Video Article

Rat Heterotopic Abdominal Heart/Single-lung Transplantation in a Volume-loaded Configuration

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Abstract

Herein, we describe a novel technique for heterotopic abdominal heart-lung transplantation (HAHLT) in rats. The configuration of the transplant graft involves anastomosis of donor inferior vena cava (IVC) to recipient IVC, and donor ascending aorta (Ao) to recipient abdominal Ao. The right upper and middle lung lobes are preserved and function as conduits for blood flow from right heart to left heart.

There are several advantages to using this technique, and it lends itself to a broad range of applications. Because the graft is transplanted in a configuration that allows for dyamic volume-loading, cardiac function may be directly assessed *in vivo*. The use of pressure-volume conductance catheters permits characterization of load-dependent and load-independent hemodynamic parameters. The graft may be converted to a loaded configuration by applying a clamp to the recipient's infra-hepatic IVC. We describe modified surgical techniques for both donor and recipient operations, and an ideal myocardial protection strategy. Depending on the experimental aim, this model may be adapted for use in both acute and chronic studies of graft function, immunologic status, and variable ventricular loading conditions. The conducting airways to the transplanted lung are preserved, and allow for acute lung re-ventilation. This facilitates analysis of the effects of the mixed venous and arterial blood providing coronary perfusion to the graft.

A limitation of this model is its technical complexity. There is a significant learning curve for new operators, who should ideally be mentored in the technique. A surgical training background is advantageous for those wishing to apply this model. Despite its complexity, we aim to present the model in a clear and easily applicable format. Because of the physiologic similarity of this model to orthotopic transplantation, and its broad range of study applications, the effort invested in learning the technique is likely to be worthwhile.

Video Link

The video component of this article can be found at https://www.jove.com/video/52418/

Introduction

The first rodent model of heterotopic abdominal heart transplantation (HAHT) was described by Abbott and colleagues in 1964[1]. This technique, and subsequent modifications have been widely applied to characterize transplant graft function and immunologic status. The majority of HAHT techniques described involve a non-volume loaded heart[2,3]. Models of HAHT involving volume-loaded ventricles have been described, but they are frequently limited in one or more respects.

Heterotopic abdominal heart-lung transplantation (HAHLT) with a volume-loaded left ventricle (LV) has been described previously. Chen and colleagues[4], and subsequently Ibrahim and colleagues[5] described HAHLT with a single aorto-aortic (donor ascending to recipient abdominal aorta) anastomosis. The only volume load presented to the ventricle in this circulation is the coronary venous return. Asfour and colleagues described a HAHT technique in which the lung circuit was eliminated by anastomosing donor pulmonary artery (PA) to donor left atrium (LA)[6]. In this circulation, venous inflow to right ventricle (RV) occurs via a donor SVC to recipient IVC anastomosis, and the subsequent LV load is ejected into the aorto-aortic anastomosis. Cardiac function was partially assessed *in vivo*, and also *in vitro* using a Langendorff rig. Figueiredo and colleagues described a HAHLT model similar to our own[7], but in mice. Venous inflow to the RV occurs via donor SVC to recipient IVC anastomosis. Blood subsequently passes through the single lung circulation and LV load is ejected into the aorto-aortic anastomosis. Cardiac function in their study was assessed by magnetic resonance imaging (MRI). Wen and colleagues described a unique HAHT technique in which the LV is loaded by means of a recipient aorta to donor LA anastomosis[8]. The LV, therefore, fills at systemic pressures. Cardiac function, and whether LV stroke volume is ejected antegradely in their model was not assessed.

Many of the techniques referenced above involve non-physiologic LV loading conditions, including the techniques whose partial LV load is represented only by coronary venous return. On the other hand, many techniques do approach physiologic LV loading. The majority of these techniques, as with the technique of Asfour and colleagues, omit the pulmonary circulation and utilize a donor PA to donor LA

anastomosis[6,9]. The circulation described by Galinanes and colleagues[10] employs a direct recipient cava to donor LA anastomosis, omitting the pulmonary circulation and the right heart. Yokoyama and colleagues achieve the same effect by ligating the donor PA and creating an interatrial communication in the donor heart (omitting donor lung and right heart circulations)[11]. The circulation of Maruyama and colleagues[12] involves an anastomosis between donor left PA and recipient Ao, which permits LV filling via the pulmonary circulation as a conduit, but effectively excludes the right heart.

In cases where near physiologic loading conditions were met, we advance the technique of HAHLT in 2 major respects. First, to our knowledge, the exact configuration we report has not been described in rats. It is possibly the most versatile circulation for investigators wishing to study the physiology, structure, and immunology of the transplanted heart-lung graft. Second, we describe how the function of the transplant graft can be directly characterized *in vivo.* For this application, pressure-volume conductance catheters can be introduced directly into the LV apex of the transplant graft, which allows for complete cardiac functional characterization.

The technique described here can be applied to both acute and chronic studies of transplant graft function, while the functional assessment may be performed either *in vivo* or *in vitro*. We present a model in which the loading conditions can be near physiologic, however the degree of ventricular loading may be manipulated both acutely and chronically by diverting venous return towards or away from the graft. Afterload conditions can also be manipulated. Because the lung and its airway are retained in this transplant configuration, investigators can re-ventilate the donor lung acutely. Uniquely, lung re-ventilation changes the composition of blood perfusing the transplant coronary arteries. Under non-ventilated conditions, blood ejected from the donor aorta is deoxygenated, and mixes with oxygenated blood in the recipient aorta. Under acutely ventilated conditions, ejected blood becomes oxygenated. Thus, transplant graft function can be compared under ventilated and non-ventilated conditions, and also under variably loaded conditions.

The protocol below describes important modifications to previously described HAHLT donor and recipient operations. It also describes an optimal technique for protecting the transplant graft throughout the period of ischemia (time between donor explant and recipient implant). Advantages of this technique include physiologic conditions potentially approaching that of an orthotopically transplanted graft, and a wide range of investigative applications. An important limitation is its technical complexity. With adequate mentoring and practice, the advantages of this technique will likely outweigh the challenges in adopting it.

Protocol

All animals were housed and cared for in accordance with National and Institutional guidelines for the care and use of laboratory animals. Ethics approval for this protocol was granted by the University of British Columbia's Animal Care Committee. Male, Sprague-Dawley rats weighing between 300 - 450 g were used for this protocol.

1. Donor Operation

1. Have approximately 100 ml of cardioplegia (RT) in a cylindrical flask connected to long intravenous (IV) catheter tubing by a 3-way stopcock. Use a stand to elevate the flask to approximately 80cm above the operative surface, enabling cardioplegia delivery by gravity.
2. To visualize structures adequately, use either a pair of surgical loupes or a dissecting microscope.
   NOTE: We currently use a binocular operating microscope with 3.4 - 21.3X magnification.
3. Place the donor in an anesthetic chamber and induce anesthesia with 4 - 5% isoflurane.
4. Transfer the rat to an operating platform and maintain anesthesia by nose-cone with 1 - 2% isoflurane. Apply vet ointment to the animal's eyes to prevent dryness. Administer midazolam (2 mg/kg) intra-peritoneally with a 25 G needle.
5. Using surgical clippers, shave the donor from xiphisternum to mandible. Shave a small region of the left groin (for subsequent IV access). Apply a depilating agent to the operative surfaces, wait approximately 5 min, and remove hair with a piece of gauze.
6. Prep the surgical sites with a povidone-iodine or chlorexidine-based solution (we use only chlorhexidine). Infiltrate the incisional sites with 0.1 - 0.5% lidocaine subcutaneously.
7. Secure the forelimbs and left hindlimb to the operating platform with adhesive tape, leaving the right hindlimb free for monitoring of anesthetic depth and vital signs.
8. After ensuring appropriate anesthetic depth by pedal pinch, make an incision in the left groin crease using a 22-blade scalpel. By blunt dissection, expose the left common femoral vein and obtain IV access as follows:
   1. Gently retract the tissue overlying the femoral vein, and cannulate the vein with a 24 G IV. Connect the IV to a short length of saline-filled IV tubing, and secure the tubing in place with adhesive tape.
      NOTE: The procedure for femoral venous cannulation in rats is described elsewhere by Jespersen and colleagues[14].
   2. Connect a saline-filled 10 ml syringe to the IV tubing, and gently aspirate blood to ensure correct positioning of the IV.
   3. Inject 300 - 500 IU of unfractionated heparin through the IV tubing, and subsequently flush the tubing with 3 - 5 ml of saline.
9. Next, tracheotomize the donor as follows:
   1. Make a midline incision in the soft tissues between the jugular notch and mandible using a 22-blade scalpel. Penetrate the capsule of the thyroid gland in the midline using Metzenbaum scissors, and separate its lobes using blunt dissection.
   2. Using blunt dissection, separate the strap muscles of the neck in the midline to expose the anterior surface of the trachea.
   3. Use a curved forceps to bluntly dissect a circumferential plane around the trachea. Encircle the trachea with a 4-0 silk tie.
   4. Using iris scissors, make a transverse incision in the anterior trachea, approximately 5mm inferior to the thyroid cartilage. Gently introduce the tracheal cannula (a 14 G IV) and secure it in place using the 4-0 silk tie.
   5. Connect the tracheal cannula to a mechanical ventilator. Redirect the flow of oxygen and isoflurane through the ventilator circuit, and ventilate the donor at a rate and tidal volume predicted by its weight[13].
10. Make an incision in the midline of the chest (using a 22-blade scalpel), extending the incision at the jugular notch to below the xipisternum.
11. Staying in the midline, Perform a median sternotomy using a bone cutter. Retract the edges of the sternum with a self-retaining retractor. Enter the pericardium and pleural cavities.

12. Perform a thymectomy. It is easiest to first bluntly divide the thymus in the midline, and then separate it from surrounding structures using a combination of blunt and sharp dissection.
    NOTE: The origin of the internal thoracic arteries may be injured when dissecting the thymus away from the superior sternal edges. To prevent bleeding, hemostatic clips may be applied before removing the thymus at these points.
13. Using Metzenbaum scissors and/or a sharp Lauer, dissect peri-caval fat away from the inferior vena cava (IVC). Ensure that the IVC is relatively free of fat and connective tissue from the cavo-atrial junction superiorly, to the diaphragm inferiorly.
14. Using a sharp Lauer, circumferentially free the superior vena cava (SVC) and encircle it with a 4-0 silk tie.
15. Next, dissect the left vena cava free from surrounding structures, and ligate it proximally and distally with 4-0 silk ties. Resect the intervening portion of cava to expose the left subclavian artery.
16. Circumferentially free the aortic arch vessels using a sharp Lauer. Apply proximal and distal surgical clips to the innominate artery, and divide it between the clips. Leave the left common carotid artery and left subclavian artery un-clipped.
17. Next, cannulate the SVC with a 24 G IV catheter. Secure the catheter with the previously placed 4-0 silk tie.
18. Prepare for an expedient, but gentle graft harvest.
19. Using sharp scissors, divide the IVC just superior to the diaphragmatic surface. Divide the SVC superior to its cannulation site, ensuring that nearby airway structures aren't injured.
20. Turn off the ventilator and disconnect the tracheal cannula. Transect the trachea proximally.
21. Grasp the transected trachea with forceps, and remove the heart and lungs en-bloc. This will require gentle traction with sharp dissection as the heart-lung graft is removed. Separate the trachea from the underlying esophagus. Avoid injuring the descending aorta, so that a long portion of it remains intact after harvest.

2. Preparation of the Heart-lung Graft

NOTE: while completing this portion of the procedure, an assistant should be anesthetizing the recipient rodent and preparing for graft implantation.

1. Place the heart-lung graft on surgical gauze with the anterior surface of the heart facing down. Using sharp dissection, remove any residual esophagus, avoiding injury to the posterior airways.
2. Locate the descending thoracic aorta and insert a 16 G blunt-tipped cannula. Secure the cannula in place with a 4-0 silk tie.
3. Locate the aortic arch vessels, and apply a surgical clip to the left subclavian artery. Leave the left carotid artery un-clipped for subsequent de-airing.
4. Administer the first dose of cardioplegia by connecting the cardioplegia tubing to the 16 G aortic cannula. The carotid artery must be temporarily occluded with forceps to ensure adequate delivery of cardioplegia.
   NOTE: A benefit to having the aortic cannula attached is that cardioplegia may be administered continuously and/or intermittently as desired. For intermittent dosing, we typically deliver cardioplegia every 10 - 15 min in 5 ml boluses over 30 - 45 sec.
5. Using Metzenbaum scissors and/or a sharp Lauer, dissect peri-aortic fat away from the aorta. Ensure that the aorta is relatively free of fat and connective tissue from the left subclavian artery (distal arch) to its cannulation site.
6. Next, expose the left mainstem bronchus using a sharp Lauer, and ligate it proximally with a 4-0 silk tie. Transect the left bronchus distal to the ligature using iris scissors. Perform a left pneumonectomy by ligating the left pulmonary artery and vein with 4-0 silk ties. Transect distal to the ligature and remove the left lung.
7. As above, remove all other lung lobes with the exception of the right upper and middle lobes. Avoid injuring the IVC in the process of performing lobectomies.
8. Connect the cardioplegia tubing to the aortic cannula and administer continuous cardioplegia while the recipient is being prepared. Place the heart-lung graft in a sterile container (*e.g.,* a beaker).

3. Recipient Operation

1. Anesthetize the recipient as per the donor operation, above. Use vet ointment to protect the animal's eyes from dryness. Confirm anesthesia frequently by pedal pinch.
2. Position the animal as per the donor operation, this time leaving the right forelimb free to monitor vital signs and anesthetic depth.
3. Shave the abdomen from xiphisternum to penis. Shave a small region of the left groin (for subsequent IV access). Apply a depilating agent to the operative surfaces, wait approximately 5 min, and remove hair with a piece of gauze.
4. Prep the surgical sites with a povidone-iodine or chlorexidine-based solution. Infiltrate the incisional sites with 0.1 - 0.5% lidocaine subcutaneously.
5. Tracheotomize and ventilate the recipient as directed above. Alternatively, maintain the recipient under nose-cone anesthesia.
6. Introduce a catheter into the femoral vein as described above. Inject 300 - 500 IU of unfractionated heparin through the IV tubing, and subsequently flush the tubing with 3 - 5 ml of saline.
7. Perform a laparotomy by making a midline abdominal incision with a 22-blade scalpel from xiphisternum to penis. Retract the abdominal wall using a self-retaining retractor. Next, retract the bowels superiorly and to the rat's left side. Wrap them in warm, saline-soaked gauze.
8. Expose the IVC and abdominal aorta by sharply dissecting through the overlying retro-peritoneal fat.
9. Have a curved vessel clamp available and ready.
10. Circumferentially free the IVC and aorta proximally and distally and encircle them with 4-0 silk ties. There should be approximately 2 - 3 cm of space between these sites.
11. Carefully apply the curved vessel clamp, ensuring that a sufficient portion of both IVC and aorta are exposed above the jaws of the clamp.
12. Make an incision in the anterior wall of the IVC with a 25 G needle connected to a saline-filled 1 ml syringe. Extend the incision with Potts scissors to match the length of the donor IVC orifice.
13. Remove the heart-lung graft from its container and disconnect it from cardioplegia. Place the graft in the recipient's abdomen in an optimal position to perform the venous anastomosis.
    NOTE:The heart-lung graft will ultimately be oriented slightly obliquely, with the apex pointing towards the abdomen's left lower quadrant.

14. Secure the heel and toe ends of the anastomosis with 9-0 nylon suture. Tie a secure knot at each end, leaving the needle attached to a long arm of suture, and a short end of suture to be tied to later. Administer a dose of cardioplegia.
15. In running fashion, complete one-half of the suture line and tie to the opposing short suture arm. Administer a dose of cardioplegia.
16. Complete the other half of the suture line and tie it down. The venous anastomosis is complete. Administer a dose of cardioplegia
17. Orient the heart-lung graft with the heart's apex pointing toward the abdomen's left lower quadrant. Ensure that the venous anastomosis is not kinked or twisted.
18. Assess the length of donor aorta that will be required to reach the recipient aorta, maintaining both IVC and aortic anastomoses in tension-free and un-kinked configurations.
19. Divide the descending aorta (distal to the left subclavian artery) with an iris scissors.
20. Make an incision in the anterior wall of the recipient aorta with a 25 G needle connected to a saline-filled 1 ml syringe. Extend the incision with Potts scissors to match the length of the donor aortic orifice.
21. Anchor the toe (superior aspect) of the donor aorta to the recipient aorta using a 9-0 nylon suture. Then, pass the needle to the medial aspect of the aorta and complete one-half of the anastomosis in running fashion.
22. At the heel of the aortic anastomosis, reverse the orientation of the suture line (a transition in the direction of stitching), and complete the lateral half of the anastomosis in running fashion.
23. Slowly remove the IV catheter in the SVC, and ligate the SVC with a surgical clip.
24. De-air the aorta by locating the un-clipped left common carotid artery. Hold the artery open, in an upright position that will allow air to be freely evacuated.
25. Briefly open the jaws of the curved vessel clamp and allow the carotid artery to bleed (de-air) for 2 - 3 sec. Re-apply the vessel clamp.
26. Apply a surgical clip to the carotid artery. Remove the curved vessel clamp.
27. Check for bleeding at the suture lines. If present, apply gentle compression with surgical gauze or repair with a length of 9-0 nylon suture (depending on the severity of bleeding).
28. The donor heart should resume beating within minutes.
29. Pay close attention to the recipient's vital signs, depth of anesthesia, and administer volume resuscitation as needed.
30. Depending on the experimental endpoints, either close the animal's abdomen and let it recover from anesthesia, or prepare the animal for cardiovascular assessment.
    NOTE: Examples of graft assessment include *in vivo* measures of load-dependent and load-independent hemodynamics, *in vitro* measures of function in Langendorff and working heart modes, and (in survival surgery) echocardiographic or MRI investigations.
31. At the end of acute terminal experiments, animals are euthanized by exsanguination.

Representative Results

The HAHLT technique described above is highly technical and requires close attention to detail. **Table 1** highlights some of the key factors associated with successful versus unsuccessful procedures, and can be used as a guide for troubleshooting technical difficulties.

After the recipient aorta is unclamped, the graft coronary arteries should be seen to fill with oxygenated blood. Accordingly, the myocardium should become pink and well perfused. In technically successful experiments, the heart will begin to beat shortly after graft reperfusion. The graft should be left in an unloaded state (with recipient IVC clamped) for a period of at least 20 - 30 min to allow for functional recovery. Following that, the graft's loading conditions may be altered to suit experimental aims. More quantitative measures of graft function (and a successful outcome) can be employed as desired. As noted in the protocol, *in vivo* and *in vitro* functional studies, as well as echocardiographic and MRI investigations can provide such information. **Figures 1** and **2** are examples of *in vitro* baseline and preload-occlusion pressure-volume data that can be derived acutely with this methodology. Hemodynamic data from these studies can provide investigators with cardiac output, stroke volume, chamber volumes, heart rate, ejection fraction, end-systolic elastance, and preload recruitable stroke work. Several other parameters can be quantified, as desired.

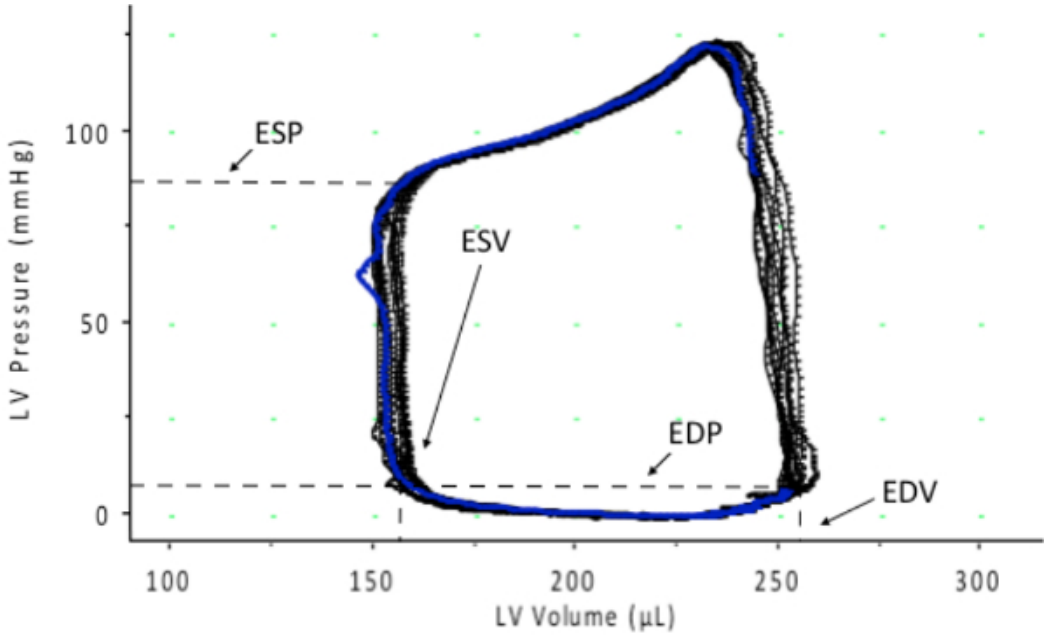

Figure 1. Baseline Pressure-volume Data. Please click here to view a larger version of this figure.

Figure 1 shows baseline pressure-volume data from an *in vitro* working heart assessment of cardiac function. EDV, end-diastolic volume; EDP, end-diastolic pressure; ESV, end-systolic volume; ESP, end-systolic pressure.

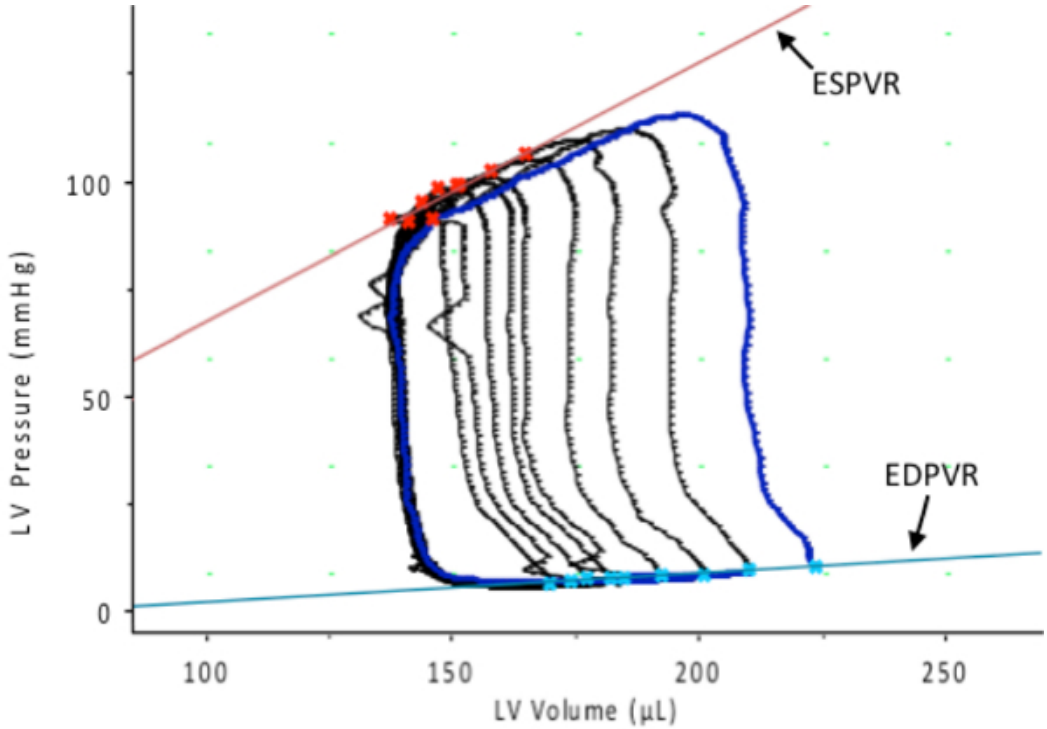

Figure 2. Preload-occlusion Pressure-volume Data. Please click here to view a larger version of this figure.

Figure 2 shows preload-occlusion pressure-volume data from an *in vitro* working heart assessment of cardiac function. EDPVR, end-diastolic pressure-volume relationship; ESPVR, end-systolic pressure-volume relationship.

	Successful Procedure	**Unsuccessful Procedure**
Donor		
Donor Stability	Stable	Unstable
Preparatory Dissection	Efficient, Limited	Inefficient, Excessive

Blood Loss	Minimal	Excessive
Time to Explant	Minimized	Prolonged
Cardioplegic Arrest	Rapid	Delayed
Graft		
Total Ischemic Time	<90 min	>90 min
Cardioplegic Arrest	Maintained Periodically	Incomplete
Recipient		
Recipient Stability	Stable	Unstable
Preparatory Dissection	Efficient	Inefficient
Clamp Times	<45 min	>45 min
Anastomosis Time	<30 min	>30 min
Blood Loss	Minimal	Excessive
Graft De-airing	Adequate	Inadequate
Volume Resuscitation	Adequate	Inadequate
Successful Reperfusion	Yes	No
Return of Stable Cardiac Function	Yes	No

Table 1. Characteristics of Successful Versus Unsuccessful HAHLT Procedures.

Table 1 provides examples of factors associated with successful and unsuccessful donor, graft, and recipient operations.

Discussion

Success with the technique described here will be predicated on several factors. Key among them will be ensuring stability of both donor and recipient animals, adopting meticulous operative technique that is safe and associated with minimal blood loss, ensuring complete cardioplegic arrest with uniform graft cooling, minimizing total ischemic time, and adequately de-airing the graft. As acknowledged above, the technique's technical complexity is its chief limitation.

We have advanced previous HAHLT techniques in several respects. The modifications described in donor and recipient operations provide a means of performing necessary operative steps in a controlled and efficient manner. The myocardial protection protocol described is an ideal means of minimizing injury during the ischemic period. The end result is a HAHLT graft in which geometry has been optimized, injury has been minimized, and intrinsic graft function has been preserved to the greatest extent possible.

The technique described above yields a HAHLT graft with dynamic and potentially near-physiologic ventricular-loading conditions. Once the technique has been mastered, the transplant configuration permits complete *in vivo* characterization of graft function. As noted, the preload and afterload conditions can be altered acutely or chronically, and donor lung may be acutely re-ventilated. Investigators can readily and broadly apply this model to the study of many medical conditions, while retaining the ability to study graft structure and function.

Disclosures

The authors have nothing to disclose.

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