Donor Pretreatment With Hypertonic Saline Attenuates Primary Allograft Dysfunction
A Pilot Study in a Porcine Model

Mitesh V. Badiwala, MD; Danny Ramzy, MD, PhD; Laura C. Tumiati, BSc; Elissa D. Tepperman, MSc; Rohit Sheshgiri, MSc; Jessica L. Prodger, MSc; Christopher M. Feindel, MD; Vivek Rao, MD, PhD

Background—Hypertonic saline (HTS) has been previously demonstrated to have immune modulatory and vascular protective effects. We assessed the effect of donor pretreatment with HTS on allograft preservation in a porcine model of orthotopic heart transplantation.

Methods and Results—Orthotopic transplants were performed after 6 hours of cold static allograft storage. Donor pigs were randomly assigned to pretreatment with (n=7) or without (n=6) HTS (4.5 mL/kg of 7.5% NaCl) administered 1 hour before donor heart arrest. Administration of HTS increased serum sodium level from 138±2 mmol/L to 154±6 mmol/L, which normalized to 144±3 mmol/L 1 hour after infusion. Successful weaning from cardiopulmonary bypass was significantly greater in HTS-treated hearts (6/7 vs 1/6; P=0.029). Preload recruitable stroke work after transplantation was improved compared to control (88±21% vs 35±8% of baseline; P=0.0001). Similarly, end-systolic elastance was improved compared to control (85±17% vs 42±12% of baseline; P=0.0002). Posttransplantation systolic blood pressure was significantly higher in the donor HTS group (60±9 mm Hg vs 35±6 mm Hg; P=0.04). Donor HTS treatment improved coronary artery endothelial-dependent vasorelaxation compared with control (Emax: HTS, 71±3%; control, 59±4%; P=0.03; ED-50: HTS, 0.56±10 to 6±0.23 mol/L; control, 2.5±10 to 6.10± mol/L; P=0.04). Sensitivity to endothelin-1-induced vasospasm was reduced with HTS pretreatment (% maximum contraction [Cmax]: HTS, 338±15%; control, 419±40%; P=0.01).

Conclusions—Donor HTS pretreatment attenuates posttransplantation cardiac allograft myocardial dysfunction, improves posttransplantation systemic hemodynamic function, and preserves posttransplantation cardiac allograft vascular function. HTS may be a novel organ donor intervention to prevent primary graft dysfunction. (Circulation. 2009;120[suppl 1]:S206–S214.)

Key Words: endothelium • ischemia • transplantation • reperfusion

Cardiac transplantation remains the gold standard for the treatment of end stage cardiac disease. Unfortunately, the discrepancy between the number of donor organs available and the number of patients awaiting transplantation continues to persist. Improved methods of myocardial protection can increase the donor pool by allowing for the procurement of cardiac allografts from more distant sites and by using donor hearts currently deemed marginal.

With conventional cold storage techniques, the safe duration of allograft storage is restricted and prolonged storage times (>6 hours) continue to be associated with compromised short-term and long-term outcomes. Specifically, myocardial injury caused by prolonged storage compromises contractile performance, resulting in low output syndrome and sometimes death. In addition, endothelial injury as a result of prolonged storage is increasingly recognized as an important cause of poor outcome after cardiac transplantation, principally because it might lead to transplant vasculopathy.1 Allograft preservation might be optimized if both the myocardium and endothelium are simultaneously targeted.

Hypertonic saline (HTS), a volume expander, has been previously shown to possess immunomodulatory effects.2,3 Several investigators have demonstrated that HTS directly affects neutrophil, macrophage, and T-cell function.2,4,5 Importantly, HTS infusion before prolonged ischemia and reperfusion has been demonstrated to protect against myocardial stunning.6

From Division of Cardiovascular Surgery, Peter Munk Cardiac Centre, Toronto General Hospital-University Health Network, Toronto, Ontario, Canada.


Correspondence to Vivek Rao, MD, PhD, Associate Professor of Surgery, Alfredo and Teresa DeGasperis Chair in Heart Failure Surgery, Division of Cardiovascular Surgery, Toronto General Hospital, University of Toronto, 4N-464, 200 Elizabeth Street, Toronto, Ontario M5G 2C4. E-mail vivek.rao@uhn.on.ca

© 2009 American Heart Association, Inc.

Circulation is available at http://circ.ahajournals.org

DOI: 10.1161/CIRCULATIONAHA.108.843169
In summary, HTS has the potential to significantly improve the results of cardiac transplantation via a myriad of beneficial effects. We hypothesized that donor pretreatment with HTS would provide a novel method of myocardial and endothelial protection during donor heart arrest and storage and, in so doing, would limit early endothelial dysfunction and improve myocardial performance after transplantation.

### Materials and Methods

All experimental protocols were approved by our institutional animal care committee and animals were treated in accordance with the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources, National Research Council, 1996.

Female Yorkshire pigs (approximately 70 kg, Riems Fur Ranch, St. Agatha, Ontario) were used as both donor and recipient animals to perform 13 orthotopic cardiac transplants. One hour before cardiopulmonary arrest and storage of allografts, donor animals received either an intravenous infusion of 4.5 mL/kg of 7.5% NaCl at 12.5 mL/min (donor HTS group, n = 7) or 4.5 mL/kg of 0.9% NaCl at 12.5 mL/min (control group, n = 8) via a marginal ear vein. All allograft donor hearts were stored under static hypothermic (4°C) storage conditions in crystalloid cardioplegic solution. The size mismatch between donor and recipient animals was <10% of body weight in all experiments.

### Donor Operation

The animals were anesthetized with intramuscular ketamine (30 mg/kg) and inhalational isoflurane (1%–5%). The animals were then intubated and ventilated with 100% oxygen to maintain normocarbia. After median sternotomy, the heart and great vessels were exposed. Systemic anticoagulation was achieved with an intravenous injection of 30 000 U heparin.

An arterial line was inserted into the right common carotid artery and a Swan-Ganz catheter was inserted via the right atrium and directed into position beyond the pulmonary artery bifurcation. Umbilical tapes were placed around the superior vena cava and inferior vena cava. A Millar micromanometer catheter and a Millar Mikro-Tip conductance catheter were inserted into the left ventricle through a small apical ventriculotomy to permit continuous measurements of left ventricular pressure–volume relations.

A purse string suture was placed in the ascending aorta to permit placement of a cardioplegia cannula. Arterial and coronary sinus blood samples were obtained just before aortic cross clamping. After cross clamping, 1 L of a hyperkalemic crystalloid solution (composition in mmol/L: Na⁺ 127, K⁺ 20, Mg²⁺ 6, Cl⁻ 7, SO₄⁻ 6, tris-hydroxymethyl-aminomethane 4, dextrose 135) was infused into the aortic root at 4°C to achieve cardioplegic arrest. After cardioplegic arrest, the donor heart was extracted, placed in a jar containing 1 L hypothermic cardioplegic solution, and stored on ice.

### Recipient Operation

Sedation and anesthesia were performed as in the donor protocol. After median sternotomy, the heart and great vessels were exposed. An arterial line was inserted into the right common carotid artery and a Swan-Ganz catheter was inserted via the right atrium and directed into position beyond the pulmonary artery bifurcation. The superior vena cava and the inferior vena cava were encircled with umbilical tape. Systemic anticoagulation was achieved with a 10 000-U heparin injection into the pump prime in addition to a 10 000-U intravenous dose. No immunosuppressive agents were administered to the recipient animals.

Ascending aortic and bivacal cannulation were used to place the recipient animal on cardiopulmonary bypass (CPB). Flow rates were adjusted to maintain a mean arterial pressure of 30 mm Hg. No vasoactive medications were administered during CPB. Systemic perfusion was maintained at 37°C for both groups.

After aortic cross-clamping, the recipient heart was extracted so that a cuff of right atrium and left atrium was maintained. The left hemiazygous vein was suture-ligated at its insertion into the coronary sinus. The anastomotic margins were inspected and trimmed in preparation for orthotopic transplantation by a standard atrial-to-atrial technique.

The donor heart was removed from hypothermic conditions after 6 hours of storage and an initial 350 mL blood cardioplegic dose was infused at a flow rate of 130 mL/min into the donor aortic root. Cardioplegic protection consisted of a 2:1 mixture of blood/crystalloid and was delivered at 10°C after the completion of each atrial anastomosis. After the completion of the pulmonary arterial anastomosis, 350 mL of blood cardioplegia was delivered at 37°C. Arterial and coronary sinus blood samples were obtained after both storage and cross-clamp removal.

Once all anastomoses were completed, the aortic cross-clamp was removed and all hearts were reperfused for 60 minutes. If ventricular fibrillation occurred during reperfusion, 3 attempts were made to defibrillate the heart. If unsuccessful, 100 mg lidocaine was given to the animals through the CPB circuit and defibrillation attempted again. If required, ventricular epidermal pacing was used to maintain a heart rate of 80 beats per minute. After 60 minutes of reperfusion, 1 g calcium chloride was administered to all animals and they were weaned from CPB. Isoproterenol infusion (4 μg/min) was used, if needed, to assist in weaning from CPB. Weaning was deemed successful if the animals maintained a systolic arterial pressure of 60 mm Hg for 30 minutes after the discontinuation of CPB.

Millar micromanometer and conductance catheters were inserted into the left ventricle through a small apical ventriculotomy to permit continuous measurements of left ventricular pressure-volume relations. Immediately after weaning completely from CPB (and before the administration of any vasoactive agents), hemodynamic parameters were recorded and assessments of left ventricular function were made as described. After 30 minutes off CPB, the hearts were excised under general anesthesia for assessment of coronary endothelial function and the animals were euthanized by an intravenous potassium chloride injection and exsanguination.

### Assessment of Left Ventricular Function

The outputs from the micromanometer and conductance catheters were linked to an analog digital converter and then transferred to a laptop computer for construction of pressure–volume loops. Measurements were taken using the methods of Baan et al² and parallel conductance was calculated by the method of Szwarz et al.³ Once baseline pressure–volume loops were constructed, the vena cavae were snared for a minimum of 6 cardiac cycles to allow for the recording of pressure–volume loops at varying preloads. Preload recruitable stroke work was evaluated by calculating the relation between stroke work and the end-diastolic volume determined by our conductance catheter technique.³ IS08 v1.8.9.13 software (EMKA Technologies, Inc., Falls Church, Va.). End-systolic elastance was similarly calculated from pressure–volume loops by determining the pressure–volume slope at end-systole at varying preloads.

### Assessment of Endothelial Function

Endothelium-dependent and endothelium-independent vascular relaxation was assessed in vitro by constructing concentration–response curves with a small-vessel myograph for isometric tension recording. Our preliminary investigations determined that endothelial function was similar immediately after cardiac transplantation compared with that of normal, freshly isolated control arterial segments (without storage and cardiac transplantation). Jeannart et al.⁶ previously determined that 48 hours of cold storage does not itself induce endothelial dysfunction in normal porcine epicardial coronary segments but can be used to study the effects of late reperfusion. Accordingly, to expose the progressive endothelial dysfunction that occurs after transplantation, we stored our vascular segments for 48 hours at 4°C after transplantation. Normal, freshly isolated control arterial segments were obtained from coronary segments of the recipient’s native heart, which was harvested after cardiopulmonary arrest and stored similarly for 48 hours.

In brief, the left anterior descending coronary artery was cleaned of fat and connective tissue and placed into Krebs-Henseleit solution
AcqKnowledge software (Biopac Systems Inc.). The rings were connected to an isometric force transducer. Data were collected with the tissue rings were precontracted with a thromboxane A2 analog. U46619 compound (30 nmol/L). Endothelium-dependent vasorelaxation was assessed on the basis of a dose response to analog, U46619 compound (30 nmol/L). Endothelium-dependent vasorelaxation was assessed on the basis of a dose response to bradykinin (0.25 nmol/L–1.0 mol/L). The tissue was stretched to 3 grams and allowed to stabilize for 90 minutes. The rings were contracted with 100 mmol/L KCl and, after washout, stabilized once again for 30 minutes.

The tissue rings were cut into 5.0-mm segments and placed into a 25-mL organ chamber. The rings were suspended between 2 wires, one of which was bathed at all times with Krebs solution containing 11.0 mmol/L glucose at 37°C and oxygenated with a 95% oxygen/5% carbon dioxide mixture. The tissue was stored at 4°C for 48 hours. Vessels were placed into a 25-mL organ chamber. The rings were suspended between 2 wires, one of which was bathed at all times with Krebs solution containing 11.0 mmol/L glucose at 37°C and oxygenated with a 95% oxygen/5% carbon dioxide mixture. The tissue was stretched to 3 grams and allowed to stabilize for 90 minutes. The rings were contracted with 100 mmol/L KCl and, after washout, stabilized once again for 30 minutes.

The tissue rings were precontracted with a thromboxane A2 analog, U46619 compound (30 nmol/L). Endothelium-dependent vasorelaxation was assessed on the basis of a dose response to bradykinin (0.25 nmol/L–1.0 mol/L) and, similarly, endothelium-independent vasodilation was assessed on the basis of a dose response to sodium nitroprusside (10 mmol/L to 5 μmol/L). The percentage of maximum relaxation from preconstriction was calculated as the maximum increase in tension from baseline. Two segments of left anterior descending coronary artery were obtained from each heart and the data were averaged, provided the variability between segments was <10%; otherwise, the data were excluded from analysis.

Biochemical Analysis

The serum concentrations of sodium and lactate were determined by analysis of arterial and coronary sinus whole blood samples using an i-STAT point of care whole blood analyzer (Abbott Laboratories) with EGM® and C4® cartridges, respectively. Coronary sinus blood plasma TNF-α level was measured using a porcine specific ELISA kit (R&D Systems). Myocardial 8-isoprostane level was assayed with an EIA kit (Cayman Chemical Company), following the manufacturer’s directions. Lung tissue TNF-α, IL-2, IL-6, and IL-10 were detected by standard Western blot techniques using porcine specific monoclonal antibodies (R&D Systems, Minneapolis, Minn). Each of the primary antibodies was used at a concentration of 0.05 μg/mL (in a 1% milk solution). Secondary horseradish peroxidase conjugated antibodies were used at a concentration between 1:10 000 and 1:15 000 (Santa Cruz Biotechnology, Santa Cruz, Calif) with ECL Plus used as a substrate (GE Healthcare, UK). Beta-actin was detected as a loading control for all blots. X-ray films were analyzed using a Bio-Rad GS-800 calibrated densitometer and Bio-Rad Quantity One software (Bio-Rad Laboratories, Hercules, Calif).

Statistical Analysis

Statistical analysis was performed using the SAS statistical software (Version 9.0; SAS Institute, Cary, NC). Categorical data were analyzed using a 2-tailed Fisher exact test when appropriate. Continuous data are expressed as the mean±SD and were analyzed by 2-way repeated measures of analysis of variance evaluating the main effects of group and time as well as the interactive effect (group–time).

Statement of Responsibility

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Operative Parameters

There were no differences in the total time to initial blood cardioplegic perfusion before organ implantation (storage time) or in the total ischemic time, because these were carefully controlled by the experimental design (data not shown). Similarly, there were no significant differences in CPB times or recipient clamp times between groups (data not shown). No episodes of arrhythmia occurred during or after infusion of either 0.9% or 7.5% NaCl in donor animals.

Biochemical Parameters

Figure 1 illustrates the arterial blood serum sodium concentration at baseline and during the hour after HTS infusion was completed. Administration of HTS increased the serum sodium level from 138±2 mmol/L to 154±4 mmol/L, which normalized to 144±3 mmol/L 1 hour after infusion. Myocardial lactate release (measured in coronary sinus effluent) was no different between the control and donor HTS groups at baseline before donor heart cardioplegic arrest (1.14±0.13 mmol/L vs 1.39±0.54 mmol/L; P=0.3) and after initial blood cardioplegic perfusion (5.30±1.93 mmol/L vs 5.45±1.70 mmol/L; P=0.9). Myocardial 8-isoprostane levels were not significantly different between the control and donor HTS groups at any time points (data not shown). The Table

Figure 1. Baseline (preinfusion) and post-HTS infusion serum sodium levels. Administration of hypertonic saline acutely increased serum sodium level that subsequently normalized by 1 hour after infusion.
summarizes the levels of TNF-α, IL-2, IL-6, and IL-10 in recipient lung tissue at 5 and 60 minutes of reperfusion as compared to baseline. Donor treatment with HTS significantly reduced coronary sinus blood levels of TNF-α at the end of allograft storage, as well as at 5 and 60 minutes of reperfusion.

### Weaning From CPB and Hemodynamics
A significantly higher proportion of animals in the donor HTS group were successfully weaned from cardiopulmonary bypass compared to the control group (86% vs 17%; $P=0.029$). No transplanted hearts in either group exhibited signs of hyperacute rejection such as cyanosis or coronary thrombosis. All hearts in both groups required initiation of an isoproterenol infusion to assist with weaning from cardiopulmonary bypass. Figure 3 illustrates hemodynamic values measured in the recipient animals pretransplantation and postreperfusion once cardiopulmonary bypass was discontinued. No significant differences in posttransplantation central venous, pulmonary artery, or pulmonary capillary wedge pressures were observed between groups. However, posttransplantation systolic systemic blood pressure was significantly higher in the donor HTS group (60±9 mm Hg vs 35±6 mm Hg; $P=0.04$).

### Left Ventricular Function
Left ventricular function, as assessed by pressure–volume loop analysis, revealed significant differences between the donor HTS and control groups. Figure 4 illustrates 2 independent and load-insensitive measures of left ventricular performance that indicated improved functional recovery of hearts from donors treated with HTS. Preload recruitable stroke work posttransplantation was improved compared to control (88±21% vs 35±8% of baseline; $P=0.0001$). Similarly, end-systolic elastance was improved compared to control (85±17% vs 42±12% of baseline; $P=0.0002$).

### Coronary Endothelial Function
Figure 5 illustrates concentration–response curves of coronary arterial segments to bradykinin, sodium nitroprusside, and endothelin-1 as measures of endothelial-dependent vasorelaxation, endothelial-independent vasorelaxation, and vasospasm, respectively. Organ donor treatment with HTS resulted in improved endothelial-dependent vasorelaxation compared with control (Emax: HTS, 59±4%; control, 47±3%; $P=0.04$; Figure 6A). Donor treatment with HTS also resulted in improved endothelial-independent vasorelaxation compared with control (Emax: HTS, 71±3%; control, 59±4%; $P=0.03$; Figure 6B), as well as improved sensitivity to sodium nitroprusside (ED$_{50}$: HTS, 0.56×10$^{-6}$±0.23 mol/L; control, 2.5×10$^{-6}$±1.0 mol/L; $P=0.04$; Figure 6B). Sensitivity to endothelin-1 induced vasospasm was reduced with donor HTS treatment (% maximum contraction [Cmax]: HS, 338±15%; control, 419±40%; $P=0.01$; Figure 6C).

### Discussion
Primary graft dysfunction continues to be the predominant cause of early mortality after isolated heart transplantation.$^{11}$

---

**Table.** Lung Tissue Cytokine Data

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Control (n=6)</th>
<th>Donor HTS (n=7)</th>
<th>$P$</th>
<th>Control (n=6)</th>
<th>Donor HTS (n=7)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>144±21%</td>
<td>158±37%</td>
<td>0.4</td>
<td>146±39%</td>
<td>156±38%</td>
<td>0.7</td>
</tr>
<tr>
<td>IL-2</td>
<td>158±33%</td>
<td>125±24%</td>
<td>0.08</td>
<td>138±30%</td>
<td>140±44%</td>
<td>0.9</td>
</tr>
<tr>
<td>IL-6</td>
<td>135±13%</td>
<td>133±31%</td>
<td>0.9</td>
<td>107±22%</td>
<td>129±44%</td>
<td>0.3</td>
</tr>
<tr>
<td>IL-10</td>
<td>145±22%</td>
<td>143±13%</td>
<td>0.8</td>
<td>146±28%</td>
<td>167±36%</td>
<td>0.3</td>
</tr>
</tbody>
</table>

---

**Figure 2.** Coronary sinus blood plasma TNF-α levels. Plasma TNF-α levels in coronary sinus effluent at the end of the allograft storage period, at 5 minutes, and at 60 minutes of reperfusion were significantly reduced with donor HTS treatment (end of storage: 11.8±4.1 ng/mL vs 2.3±0.6 ng/mL, *$P<0.0001$; 5 minutes after reperfusion: 15.3±5.1 ng/mL vs 5.8±2.0 ng/mL, §$P=0.004$; 60 minutes after reperfusion: 8.9±3.0 ng/mL vs 3.3±1.2 ng/mL, ¥$P=0.0004$).
Endothelial damage sustained during allograft preservation may increase antigen expression and lead to the development of immunologically mediated late allograft coronary vasculopathy. Ischemia and reperfusion injury during organ retrieval and transplantation has several direct effects on the myocardium. Myocardial contractile function is impaired as a result of decreased energy substrate; there is injury to the cell membrane, cell swelling, intracellular calcium accumulation, impaired protein function, and myocardial cell death and apoptosis. Ischemia and reperfusion can exacerbate myocardial injury indirectly by activating both leukocytes and endothelial cells, resulting in a liberation of reactive oxygen species and a wide variety of inflammatory cytokines. Thus, improved strategies aimed at combined myocardial and endothelial protection during allograft storage are necessary to improve the short-term and long-term outcomes of clinical heart transplantation.

HTS, a volume expander, is well-known to reverse hypotensive states such as hemorrhagic shock. The use of HTS in the trauma setting has been shown to be safe and efficacious. Recent studies have demonstrated that HTS directly affects neutrophil, macrophage, and T-cell function. We hypothesized that the immunomodulatory effects of HTS treatment may provide a novel strategy of combined myocardial and endothelial protection during cardiac allograft transplantation.

Enhanced Allograft Functional Recovery

HTS has been previously studied in the setting of cardiac surgery and has been demonstrated to improve cardiac index without a concomitant rise in pulmonary capillary wedge pressure when administered early postoperatively. HTS infusion before prolonged ischemia and reperfusion protects against myocardial stunning. Reperefusion injury to the myocardium results in intracellular calcium accumulation, predisposing the myocardium to myocyte dysfunction and death. High extracellular sodium concentrations result in the hyperpolarization of the plasma membrane, leading to a forward mode of the Na⁺/Ca²⁺ exchange channel. This forward mode results in a reduction of intracellular calcium stores in the cardiomyocyte. In a rat model of regional ischemia induced by 45 minutes of left anterior descending coronary artery occlusion, Waagstein et al found that HTS infusion improved hemodynamic and myocardial performance during reperfusion. Furthermore, in a porcine model of ischemic left ventricular dysfunction induced by 15 minutes of proximal left anterior descending coronary artery occlusion, Sidi et al found that hypertonic saline administered 5 minutes after reperfusion improved left ventricular contractility and lowered systemic vascular resistance. Given these beneficial effects, we hypothesized that HTS may improve the functional recovery of cardiac allografts from ischemia-reperfusion injury.

We evaluated allograft contractile performance by using load-insensitive measures of left ventricular systolic function from pressure–volume loop analysis after caval occlusion. Donor HTS treatment enhanced the recovery of left ventricular function after transplantation, as determined on the basis of both preload recruitable stroke work and end-systolic elastance. Importantly, the preload recruitable stroke work relationship is a sensitive measure of porcine allograft left ventricular functional recovery after orthotopic transplantation. The decrease of preload recruitable stroke work was attenuated in the allografts from donors treated with HTS, indicating beneficial effects on left ventricular recovery. Furthermore, the ability to wean from cardiopulmonary bypass was significantly greater in the donor HTS-treated group compared with control. Similar to previous studies published from our group, the porcine heart is exquisitely sensitive to ischemia and standard organ preservation techniques fail to preserve the porcine heart even after 4 hours of hypothermic storage. The ability to wean these hearts after 6 hours of static hypothermic storage (compared to intermittent blood perfusion as assessed in previous experiments) further emphasizes the efficacy of this treatment. Improved left ventricular functional recovery also had a positive impact on
Donor HTS treatment enhances left ventricular functional recovery after orthotopic transplantation. Two independent and load-insensitive measures of left ventricular performance indicated improved functional recovery of hearts from donors treated with HTS. A, Preload recruitable stroke work after transplantation was improved compared to control (88±21% vs 35±8% of baseline; *P=0.0001). B,Similarly, end-systolic elastance was improved compared to control (85±17% vs 42±12% of baseline; §P=0.0002). C, D, and E, Representative pressure-volume loops used to determine these parameters from baseline (donor heart before arrest and storage), control, and donor HTS-treated animals (after transplantation), respectively.
systemic blood pressure posttransplantation, because we observed that systolic blood pressure was significantly greater in the donor HTS-treated group compared with control. Therefore, donor HTS treatment before allograft cardioplegic arrest and storage can enhance myocardial preservation with improved recovery of left ventricular function after transplantation.

Enhanced Endothelial Protection
In vitro studies have demonstrated that ischemia-reperfusion activates the endothelial cell leading to the expression of cell surface adhesion molecules such as intracellular adhesion molecules, vascular cell adhesion molecule, and E-selectin, among others.15,23 In addition, activated endothelial cells produce and release cytokines such as IL-1 and IL-8, which promote the adhesion of circulating leukocytes in conjunction with the increased expression of cellular adhesion molecules. During reperfusion, circulating leukocytes bind to the acti-

![Figure 5](image)

**Figure 5.** A. Endothelial-dependent vasodilation in porcine left anterior descending coronary arteries. The graph depicts the cumulative dose–response curves to bradykinin (Bk) in coronary artery segments. Donor heart pretreatment with HTS results in improved endothelial dependent vasorelaxation compared with controls (*P<0.04). B. Endothelial-independent vasodilation of porcine left anterior descending coronary arteries. The graph depicts the cumulative dose–response curves to sodium nitroprusside (SNP) in coronary artery segments. Donor heart pretreatment with HTS results in improved endothelial-independent vasorelaxation compared with controls (§P<0.03). C. Sensitivity to vasospasm. Cumulative dose–response curves to endothelin-1 (ET-1) in coronary artery segments. Donor heart pretreatment with HTS decreased vasosensitivity to ET-1 compared with controls (¥P<0.01).

![Figure 6](image)

**Figure 6.** A. Organ donor pretreatment with HTS resulted in improved endothelial-dependent vasorelaxation compared with control (Emax: HTS, 59±4%; control, 47±3%; *P<0.04). B. Pretreatment with HTS also resulted in improved endothelial-independent vasorelaxation compared with control (Emax: HTS, 71±3%; control, 59±4%; §P<0.03; ED50: HTS, 0.56×10⁻⁶±0.23 mol/L; control, 2.5×10⁻⁹±1.0 mol/L; **P<0.04). C. Sensitivity to endothelin-1 induced vasospasm was reduced with HTS pretreatment (% maximum contraction [Cmax]: HTS, 338±15%; control, 419±40%; ¥P<0.01).
vated endothelial cells, migrate through the endothelial layer into the media, and start to produce oxygen-derived free radicals and toxic cytokines such as TNF-α and TGF-β. Although we were unable to demonstrate differences in oxidative injury by measuring myocardial 8-isoprostanate levels, we did observe significant attenuation of coronary sinus blood TNF-α levels at the end of storage and during allograft reperfusion after donor HTS treatment. This suggests that donor HTS treatment may be protective against activation of endothelial cells within the allograft that results in production of cytokines such as TNF-α. We believe that if this endothelial activation can be attenuated, it may be possible to obviate much of the myocardial dysfunction seen early after cardiac transplantation.

HTS has several effects on neutrophils after either ischemia and reperfusion or hemorrhagic shock by decreasing their ability to be sequestered in the lung and by decreasing rolling, adherence, and migration through the endothelium. Hyper-tonicity results in decreased expression of both CD11b and L-selectin on neutrophils resulting in decreased adherence to the endothelium. Adherence to the endothelium is also inhibited by HTS via the decreased expression of intracellular adhesion molecule-1 on endothelial cells. Zakaria et al. have demonstrated that the endothelial protective effects of HTS demonstrated in cellular studies translates into improved vascular function in a rat model of hemorrhagic shock. A small volume infusion of HTS significantly improved intestinal microcirculation by vasodilation of precapillary arterioles and was associated with significant improvement of endothelial function. Thus, there is both in vitro and in vivo evidence that HTS protects against endothelial activation and improves endothelial function, which may protect against endothelial dysfunction in the setting of transplant-related ischemia-reperfusion injury.

We observed that allograft coronary endothelial dysfunction was a late consequence of reperfusion after prolonged storage. However, it should be noted that animals were euthanized immediately after functional assessments made after the surgical reperfusion period. Thus, we did not assess endothelial function after prolonged reperfusion in vivo. We stored our vascular rings ex vivo in a solution for 48 hours to enhance our ability to detect endothelial dysfunction after transplantation, recognizing that this might not necessarily reflect changes consistent with in vivo reperfusion.

We evaluated coronary artery vasomotor function, ex vivo, in response to bradykinin and sodium nitroprusside as measures of endothelial-dependent and endothelial-independent vasorelaxation, respectively. Additionally, we assessed sensitivity to endothelin-1 induced vasospasm. Donor HTS treatment significantly improved both coronary artery endothelial-dependent and independent vasorelaxation. Furthermore, coronary artery sensitivity to vasospasm after transplantation was significantly attenuated by donor HTS treatment. These findings suggest that donor HTS treatment protects not only endothelial function, but also vascular smooth muscle cell functional integrity.

Limitations
These data are limited to the acute setting after transplantation and the vascular rings were not exposed to 48 hours of in vivo reperfusion. Although we have reason to believe that combined myocardial and endothelial protection will provide long-term benefits, these issues remain to be evaluated. In addition, our technique for the assessment of endothelial function is an ex vivo method and is limited to the epicardial coronary vasculature. Myocardial perfusion was not measured and it is conceivable that regional perfusion differences could result from enhanced endothelial function. We also cannot exclude a sex-related effect because only female swine were used in this study.

HTS administration protocols similar to the one used in the present study have been safely and effectively applied in cardiac surgical patients as mentioned previously. Currently, there is some concern particularly about the presence of hypernatremia in liver organ donors as previous studies have associated organ donor hypernatremia with poor liver graft outcomes. Our pilot study was not designed to assess the outcomes after transplantation of other organs. However, we demonstrated that with our protocol of HTS administration, peak serum sodium levels reached 154±2 mmol/L and returned to normal levels within 1 hour of administration. Because previous studies have only associated donor serum sodium levels >155 mmol/L with poor liver graft survival and function, we believe that the our protocol of donor HTS administration is unlikely to negatively impact the outcomes of liver transplantation. Furthermore, Jawan et al. have demonstrated that a protocol of establishing acute donor hypernatremia by administering a 10% NaCl solution to liver organ donors does not affect the survival of recipients in a rat model of liver transplantation.

Summary
Using a preclinical porcine model of orthotopic heart transplantation, we determined that organ donor treatment with hypertonic saline immediately before allograft harvest and preservation limited endothelial injury and enhanced ventricular recovery after transplantation. We believe that HTS infusions may represent a simple, cost-effective, and efficacious method to improve clinical outcomes after cardiac transplantation. Improved methods of allograft preservation may lead to improved early and late survival after cardiac transplantation.

Sources of Funding
This work was supported by a Heart and Stroke Foundation of Ontario grant-in-aid (T-5346) to Dr Feindel and Dr Rao.

Disclosures
None.

References


5. Cuccieri J, Gourlay D, Garcia I, Jelacic S, Maier RV. Hypertonic preconditioning inhibits macrophage responsiveness to endotoxin. 


14. Ma XL, Weyrich AS, Lefer DJ, Lefer AM. Diminished basal nitric oxide release after myocardial ischemia and reperfusion promotes neutrophil adherence to coronary endothelium. 

15. Ginis I, Mentzer SJ, Li X, Fuller DV. Characterization of a hypoxia-responsive adhesion molecule for leukocytes on human endothelial cells. 


Donor Pretreatment With Hypertonic Saline Attenuates Primary Allograft Dysfunction: A Pilot Study in a Porcine Model
Mitesh V. Badiwala, Danny Ramzy, Laura C. Tumiatil, Elissa D. Tepperman, Rohit Sheshgiri, Jessica L. Prodger, Christopher M. Feindel and Vivek Rao

Circulation. 2009;120:S206-S214
doi: 10.1161/CIRCULATIONAHA.108.843169

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/120/11_suppl_1/S206