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Stabilizing the endothelium of donor hearts with fusogenic liposomes reduces myocardial injury and dysfunction

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Abstract

Background—Myocardial injury after heart transplantation is a consequence of pathophysiological events initiated by local ischemia/reperfusion (IR) injury that is further aggravated by the inflammatory response due to blood exposure to the pump's artificial surfaces during cardiopulmonary bypass (CPB). The purpose of this study was to determine the effectiveness of fusogenic lipid vesicles (FLVs) in enhancing the cardioprotective effect of St. Thomas organ preservation solution (ST). We hypothesized that donor hearts preserved with ST-FLVs will stabilize the endothelium during reperfusion, which in turn will reduce both endothelial barrier dysfunction and myocardial damage.

Materials and methods—To examine the effect of ST-FLVs therapy *in vitro*, C3b deposition and adhesion molecule expression studies were performed on human umbilical vein endothelial cells (HUVECs) challenged with plastic-contact-activated plasma. To assess the therapy *in vivo*, a cervical heterotopic working heart transplantation model in rats was used. Donor hearts were preserved for 1 h at 27°C (15min) and 4°C (45min), and after transplant were followed for 2 h. Left ventricular (LV) function and blood cardiac troponin I (cTnI) levels were quantified.

Results—HUVECs treated with ST-FLVs had reduced C3b deposition and expression of adhesion molecules compared to ST alone ($P < 0.05$). Donor hearts receiving ST-FLVs therapy had reduced LV dysfunction and cTnI compared to ST alone

Conclusion—We conclude that FLVs enhance the cardioprotective effect of ST and reduce post-ischemic LV dysfunction and myocardial damage. The mechanism of protection appears to be associated with the stabilization of endothelial cell membranes due to incorporation of FLV-derived lipids.

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Keywords

Ischemia-reperfusion injury; complement; transplantation; heart; liposomes; endothelium; rat

Introduction

Heart transplantation and cardiopulmonary bypass (CPB) are commonly performed procedures but have potential for significant morbidity and mortality induced by the post-ischemic inflammatory response. When cardiac tissue is subjected to reversible ischemic stress, endogenous mechanisms of cell survival are activated resulting in physiological adaptations that produce what is known as myocardial stunning [1]. Stunning is not only the result of myocardial ischemia, but is also associated with the pathophysiological milieu triggered by reperfusion.[2] [3] The clinical observance of cardiac reperfusion injury has been investigated for some time and is most commonly manifested by dysrhythmia and myocardial dysfunction. While abundant success has been achieved in the laboratory in attenuating reperfusion injury, few advances have taken hold in clinical scenarios due in part to multi-factorial causality. Alterations to the composition of perfusate (i.e., the addition of glutamate) and controlled reperfusion strategies, however, have been shown to lower the incidence of clinical morbidity and mortality in patients following acute myocardial ischemia [4,5].

In heart transplantation, the obligatory ischemia/reperfusion (IR) and the systemic inflammatory reaction induced by blood contact with CPB pump components induce the release of proinflammatory cytokines and activate complement [6–8]. As blood circulates back from the pump into the patient, increased levels of cytokines and complement will interact and activate endothelial cells in heart and other tissues, initiating a systemic inflammatory response [6,9,10]. Tumor necrosis factor- α (TNF- α) is produced in a positive feedback loop by activated monocytes and endothelial cells.[11] Abundant in vitro data demonstrate a role for TNF- α and interleukin-1 β (IL-1 β) in up-regulation of adhesion molecules intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1). Activated endothelial cells and leukocytes express adhesion molecules, which facilitate the migration of leukocytes into the interstitial space, where they release toxic metabolites that lead to myocardial dysfunction[12,13]. In heart transplantation, inappropriate complement activation during CPB and after IR is responsible for a substantial amount of myocardial damage. Tissue injury from complement activation is directly mediated by the MAC, and indirectly by anaphylatoxins (C3a and C5a), which induce early damage through their effects on monocytes/macrophages, polymorphonuclear cells (PMNs), and mast cells[14]. C3a activates leukocytes and endothelial cells causing the release of proinflammatory mediators including TNF- α and IL-1 β . [15] C3b binds to complement receptor 1 (CR1) and its sequential cleavage fragments, iC3b and C3d, bind to complement receptor 2 (CR2) on the surface of endothelial cells, and serve as ligands for β_2 integrins (CD11b/CD18 and CD11c/CD18) present on various inflammatory and immune accessory cells [14,16]. C3b acts as an acceptor site for C5, which is cleaved by C5 convertase to C5b and C5a. C5a also mediates endothelial cell activation by binding to C5a receptors, and also promotes recruitment, and activation of neutrophils and monocytes [17,18]. Binding of C5b to target cell membranes initiates assembly of the MAC.

FDA-approved heart preservation solutions are designed to protect organs during ischemia but provide little protection during reperfusion since they are flushed out by returning blood flow. Our group has focused on developing liposomal-based therapeutic strategies that protect against reperfusion injury by decorating the endothelial barrier of organs with protective agents during *ex vivo* preservation [19,20]. We have developed specially

formulated fusogenic lipid vesicles (FLVs) that, when infused into organs intra-arterially, rapidly incorporate lipids into endothelial cell membranes, which appear to interfere with cell activation. These observations are supported in the literature by infrequent reports indicating that certain liposomes restore the lipid content of cell membranes and inhibit activation of endothelial cells and expression of adhesion molecules [21].

The purpose of the present study was to determine the effectiveness of FLVs in enhancing the cardioprotective effect of St. Thomas organ preservation solution (ST). We hypothesized that donor hearts preserved with FLVs-ST have a more stable endothelium during reperfusion that will reduce endothelial barrier dysfunction and myocardial damage. Here we report that FLV treatment enhances the ST cardioprotective effect by reducing left ventricular (LV) dysfunction and myocardial injury compared to hearts preserved with ST alone.

Materials and Methods

Animals

Male Fisher rats weighing ~350g were used as donors and recipients. All animals received humane treatment in compliance with the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1985). The experiment was commenced after appropriate approval from the University of Louisville IACUC committee.

Preparation of FLVs

Preparation of FLVs was performed as previously reported by Goga et al. with a few minor modifications [19]. Briefly, 1,2-Dioleoyl-*sn*-glycerol-3-phosphocholine (DOPC), 1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphate (DOPA), and/or 1,2-Dioleoyl-*sn*-Glycerol-3-[N(5-Amino-1-Carboxypentyl)iminodiAcetic Acid]succinyl zinc salt (DOGS-NTA-Zn²⁺) were mixed at the appropriate ratio. The lipid material was hydrated in PBS to a concentration of 2mg/mL and diluted 1:10 using ST. The resulting solution of lipid vesicles was sonicated with a Branson Sonifier® 450A (Branson Ultrasonics Corp, Danbury, CT) at 50% duty cycle for 1 minute per mL to form smaller unilamellar FLVs, and the solution was extruded using a Northern Lipids extruder (Toronto, Canada) with a 100nm pore size membrane.

Plastic Contact Activation of Blood

After obtaining informed consent from normal volunteers ~12mL of venous blood was collected in vacutainers containing 0.5mg/mL of lepirudin (Bayer Corp, Pittsburgh, PA). The study was conducted according to the NIH guidelines for human studies research, and was approved by the University of Louisville’s Institutional Review Board (IRB). Volunteers were required to fast overnight prior to venapuncture. After blood was collected, samples were incubated at 37 C and placed on a mechanical rocker for 30 min or 3 h to activate blood. Blood samples were centrifuged at 4500 rpm, and plasma was collected for experiments. Assessment of TNF and complement activation levels was performed in a separate study, and results demonstrated that our plastic-contact-activation method produced significant increases in both to elicit biologic responses in cultured cells (unpublished data).

Endothelial Cell Complement Deposition and Adhesion Molecule Studies

Complement deposition and adhesion molecule expression studies were performed on human umbilical vein endothelial cells (HUVECs) challenged with plastic-contact-activated plasma. Briefly, HUVECs seeded in 96-well plates at a concentration of 8,000 cells/well

were grown to ~90% confluence. Studies were performed in quintuplicate per experimental condition. The experimental groups were as follows: Group 1 was vehicle control; Group 2 was the background control with FITC-labeled antibody; Group 3 was FLVs control; Group 4 was the VCP (0.23 μ M) control; Group 5 was untreated cells incubated with non-activated human plasma; Group 6 was untreated cells challenged with plastic-contact-activated plasma; Group 7 was cells treated with FLVs (0.2mg of lipid /mL) and challenged with plastic-contact-activated plasma; Group 8 was cells treated with FLVs (0.2mg of lipid /mL) and 0.23 μ M vaccinia virus complement control protein (FLVs+VCP) and challenged with plastic-contact-activated plasma. Groups 1–4 were background controls, and Groups 5–8 were incubated for 60 min with plasma diluted 10:1 in either partial cell media containing 2% fetal bovine serum (complement deposition study) or in ST (ICAM-1 and VCAM-1 studies). FLVs (0.2mg of lipid/mL) were incubated for 40 min, and VCP (0.23 μ M) was incubated for 30 min. All incubations were performed at 37°C. In complement deposition experiments a FITC-labeled α -C3 antibody (Cappel MP, Solon, OH) diluted 1:400 was incubated for 40 min at 37°C. In adhesion molecule expression experiments, FITC-labeled ICAM-1 antibodies (Millipore, Billerica, MA) diluted 1:400 or FITC-labeled VCAM-1 antibodies (SouthernBiotech, Birmingham, AL) diluted 1:400 were incubated for 45 min at 37°C. Cells were washed thrice between steps, and after the last wash fluorescent intensity was quantified using a SpectraMax M2^e microplate reader running Softmax Pro software (Molecular Devices, Sunnyvale, CA) with filters set at excitation 494nm, emission 518nm and cutoff at 515nm.

Working Heart Transplantation Studies

Two groups were studied: In the control group, 2mL of ST were infused (n=8), and in the treatment group 2mL of ST-FLVs (0.2mg of lipid/mL) were infused (n=8). During the first 15 min, hearts were preserved at 27°C, and in the subsequent 45 min hearts were placed in ST at 4°C. Hearts were instrumented with latex balloons inserted into the left ventricle connected to pressure transducers and were transplanted heterotopically to the cervical area of recipients using polyethylene cuffs for anastomosis of vessels. LV function at various increasing pre-load volumes was measured after 15, 60 and 120 min of reperfusion. Donor and recipient animals were anti-coagulated with 1.5mg lepirudin diluted in 1mL of ST. Donor hearts were paced at 180 bpm using a 310 Accupulser pulser generator (World Precision Instrument, Sarasota, FL) and a CCSIU isolator unit (Bloom Associates, Ltd., Reading, PA). Transplanted heart function measurements included: LV pressure, dP/dt_{MAX}, heart rate, and donor heart surface electrocardiogram. Recipients were monitored using a Starr pulse-oxymetry system (Life Science Corporation, Oakmont, PA) for pulse, breath distention, oxygen saturation, heart and respiratory rates.

Cardiac Troponin I Analysis

To analyze damage to donor hearts during reperfusion, serum cardiac troponin I levels were measured. Blood samples were taken from recipients prior to transplantation (baseline) and at the end of the 2 hours of reperfusion. A 1mL blood sample was withdrawn each time from the femoral veins of recipients. Samples were centrifuged at 1000 rpm for 10 minutes and plasma was removed and stored at -20°C. Samples were analyzed using Access® AccuTnI™ cardiac troponin I assay (Beckman Coulter, Brea, CA).

Heterotopic Heart Transplantation Procedure

Heterotopic heart transplantation was performed as described by Heron et al. with a few modifications including instrumentation of LVs with a balloon to measure LV function [22]. Briefly, donor hearts were harvested from rats anesthetized with 50mg/kg i.p. of sodium pentobarbital and immediately submersed in ST at 27°C. The heart then received two antegrade infusions of preservation solution via the aorta. The first infusion consisted of

1mL of ST with 1.5mg of lepirudin, and the second consisted of 2mL of ST or ST-FLVs. The vascular pedicles were prepared as follows: The superior and inferior vena cava were ligated along with the left and right pulmonary arteries and veins with silk 6-0. Then, the main pulmonary artery was identified and isolated for posterior anastomosis with the recipient's right internal jugular vein. The left pulmonary veins were ligated with surgical staples. The aorta was clamped distal to the origin of the left carotid artery, which was used in the anastomosis with the recipient's right common carotid, all other aortic branches were ligated with surgical staples. Recipient rats were anesthetized with a mixture of isoflurane (1.5–5%) and oxygen, the skin of the right cervical area was prepared and an transverse incision was performed to allow isolation of the right internal carotid and right internal jugular vein. These vessels were anastomosed to the donor's the left carotid and the common pulmonary artery using custom pre-shaped polyethylene cuffs. The cuffs allowed the use of surgical glue to perform a rapid endothelium-to-endothelium donor-recipient vessel anastomosis.

Instrumentation of LV

To quantify LV function, a balloon was constructed from latex extra thin condoms. The empty balloon was introduced into the LV chamber, and was gradually filled with fluid using an ultra low volume syringe (Harvard Apparatus, Holliston, Massachusetts) until an internal volume of 0.06mL was achieved. The balloon was connected to a pressure sensitive transducer and amplifier (Biopac System, Santa Barbara, CA) via a silicone tube with an internal diameter of 1mm. Data were recorded using a BioPac MP100 recording system with Acknowledge software (Biopac Systems, Goleta, CA) running on a MAC mini-computer (Apple, Cupertino, CA).

Statistical Analysis

Statistics were performed using GraphPad InStat software (GraphPad Software, La Jolla, CA). A oneway analysis of variance (ANOVA) was performed in complement deposition and adhesion molecule studies. A pairwise post-hoc Tukey's test was performed to determine differences between groups. A repeated measures ANOVA was performed in pressure-volume studies. Pairwise post-hoc tests were performed to determine differences between groups and baseline controls. When other variables were compared between two groups unpaired *t*-tests were performed to determine significant differences. Data are presented as means±standard error of the mean (SEM). A *P*-value of <0.05 was considered to be significant.

Results

Effect of FLVs on Complement Deposition and Activation of Endothelial Cells

To determine the effect of FLV therapy on reducing complement activation, HUVECs were incubated with plastic-contact-activated plasma, and assessed for C3b deposition (Fig. 1). FLV treatment reduced complement deposition significantly ($P<0.05$) suggesting that FLV fusion and incorporation of lipids into cell membranes reduces the ability of C3b to bind CR1 receptors on the cell membrane. FLV treatment also appeared to have a mitigating effect on the activation of HUVECs as demonstrated by the reduced expression of ICAM-1 and VCAM-1 compared to controls following a 1-hour incubation with plastic-contact-activated plasma (Fig. 2-A and -B). Levels of ICAM-1 expression increased by 87% and levels of VCAM-1 expression increased by 72% compared to background controls.

Effect of FLVs on Heart Graft Function Following Ischemia

LV pressures were measured at increasing preload volumes to assess the effect of preserving donor hearts with ST-FLVs to reduce post-ischemic myocardial dysfunction. Systolic and diastolic pressure-volume curves were plotted and analyzed to determine LV recovery after ischemic preservation. Figure 3 shows post-ischemic peak systolic LV pressures recorded during 2 h of reperfusion. The results demonstrated that after 15 min of reperfusion ST controls and ST-FLVs-treated hearts generated similar pressures at a given volume (Fig. 3-A). After 60 min of reperfusion (Fig. 3-B), ST-FLVs treated isografts maintained systolic LV pressure whereas pressures in controls significantly declined. After 120 min of reperfusion, ST-FLVs treated isografts maintained peak systolic LV pressure, whereas in controls pressure continued to decline (Fig. 3-C).

Analysis of diastolic LV pressure over 2 h of reperfusion in hearts treated with ST with and without FLVs were similar between the two groups, except after 15 minutes of reperfusion (Fig. 4-A), ST-FLVs-treated hearts generated significantly higher diastolic pressures at the two highest balloon inflation volumes than ST controls ($p<0.05$). However, these pressures fall within the normal physiological range and do not suggest an abnormality. After 60 min and 120 min of reperfusion (Fig. 4-B and -C), there was no significant difference between ST-FLVs treated isografts and ST controls at any of the balloon inflation volumes.

Analysis of dP/dt_{MAX} as an index of LV contractility of grafts followed a similar pattern as peak systolic LV pressures. After 15 minutes of reperfusion (Fig. 5-A), ST-FLVs-treated hearts generated similar dP/dt_{MAX} indices as ST controls at a given volume ($p=NS$). However, after 60 min of reperfusion (Fig. 5-B), there was a significant difference in dP/dt_{MAX} at maximal balloon inflation between ST-FLVs treated isografts and ST controls ($p<0.05$). After 120 min of reperfusion (Fig. 5-C), ST-FLVs treated isografts maintained mean dP/dt_{MAX} whereas dP/dt_{MAX} in controls continued to decline significantly ($p<0.05$).

To quantify level of injury in grafts, cardiac troponin I was measured in recipient animals before transplantation and at the end of 2 h of reperfusion (Fig. 6). Cardiac troponin I levels increased significantly in both ST and ST-FLVs treated groups compared to baseline. However, the levels in controls were significantly higher than in FLV-treated isografts ($P<0.05$). These results suggest that myocardial injury sustained by ST control isografts was considerably greater than those treated with ST-FLVs.

Discussion

The major finding of this study was that FLV therapy added to ST increased the cardioprotective effect of the preservation solution in transplanted hearts. ST-FLVs reduced the level of post-ischemic myocardial injury as demonstrated by decreased blood levels of cardiac troponin I and reduced LV dysfunction. Another important finding in this study was that HUVEC pre-treatment with ST-FLVs was just as effective in reducing endothelial cell activation as FLVs+VCP. This was an unexpected finding since VCP is a potent anti-complement agent. The precise mechanisms by which FLVs inhibit endothelial cell activation are not clear. However, HUVECs pre-treated with ST-FLVs had reduced C3b deposition, ICAM-1 and VCAM-1 expression after exposure to plastic-contact-activated plasma, suggesting that FLV fusion and incorporation of lipids into cell membranes interfere with endothelial cell activation. Furthermore, a review of the literature regarding liposome treatment of cultured cells demonstrate that particular lipids appear to modify the functional properties of membranes, and also have the ability to restore lipid content in old plasma membranes [23]. Treatment of cells with phosphatidylcholines interferes with pro-inflammatory signaling, and therefore may reduce injury [18]. Additionally, there is evidence that liposome treatment restores lipid content of cell membranes and inhibits the

expression of adhesion molecules [18,21]. Therefore, it is plausible that the increased cardioprotective effect of ST-FLVs is primarily associated with the alteration/stabilization of endothelial cell membranes caused by the insertion of FLV derived lipids. In a previous study, utilizing an ischemic hindlimb rat model, we demonstrated that a femoral arterial infusion of FLVs formulated with biotinylated lipids that also was treated with FITC-labeled streptavidin, had a strong fluorescent signal 48 h post-reperfusion in the endothelial layer of femoral vessels suggesting the incorporation of FLV lipids [19]. With these findings in mind, we propose two potential speculative hypotheses regarding the mechanism of action of FLVs that enhance the ST cardioprotective effect.

The first hypothesis proposes a molecular scenario whereby FLV fusion with endothelial cells interferes with the G_i -, nuclear factor (NF)- κ B-, and Rac-dependent mechanism of adhesion molecule expression [24,25]. The key element of this pathway is NF- κ B, which serves as a transcription factor that is activated by signaling from various cell surface receptors, including Toll-like receptors (TLRs) and TNF- α receptors, leading to rapid modifications in gene expression [25–27]. TLRs and TNF- α receptors span the endothelial cell membrane and following FLV fusion the cascade of events associated with NF- κ B activation may be disrupted. The incorporation of FLV derived lipids into cell membranes may alter bilayer stiffness/fluidity, which interferes with adhesion molecule externalization, receptor function, or function of membrane associated proteins required for adhesion molecule expression. Pre-treatment of endothelial cells with cationic liposomes is reported to have an inhibitory effect that is associated with activator protein-1 (AP-1) an important multiprotein complex that forms a trans-activating protein complex with NF- κ B involved in TNF- α induced VCAM-1 expression [21]. Our experiments demonstrated that FLV-treated HUVECs exposed to plasma with high levels of TNF- α and activated complement had reduced C3b binding to CR1, and reduced expression of adhesion molecules. These findings suggest that FLV fusion may primarily interfere with upstream events since C3b was prevented from accessing its target receptor on membranes, and it is plausible that FLV fusion may have interfered with TNF- α receptor function and subsequent signals that lead to expression of adhesion molecules.

The second hypothesis proposes a scenario where incorporation of FLV derived lipids into blood vessel endothelial cell membranes has a beneficial effect on pathogenetic mechanisms associated with endothelial nitric oxide (NO) synthase (eNOS) dysfunction following IR injury. Normal eNOS function generates adequate NO levels to maintain homeostatic smooth muscle relaxation of blood vessels and also to inhibit platelet aggregation. [28] eNOS is only effective when associated with the endothelial cell membrane [29,30], and there is evidence that liposome treatment repairs endothelial cell dysfunction by restoring normal synthesis of NO in hypertensive rat blood vessels [23]. Therefore, we postulate that incorporation of FLV derived lipids into endothelial cell membranes prior to reperfusion, may prevent eNOS from being disengaged or internalized away from cell membranes inducing the cardioprotective effect.

Our syngeneic working heart transplantation model allowed us to examine the effect of ST-FLVs on myocardial stunning following hypothermic preservation. Our FLVs were formulated with lipids that have transition temperatures below 0°C, and therefore, this property prevents FLVs from congealing at 4°C the most frequently utilized organ preservation temperature. The addition of FLVs to ST appears to enhance and prolong the cardioprotective effect beyond the ischemia phase. Our results appear to be in agreement with the observation by others that myocardial stunning is not an outcome strictly related to ischemia, because pharmacological therapy initiated at the time of reperfusion reduces myocardial infarct size, suggesting an association to post-ischemic pathophysiological events triggered by reperfusion [3]. In the clinical setting, prolonged myocardial dysfunction

due to stunning can cause serious hemodynamic instability, which requires pharmacological and/or mechanical support following heart transplantation with CPB. Furthermore, patients with an exaggerated complement and pro-inflammatory cytokine response following CPB have increased postoperative hemorrhage and prolonged ventilator support [31]. The use of FLVs in combination with crystalloid cardioprotective solutions can potentially reduce these complications and reduce hospital stays.

Conclusions

We conclude that FLV fusion to vascular endothelium during ischemia enhances the cardioprotective effect of ST and reduces post-ischemic LV dysfunction. The mechanism of protection appears to be associated with the stabilization of endothelial cell membranes due to incorporation of FLV lipids into cell membranes, which during reperfusion reduce: endothelial cell activation, barrier dysfunction and ultimately myocardial injury. Future mechanistic studies need to be performed to gain a better understanding of signaling involved in the FLV protective effect.

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References

1. Rashed E, Depre C. Cardiac cell survival and reversibility of myocardial ischemia. *Arch. Mal Coeur Vaiss.* 2006; 99:1236. [PubMed: 18942527]
2. Bolli R, Jeroudi MO, Patel BS, Aruoma OI, Halliwell B, Lai EK, McCay PB. Marked reduction of free radical generation and contractile dysfunction by antioxidant therapy begun at the time of reperfusion. Evidence that myocardial "stunning" is a manifestation of reperfusion injury. *Circ. Res.* 1989; 65:607. [PubMed: 2548761]
3. Appleyard RF, Cohn LH. Myocardial stunning and reperfusion injury in cardiac surgery. *J. Card Surg.* 1993; 8:316. [PubMed: 8461524]
4. Allen BS, Buckberg GD, Fontan FM, Kirsh MM, Popoff G, Beyersdorf F, Fabiani JN, Acar C. Superiority of controlled surgical reperfusion versus percutaneous transluminal coronary angioplasty in acute coronary occlusion. *J. Thorac. Cardiovasc. Surg.* 1993; 105:864. [PubMed: 8487565]
5. Cimochoowski GE, Harostock MD, Foldes PJ. Minimal operative mortality in patients undergoing coronary artery bypass with significant left ventricular dysfunction by maximization of metabolic and mechanical support. *J. Thorac. Cardiovasc. Surg.* 1997; 113:655. [PubMed: 9104974]
6. Miller BE, Levy JH. The inflammatory response to cardiopulmonary bypass. *J. Cardiothorac. Vasc. Anesth.* 1997; 11:355. [PubMed: 9161905]
7. Nilsson B, Ekdahl KN, Mollnes TE, Lambris JD. The role of complement in biomaterial-induced inflammation. *Mol. Immunol.* 2007; 44:82. [PubMed: 16905192]
8. Kirklin JK, Westaby S, Blackstone EH, Kirklin JW, Chenoweth DE, Pacifico AD. Complement and the damaging effects of cardiopulmonary bypass. *J. Thorac. Cardiovasc. Surg.* 1983; 86:845. [PubMed: 6606084]
9. Wachtfogel YT, Harpel PC, Edmunds LH Jr, Colman RW. Formation of C1s-C1-inhibitor, kallikrein-C1-inhibitor, and plasmin-alpha 2-plasmin-inhibitor complexes during cardiopulmonary bypass. *Blood.* 1989; 73:468. [PubMed: 2917186]
10. Motte S, van BR, Mottet J, Rondelet B, Mathieu M, Havaux X, Lause P, Clercx C, Ketelslegers JM, Naeije R, McEntee K. Early activation of cardiac and renal endothelin systems in experimental heart failure. *Am. J. Physiol Heart Circ. Physiol.* 2003; 285:H2482. [PubMed: 14613913]

11. Pober JS. Endothelial activation: intracellular signaling pathways. *Arthritis Res.* 2002; 4(Suppl 3):S109. [PubMed: 12110129]
12. He P. Leucocyte/endothelium interactions and microvessel permeability: coupled or uncoupled? *Cardiovasc. Res.* 2010; 87:281. [PubMed: 20472564]
13. Lum H, Roebuck KA. Oxidant stress and endothelial cell dysfunction. *Am. J. Physiol Cell Physiol.* 2001; 280:C719. [PubMed: 11245588]
14. Brown EJ. Complement receptors and phagocytosis. *Curr. Opin. Immunol.* 1991; 3:76. [PubMed: 1675856]
15. Hugli TE. Biochemistry and biology of anaphylatoxins. *Complement.* 1986; 3:111. [PubMed: 3542363]
16. Holers VM. The complement system as a therapeutic target in autoimmunity. *Clin. Immunol.* 2003; 107:140. [PubMed: 12804527]
17. Woodruff TM, Nandakumar KS, Tedesco F. Inhibiting the C5-C5a receptor axis. *Mol. Immunol.* 2011; 48:1631. [PubMed: 21549429]
18. Baker PW, Rye KA, Gamble JR, Vadas MA, Barter PJ. Phospholipid composition of reconstituted high density lipoproteins influences their ability to inhibit endothelial cell adhesion molecule expression. *J. Lipid Res.* 2000; 41:1261. [PubMed: 10946014]
19. Goga L, Pushpakumar SB, Perez-Abadia G, Olson P, Anderson G, Soni CV, Barker JH, Maldonado C. A novel liposome-based therapy to reduce complement-mediated injury in revascularized tissues. *J. Surg. Res.* 2011; 165:e51. [PubMed: 21074780]
20. Pushpakumar SB, Perez-Abadia G, Soni C, Wan R, Todnem N, Patibandla PK, Fensterer T, Zhang Q, Barker JH, Maldonado C. Enhancing complement control on endothelial barrier reduces renal post-ischemia dysfunction. *J. Surg. Res.* 2011; 170:e263. [PubMed: 21816416]
21. Maus U, Rosseau S, Mandrakas N, Schlingensiepen R, Maus R, Muth H, Grimminger F, Seeger W, Lohmeyer J. Cationic lipids employed for antisense oligodeoxynucleotide transport may inhibit vascular cell adhesion molecule-1 expression in human endothelial cells: a word of caution. *Antisense Nucleic Acid Drug Dev.* 1999; 9:71. [PubMed: 10192291]
22. Heron I. A technique for accessory cervical heart transplantation in rabbits and rats. *Acta Pathol. Microbiol. Scand. A.* 1971; 79:366. [PubMed: 4943572]
23. Soloviev AI, Stefanov AV, Bazilyuk OV, Sagach VF. Phospholipid vesicles (liposomes) restore endothelium-dependent cholinergic relaxation in thoracic aorta from spontaneously hypertensive rats. *J. Hypertens.* 1993; 11:623. [PubMed: 8397241]
24. Rizza C, Leitinger N, Yue J, Fischer DJ, Wang DA, Shih PT, Lee H, Tigyi G, Berliner JA. Lysophosphatidic acid as a regulator of endothelial/leukocyte interaction. *Lab Invest.* 1999; 79:1227. [PubMed: 10532586]
25. Lee H, Lin CI, Liao JJ, Lee YW, Yang HY, Lee CY, Hsu HY, Wu HL. Lysophospholipids increase ICAM-1 expression in HUVEC through a Gi- and NF-kappaB-dependent mechanism. *Am. J. Physiol Cell Physiol.* 2004; 287:C1657. [PubMed: 15294853]
26. Chandel NS, Budinger GR. The cellular basis for diverse responses to oxygen. *Free Radic. Biol. Med.* 2007; 42:165. [PubMed: 17189822]
27. Zhang FX, Kirschning CJ, Mancinelli R, Xu XP, Jin Y, Faure E, Mantovani A, Rothe M, Muzio M, Arditi M. Bacterial lipopolysaccharide activates nuclear factor-kappaB through interleukin-1 signaling mediators in cultured human dermal endothelial cells and mononuclear phagocytes. *J. Biol. Chem.* 1999; 274:7611. [PubMed: 10075645]
28. Massion PB, Balligand JL. Modulation of cardiac contraction, relaxation and rate by the endothelial nitric oxide synthase (eNOS): lessons from genetically modified mice. *J. Physiol.* 2003; 546:63. [PubMed: 12509479]
29. Feron O, Michel JB, Sase K, Michel T. Dynamic regulation of endothelial nitric oxide synthase: complementary roles of dual acylation and caveolin interactions. *Biochemistry.* 1998; 37:193. [PubMed: 9425039]
30. du Toit EF, Genade S, Carlini S, Moolman JA, Brunner F, Lochner A. Efficacy of ischaemic preconditioning in the eNOS overexpressed working mouse heart model. *Eur. J. Pharmacol.* 2007; 556:115. [PubMed: 17157294]

31. Holmes JH, Connolly NC, Paull DL, Hill ME, Guyton SW, Ziegler SF, Hall RA. Magnitude of the inflammatory response to cardiopulmonary bypass and its relation to adverse clinical outcomes. *Inflamm. Res.* 2002; 51:579. [PubMed: 12558191]

Figure 1.

Complement deposition assay using HUVECs treated with FLVs only and FLVs+VCP, using partial cell media (2% FBS) as the vehicle. All treatments were performed at 37°C. White bars represent background controls, all groups except for group 1 received FITC-labeled anti-C3b monoclonal antibody (Anti-C3b mAb). Hatched bar (group 5) represents cells incubated in non-activated plasma. Gray bars represent cells incubated with plastic-contact-activated plasma. Group 6 is the untreated positive control group. *P < 0.05 vs. all groups. †P < 0.05 vs. groups 1–6. ‡P < 0.05 vs. groups 1–6. FIU= fluorescence intensity units, FLVs= fusogenic lipid vesicles, VCP= Vaccinia virus complement control protein.

A.

B.

Figure 2.

ICAM-1 (A) and VCAM-1 (B) expression assays using HUVECs treated with ST. Thomas solution and FLVs only or FLVs+VCP. All treatments were performed at 37°C. White bars represent background controls, all groups except for group 1 received FITC-labeled anti-ICAM-1 monoclonal antibody (Anti-ICAM mAb) or FITC-labeled anti-VCAM-1 monoclonal antibody (Anti-VCAM mAb). Hatched bar (group 5) represents cells incubated in non-activated plasma. Gray bars represent cells incubated for 1 h with plastic-contact-activated plasma. Group 6 is the untreated positive control group. In (A) *P < 0.05 vs. all groups. In (B) *P < 0.05 vs Groups 1–5 and 7. FIU= fluorescence intensity units, FLVs= fusogenic lipid vesicles, VCP= Vaccinia virus complement control protein.

Figure 3.

Left ventricular systolic pressure-volume curves generated by heart isografts after (A) 15 min, (B) 60 min and (C) 120 min post ischemia. Grafts were preserved for 1 h of ischemia (15 min at 27°C and 45 min at 4°C) with St. Thomas solution with fusogenic lipid vesicles (FLVs, 0.2mg of lipid/mL) and without (control). Left ventricles of isografts were instrumented with an inflatable balloon that was gradually filled with saline at 0.01mL increments. Balloons were connected to a pressure transducer. Data represents mean±SEM, n=8 per group; *p<0.05 vs. control.

Figure 4.

Left ventricular diastolic pressure-volume curves generated by heart isografts after (A) 15 min, (B) 60 min and (C) 120 min post ischemia. Grafts were preserved for 1 h of ischemia (15 min at 27°C and 45 min at 4°C) with St. Thomas solution with fusogenic lipid vesicles (FLVs, 0.2mg of lipid/mL) and without (control). Left ventricles of isografts were instrumented with an inflatable balloon that was gradually filled with saline at 0.01mL increments. Balloons were connected to a pressure transducer. Data represents mean±SEM, n=8 per group; *p<0.05 vs. control.

Figure 5.

Left ventricular dP/dt_{MAX} -volume curves generated by heart isografts after (A) 15 min, (B) 60 min and (C) 120 min post ischemia. Grafts were preserved for 1 h of ischemia (15 min at 27°C and 45 min at 4°C) with St. Thomas solution with fusogenic lipid vesicles (FLVs, 0.2mg of lipid/mL) and without (control). Left ventricles of isografts were instrumented with an inflatable balloon that was gradually filled with saline at 0.01mL increments. Balloons were connected to a pressure transducer. Data represents mean $\pm$ SEM, n=8 per group; *p<0.05 vs. control.

Figure 6.

Change in cardiac troponin I serum levels of recipients before and after 120 min post-transplantation. Grafts were preserved for 1 h of ischemia (15 min at 27°C and 45 min at 4°C) with St. Thomas solution with fusogenic lipid vesicles (FLVs, 0.2mg of lipid/mL) and without (control). Data represents mean±SEM, n=8 per group; *p<0.05 vs. control; †p<0.05 vs. baseline.