Remifentanil and Desflurane as a Myocardial Protective Regimen for Donor Hearts in the Perioperative Procurement Phase of the Brain-dead Patient

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Abstract

The proposed experiment will consist of surgical heart-transplants between a healthy adult rat and an adult rat with a life history of dilated cardiomyopathy to test organ-protective effectiveness of anesthetics for heart-transplant. During the procurement phase of the heart-transplant procedure, four independent conditioning regimes will be applied to the donor heart with: desflurane, remifentanil, desflurane and remifentanil or a regime of no anesthetic conditioning. Each regime will be tested on their efficacy by data analysis of the length of life after the procedure as well as the amount of cell damage markers following the procedure to determine which treatment is best effective in preserving the long-term survival of the rats with the anesthetically preconditioned donated heart. The proposed research is taken as a first step towards utilizing the organ protective effects of anesthetics to an organ procurement procedure that will benefit patients receiving an organ transplant.

Keywords: heart-transplant, pre-conditioning, desflurane, remifentanil, organ procurement

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Chapter I: Introduction

In surgery, a patient is placed under "controlled trauma" where the surgical team manipulates the body in hopes of improving the quality of life of the patient while the anesthesia team serves to sustain the patient and preserve the vitals of the patient. It is known that anesthetic drugs can serve as tools for this preservation by their organ protective properties by different mechanisms (Oei, Weber, Hollmann & Preckel, 2010). These protective properties can be especially useful in the special case of heart transplant surgeries because it is of the utmost importance to maintain organ function from the transfer between the donor to the acceptor patient. There are many anesthetic drugs in use today; and exploring combinations of these drugs and creating a regimen that maximizes myocardial protection is the goal of this study to provide better organ acceptance and functionality for ultimately a better quality of life for the accepting patient.

There is currently little research on the management of organ procurement for brain-dead donor patients and on the beneficial protective properties of applying anesthetic agents on these patient's organs (Elkins, 2010). Current research points to inhaled anesthetic gases and analgesic drug therapies to have protection against ischemic-reperfusion injury. It has been demonstrated that opioids (Tsutsumi, Kawaraguchi, Niesman, Patel & Roth, 2010) especially remifentanil protects the myocardium when used as a preconditioning agent to prevent cellular death (Kim et. al., 2010; Xu et. al., 2009). Desflurane has been shown to have myocardial protective properties when used in certain time windows as a preconditioning agent (Smul et. al., 2010). Combining these two anesthetic agents can potentially be applied to precondition the donor heart because of their beneficial properties and improve long-term outcomes of the acceptor patient's new heart.

There is only one viable heart in these procedures and it is important to keep the organ as preserved as possible.

Statement of Problem

The purpose of this study is to identify the myocardial protecting effects of remifentanil and desflurane on the brain-dead donor heart for long-term heart transplant success.

Significance of the Study

In 2005 there were about 89,000 patients that were in need of donor organs. Even when the organ accepting patients were stabilized and prepared for transplant, 17% to 25% of the donor patients ended up having unsuitable organs for transplant. These organ donors are brain-dead and in the procurement procedure only receive fluid resuscitation and hormonal therapy to stabilize and protect the donor organs (Elkins, 2010).

Heart transplantation is the third most common transplant procedure in the US behind liver and kidney transplants with a five-year success rate for heart transplants is about 73.1% for males and 69.0% for females. The greatest limiting factor to the procedure is the amount of available hearts for transplanting because of the scarcity of viable donor hearts. It is paramount to preserve these hearts long-term because more patients are in a situation needing a transplanted heart with rising trends of heart disease (American Heart Association, 2011).

Mice organ transplantation is the most commonly used model for transplant immunology and it is economically sensible to continue the use of this model for research into heart transplant organ conditioning (Babany, Morris, Babany, Morris & Kates, 1989). Previous research implemented anesthetic agents for preconditioning myocardial protection to prevent ischemic damage in rat hearts and the next step is to apply it to transplanted hearts. This study will consist of a population of paired mice with compatible cross-matched hearts. Desflurane and

remifentanil will be applied to the donor mice during the procurement phase and a heart transplant will be carried out to see if there is improvement in long-term function from the pharmacological regime. Long-term function will be assessed by total life-span after the procedure and testing of serum myocardial damage markers.

Delimitations

1. The study will have 120 mature mice participants (9-10 weeks after birth) with 60 pairs of mice. In each pair of mice, one mouse will be healthy (donor group) with brain and spinal-cord trauma to induce brain-damage and another mouse will have a given genetic dilated cardiomyopathy (acceptor group) which simulates the heart-failure human patient in need of a new heart (Ikeda & Ross, 2000). The pair of mice will be compatibly heterogeneously cross-matched.

2. Each pair will be randomly placed in one of four groups: (a) A control with a normal heart transplant given only standard cardiothoracic donor management, (b) donor preconditioned with a combination of desflurane and remifentanil, (c) donor preconditioned only with remifentanil and (d) donor preconditioned only with desflurane. There will be 15 pairs in each group each given with an even distribution of gender between groups. Each donor patient will receive at least standard cardiothoracic donor management and titrated rocuronium.

3. Desflurane will be administered in a constant 1.0 MAC for all donor patients receiving desflurane (Smul et. al., 2010). Remifentanil will be administered in 6 ug kg^{-1} min^{-1} doses for all donor patients receiving remifentanil (Kim et. al., 2010).

4. Preconditioning will be carried out prior to the transplant procedure as needed, and a heart transplant will be carried out by a single skilled surgeon.

5. Long-term assessment of the myocardium will be tested immediately after the transplant with follow-up weekly serum CK/CK-MB ratio and Troponin-1 marker tests as well as total life-span of the acceptor mouse following the procedure including determination of cause of death.

Limitations

1. The skill and consistency of the surgeon conducting the heart transplant may affect the transplant procedure. The number of procedures will eliminate this variation.

2. Mice must be heterogeneously matched between donor and acceptor group mice and potential spontaneous organ rejection may still occur and the mice may not survive for long-term assessment.

3. Mice given the congenital cardiomyopathy may not survive far into maturity without the transplant. The mice must be mature at the time of transplant to simulate adult patients needing transplant as well as minimizing variations within juvenile development.

4. If a co-existing disease accompanies the mouse systemically it may alter the life-span of the mouse. The mouse cannot have any non-cardiac disease accompanying when the heart transplant is being conducted.

5. In measuring the amount of time until demise, the end-stage cause of death may not be due to cardiac reasons. The amount of subjects mitigates this and may uncover trends in cause of death from heart transplant procedures.

Assumptions

1. The congenital cardiomyopathy in the mouse heart simulates a patient in heart failure that is similar to the failing hearts of humans needing a heart transplant.

2. The donor mouse heart represents the normal healthy donor human patient that receives nervous system damage to become brain-dead.

3. The acceptor mouse group behavior after transplant is consistent with all mice in terms of feeding, drinking and exercise habits.

4. The decided CK/CK-MB and troponin-1 tests accurately correlate to myocardial viability and eventual long-term transplant success.

5. The end cause of death is ultimately caused in part by cardiac transplant related reasons and not a non-physiological cause.

Hypothesis

With heart transplants it is vital to protect as much myocardial tissue as possible to provide long term survival for the organ-acceptor patient; this can be difficult and unreliable using non-pharmacological support. Based on the recent current research support that remifentanil and desflurane can protect myocardial cells from ischemic damage through preconditioning, the following hypothesis is proposed: If desflurane and remifentanil are applied to the donor heart during the procurement phase of heart transplantation, then myocardial protection will be greater than in a donor heart receiving no anesthetic preconditioning. Desflurane alone or remifentanil alone will improve long-term heart transplant success over a normal heart transplant procedure without preconditioning but not more than the combination regime of desflurane and remifentanil applied together to the donor heart.

Definition of Terms

1. Brain-dead – In this experiment, brain-dead is a state of non communicable brain induced by controlled nervous system damage through spinal cord and brain trauma.

2. CK – Total creatine kinase test measures the amount of creatine in the blood released my muscles and heart cells due to cellular damage.

3. CK-MB – Creatine kinase MB is a specific CK test for myocardial cell damage.

4. CK/CK-MB Ratio – Ratio of total CK to CK-MB of 2.5-3.0 represents myocardial damage and is used to assess damage due to myocardial infarctions.

5. Desflurane – Desflurane is a general inhaled anesthetic with human 1.0 MAC of 6.0vol% and mouse MAC varies by mouse strain and a 1.0 MAC will be considered 7.5vol% (Sonner, Gong, Li, Eger, & Laster, 1999).

6. Minimum Alveolar Concentration (MAC) – Minimum alveolar concentration is the percent concentration of anesthetic gas needed to suppress movement in response to surgical stimuli in 50% of patients.

7. Procurement phase – In this experiment, the procurement phase is defined as the time before harvesting the organs and after inducing brain death in the donor group.

8. Remifentanil - a potent opioid analgesic used in anesthetic technique, 0.2 - 0.8 ug kg^{-1} min^{-1} is the standard dose (Smul et. al., 2010).

9. Rocuronium – A muscle relaxant used in surgery to prevent movement. Dose will be administered titrated to a train of four count of 0.

10. Standard Cardiothoracic Donor Management – As recommended by the United Network of Organ Sharing (UNOS), recommended hormonal therapy used in the donor patient consisting of fluid and various hormones (see Figure 1).

11. Troponin-1 – Troponin-1 serum test for myocardial damage due to myocardial infarctions, 10 ug L^{-1} signifies myocardial damage.

Chapter II: Literature Review

Introduction

It has been shown that pharmacological agents used in anesthesia can provide protection from ischemic damage in addition to their analgesic and general sedative properties. These anesthetics often exert their benefit on organs that receive a limited supply of blood such as the spinal cord or organs that utilize a great amount of oxygen such as the brain or heart (Ding et. al., 2009). The purpose of this experiment is to utilize the protective properties of anesthetic pharmacological agents in order to protect the heart in a heart transplant procedure from a donor to acceptor patient.

Recent research uncovered the myocardial protective properties of anesthetics desflurane and remifentanil in the animal model when placing the heart under ischemia and reperfusion. In these experiments myocardial damage markers and cell death size is reduced (Xu et. al., 2009). This benefit can be directly applied to the heart of the donor patient in the proposed experiment and translate to better long-term outcomes.

The previous research experiments established in literature will be explored and applied to the proposed experiment based on the maximum benefit of desflurane and remifentanil on myocardial cells. Both desflurane and remifentanil have been experimented on to provide data on their organ protective properties; and a critical analysis of these previous experiments is developed to reveal the peak benefits of both drugs that can be adopted for the heart-transplant research study. Both drugs will be assessed for the compatibility and application in improving the outcomes for heart-transplant surgery.

Previous Experiments.

Desflurane experiments. Desflurane is an inhaled general anesthetic utilized for cardio-protection through ischemic protection of the myocardium. It is usually applied through positive pressure ventilation and can be used in conjunction with intravenous agents such as remifentanil. The following studies utilize desflurane and present their benefit as a preconditioning agent.

Time window application of desflurane. Smul et. al. (2010) conducted an experiment on 75 rabbits by administering 1.0 minimum alveolar concentration (MAC) desflurane for 30 minutes then discontinuing for a period of time (0.5, 2.0, 3.0, 24.0, 48.0, 72.0 and 96.0 hours). The rabbits were then subjected to 30 minutes of ischemia through coronary artery occlusion then reperfusion therapy for 3 hours after. For 3.0, 12.0 and 96.0 hours of discontinuation time, infarct size was not significantly reduced; while for 0.5, 2.0, 24.0, 48.0 and 72.0 hours infarct size was significantly reduced. Desflurane therefore has two ideal preconditioning time windows of 0.5-2.0 hours and 24.0-72.0 hours that prevent acute ischemic damage to the myocardium. This study has a strong experimental background because of the consideration of the time effects of the protective benefits from desflurane; the anesthetic time window is different from the protective time window because the anesthetic is quick onset and quick offset of effect.

These time windows seem to be important to follow in the proposed heart-transplant experiment. Applying desflurane within 0.5-2.0 hours before surgery for 30.0 minutes before the surgical heart procedure seems to be best time in economical sense rather than applying desflurane in the 24.0-72.0 hour time window. However, a weakness in the experiment by Smul et. al. (2010) was that the 30.0 minute application of desflurane seems to be arbitrarily picked

and was not explained in the methods; perhaps only 10.0 minutes or even 5.0 minutes of preconditioning is needed for the same effect.

In an adjunct experiment also performed by Smul et. al. (2010), nitric oxide synthase inhibitor N-omega-nitro-L-arginine (L-NA) was administered 72 hours after administering 1.0 MAC desflurane in the same perfusion/reperfusion experiment to determine that the myocardial preconditioning is mediated by nitric oxide (NO) when L-NA inhibited desflurane ischemic damage protection. This cellular mediated pathway for ischemic protection is only applied to the second window of preconditioning (24.0-72.0 hours). The chosen application of L-NA has a weakness in the study because NO is a biologically important paracrine signaling agent that is utilized in many other pathways that can be protective to the myocardium, however the study states this concern. The L-NA inhibitor only disabled the protective effects of desflurane for the long time window (24.0-72.0 hours) and may not affect the heart-transplant experiment. The mediated NO pathway only explains the mechanism for the second time window and fails to explain the mechanism for the first time window that will be utilized in the heart-transplant study. This can be answered through a study done by the same group in the following study analysis.

Repetitive administration of desflurane. Lange et. al. (2009) tested the repetitive application of desflurane and its action on β2 adrenergic receptors also mediate myocardial cell injury during ischemia.

96 rabbits were randomly placed in different desflurane preconditioning groups: desflurane continuously for 30 minutes at 0.5, 1.0 or 1.5 MAC; continuously for 90 minutes at 0.5 or 1.5 MAC; repetitively for three 10 minute periods at 0.5, 1.0, or 1.5 MAC (with two 10 minute breaks in between). The rabbits were then subjected to 30.0 minutes of coronary artery

occlusion and then 3 hours of reperfusion after 30.0 minutes of waiting. The success of desflurane was assessed by the infarct size of the myocardium expressed by the percent area available to potentially be infracted. In controls with no desflurane preconditioning, infarct size was 61% +/- 5%. Continuous desflurane for 30.0 minutes at 0.5 MAC did not affect infarct size significantly; however 1.0 and 1.5 MAC reduced infarct size to 35% +/- 5% and 39% +/- 4%. Repetitive administration of desflurane resulted in infarct size 37% +/- 6% for 1.0 MAC and 29% +/- 4% for 1.5 MAC. The repetitive administration of desflurane and the 90 minute administration of desflurane reduced infarct size, but not significantly different than the continuous 30.0 minute regime of desflurane. ICI 118,551 (0.2mg/kg), a β2 adrenergic receptor blocker was also given by Lange et. al. (2009) against a normal saline placebo in this experiment to determine the mechanism of cardio-protection. ICI 118,551 given alone did not affect the infarct size of the control, but abolished the beneficial effects of 1.0 MAC desflurane continuous for 30.0 minutes to a 51% +/- 5% infarct size. β2 adrenergic receptors seem to be mediator for the protection against infarction and may explain the mechanism for the time window experiment done by Smul et. al. (2010) discussed earlier; however this idea can only be hypothesized at this point. Understanding the mechanism will help determine the effects the other agents will have in supporting myocardial protection and we can experiment to find the benefit as additive or synergistic.

It is noted that the study by Lange et. al. (2009) conducts the experiment using the 30.0 minute time window discussed earlier and doesn't establish if the effect of the longer time window of 24.0-72.0 hours is mediated by β2 adrenergic receptors as well. However, Smul et. al. (2010) already establishes that NO takes effect in that time window, but we can't determine if

it masks the action of β2 or if the effect of β2 is even present in that time window and a separate experiment may need to be conducted to verify this.

Between the studies by Lange et. al. (2009) and Smul et. al. (2010), the greatest sources of error in the experiments are not agreed upon in the use of desflurane preconditioning. Smul et. al. (2010) considers the anatomy of collateral coronary perfusion to provide variations in the control group infarct size to cause most of the differentiating data. Lange et. al. (2009) suggests that most of the possible error is caused by overall body metabolism and attributes changes based on the ages of heart with more physiological dependence as opposed to anatomical dependence. It can be hypothesized that both great sources of errors can be a combination of both body metabolism and variations in the coronary anatomy. The proposed experiment will mitigate both of these sources of error by conducting the heart-transplants at similar close age groups in the mice as well as assigning genetically similar participants over the different experimental groups.

Both experiments favor the benefit of 1.0 MAC of desflurane applied continuously and will be considered as the core method for applying desflurane on the brain-dead mouse to provide the best myocardial protection for heart-transplant. Lange et. al. (2009) effectively establishes desflurane to be effective administered either continuously or in repeated administrations. The data from the study by Lange et. al. (2009) is usable for the proposed experiment because it uncovers the mechanism behind the cardio-protection as well as the best way to administer desflurane through its many test groups. In applying the study to the proposed heart-transplant experiment, a continuous administration seems to be the best way to economically apply desflurane for the proposed benefit. A repeated administration approach to desflurane will be time consuming and not economically favored. A MAC of 1.0 seems to have

slightly better protection against infarction versus a MAC of 1.5 and uses resources more efficiently and is the reason for selection of 1.0 MAC for the proposed experiment.

Remifentanil experiments. Remifentanil is an ultra potent opioid used intra-operatively as an analgesic intra-operatively as a continuous infusion because it is short acting and commonly used in cardiac surgery for myocardial damage protection (Wong, Huang, Ji & Irwin, 2010). The proposed heart-transplant experiment will utilize remifentanil intravenously as a continuous infusion while desflurane discussed above will be administered by positive pressure ventilation. The following study establishes remifentanil as a myocardial protecting agent and uncovers the pathways it functions by.

Anti-apoptotic pathways activation from remifentanil. Kim et. al. (2010) explores the use of remifentanil and the effect on myocardial protection against ischemia using a rat model. In this experiment, 34 rats were randomly placed in one of five groups; (a) placed under 30 minutes of ischemia then re-perfusion (I/R), (b) remifentanil preconditioned and placed under I/R, (c) I/R then post conditioning, (d) continuous infusion during I/R and (e) a group where neither I/R nor remifentanil were introduced. Remifentanil preconditioning was done at 0.6 ug kg^{-1} min^{-1}.

Infarct size was assessed along with serum levels of pro-apoptotic protein, Bax and cytochrome C which signal cell death and can be attributed to destruction of myocardium. It was found that regardless of timing and duration of remifentanil application; expression of the signaling proteins was reduced showing a cardio-protective effect of remifentanil. Infarct size was assessed and remifentanil reduced infarct size from 40% to consistently around 20% as long as remifentanil was administered independent of administration duration. Protein ERK 1/2 and anti-apoptotic protein Bcl2 were found to be increased in expression in the remifentanil

conditioned rats and signaled that remifentanil is cardio-protective through anti-apoptotic pathways.

The experiment by Kim et. al. (2010) is an important basis for the application of remifentanil to protect the myocardium in the proposed heart-transplant procedure. The results of the experiment establish remifentanil as a cardio-protective pharmacological agent and shows that anti-apoptotic pathways are enhanced and pro-apoptotic pathway indicators are reduced. This uncovering of mechanism is important in combining remifentanil with desflurane for the protection of the heart tissue. As discussed previously, Lange et. al. (2009) found β2 adrenergic receptors to be the primary regulator for the proposed experiment and may be different than the cellular expressions of apoptotic pathways that remifentanil uses. We can infer that one of three things may happen when both pharmacological agents are applied to the myocardium at the same time: (a) both mechanisms cancel each other out and no benefit will arise from the combination; (b) both mechanisms will benefit independently and the protective effect will be maximally expressed by the best anesthetic agent; or (c) the two pathways will support each other in an additive protective effect and have more benefits than either agent alone or more benefit than no intervention at all against the control.

Kim et. al. (2010) found that as long as remifentanil is used to condition the heart, the protective effect will be expressed; the infusion of remifentanil is done at least 20.0 minutes in this experiment and no other lower time is considered. A lower application time of remifentanil could have been tested and selected if that conclusion is to be solidified. The experiment however does cover many of the common cellular expression markers for myocardial damage as well as many of the scenarios that remifentanil can be utilized in. For the proposed heart-transplant experiment, a 30.0 minute infusion of remifentanil will be utilized because it satisfies

the lowest 20.0 minute regime determined by Kim et. al. (2010) and will match up with the desflurane time this study will adopt from the Lange et. al. (2009) and Smul et. al. (2010) studies discussed earlier.

Application to Proposed Heart-Transplant Procedure

The studies analyzed above describe the usage of the anesthetics remifentanil and desflurane to have better protection of the myocardium than without pharmacological intervention at all. Currently the regimen used to prep the donor heart does not include anesthetic agents to protect the integrity of the heart and long term outcomes are not perfect with 5-year successes rounded around 70% (American Heart Association, 2011). Combining these two anesthetic agents and using them in preconditioning the donor heart in the procurement phase will hopefully improve long-term outcomes of the acceptor patient's new heart. There is only one viable heart in these procedures and it is important to keep the organ free from infarction and apoptotic damage. The previous research was done on animal models to improve organ viability and now it is logical to apply it to a clinical scenario: heart-transplants.

Chapter III: Methodology

Introduction

Within the heart-transplant procedure it is vital to protect as much myocardial tissue as possible to provide long term survival for the organ-acceptor patient; this can be difficult and unreliable using non-pharmacological support.

With current rising trends of heart disease and more patients being put in a situation requiring a transplanted heart it is paramount to preserve these hearts as best as possible especially with success rates only at around 70% for a 5-year success rate (American Heart Association, 2011). The purpose of this experimental study is to utilize the myocardial protecting effects of remifentanil and desflurane on the brain-dead donor heart in the procurement phase for long-term heart transplant success in the acceptor patient.

The proposed experiment will be appropriately overviewed with description of the experiment participants, their role in the heart-transplant experiment, and ethical considerations and the proposed procedures for the experiment. Data collection and analysis methods will be assessed as well as a discussion of the ultimate usefulness and contributions to conclusions and future studies on the usefulness for heart-transplants.

Research Design

The proposed experiment will consist of professionally performed surgical heart-transplants between a healthy adult rat and an adult rat with a life history of dilated cardiomyopathy. During the procurement phase of the heart-transplant procedure four independent conditioning regimes will be utilized and tested on their efficacy in prolonging the life of the rat that received the donor heart. The length of life after the procedure will be measured as the dependent comparative factor as well as cell damage markers following the

procedure to determine which independent treatment is best effective in preserving the long-term survival of the rat patients with the donated heart.

Research Participants. All experimental participants will be Sprague-Dawley rats (250-300 g) aged to 9-10 weeks old. These rats will be selected for the experiment based on their genetic similarities to each other to mitigate behavioral and physiological differences as best as possible. There will be 120 participants in total matched up to 60 pairs: each pair will have (a) one "donor patient" healthy rat with eventual deliberately induced brain and spinal-cord trauma done by surgical intervention and (b) one "acceptor patient" rat given an α-cardiac actin knockout genetic cardiomyopathy defect in vivo development and maturation to simulate the human patient needing a donor heart (Ikeda & Ross, 2000).

Each pair will be randomly placed in one of four groups: (i) a control with a normal heart transplant given only standard cardiothoracic donor management, (iii) donor preconditioned with a combination of desflurane and remifentanil, (iii) donor preconditioned only with remifentanil and (iv) donor preconditioned only with desflurane.

There will be 15 pairs in each group. Each participant within the pair will be the same gender and an approximate equal gender distribution will be created within groups. Between each pair a heterogeneous type and cross will be verified to allow immunological compatibility as well as reduce incidence of rejection (Horuk et. al., 2001).

Proposed Procedure. The following describe the experiment and care of the patient subjects from pre-procedure care, to the heart-transplant procedure, to the final post-experimental care.

Pre-procedure. To best prepare for the heart-transplant procedures all of the participating rats will be raised and cared for in a neutral environment to reduce effects of these

extraneous variables. All rats will be provided with three 1.0 g nutrient pellets and 500 mL water trays per day. Each housing room will be 4' x 4' and fitted with neutral gray walls, standard barn hay floors and a 1' diameter exercise wheel. Blood draws will be assessed weekly for both the acceptor and donor rats for baseline complete metabolic panel (CMP), CK/CK-MB ratios and troponin-1 levels. This will be done until a suitable acceptor and donor pair meets the experimental requirement and assigned to an experimental group (i-iv).

Procedure. The same certified surgeon and anesthesiologist will perform the heart-transplant procedures to mitigate differences in heart-transplant skill. The healthy donor rat patient will be given a controlled traumatic spinal cord injury 24 hours before the surgery at the C6 level as well as carotid occlusion for 2.0 minutes creating a brain-dead state mimicking a brain-dead human patient available to donate a heart. The donor patient will be preconditioned based on the assigned group according to the following.

Group i, the control, will be given the standard cardiothoracic donor care for 1.0 hrs before surgery is performed. Group ii will be conditioned with both desflurane at 1.0 MAC (7.5 vol%) and remifentanil at 6 ug kg^{-1} min^{-1}. The preconditioning will be performed 1.0 hour before the surgical time and both administered for exactly 30 minutes (Smul et. al., 2010). Group iii will only be preconditioned only with remifentanil 6 ug kg^{-1} min^{-1} and be applied similarly for 1.0 hour before the surgical time for 30 minutes. Group iv will be preconditioned only with 1.0 MAC at 7.5 vol% for 1.0 hour before surgery and for 30 minutes (Sonner, Gong, Li, Eger, & Laster, 1999; Kim et. al., 2010).

The acceptor patient with the genetic cardiomyopathy will be stabilized enough for heart transplant surgery. Both the preconditioned donor and acceptor patient will be induced with anesthesia with 3 mg kg^{-1} phenobarbital and rocuronium with dose titrated to a train of four of 0.

The ventilator will be set at volume mode 10 ml kg^{-1} and 50% FiO_2. Lactated ringers solution will be used as needed. Labetalol and/or ephedrine will be utilized to reduce the surgical stress response and titrated as needed as decided by the attending anesthesiologist.

The acceptor patient will have the failed heart removed and immediately put on a cardiopulmonary bypass machine with cardiac output titrated as needed to the metabolic demand. When the preconditioning phase of the donor patient is finished, the donor heart will be removed and transferred to the acceptor patient. Immediately following the procedure, CMP, CK/CK-MB ratios and troponin-1 levels will be taken from blood draw on the patient with the new heart to assess any changes from baseline from the pre-procedure. The patient will be taken to an intensive recovery unit until vitals stabilize.

Post-procedure. After the initial intensive care recovery, the acceptor patient will be cared for with standard care as described in the pre-procedure section. Bi-weekly CMP, CK/CK-MB ratios and troponin-1 levels will be taken from blood draw to assess the success of the surgery. An overall life-span time count will be started when the acceptor patient is taken out of intensive care. When all acceptor rats have expired the experiment is concluded and the data analysis will be carried out.

Experimental Data

Data Collection Methods. The data will be collected and analyzed through standard blood draw from a vein in needle withdrawal and metabolic analysis with protein count of the blood sample in the blood analysis lab. Each of the four independent test groups with different pre-conditioning regimes will have their cardiac damage marker levels tested against each other (CK/CK-MB ratios and troponin-1 levels) based on mass spectrometer readings.

By blood draw and chemical analysis the CMP will also be followed and compared to baseline as the rat patients progress in age. The time following the heart-transplant procedure will be counted and taken as the ultimate dependent indicator of long-term success.

Proposed Data Analysis. The collected data will be compared against both the baseline standards taken right before the heart-transplant procedure is carried out and the post-transplant levels. The cardiac damage markers will be assessed against all groups on a direct comparative trend basis. The troponin-1 levels and CK/CK-MB ratios will be averaged within the independent groups and compared between groups for a positive or negative % deviation of the average.

The CMP will be used as a guide for the long-term life expectancy of the rat patients to identify any outstanding abnormalities or outliers within the independent group of the rat vitals. A direct comparison of the time averaged within the groups will be done with note of any outlying metabolic indicators for cause of death from the CMP

Ethical Considerations

The greatest ethical concern for the proposed experiment is based on the fair treatment and handling of the animal test subjects. All experimental procedures and protocols will be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and follow the United States Animal Welfare Act as well as any state laws to conform to legal animal ethics (Institutional Animal Care and Use Committee, 2002). Additional care will be provided to the experimental subjects to ensure metabolic homeostasis and cognitive stability. The limit of ethical concern in this experiment extends as far as animal test-subject care as similar to human study care as possible.

Expected Medical Contribution

The proposed research is taken as a first step towards utilizing the organ protective effects of anesthetics to an organ procurement procedure that will benefit patients receiving an organ transplant. While this research is done on animals it will hopefully prove worthy to try the anesthetic preconditioning techniques on human patients to improve long-term survival outcomes to better than 70% for heart-transplant patients (American Heart Association, 2011). Not only will this research allow progress towards human patients for heart-transplants, but it may open up the possibilities of improvement for other organs such as kidney or liver transplants. This application of anesthetic drugs in this research has not yet been tested on the brain-dead organ donor to improve viability of organs to be transplanted; and this research serves as a first step to opening up many other possibilities for improving organ-transplant technique (Elkins, 2010).

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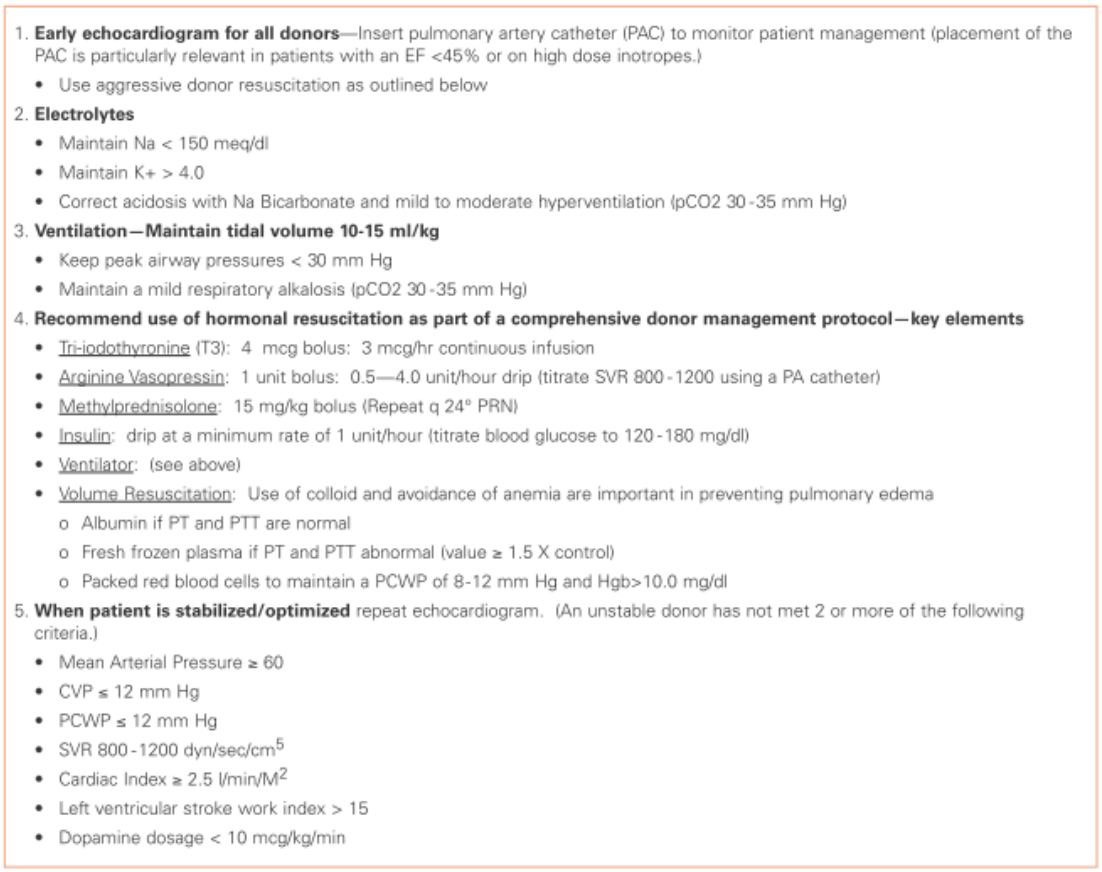

1. **Early echocardiogram for all donors**—Insert pulmonary artery catheter (PAC) to monitor patient management (placement of the PAC is particularly relevant in patients with an EF <45% or on high dose inotropes.)
   - Use aggressive donor resuscitation as outlined below
2. **Electrolytes**
   - Maintain Na < 150 meq/dl
   - Maintain K+ > 4.0
   - Correct acidosis with Na Bicarbonate and mild to moderate hyperventilation (pCO2 30-35 mm Hg)
3. **Ventilation—Maintain tidal volume 10-15 ml/kg**
   - Keep peak airway pressures < 30 mm Hg
   - Maintain a mild respiratory alkalosis (pCO2 30-35 mm Hg)
4. **Recommend use of hormonal resuscitation as part of a comprehensive donor management protocol—key elements**
   - Tri-iodothyronine (T3): 4 mcg bolus: 3 mcg/hr continuous infusion
   - Arginine Vasopressin: 1 unit bolus: 0.5—4.0 unit/hour drip (titrate SVR 800-1200 using a PA catheter)
   - Methylprednisolone: 15 mg/kg bolus (Repeat q 24° PRN)
   - Insulin: drip at a minimum rate of 1 unit/hour (titrate blood glucose to 120-180 mg/dl)
   - Ventilator: (see above)
   - Volume Resuscitation: Use of colloid and avoidance of anemia are important in preventing pulmonary edema
     - Albumin if PT and PTT are normal
     - Fresh frozen plasma if PT and PTT abnormal (value ≥ 1.5 X control)
     - Packed red blood cells to maintain a PCWP of 8-12 mm Hg and Hgb>10.0 mg/dl
5. **When patient is stabilized/optimized** repeat echocardiogram. (An unstable donor has not met 2 or more of the following criteria.)
   - Mean Arterial Pressure ≥ 60
   - CVP ≤ 12 mm Hg
   - PCWP ≤ 12 mm Hg
   - SVR 800-1200 dyn/sec/cm^5
   - Cardiac Index ≥ 2.5 l/min/M^2
   - Left ventricular stroke work index > 15
   - Dopamine dosage < 10 mcg/kg/min

Figure 1. As recommended by the United Network of Organ Sharing (UNOS), this is the comprehensive hormonal management of the donor patient for cardiothoracic donor surgery. Na indicates sodium; K+, potassium ion; pCO2, partial pressure of carbon dioxide; PA, pulmonary artery; SVR, systemic vascular resistance; q 24° PRN, every 24 hours as needed; PT, prothrombin time; PTT, partial thromboplastin time; CVP, central venous pressure; and PCWP, pulmonary capillary wedge pressure. Adapted from "Inhalational anesthesia for organ procurement: potential indications for administering inhalational anesthesia in the brain-dead organ donor," by Elkins, L. J., 2010, American Association of Nurse Anesthetists Journal, 78, 293-299. Copyright 2010 by the United Network of Organ Sharing.