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Ex Vivo Heart Perfusion

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Abstract

Heart transplantation offers the best prognostic results for patients with end stage heart failure. However, there is a much greater demand for donor hearts than there is adequate supply. Cold static storage (CSS) is the current standard protocol for donor heart procurement. CSS has excellent prognosis but subjects the organ to ischemic reperfusion injury (IRI) and induces tissue inflammation due to anoxic conditions and oxidative stress. Hearts from older donors or patients that have a history of previous heart disease can't withstand the anoxic stressors and make for poor donor candidates with the CSS protocol since they are associated with worse prognostic outcomes, which restricts the donor pool for acceptable hearts. Ex vivo heart perfusion, a novel method for heart transport, is a potential solution to expanding the donor pool and reducing the IRI and anoxic insults. This protocol continuously perfuses the donor heart and has been shown to reduce ischemic injury, increase ex vivo viability time and improve the biochemical and cellular profile of the donor heart. These factors collectively open the door for the possibility of expanding the acceptable pool of donor hearts since this protocol places fewer stressors on the myocardial tissue. We review the limitations of the cold static storage protocol and evaluate the benefits, drawbacks and practicality of the ex vivo heart perfusion for use in clinical practice by examining both human and animal studies.

Keywords: Cardiothoracic, surgery, ex vivo, heart, perfusion, transplantation

Introduction

Heart transplantation has been and remains the best avenue of clinical treatment for patients with end stage heart failure. When pharmacological and resynchronization therapies fall short, heart transplants become the desired form of care to treat patients. The prognosis of transplant recipients is excellent, such that as of 2014 there is an 84.5% 1-year survival rate post-transplant and a 72.5% 5-year survival rate post-transplant.[1] With the right cocktail of immunosuppressants and close monitoring of patient's life and activities, 21% of patients have been documented are alive and healthy after 20 years post-transplant.[1]

The issue lies with the fact that there is a significant disparity between the supply of donor hearts and the magnitude of qualifying recipients. To meet such high demands, alternative avenues have been explored including the use of total artificial hearts, ventricular assist devices and stem cell therapy, but they are by no means comparable to treatment via transplant.[2] Thus, research efforts have been redirected towards expanding the possible donor pool since currently many potential donors are unable to donate due to the age of the organ, smoking and drug history, IV drug use and those with previous instances of heart disease.[2] These "extended criteria hearts" (Table 1) are often refused because of their ability to induce primary graft failure in the recipient. These hearts often cause single or biventricular dysfunction and result in low cardiac output and hypotensive characteristics, regardless of their ability to maintain adequate atrial and ventricular

filling pressures.[3, 4] During the transplant procedure, there are repeated instances of ischemic stress experienced by the donor heart and it has been documented that the myocardial tissue of older donors are not only more susceptible to primary graft failure, but have a higher risk of experiencing ischemia-reperfusion injury and subsequent recovery from the injury is reduced relative to younger donor organs. As such, younger donor hearts are preferred for their ability to withstand ischemic stresses for longer periods of time without experiencing irreversible damage to the myocytes in the process.[3, 5, 6]

Researchers and clinicians have looked to different means of expanding the donor pool to not only include these extended criteria hearts, but also to increase the time that a donor heart remains viable once it has been explanted. The current standard protocol of cold immersion, which has wonderful prognostic statistics, only has a 4-6-hour window between explantation and re-implantation from the donor to the recipient respectively.[7] Ex vivo heart perfusion and donation after circulatory death (DCD) are two potential routes for expanding the donor pool that are being considered right now. This review will analyze recent findings on cold static storage and ex vivo perfusion as they relate to heart transplants.

Traditional cardiac donor selection parameters
Age < 55 years old
No history of chest trauma or cardiac disease
No prolonged hypotension or hypoxemia
Appropriate hemodynamics
Mean arterial pressure > 60 mmHg
Central venous pressure 8 to 12 mmHg
Inotropic support less than 10 mg/kg/min (dopamine or dobutamine)
Normal electrocardiogram
Normal echocardiogram
Normal cardiac angiography (if indicated by donor age and history)
Negative serology (hepatitis B surface antigen, hepatitis C virus and HIV)

Table 1. Traditional Cardiac Donor Selection Parameters. Adapted from Sabiston & Spencer surgery of the chest, 8th ed. Sellke FW, del Nido PJ, Swanson SJ, *et al.* eds. Philadelphia: Saunders Elsevier, 2010). Extended criteria hearts naturally are hearts that do not fall within these parameters.

Cold Static Storage

The current gold standard for heart transplantation is via cold static storage. This method involves inducing cardiac arrest in the donor heart via the usage of cold crystalloid cardioplegic solution that serves to also flush out any leftover blood within in the heart. This is then followed by a cardiectomy and storage in an ice chest with the intent of placing the heart in a hypothermic environment to prevent metabolism. In doing so, this reduces the build of reactive oxygen species, accumulation of metabolic and toxic wastes and reduces ATP depletion.[8] To date this strategy has proved both reliable and cost effective, however there are several cellular and systemic issues associated with this method. Despite effort to shut down metabolism, there is still sub physiologic levels of metabolism taking place and this results in steady depletion of ATP and build of ROS and toxic metabolic wastes effectively inducing acidosis in the myocardium.[2, 9]

Because of these factors as well as ischemic and reperfusion stressors (during ex vivo transport and implantation into recipient respectively), the viability of the donor heart using this method is restricted to 4 – 6 hours.[7, 9, 10]

In addition to the short viability window, hearts having undergone cold static storage experience ischemia-reperfusion injury (IRI) when the heart is re-introduced into the recipient as well as the heat-cold shock of returning to body temperature. IRI has been characterized to induce multiple inflammatory and cell apoptotic pathways, namely complement 3, caspase-3, caspase-8 and NFkB pathways.[11]

The myocardium constitutively expresses TLR4 which activates the nuclear factor κB (NFκB) transcription factor. This then induces the expression of several pro-inflammatory cytokines such as TNF-α and IL-1β. Hypoxia resulting from the cold static storage induces expression of heat shock proteins (HSP60 specifically in this case) which activates TLR receptors.[12] Murine models have shown that TLR knockout mice have reduced infarct sizes and suggest that reducing TLR activation during organ transplant can facilitate reduction in tissue damage.[12] Since the cold static storage mechanism isn't able to completely shut down metabolic processes, there is a slow but definite build-up of reactive oxygen species (ROS) which have also been shown activate TLR receptors and instigate the pathway. Effectively, the significant activation of TLR receptors eventually cumulates in substantial inflammatory effects in the myocardium.

The activation of the pro-inflammatory cytokines (IL-6, TNF-α and IFN-γ) in turn activates the complement system. Complement has also been shown to be activated via the mannose binding lectin pathway in renal reperfusion models, suggesting that similar modes of activation in other tissues is also likely. Complement activation not only activates the innate immune system, causing inflammation, but also recruits and helps activate the adaptive immune system by increasing antibody secretion from B cells due to C3a and C5a.[12] In a transplanted organ where immunosuppression is desired, complement activation and adaptive immune system recruitment work to hinder the host's ability to accept the organ.

By incorporating siRNAs against these molecular targets into the Celsior solution used to perfuse the heart has been shown to limit myocardial damage and prolong tissue preservation in cold storage environments in porcine models.[11] Treatment with siRNAs were associated with reduced apoptotic markers such as BAX and reduced levels of tissue damage markers (creatine kinase, lactate dehydrogenase and malonaldehyde) while increasing the activity of superoxide dismutase.[11] Effectively, treatment with siRNAs have provided insight into the inflammatory and apoptotic pathways that are activated in cold static storage and resultant IRI and provided a means to limit the damage and prolong preservation and limit tissue damage without compromising post-transplant organ functionality. Part of the novelty of using siRNAs is that they are highly specific towards their targets and are short lived. As such there are limited side effects, and since the effects are only short lived, the effects don't persist for too long post-transplant.[11]

Treatment with siRNAs should be supplemented with activation of pro-survival signaling pathways. The akt pathway is one of the key pathways in regulating cardiomyocyte survival after ischemic stress and can be a pharmacological target to supplement siRNA treatment.

Phosphorylation of akt and resulting downstream effects have muted the apoptotic response of the myocytes after being exposed to hypoxic environments.[13] By manipulating this pathway, the infarct size and apoptotic events in cardiomyocytes have been reduced. Doxycycline use in the cardioplegic solution has been shown to increase the phosphorylation of the pro-survival kinase responsible for activating akt. As a result, doxycycline usage has facilitated reduction in the apoptotic events that take place after the hypoxic stressors of cold static storage and reperfusion injury.[13 – 15]

Various combinations of inhibitors and activators have been experimented with to create the optimum drug cocktails for the ideal perfusate, however since cold static storage isolates the donor heart, the perfusate cocktail is limited to post-transplant or pre-transplant administration in a single dose, as continuous flow is not possible in the protocol. This is a major roadblock in increasing the viability of donor hearts and the small window for maintaining tissue integrity.

Ex Vivo Continuous Perfusion

Overview/Potential Benefits

A growing field of interest is in studying the feasibility of continuous perfusion compared to cold storage for heart explantation and transport. Whereas cold storage arrests the heart and attempts to shut down metabolism, continuous perfusion seeks to do the opposite. Rather than shut down circulation, the heart is hooked up to a machine that circulates donor blood or synthetic perfusate through the myocardium. TransMedics has created one such machine to enable this process, referred to as the Organ Care System (OCS) which allows for both continuous perfusion of the heart tissue as well as consistent monitoring of the tissue's condition.[2] The OCS enables the transplant team to keep track of aortic pressure, coronary flow, temperature, cardiac output, heart rate, pressure and a lactate profile of the heart. The lactate profile of the donor heart is the best indicator of the patient outcome.[2] OCS has been used in Australia and recently licensed for use in Europe as well. Clinical use in the US is still pending implementation.

During cold static storage the heart is in an anaerobic environment and despite sub-physiologic conditions, the background levels of metabolism taking place results in energy consumption. These cold ischemic conditions force the heart to move from oxidative phosphorylation to lactic acid fermentation and concurrent build-up of lactic acid resulting in a state of acidosis.[16] In contrast, continuous perfusion allows the circulation of fluid through the tissue to mimic the circulation of the body and carry away the toxic metabolites. This then effectively prevents tissue acidosis hence promoting tissue survival. Myocyte viability is most dependent on pH such that even with glucose and oxygen present, an acidic environment of $pH < 6.5$ has induced massive deaths in vitro.[17] By eliminating the onset of acidic conditions, continuous perfusion is able to delay myocardial necrosis and increase the viability ex vivo. In addition, clinical cases have shown that there are over twice as many deaths post cardiac surgery in patients with pH levels below 6.3 and that there is approximately half the ATP present in acidic tissues compared to myocardium at a physiologic pH.[7, 18]

In addition, when machine perfusion was compared to static storage, myocardial tissues samples have shown reduced markers of oxidative, tissue and DNA damage, reduction in oxygen and lactate levels (indicative that there is more aerobic and less anaerobic metabolism taking place in

the mechanically perfused heart) and a more rapid recovery period after blood reperfusion during implantation of the mechanically perfused heart.[9, 19 – 21]

As mentioned previously, the use of continuous perfusion (and demonstrated with normothermic perfusion) as an alternate to cold static storage drastically reduces ischemic times. This allows for much greater ex vivo windows and increases the retrieval boundaries for donor organs, allowing geography and population density to facilitate increases in the donor pool. Australia has had success with OCS and normothermic perfusion in that the donor heart was ex vivo for over 10.5 hours, almost double that of a safe zone for cold static storage.[2] In the context of the united states, donor hearts at one coast can be considered for recipients on the other and in more dire cases, east coast patients and western Europe can engage in organ sharing due to this increase in tissue viability.

The increase in time available also allows for transplant and heart surgeons to gauge the quality of the donor heart and potentially get the donor organ typed and checked if it came from an emergent situation. The increase in time also gives the transplant team and the recipient patient's surgeon more wiggle room in terms of coordination. With cold static storage, the recipient must be prepped and ready for transplant right when the donor organ arrives, to limit time spent on anesthesia and to not require extra time compromising the viability of the donor heart.[2, 22]

An issue with both cold static storage and continuous perfusion is that the heart experiences significant edema during the process. In the case of cold static storage, the induction of ischemic conditions results in acidosis and toxic metabolite build-up which eventually compromises the cell membrane integrity and promotes fluid movement into the cell. Continuous perfusion doesn't have this issue since the perfusate is usually oxygenated (allowing for aerobic metabolism) and washes out the metabolic waste products.[9, 20] However, as soon as the heart has been explanted, it loses its lymphatic drainage system which then promotes tissue edema. This is not so problematic in the cold static treatment, but substantially more so in the continuous perfusion methodology since there is constant oncotic force being exerted on the tissue membranes. Initially constant perfusion was kept at a static pulse, which isn't representative of the circulation in vivo. Mathematical models that mimic physiologic variability in circulation have since been integrated into the perfusion machines and this has resulted in a reduction of weight gain due to edema from 17% to 5% in transplanted hearts. In addition, modulation of the perfusate with albumin facilitates oncotic pressure regulation to further alleviate the stressors associated with this edema.[23 – 25]

From a logistical and financial perspective, utilization of the Organ Care System for ex vivo heart perfusion and transplantation are more expensive and cumbersome relative to the standard cold static storage protocol. The OCS methodology necessitates a larger team and resources due to increased technical support staff, equipment and adequate transportation. Collection of donor blood is also required to prime the perfusion module of the OCS and this also requires time and preparation. Collectively these factors make ex vivo heart perfusion and transplantation more expensive than the cold static storage counterpart. However, holistically speaking, the potential reduction in costs due to fewer instances of recurrent surgery stemming from ischemic injury or primary graft failure should be considered in weighing cost benefit analyses of these two protocols. A paired study comparing two cases, one with cold static storage and the other with

normothermic ex vivo perfusion (NEVP) showed that although the NEVP protocol incurred higher retrieval expenditures than cold static storage, but these costs were offset by the reduction in follow up inpatient costs. The value of better surgical outcomes and an increased donor pool should also be considered in evaluating the protocols' financial burden on the healthcare system.[2, 26]

Perfusate/Metabolism/Cell Mo

By varying the perfusate and its additives, it is possible to increase the time spent ex vivo, increase the viability of the heart tissue, limit inflammation post-transplant and expand the donor pool even further. Celsior solution and University of Wisconsin are common perfusates used, especially in animal models. Between the two, Celsior has exhibited relatively higher oxygen consumption and glucose metabolism by the myocardium and decreased edema and reperfusion resistance compared to the University of Wisconsin solution. Both solutions however proved superior to statically preserved hearts in rat models.[27] A combination of hypothermic perfusion and oxygenated 4 degree Celsius Celsior solution showed successful heart preservation in animal models at 12 and 24 hours post explantation.[28] Rat and porcine models have explored the addition of erythropoietin (EPO), glyceryl trinitrate (a nitric oxide supplement) and zoniporide or cariporide (a sodium hydrogen exchange inhibitor), to hypothermic Celsior solution and reported dose dependent improvements in myocardial recovery after 6 hours of ischemia.[29 – 31]

After a donor heart has been implanted, there is a natural inflammatory response in the recipient body as it has just undergone significant trauma. Inflammation can have adverse effects and harm the native and foreign tissue, however varying the pulsate formula has been shown to limit the host inflammatory response. Perfusate that has been fortified with mitomycin C, pyrazolotraizine derivatives and cariporide have been shown to reduce post-transplant inflammatory responses and inhibit intracellular sodium and calcium build up. Collectively these factors have shown to promote tissue recovery after reperfusion injury and limit the damaging effects of inflammation, and as a result have shown improved tissue functionality and recovery post-surgery.[32 – 34]

Ex vivo perfusion has opened the floodgates with regards to the optimal perfusate and in doing so has also allowed for exploration of using pharmacological interventions to limit ischemic injuries and promote myocardial viability.[2] Perfusates can also be altered to include nutritional supplements so that not only are old metabolic wastes washed away, but fresh nutrients and sources of energy can be provided to the tissue so that it can sustain aerobic metabolism.[7] Adding oxygen to the perfusate allows for aerobic metabolism to continue but also increases the likelihood of superoxide radical formation which can compromise the integrity of the cell membrane. Administration of superoxide dismutase and other enzymes that regulate free radicals facilitate tissue integrity. Similarly, incorporating vasodilators such as nitric oxide and sodium nitroprusside help establish perfusion of all the tissue. This is particularly important, since the harvesting process can be traumatic on the donor organ and a reduction in endothelial nitric oxide takes place, making it harder for homogenous tissue perfusion to take place using machine perfusion techniques. Incorporating vasodilators can help ameliorate that process. Other components of the perfusate that have been experimented with include albumin to reduce edema, hypocalcemic solutions to prevent intracellular accumulation, magnesium cation to maintain the

integrity of the myocardial mitochondria and lidocaine to stabilize conduction through the tissue and stabilize the membrane potential.[7, 9]

There have been some efforts to use blood as the perfusate, but issues arise with obtaining adequate blood volume from the donor and the efficacy of the blood under hypothermic conditions. Concerns with inflammatory responses and activation of coagulation factors in using donor blood exist and how these factors would play a role in tissue preservation and host rejection.[7] Nonetheless, canine models have showed that reperfusion of excised heart with warm blood have a higher likelihood of successfully being weaned off cardiopulmonary bypass and to resume beating relative to cold static storage.[35]

Hypothermic and Normothermic Perfusion

Aside from considering the constitution of the perfusate in ex vivo perfusion, the optimum temperature of the perfusate should also be considered. Currently, hypothermic perfusate is the most prevalent protocol for the perfusate. It has been documented that mild hypothermia (32 – 35 degrees Celsius) is protective against myocardial infarctions post ischemic conditions.[36]

The rationale behind using hypothermic conditions is to protect the organs of the body from ischemic conditions and subsequent injury. Cooling the body and the perfusate reduces the metabolic rate and the oxygen consumption to free radicals and toxic metabolites form in smaller concentrations due to reduced flux through metabolic pathways.[37] However, use of hypothermic conditions also can compromise the three-dimensional cellular structure and the tissue structure, effect enzymatic functions and reduce the ability to generate energy which can lead to apoptotic conditions.[37]

Hypothermic perfusion studies have shown to be successful in the transport of extended criteria hearts (typically hearts that are older or come from donors with other disease). The continuous perfusion allows maintenance of aerobic metabolism and limits ischemic conditions which are less tolerated by donor hearts that are older. Continuous perfusion also helps these organs by providing them with a supply of metabolites which alleviates the stress of the donor heart relying on its own stores (which can be limited in the extended criteria organs). This effectively increases the donor pool not only by increasing the radius from where they can be retrieved, but also relaxes the criteria for a donor organ.[9, 37]

Normothermic perfusion closely mimics physiologic conditions and thus provides an environment most native to the donor heart. The use of normothermic or warm blood perfusion has been shown to be superior to the traditional crystalloid based perfusions used to induce cardioplegia.[38] Furthermore, studies have demonstrated that warm blood circulated through the donor heart tissue can be safely done, however interruption to warm perfusion can result in warm ischemic conditions which often results in myocardial infarctions.[39, 40] For ex vivo perfusion and heart transplantation this is problematic when the heart is being both explanted and implanted as there is a natural interruption of the perfusion during these times, raising the potential for adverse outcomes and heart attacks.

Clinical trials using normothermic solutions used for perfusion and cardioplegic solutions during

cardiopulmonary bypass reported lower rates of infarction and higher rates of spontaneous defibrillation after removing the aortic cross clamping relative to the hypothermic procedures.[41] A concern for switching to normothermic solutions is the potential loss of neuroprotective capabilities of the hypothermic solutions. These results are inconclusive since other clinicians have observed in clinical trials that there was no significant difference in cognitive performance post-surgery between hypothermic and normothermic solutions.[41]

A review of 6731 patients undergoing heart surgery, compared the risks and benefits of hypothermic and normothermic cardiopulmonary bypass. They concluded that there is no difference in the risk of stroke, cognitive decline, atrial fibrillation, myocardial infarction, or acute kidney injury between these two methods.[42] They further stated that using normothermia during cardiopulmonary bypass is just as safe as using hypothermic conditions during cardiac surgery and even offer a reduced risk of experiencing allogeneic blood transfusions in normothermic conditions.[42]

Stem Cells

Ex vivo perfusion allows novel approaches to treating donor hearts, specifically with regards to mesenchymal stem cell therapy and studies have determined that use of lineage negative bone marrow stem cells locally delivered on infarcted cardiac tissue resulted in de novo myocardium formation in murine models.[43] In vivo stem cell treatments are diluted, washed out and circulated throughout the body, preventing the stem cells to properly adhere to and occupy the target tissue. With ex vivo perfusion, the blood or perfusate is continually being recycled into the same organ, as such high dose stem cell concoctions can be delivered with greater efficacy.[2] One of the goals of stem cell therapy in heart transplantation is to restore donor hearts that have been damaged due to myocardial infarctions, limit left ventricular remodeling and restore lost cardiomyocytes. Not only is ex vivo treatment with stem cells more feasible, but ex vivo delivery of transgenes and modified stem cells also attainable. Like stem cells, transgenes are at risk of being washed out and diluted if given in vivo or taken up by non-target cells resulting in tumors, hence it is preferred for a higher concentration to be administered in a smaller circuit in the ex vivo setting to overcome this.[44] Cardiomyocytes are generally resistant to transfection protocols, however recent work in rat and porcine models have shown promise in delivery of the transgenes as well as uptake and production of the gene products of these transgenes. Specifically, vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2). Ex vivo local administrations has limited the likelihood of tumor formation.[45, 46]

Conclusion

There is no doubt that there is a huge shortage of donor hearts compared to the long waiting list of recipients. Ex vivo perfusion would vastly improve this gap between the diminishing supply and the increasing demand. Ex vivo heart perfusion would effectively increase the retrieval radius for a heart, lower the restrictive criteria for acceptable hearts and with the volume of research being dedicated to creating the ideal perfusate, the prospect of receiving hearts with minimal damage and trauma is becoming increasingly feasible. Novel approaches to stem cell therapy and inducing protective cellular mechanisms are easier with the ex vivo perfusion protocol compared to the cold static storage protocol. There are numerous benefits to using this method for organ transport and transplant and as the protocol is further optimized, it will become

increasingly apparent that ex vivo organ perfusion for heart transplants is the logical next step in improving the organ crisis for patients and health care providers.

Disclosures

There are no disclosures or conflicts of interest.

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