to re-establish circulation in the donor post-death, thereby allowing cardiac reanimation and assessing viability of the allograft following warm ischaemia. This approach has been used in assessing human DCD hearts for research and more recently a clinical DCD heart transplant (27,28). This adds fuel to the debate, with the irreversibility of circulatory cessation now questioned. It is felt that while this approach allows organ viability assessment in vivo, it raises several ethical concerns and questions the legal definition of death. Our approach to DCD organ procurement respects the finality of circulatory cessation in the donors whether there is potential to re-establish it or not. It is felt that the optimal approach to respect the ethics and community opinion, while maintaining the viability of these cardiac allografts is for organ procurement and reanimation only in an ex vivo setting. As several DCD heart programs worldwide embark on different approaches, it will continue to be a source of ongoing ethical discussion.

With the establishment of strong pre-clinical evidence and the successful clinical translation, DCD cardiac transplantation has gained widespread media attention (29) and has started to establish itself as an additional source of cardiac allografts. The implementation of DCD heart programs around the world will provide additional organs in the battle against organ scarcity, and help limit recipient mortalities on the waiting list. While early outcomes with DCD cardiac transplantation appear acceptable,

the medium- to long-term outcomes post-transplantation remains to be seen. Several challenges remain in progressing the science of DCD cardiac transplantation. IRI remains an area where further progress is required. While relevant pharmaceutical companies are being approached about the use of certain withdrawn sodium-hydrogen exchange inhibitors in the donor, work in identifying other pharmacological agents that exert a similar action to these agents is required. Ex vivo perfusion offers an excellent platform for organ resuscitation. Agents to address immunomodulation and limit rejection, gene therapy and targeted treatments are all areas of potential progress with EVP. The challenge of limiting organ oedema remains, and lies in further understanding EVP science and improving the technology. In addition, methods of functional organ assessment in addition to metabolic assessment need to be considered and evaluated. As seen in the early clinical results, it is likely that following a warm ischaemic insult these stunned hearts will result in a higher rate of short-term mechanical support post-transplant. The limited clinical numbers so far suggest rapid recovery of ventricular function. Moreover, with increasing experience and improving ICU care, there is growing evidence that the use of mechanical support has little consequence to short- and long-term outcomes (30). Finally, the ethics of DCD heart transplantation is of vital importance—education, debates and discussions with medical staff and the general community are paramount for the long-term survival of such a program. With further understanding of DCD allografts and progress in EVP science it is felt that DCD hearts transplantation can continue to make a large and significant contribution to the treatment of patients with severe ESHF.

Chapter 7 References

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