The Basic Science Aspect of Donor Heart Preservation: A Review

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Abstract: In cardiac transplantation, the transport time between harvest and recipient is limited by the viability of the donor heart. The problem of viability is a consistent limitation in cardiac transplantation. Since the 1960s, techniques, including hypothermia, perfusion, oxygenation, and hyperbaria, have been used to prolong the preservation of the transplantable heart. Continuing development of cardioplegic solutions has minimized edema and oxygen radical formation, which have resulted in extension of the donor heart viability. New research into the events leading to necrosis, oncosis, and apoptosis may allow further advancement of protective cardioplegic solutions in combination with technology of transporting the heart. With a prolonged preservation time there is potential to increase the donor pool and ultimately improve post-operative outcomes. Key words: oxygen radicals, myocardial edema, transplantation, apoptosis, cardiac.

Soon after the first heart transplantations were performed in the 1960s by Christiaan Barnard and Norman Shumway, the race was on to develop the optimal solutions for donor heart preservation. Cardioplegic solutions are supplemented with various metabolic precursors to prevent necrosis and oncotic agents to prevent edema formation. The composition of commonly used solutions today address problems related to cellular metabolism in the context of organ ischemia, cold storage, and reperfusion. In addition, programmed cell death (apoptosis) may be an important factor in ischemic donor hearts and an active area of research. Additional techniques have been developed to extend donor heart preservation, such as: continuous or intermittent cardioplegic flow, oxygenation, hyperbaric chambers, and the working heart.

METABOLITE CHANGES IN THE DONOR HEART

Normally, a balance exists in cellular metabolism that maintains an active supply of energy to meet the respective consumption. After heart procurement for transplantation, the heart becomes ischemic and the energy supply is diminished. Metabolites are formed in the cell and the primary energy source, adenosine triphosphate (ATP), is depleted in catabolic action.

As a product of ATP catabolism, adenine nucleotide metabolites such as adenosine, inosine, hypoxanthine, and xanthine are generated. These metabolites, called dephosphorylated purines, can permeate through the cell membrane as illustrated in Figure 1. As a result of the ATP catabolism, there exists a drain on adenine nucleotides and without their replenishment into the Cori cycle, the electron transport chain fails and cell necrosis occurs.

Necrosis that occurs with prolonged hypothermic storage is pronounced in organs with a high metabolism, such as the heart. Prevention of cell necrosis is important. Experiments with kidney perfusate solutions at the University of Wisconsin have shown that ATP levels remained higher with adenine and ribose supplemented solutions (1). The conclusion of the experiments was that the hypothermic solutions used to preserve the kidneys provided a constant source of nucleotides for maintenance of ATP levels. The benefit of nucleotide supplementation is that it theoretically serves as a substrate source for ATP catabolism during storage and upon rewarming of kidneys; thus,
it prevents an energy crisis due to a rapid increase in mitochondrial oxidative phosphorylation.

**SEQUELAE OF CARDIOMYOCYTE ENERGY DEPLETION**

ATP plays a central role by providing a source of energy to stabilize the outer cellular membrane as well as the sarcoplasmic reticulum in the myocardium. Without ATP, outer cellular membrane pumps such as the Na⁺/K⁺ ATPase are paralyzed and failure of those pumps allows Na⁺ to accumulate in the cytoplasm causing increased cell osmolarity and phospholipid breakdown. Water then follows the osmotic gradient and causes the cell to swell and rupture. Furthermore, ATP is also the primary energy source used by the sarcoplasmic reticulum to sequester Ca²⁺ via the Ca²⁺/ATPase membrane pump. Depletion of ATP leads to the release of the sequestered Ca²⁺, which is known to activate intracellular lipases such as phospholipase A₂ and lysophospholipids in the process of necrosis (2).

An increase in intracellular calcium also decreases overall protein synthesis and activates calcium dependent proteases such as calpain. Normally, xanthine dehydrogenase enzymatically converts xanthine to uric acid, as illustrated in Figure 1. However, in the presence of calpain, xanthine dehydrogenase is converted to xanthine oxidase (Figure 2). This functional change is detrimental to the integrity of the cell because xanthine oxidase catalyzes the conversion of xanthine with cytotoxic byproduct: superoxide anion (·O₂). In side reactions, superoxide anion can oxidize surrounding proteins and phospholipids.

Superoxide anion (·O₂⁻) is created as a byproduct of normal mitochondrial energy production. Superoxide anion oxidizes critical cellular enzymes, nucleic acids, and cellular membranes. This cytotoxicity of superoxide anion is normally mitigated by the enzymatic action of superoxide dismutase which creates the slightly less toxic product of hydrogen peroxide (H₂O₂). In the presence of transition metal ions (Fe²⁺), hydrogen peroxide is then catalyzed to hydroxyl radicals (·HO⁻) that can also damage the cell. Protection from the damage caused by these products can be achieved by maintenance of redox precursors such as glutathione in cardioplegic solutions. Glutathione is used by the enzyme glutathione peroxidase to convert H₂O₂ to the non-reactive molecules of H₂O and O₂ (Figure 3).

**CARDIAC APOPTOSIS**

The etiology behind cardiomyocyte death during donor heart storage is complex and likely involves other mechanisms such as apoptosis. Apoptosis is usually associated with pathophysiological situations of noncardiac cell death (cancer, embryonic development, aging), however an increasing body of evidence exists for the role apoptosis plays in myocardial ischemia and heart failure (3). Apoptosis is classically defined as programmed cell death. The cell undergoes a suicidal process that preserves the mitochondria and sarcolemmal membranes. In this regular process, the nuclear chromatin condenses (pyknosis); the cell shrinks; the nucleus fragments (karyorhexis); and the

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**Figure 1.** Drainage of membrane-permeable metabolites to the extracellular space. Intracellular ATP catabolism produces membrane permeable metabolites that drain out from the cell. Of those metabolites, xanthine is converted to the nonpermeable uric acid.

**Figure 2.** Reaction pathway of free calcium to activated superoxide anion. Calcium that is released from the sarcoplasmic reticulum activates the protease calpain, which then converts xanthine dehydrogenase to xanthine oxidase. Superoxide, a byproduct from the oxidation of xanthine is produced which is cytotoxic.

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Xanthine + H₂O → Xanthine oxidase

Ca²⁺ + Calpain + xanthine dehydrogenase → xanthine oxidase
DNA are cleaved at uniform lengths. While in oncosis, cells swell edematously; membranes budding occurs early; chromatin clumps irregularly; and the mitochondria are randomly damaged. Both pathways of oncosis and apoptosis lead to cell death and irreversible changes afterwards (necrosis).

Unfortunately, the role of apoptosis in cardiac cell death is not completely elucidated in the ischemic myocardium (4). The apoptotic cascade consists of an undetermined death signal and other factors occurring at the same time. Several studies have shown that acidosis, reoxygenation, and reperfusion are necessary for induction of apoptosis and that hydrogen peroxide and free radicals can indeed initiate the apoptotic cascade (5).

Methods to identify apoptotic cells include DNA staining for terminal deoxynucleotidyl transferase mediated dUTP-biotin nick end labeling (TUNEL). The TUNEL method detects single- and double-strand DNA fragments with a 3'-OH terminal end. TUNEL positive staining indicates late stages of apoptosis because the DNA damage already has occurred. Another marker of apoptosis is the cleavage of a DNA repair enzyme PARP (poly ADP-ribose polymerase). Cleavage of PARP prevents repair of ongoing DNA damage by activated apoptotic enzymes. An apoptotic cell also releases mitochondrial contents such as cytochrome c and caspases into the cytosol, furthering self-destruction. Information on apoptosis and its relation to necrosis is widely available (6); however, the study of apoptosis in cardiomyocytes is an important area that deserves further exploration.

DONOR HEART HYPOTHERMIA

In general, hypothermia of the arrested ischemic heart prolongs its preservation and improves its function when compared with the normothermic arrested ischemic heart. In addition, hypothermic blood cardioplegia as used in bypass surgery, appears to be advantageous when compared with warm blood cardioplegia in that the hypothermia is thought to have a protective effect on the microvasculature (7).

Currently there is an active debate on whether or not warm cardioplegic solutions are equivalent to cold cardioplegic solutions for surgery (8). In prospective randomized clinical trials warm cardioplegic solutions have had satisfactory outcomes for coronary artery bypass surgery with an aortic cross-clamp time shorter than 60 min (9–11). However, the time spans involved in coronary artery bypass surgery are less than what is involved in organ preservation. On the other hand, cold cardioplegic solutions are associated with improved outcomes for cardiac surgery requiring aortic cross clamp time lasting greater than 60 min (12,13). In the animal model, continuous retrograde infusion of warm blood cardioplegia in 3 h of simulated bypass surgery did not prevent ischemic related changes (14). Even though there is an active debate about the best method of cardiac protection, hypothermia continues to be used for preservation of the donor heart.

The hypothermic cold storage method has been very successful and reproducible since the beginning of human heart transplantation in the 1970s.

The "4°C" hypothermic temperature generally leads to reduced metabolic demands. However, cold storage is associated with endothelial dysfunction. In a preservation study, hearts stored in an antioxidant cardioplegic solution called Celsior for up to 30 h. They were compared with hearts stored in solutions not containing the antioxidants. Celsior preserved hearts had significantly improved endothelial function (15). There may be a relationship between improved endothelial function and antioxidant-containing solutions. The chemical reaction pathway that produces nitrites (\(\cdot\)NO2), nitrates (\(\cdot\)NO3), and hydroxyl radicals (\(\cdot\)OH) from endothelial relaxing factor (NO•) is blocked (16–18).

During cold storage, nitrites (\(\cdot\)NO2) as well as hydroxyl radicals (\(\cdot\)OH) may cause cellular damage via the mechanisms as illustrated in Figure 4. Endothelial relaxing factor
and its oxidation within the endothelial cell may determine a manifestation of post cardioplegia contractile dysfunction secondary to ischemia and reperfusion. This “stunning” of the myocardium is thought to be due to a burst of oxygen radicals upon perfusion of the heart with oxygenated blood after an ischemic period. The oxygenated blood may provide enough molecular oxygen as a reagent in catalysis by tissue oxidases to produce cytotoxic metabolites such as the nitrates and hydroxyl radicals that further degrade the donor heart.

**MYOCARDIAL EDEMA**

At the cellular level, membrane disruption from free radical lipid peroxidation and membrane protein damage is the primary cause for the influx of ions as well as water that follows the osmotic gradient. This osmotic influx of water into the cell is a possible cause of myocardial edema, noticed in hearts after ischemic conditions and reperfusion.

Myocardial edema formation is thought to be due to an in vivo “reperfusion injury” (19) that occurs after regional and global ischemia and subsequent restoration of oxygenated blood flow. Because metabolically active cells continue to use up vital metabolic precursors (i.e., ATP, creatine phosphate) during the ischemic period, demand for energy can not be matched by the lack of continuous supply of nutrients. The re-establishment blood flow and oxygen provides a resource for cellular oxidation and subsequent free radical formation leading to cellular damage (20).

Myocardial edema formation also occurs in situ with ischemic hearts preserved with coronary perfusion during cold storage (21). The edema formation under this condition is not related to “reperfusion injury” because the edema is formed prior to the re-establishment (reperfusion) of normal blood flow. In an attempt to prevent the problem of edema formation, impermeable solutes have been added to some cardioplegic solutions. The addition of impermeable solutes, such as mannitol, albumen, or lactobionate was performed to ameliorate edema formation. Unfortunately, balancing the oncotic pressures is not as effective as it is originally intended. Experiments have shown that the addition of impermeable solutes as adjuncts in cardioplegic solutions does not reduce the edema formation (22,23).

Another hypothesized mechanism for edema formation in ischemic hearts focuses on the generalized nutrient and energy depletion. This depletion is thought to cause free radical formation damaging cellular membranes. The membrane damage then leads to increased permeability to extracellular fluid. One method that has been used commonly in clinical cardiac surgery involves using whole oxygenated blood to provide an adequate source of oxygen and nutrients to the myocardium. The hypothesis of normothermic continuous antegrade oxygenated blood cardioplegia to prevent edema formation has been proposed and tested. Theoretically the whole oxygenated blood at normothermic conditions should serve as a cardioplegic solution that addresses every possible energy supply variable. Unfortunately results from a series of experiments demonstrated that using the whole blood cardioplegia in fact does not necessarily prevent myocardial edema (24).

A final mechanism of myocardial edema formation is the prolonged diastole of the arrested heart, which impairs myocardial lymph removal (25). Evidence of impaired lymphatic drainage of the heart in asystole is documented histologically and has been associated dysfunction of the heart (26). It is also evident that repeated coronary artery perfusions in the normothermic non-beating heart are also associated with progressive heart weight increases (27).

### ADDITIONAL METHODS TO PROLONG DONOR HEART VIABILITY

The use of the donor heart has been limited by the ischemic time of 4–6 h. This time limitation places critical

<table>
<thead>
<tr>
<th>Study</th>
<th>Model</th>
<th>Storage Time</th>
<th>Type of Perfusate</th>
<th>Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Watson (28)</td>
<td>Foxhound</td>
<td>24 h</td>
<td>Oxygenated solution</td>
<td>N/a</td>
</tr>
<tr>
<td>Wicomb (21)</td>
<td>Baboon</td>
<td>24 h</td>
<td>Oxygenated solution</td>
<td>Continuous 60–120 mL/min</td>
</tr>
<tr>
<td>Wicomb (29)</td>
<td>Human</td>
<td>7–15 h</td>
<td>Oxygenated solution</td>
<td>Continuous 60–120 mL/min</td>
</tr>
<tr>
<td>Qayumi (30)</td>
<td>Swine</td>
<td>5 h</td>
<td>Oxygenated solution</td>
<td>N/a</td>
</tr>
<tr>
<td>Kitamura (31)</td>
<td>Canine</td>
<td>5 h</td>
<td>UW solution</td>
<td>Continuous 3–6 mL/min</td>
</tr>
<tr>
<td>Calhoon (32)</td>
<td>Canine</td>
<td>12 h</td>
<td>Oxygenated UW solution</td>
<td>Continuous 0.22–0.39 mL/min/g</td>
</tr>
<tr>
<td>Hill (33)</td>
<td>Human</td>
<td>3 h</td>
<td>Cardiosol I</td>
<td>Continuous 86 mL/min</td>
</tr>
<tr>
<td>Oshima (34)</td>
<td>Canine</td>
<td>12 h</td>
<td>UW solution</td>
<td>Continuous 35–50 mL/h</td>
</tr>
<tr>
<td>Tsutsumi (35)</td>
<td>Canine</td>
<td>24 h</td>
<td>Oxygenated celsior</td>
<td>Continuous 30–40 mL/h</td>
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</tbody>
</table>
have been proposed (36). Various methods of preserving donor hearts for transplantation have been proposed in addition to the current method of heart procurement and storage. The relatively short time frame limits the distance the donor heart can be transported geographically. Business jets are used by organ procurement organizations in an attempt to maximize the distance traveled, however the maximum range is usually limited to a maximum of approximately 1000 miles. A donor heart that is transplanted beyond the 4- to 6-h limit may have abnormal inotropic and chronotropic function yielding a challenging postoperative course for the recipient. Any successful method to prolong the preservation of donor hearts will benefit the entire process of cardiac transplantation. An example would be that improved preservation may allow transplant surgeons the time to critically assess “marginal” hearts not used routinely used for transplantation.

Various methods of preserving donor hearts for transplantation have been proposed in addition to the current method of heart procurement and storage. They are available for clinical or research use (Table 1) (28–35). The methods primarily involve a selection of perfusion, oxygenation, and temperature. Perfusion of the stored heart can be done with a constant flow or intermittent flow of cardioplegic solution. Several benefits of an intermittent flow have been proposed (36–39). One of them is that intermittent flow may allow the myocardium/endothelium to relax between perfusion cycles thus delaying the rate of edema formation (7). On the other hand, a constant flow can be readily maintained by a pump set at a given rate. Donor hearts preserved up to 12–24 h with this method have been successfully implanted mainly in animals (Table 1). Despite the edema formation using a constant rate pump, transplantation hearts stored for 12–24 h have been successfully achieved using this particular method in animal models. The concern for this method is that constant flow may cause more edema formation (40). Oxygenation for heart preservation is possible using an oxygenated perfusate or in a hyperbaric chamber. The use of hollow-fiber membrane oxygenator now supplants the bubbler to raise the partial pressure of oxygen in crystalloid-, blood-, or oxygen-carrying solutions. Hyperbaric chambers can be used to increase the partial pressure of oxygen in the perfusate but their use is also limited. Experiments with normothermic perfusion of the portable isolated working heart using an oxygenated blood solution have been tried, but bacterial growth in the normothermic temperature is a serious problem (41). Unfortunately, studies to compare single perfusion methods vs. a combination of methods to preserve donor hearts are limited because of the high costs of conducting large-scale randomized studies to achieve statistically significant conclusions. However, experiments have shown that the heart stored up to 24 h is possible and survival after transplantation is achievable (Table 2).

**SUMMARY**

One of the greatest challenges in heart transplantation is how to preserve the donor heart so that the recipient receives a healthy heart that is a functioning replacement. The metabolism of the heart limits the procurement time today to 4–6 h before the heart undergoes excessive loss of ATP, production of oxygen radicals, and irreversible destruction by apoptosis, oncosis, and necrosis. Methods to prolong the preservation include hypothermia, oxygenation, and perfusion. Renewed interest to improve preservation of the heart comes from a critical lack of suitable donors and national effort to maximize donor organs. Ongoing improvements in technology and basic science research, hopefully, will lead to new breakthroughs in donor heart preservation, especially in the area of expanding the donor pool.

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