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Heart Transplant: Donation after Circulatory Death
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Abstract

Donation after circulatory death (DCD) is a potential avenue to narrow the gap between demand for donor hearts and their limited supply. DCD was abandoned after 1970 where donation of organs after brain death became the standard, however DCD has made a recent resurgence for organ donation, predominantly for lung transplants which has shown much success. Heart transplant donors primarily source from brain dead donors. Benefits of DCD for heart transplants include an expanded donor pool to address the drastic shortage of supply and reduced onerous financial burden. Drawbacks of heart DCD include substantial ischemia reperfusion injury (IRI) and inflammation experienced by the donor heart. IRI changes the metabolic phenotype of the myocardium, compromising its efficiency. Pharmacological interventions by manipulating metabolic signaling pathways can shift the myocardium towards cardioprotective phenotypes to maximize the integrity of the organ during the stressful transplantation process. Lastly, a process of ischemic pre and post conditioning of the heart tissue to facilitate an incremental adjustment to ischemic conditions as a therapeutic approach is desirable. We review the practicality of DCD along with the financial and logistical constraints of implementing this technology, including the optimization of signaling pathways and conditioning strategies to reduce IRI in heart transplant.

Keywords: Cardiothoracic, surgery, donation after circulatory death

Introduction

There is a huge shortage of donor organs on a global scale and the supply cannot hope to meet the current demands. Heart transplants are particularly problematic since they have been and remain the best avenue for clinical treatment of patients with end stage heart failure. To increase the donor pool and increase the available supply of hearts, clinicians have looked to bringing back donation after circulatory death (DCD) as a means of closing the gap between the supply and the demand for donor hearts.

Generally, there are three types of organ donors. The first is the living donor where the donor is alive and usually donating to family or a loved one. The second is a donor who has been classified as brain dead. These donors have been declared dead based on neuro criteria, however their heart beat is still intact. Organ donation is predominantly from brain dead donors. The last major category is the donor who has experienced circulatory death. These patients have experienced irreversible circulatory and respiratory arrest and are considered dead at that point. Historically DCD took place between 1951 and 1967 and included kidney, liver and heart transplants. In 1968, the Harvard Committee established brain death criteria and brain death became the standard after its acceptance in 1970, which effectively put a damper on using DCD donors for their organs. Recently there has been a move to increase the donor pool by reintroducing the DCD to meet the overwhelming demand for donor organs.

Donation after brain death (DBD) has been the traditional source of solid organ transplants. However, between 2001 and 2008, DBD transplants have gone from virtually a 100% of donations, down to 55%, not because of fewer DBD donors per se, but because of the tremendous increase in DCD donors. This trend has not manifested for heart transplants and during this period, essentially all the heart transplants came from DBD.¹

A pilot study at the University of Alberta demonstrated success using DCD for lung transplants. They reported significantly reduced cold ischemic times due to usage of ex vivo lung perfusion and reduced primary graft dysfunction 72 hours post-transplant. These results had similar mechanical ventilation times and length of stay in the hospital compared to traditional lung transplant methods.² These findings suggest that similar results can be attained using heart transplant models.

From an economic and humanitarian standpoint, patients waiting on transplant lists are often put on LVADs to prolong their ability to wait. This is traumatic and stressful for the patient, not to mention costly for the patient and the system overall, both of which could be eased with an increase in donor pool which is possible by harvesting DCD hearts.¹

DCD heart transplantation has been shown to be successful in both animal models (sheep and primate) and humans (adult case in 1967 and pediatric cases in 2007).³⁻⁵ The human studies required that warm ischemic conditions for the donor heart be limited to 30 minutes, which becomes an issue during explantation and implantation of the organ as it is cut off from circulation/perfusion. Ex vivo perfusion and close monitoring of the organ tissue in conjunction with a therapeutic perfusate can help to optimize these results.

This review will explore some of the benefits and drawbacks to using DCD for heart transplants and will focus largely on the cellular environment during ischemic-reperfusion injury, one of the main points of contention with using DCD.

Benefits of DCD Usage

Brain death accounts for a small proportion of mortality in the US, whereas circulatory and cardiovascular death account for a significantly greater fraction of deaths. As such, the donor pool is much larger with DCD and can vastly ameliorate the shortage of donor organs by as much as 40%.⁶⁻⁸ In addition, DCD hearts can be transplanted via traditional cold static storage methods or using ex vivo perfusion methods. In the latter case, the viability of the organ ex vivo is significantly longer, close to 10.5 hours as documented by Australia, while cold static storage averages around 4 – 6 hours of viability.⁵ This increase in time would increase the radius between donor and recipient for a transplant and would even make trans-Atlantic donations between countries possible, on top of having a larger donor pool from using DCD. These benefits alone warrant exploration of DCD in heart transplants as it has been adopted to for other organ transplants.

Drawbacks

There are some hurdles, both ethical and procedural, that need to be overcome before heart DCD transplants become commonplace. Ethical dilemmas include the amount of time that must be

observed between circulatory death and donor organ harvest and pre-mortem pharmacological interventions to limit damage to heart. Procedural issues relate to the onset of warm ischemia in a DCD heart, DCD physiologic effects, extubation comorbidities and ischemia-reperfusion injury.

One of the drawbacks associated with using hearts obtained from donors after circulatory death is that donors who have experienced brain death often exhibit a Cushing response. This is sympathetic system response that results in a massive cytokine release after brain death that has been shown to alter the phenotype of the donor organ. Animal studies in baboons have shown that not only can the cytokine cascade potentially damage the cardiac tissue, but the surface antigens have been altered to promote inflammation. This combination of damaged tissue and pro-inflammatory phenotypes has been observed to increase the risk of graft rejection and reduced positive long-term outcomes for kidneys.^{9, 10}

Extubation of a DCD heart donor resulted in hypoxic pulmonary vasoconstriction. This was observed with right ventricular distension and concurrent reduced cardiac output. Both events were followed by a 120-fold increase in epinephrine levels which further exacerbated the right ventricular distension. The large catecholamine release following extubation can compromise the integrity of the cardiac tissue and close monitoring and management of these physiologic changes is necessary before transplanting a DCD heart.¹¹

Ischemia and Reperfusion

One of the issues with all forms of heart transplant is ischemic-reperfusion injury. Ischemic conditions are experienced during harvest and implantation of the donor organ. As such it is important to identify the cellular factors at play during ischemic conditions and identify how to intervene to limit the damage. Extended criteria hearts are more fragile by definition, as such it is important to limit the insult that they experience during the transplant procedure to ensure maximal functionality after transplant. Characterizing the ischemia and reperfusion injury to heart tissue in the context of transplants will be the focus of the remainder of this paper.

Ischemia reperfusion injury refers to the damage that takes place in the tissue due to a lack of oxygen under ischemic conditions, followed by additional tissue damage that takes place as the oxygen is restored during reperfusion. Restoration of flow through the tissue brings with it fresh nutrients and flushes out toxic build up, but this process jump starts the cellular metabolism of the tissue and can result in many side effects that culminate in tissue damage.

AMPK Pathway and Metabolism

Approximately 60 – 80% of myocardial ATP production comes from fatty acid oxidation and the remaining 20 – 40% from glycolysis and ketone body oxidation. Ischemia and reperfusion cause fluctuations and change the metabolic phenotype of the heart and this in turn effects the myocardial function.¹² Increasing evidence has shown that enhancing glucose metabolism and inhibiting fatty acid oxidation in the ischemic heart has a beneficial effect for maintaining cardiac efficiency. Hence increasing glucose uptake and metabolism in ischemic hearts can provide protective phenotypes relative to fatty acid oxidation.¹²

Flux through fatty acid β -oxidation is dependent on several factors including the presence and concentration of free fatty acids in the plasma. During surgical stress or ischemic stress, as is the case during harvest and implantation of a donor organ, there is a surge of catecholamine release and these hormones increase the plasma concentration of free fatty acids by increasing the rates of lipolysis. β -oxidation is regulated by malonyl CoA which is formed intracellularly via the conversion of acetyl-CoA by the enzyme acetyl-CoA carboxylase (ACC). ACC is activated via the 5'AMP activated protein kinase (AMPK) pathway, a pathway that regulates many metabolic processes.¹³

In addition to ACC, Notch1 and liver kinase beta1 (LKB1) also come together to modulate the AMPK activity, specifically reducing glucose oxidation and fatty acid oxidation during ischemia reperfusion. Slowing down these biochemical processes naturally reduce the production of radical oxygen species and limits the damage of reperfusion. As such, activation of the AMPK signaling pathway via Notch1's interaction with LKB1 has been shown to induce cardioprotective properties against ischemia reperfusion injury.¹³

The AMPK pathway is also triggered when the AMP/ATP ratio is elevated. Activation of AMPK facilitates conservation of cellular energy stores, increases ATP formation and decreases ATP usage during ischemic conditions. AMPK pathway and STAT3 pathways, when activated by leptin signaling, which also limits structural and functional damage to cardiomyocytes, reduces glycolysis, cardiac hypertrophy, apoptosis, and inflammation in post myocardial infarction hearts.^{14, 15} Interestingly, leptin is associated with lipolysis and increased free fatty acid formation which is detrimental to metabolic processes in ischemic conditions, suggesting that there are complicated interactions between signaling molecules and their downstream effects. Nonetheless, these protective effects are lost in AMPK knockout mice and suggest that these are physiologic protective functions. To facilitate this protection in ex vivo perfusion during ischemic reperfusion, it might be worthwhile to induce NOTCH1 and LKB1 signaling to induce the AMPK pathway, concurrent with inhibition of ACC to limit β -oxidation, in the heart to jump start the protective facilities and limit peripheral side effects.

Since most of myocardial energy comes from fatty acid oxidation which is dependent on the presence of oxygen (for the electron transport chain in the production of ATP), ischemic conditions halt the bulk energy production. In terms of energy, a single molecule of palmitate (fatty acid) produces 105 molecules of ATP via β -oxidation, in contrast to the 31 ATP molecules produced by glycolysis of a single glucose. β -oxidation requires oxygen however, whereas glycolysis does not and as such the phosphorous/oxygen ratio, a measure of ATP produced per

atom of oxygen reduced by the electron transport chain, and a proxy for cardiac efficiency, favors glycolysis by as much as 25 – 40%.¹⁶

Furthermore, cardiomyocytes express uncoupling proteins, UCP3 specifically among a few other isoforms, which essentially break the flow of electrons in the electron transport chain. UCP3 is activated in ischemic conditions when there are higher levels of free fatty acids and exports their intermediate break down products from the mitochondria, preventing accumulation of charged species within the matrix. This process requires the action of acyl-CoA synthase which uses 4 equivalents of ATP by converting 2 ATP to AMP and PPI. Since ADP isn't being generated, this process further hinders regeneration of ATP since glycolysis requires ADP as a substrate.¹⁷ Glycolysis also produces pyruvate, a charged product, which needs to be transported into the mitochondria to enter the tricarboxylic acid cycle for further oxidation. The transport of pyruvate is coupled with protons, however when this process is uncoupled, can result in intracellular acidosis. This is particularly problematic in ischemic conditions as these toxic metabolic wastes can't be flushed out, compromising cardiac efficiency and functionality.¹²

As ATP generation becomes more difficult, cardiac efficiency plummets. What little energy is produced to maintain cellular integrity via anaerobic metabolism, i.e. glycolysis without pyruvate oxidation, results in lactic acidosis. As ATP depletion manifests, ion homeostasis is also compromised as sodium and potassium concentration gradients can't be sustained without the action of ATPases, resulting in intracellular sodium overload and potassium deficiency and inability of the cell to reuptake cytosolic Ca^{2+} into the sarcoplasmic reticulum.¹²

After ischemic conditions end, fatty acid oxidation resumes to normal levels, however glycolysis and cardiac efficiency, and as a result the mechanical function of the heart don't return to normal. Reperfusion attempts to restore the cytosolic levels of protons across the sarcolemma, but this effects the Na^+/H^+ exchanger and increases the already elevated Na^+ concentration as it returns cytosolic proton concentration to normal.¹² The Na^+ levels are attempted to be balanced by activating the $\text{Na}^+/\text{Ca}^{2+}$ exchanger which pumps out 3 Na^+ for every Ca^{2+} pumped in resulting in intracellular calcium overload. Calcium homeostasis is integral for maintaining the contractile functionality of the myocardium as the actin-myosin filament cannot properly function.¹²

As mentioned earlier, ischemia induces significant catecholamine release resulting in increased lipolysis and free fatty acids. An adverse reaction that stems from this is the development of insulin resistance systemically as this prevents GLUT transporters from making it to the myocardial surface and limits glucose transport into the cell. As such reverting to anaerobic metabolism becomes difficult even under anaerobic conditions.^{18, 19} Activated protein C (APC) increases AMPK signaling and results in increased GLUT4 transporter translocation into the plasma membrane of cardiomyocytes. This facilitates preferential glycolytic metabolism over fatty acid oxidation and can potentially counterbalance the insulin resistive effects of catecholamine release.²⁰

Since the cardiac efficiency and potassium/oxygen ratio favors glycolysis, it seems that exploring pharmacological therapies that target changing in metabolic flux to favor glycolysis can favor cardiac mechanical efficiency. Using inhibitors of beta-oxidation such as malonyl-CoA decarboxylase inhibitors, beta blockers to inhibit catecholamine release which in turn would reduce heart rate, lipolysis and increase glucose oxidation.²¹ Drugs such as ranolazine,

trimetazidine and etomoxir essentially shuttle metabolism towards glucose oxidation by shutting down fatty acid oxidation at different stages. Increasing glucose oxidation under ischemic conditions can be an effective way of improving ATP generation efficiency without sacrificing cardiac function.¹²

Role of Calcium

Increases in intracellular Ca^{2+} concentration combined with the presence of reactive oxygen species (ROS) species as is the case during ischemic conditions, triggers the opening of a mitochondrial conductance channel referred to as the mitochondrial permeability transition (MPT). This channel results in a loss of ATP, compromised mitochondrial function, induction of mitochondrial swelling resulting in loss of mitochondria membrane integrity and triggering release of cytochrome c and inducing apoptosis.²² This is in addition to the myocardial autophagy that takes place due to high levels of intracellular Ca^{2+} that resulted from ischemic conditions.²³ Intracellular Ca^{2+} stores rise by reverse reaction of the Na^+ - Ca^{2+} exchanger due to the build-up of intracellular Na^+ from the balancing out effects of proton gradients mentioned earlier. Inhibition of either the Na^+ - H^+ exchanger or the Na^+ - Ca^{2+} exchanger have both shown cardioprotective properties against reperfusion injury. The build-up of protons results in an acidic intracellular pH, and under ischemic conditions the extracellular fluid also becomes acidic. Reperfusion restores extracellular pH rapidly as the contents are readily flushed out. The intracellular pH is restored through the actions of the exchangers, largely through the Na^+ - H^+ exchanger. The intracellular Na^+ concentration is then balanced out by exchanging it with extracellular Ca^{2+} .²² Ca^{2+} is necessary to maintain and induce muscle contractions and when the intracellular concentrations rise in myocytes, it can result in arrhythmias or hyper-contractions which can result in cell death or other irreversible cellular injury during ischemia-reperfusion. Preventing the unregulated influx of Ca^{2+} has shown to be effective in protecting against this. Inhibition of Na^+ - Ca^{2+} exchanger by agents such as SEA0400 have even shown to be able to maintain mitochondrial function and cellular ATP content under ischemic conditions.²⁴

ROS

ROS species have been shown in many studies to be generated during reperfusion after ischemia.²² The current hypothesis is that ROS species increase during ischemic and reperfusion states is due to damage to the electron transport chain which results in poor electron transfers and incomplete oxidation-reduction reactions resulting in the production of superoxide radicals. ROS species are also secreted by neutrophils and monocytes that have infiltrated the tissue during early reperfusion as a part of the inflammatory response.²⁵ The ROS species can damage the cell themselves or induce the expression of mitochondrial transition pores in the inner membrane. Several animal models have been tested with the addition of antioxidants such as superoxide dismutase and catalase as well as some protective vitamins such as vitamin E and vitamin C, to limit the effect of the free radicals, as well as being treated with chemokine inhibitors such as Evasin-3.^{25, 26} Chemokine inhibition was shown to have ameliorative effects, while the results for the antioxidant treatments were split with some studies reporting reduction in infarct size,^{27, 28} while others reporting there were no significant reductions in infarct size.^{29, 30} The conflicting results could be indicative that the antioxidant agents were not able to scavenge the radicals before they had already compromised the integrity of the myocyte, and that administration of

these clinical therapies is time sensitive or must be precautionary as opposed to reactionary in order to demonstrate cardiomyocyte protection, however these findings are still unclear.

Preconditioning

Ischemic preconditioning (IPC), which is treatment of the heart with short cycles of ischemic and reperfusion periods of 5 minutes or so, was first introduced approximately 20 years ago as a means of reducing ischemic damage to tissues under prolonged periods of ischemia.³¹ Hearts that are preconditioned, experience reduced instances of anaerobic metabolism and reduced ischemic lactic acidosis. The cellular cascade that results in the cardioprotective effects of preconditioning is included below.²² The first half of Figure 1 is a cascade showing the effects of IPC while the second half of the figure is the cascade showing the effects of ischemic post conditioning. Both pathways converge to inhibit mitochondrial permeability transition pores (MPT pores) which were mentioned earlier. Inhibition of MPT seems to be an effective method of inducing cardioprotection and has been shown using drugs such as cyclosporin.³²

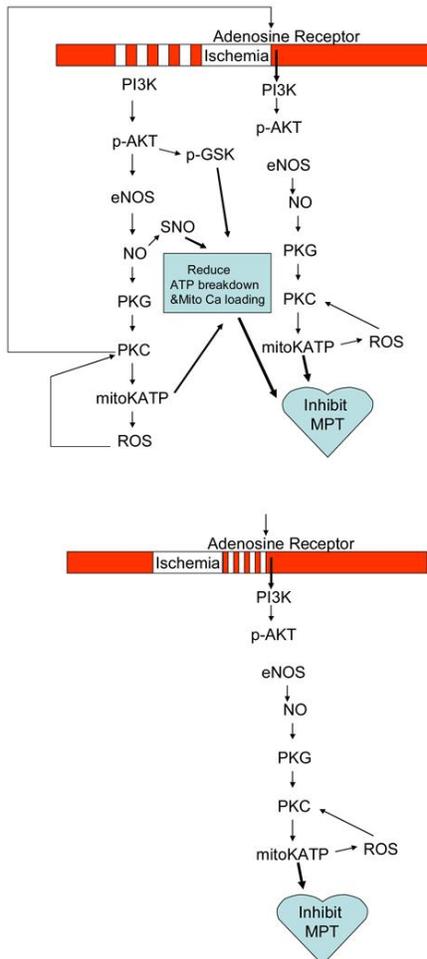


Figure 1. Ischemic pre and post conditioning signaling pathway. Taken from *Mechanisms Underlying Acute Protection from Cardiac Ischemia-Reperfusion Injury*.²²

It is suggested that the short cycles of ischemia and reperfusion slow down the rate of ATP

hydrolysis since there is less ATP production taking place (due to the intermittent anaerobic glycolysis).³³ The attenuation of the rate of ATP hydrolysis likely conditions the cell and persists into the global prolonged ischemic conditions for the tissue, likely aiding in preventing the depletion of energy stores. A second method by which IPC can be protective is by preventing ATP breakdown by the mitochondrial ATPase which functions in reverse to the ATP synthase molecule from the electron transport chain.³⁴ When the mitochondrial membrane potential is thrown off balance due to pH imbalances under ischemic conditions, the ATPase molecule can break down ATP molecules (which are charged molecules) to restore the membrane potential. There is conflicting evidence for protecting and stimulating ATP levels in relation to ATPase activity in the mitochondria after preconditioning.^{35, 36}

IPC results in phosphorylation of the PI3K/Akt pathway which propagates a pro-survival signal during times of ischemic reperfusion. The activation of this pathway releases hydrophobic mediators into the coronary circulation and has been characterized as reducing infarct size by 55% compared to controls. Inhibition of this pathway, eliminated any protective properties that were observed using the IPC treatment. Using the effluent from a heart that has undergone IPC in the coronary circulation of another heart has also protected against infarct size, namely through the PKC pathway which is just downstream of the PI3K pathway.³⁷⁻³⁹ IPC has also been characterized to induce other RISK survival pathways including the ERK pathway. These pathways branch at several levels but converge and induce cardioprotection by ultimately inhibiting MPT. Inhibition appears to be time sensitive and the most effective and pronounced reduction in infarct sizes appears to be at the beginning of reperfusion.⁴⁰ Furthermore, metabolic analyses of IPC have shown that SIRT1, a lysine deacetylase, is required in IPC treatments to target various metabolic regulatory systems. IPC induced the following changes in the myocardium: increased glycolysis, glycogen and amino acid synthesis, reduction in glutathione levels, and inhibited fatty acid oxidation. These pathways were not affected as such when SIRT1 was inhibited and the protective capacity of IPC treatments were eliminated with SIRT1 inhibition.⁴¹

Hypothermia and Post Conditioning

Mild hypothermic conditions (32 – 35 degrees Celsius) at the onset of reperfusion for a short period of time (approx. 10 minutes) has shown improvements in post ischemic heart functioning.⁴² This intervention improved left ventricular work capacity, as well as improved the pressure, heart rate, contraction rate and cardiac output compared to hearts that did not receive the hypothermic intervention. These same parameters were also improved when they treated the DCD hearts with mechanical post conditioning, i.e. 30 second cycles of ischemia and 30 seconds of reperfusion, twice, directly at the time of initial reperfusion. They also measured reduced levels of necrosis and cytochrome c release and higher levels of oxygen consumption, suggesting improvements in post ischemic metabolism when treated with hypothermia. The protective capacity of mild hypothermic conditions against infarctions was completely abrogated when extracellular signal regulated kinase (ERK) inhibitors were administered. This suggests that the protective capacity of the mild hypothermic treatments acts through the ERK signaling pathway. Inhibition of ERK pathway did not change the levels of ATP preservation, although ATP hydrolysis and metabolism are slowed because of this intervention.⁴³

Mechanical postconditioning intervention also improved hemodynamics in hearts that were exposed to high levels of free fatty acids, but reperfused with only glucose, favoring glycolysis over fatty acid oxidation. This method of post conditioning works through shutting off mitochondrial permeability transition pores.^{44, 45}

Lastly, this group reported improvements in hemodynamic functions after inducing a brief period of hypoxic reperfusion when compared with oxygenated reperfusion strategies. Specifically, they saw improvements in left ventricular work, increased pressure, contraction and relaxation rate as well as coronary flow. If the heart is perfused with glucose under hypoxic conditions, then the ability to produce reactive oxygen species is drastically reduced and protective of the myocardial tissue.

Conclusion

There have been clinical trials that have failed with the implementation of different drugs and protocols and it's important that further exploration into cardioprotective measures be taken to reduce ischemia-reperfusion injury. Both the timing of administration and different drug cocktails should be considered, i.e. MPT and ion exchange inhibitors and antioxidant treatments early on reperfusion. DCD presents a way of reducing the burden of limited organs available for donation and has been re-introduced into many transplant procedures. Heart transplants have not however, and one of the fears is the deleterious effects of ischemic-reperfusion injury. Nonetheless, by understanding the mechanisms of reperfusion injury and targeting them to maximize heart transplant viability, DCD will become a more feasible option, both using cold static storage methods as well as using ex vivo perfusion methodologies.

The UK and Australia have reintroduced DCD into their healthcare systems. St. Vincent's Hospital heart transplantation in Australia have also reported success using DCD heart transplantation protocols and OCS in 2014. Currently the PROCEED II trials for heart transplants with OCS are taking place, which is an FDA approved international trial. As of 2014, 128 patients have received an OCS ex vivo heart perfused transplant. Results of the trial were released online in 2015 and both the primary effectiveness and safety endpoints (30-day patient survival and serious adverse effects/rejection/ICU time) goals were met.^{46, 47} Success in these trials and proper manipulation of cellular processes can optimize hear transplantation and limit adverse outcomes.

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