Lazaroid (U74389G)-Supplemented Cardioplegia: Results of a Double-Blind, Randomized, Controlled Trial in a Porcine Model of Orthotopic Heart Transplantation

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Background: U74389G (16-desmethyl tirilazad), a 21-aminosteroid or "lazaroid," inhibits lipid peroxidation, which is an important element of ischemia–reperfusion injury. The aim of this study was to determine whether the addition of U74389G to the cardioplegic preservation solution could improve early cardiac allograft function.

Methods: A porcine model of donor brain death and orthotopic cardiac transplantation was used. Hearts were arrested and preserved for 6 hours in an aspartate-enriched extracellular cardioplegia that had been supplemented with either U74389G and its carrier ($n = 7$) or the carrier alone ($n = 9$). Epicardial sonomicrometry and transmyocardial micromanometry were used to obtain pressure–volume loops before and after transplantation. Left ventricular wall volume was measured by volume displacement.

Results: A higher proportion of U74389G-treated hearts were weaned successfully from cardiopulmonary bypass, but this difference did not achieve statistical significance (86% [6 of 7] vs 56% [5 of 9]; $p = 0.308$). In the hearts that were weaned successfully, preservation of left ventricular contractility, as judged by the pre-load recruitable stroke work relationship, was significantly better in the U74389G-treated hearts ($p = 0.0271$). In contrast, left ventricular compliance, as judged by the end-diastolic pressure–volume relationship, was significantly better preserved in the control group ($p < 0.0001$). U74389G-treated hearts developed less myocardial edema, as judged by the post-transplant left ventricular wall volume/baseline steady-state epicardial end-diastolic volume ratio (64 ± 9% vs 76 ± 11%; $p = 0.045$).

Conclusions: The benefit obtained from U74389G-supplemented cardioplegic preservation solution was marginal for hearts stored for 6 hours. After longer ischemic times, the benefit may be clearer. J Heart Lung Transplant 2003;22:347–356.

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Cardiac allograft transplantation remains the only definitive treatment for end-stage cardiac disease.[1] However, the inherent logistics of cadaveric organ donation subject the donor heart to a period of extracorporeal hypothermic ischemic preservation. Conventional storage solutions provide only limited protection against the associated ischemia–reperfusion injury and consequent early graft dysfunction. One-year mortality increases with each hour of ischemic preservation.[2]

Lipid peroxidation by reactive oxygen species formed on reperfusion is a significant component of the pathophysiology of ischemia–reperfusion injury.[3–5] Under experimental conditions, inhibition of lipid peroxidation by 21-aminosteroids, commonly known as "lazaroids," has been shown to improve functional recovery in a variety of organs,[6–9] including the heart.[10–13]

The present study was conducted to determine, in a clinically relevant large animal model, if the addition of the lazaroid U74389G (16-desmethyl tirilazad) to the cardioplegic preservation solution could reduce the injury associated with conventional hypothermic ischemic preservation and thus improve cardiac function post-transplant.

MATERIALS AND METHODS

The experiments were approved by our institutional animal experimentation ethics committee and conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Animals and Anesthesia

Thirty-six highly inbred Westran pigs (20 to 57 kg) were obtained in sibling pairs. Each animal was pre-medicated with an intramuscular injection of ketamine (10 mg/kg), midazolam (1 mg/kg) and atropine (1.2 mg). General anesthesia was induced with intravenous thiopentone (50 mg boluses, to effect), and maintained by inhaled isoflurane (1% to 3% inhaled gas) and intravenous fentanyl (5 μg/kg boluses). The animals were intubated and ventilated with 100% oxygen. Normal (0.9%) saline was infused intravenously at a rate of 10 ml/kg for the first hour, then 5 ml/kg thereafter. Arrhythmias were treated with internal DC countershock (10 to 30 J) and lignocaine (1 mg/kg). The donor animal received heparin (5,000 units) in preparation for harvest. The recipient animal received heparin (10,000 units) before going on cardiopulmonary bypass ("bypass"). Additional heparin (10,000 units) was added to the pump prime. Recipient animals also received methylprednisolone on induction (500 mg) and 15 minutes before reperfusion (500 mg).

Pulse, cardiac rhythm, arterial pressure and core temperature were monitored continuously in all animals. Left atrial pressure was also monitored in the transplanted heart.

Experimental Design

Orthotopic transplants were performed using hearts harvested from brain-dead donors and subjected to 6 hours of hypothermic ischemic preservation. The primary outcome measures were successful weaning from bypass, left ventricular contractility and left ventricular compliance, as determined by pressure–volume loop analysis, and the post-transplant left ventricular wall volume/baseline steady-state epicardial end-diastolic volume ratio.

Donor animals were randomly assigned to either the lazaroid (LAZ) group ($n = 9$) or control (CON) group ($n = 9$). Hearts in the LAZ group were arrested and stored in U74389G-supplemented cardioplegia. This solution is readily distinguishable from crystalloid cardioplegia because of the presence of the lipid carrier solution used with U74389G. To blind the surgical team at the time of the transplant, the hearts in the CON group were arrested and stored in carrier solution–supplemented cardioplegia (Table I).

The U74389G used in this study was provided free of charge by the manufacturer (Pharmacia Corp., North Peapack, NJ) under an independent external investigator agreement. The cardioplegic solutions were freshly prepared in our laboratory for each experiment. Intralipid 20% (Baxter Healthcare Corp., Deerfield, IL) was diluted to 10% by mixing 1:1 with normal (0.9%) saline. Thirty micromoles (21.8 mg) of U74389G was dissolved in 10 ml of 0.1 mol/liter HCl then mixed, by vortexing, with 10 ml of Intralipid 10%. That solution was then mixed, by vortexing, with 80 ml of normal (0.9%) saline to produce the "carrier + U74389G" solution. The U74389G was omitted for the "carrier − U74389G" solution. The carrier ± U74389G solutions were stored for a maximum of 48 hours at 4°C and protected from light with aluminum foil. St Vincent's cardioplegia[14] (an aspartate-enriched extracellular cardioplegia currently used in the clinical transplantation program at our institution) was prepared on the day of the experiment by our clinical perfusion service. Immediately before organ harvest, the carrier ± U74389G solution was added to St Vincent's cardioplegia and mixed by repeated inversion. We have previously demonstrated that

TABLE I Composition of cardioplegic solutions (concentrations in millimoles per liter)

Substance	St Vincent's cardioplegia (962.5 ml)	Carrier ± U74389G solution (100 ml)	Carrier ± U74389G-supplemented cardioplegia
Na^+	150	130.9	148
Cl^-	117	140.9	119
K^+	19		17
MgSO	5		4
Ca^{2+}	2		2
Bicarbonate	28		26
Aspartate	24		22
Glucose	39		35
Lactate	27		25
Intralipid		1%	0.09%
H^+		10.0	0.9
U74389G		±0.3	±0.028

the carrier solution does not alter the efficacy of the normal preservation solution.[11]

Cardiac Instrumentation and Data Acquisition

The donor heart was exposed via a median sternotomy. Hemispheric ultrasonic dimension transducers (Vernitron, Inc., Bedford, OH) were attached to the epicardium to measure the base–apex major axis and anterior-posterior minor axis dimensions of the left ventricle. These were left in situ while the heart was in storage. A transmyocardial approach was used to place a micromanometer-tipped catheter (Millar Instruments, Inc., Houston, TX) within the left ventricle. This was removed before harvest and re-introduced after transplantation. Dimension and pressure data were obtained at a sampling rate of 200 Hz and digitized (American Data Acquisition Corp., Woburn, MA).

Pressure-dimension data files were recorded immediately before and during transient occlusion of the inferior vena cava. Baseline data were obtained before induction of brain death. Post-transplant data were obtained, after a period of stabilization, in hearts that were weaned successfully from bypass. Mechanical ventilation was suspended during data acquisition.

Cardiac Data Analysis Software (CDAS; James W. Davis Consultant, Inc., Durham, NC) was used to acquire and analyze the data files. The prolate ellipsoid model was used to calculate epicardial left ventricular volume from the dimension data ($LVV = \pi \cdot a \cdot b^2/6$, where LVV is the left ventricular volume, a is the major axis length and b is the minor axis diameter). Pressure–volume loops were then constructed. End diastole was determined automatically, using the first derivative of the pressure trace (dP/dt). The pressure and volume at the end-diastolic timepoint were recorded. Stroke work was calculated as the area of the pressure–volume loop for each beat (end of protodiastole to end of protodiastole) and recorded.

All volume estimates were normalized to the baseline steady-state epicardial end-diastolic volume for each heart. Stroke work was similarly normalized to the baseline steady-state stroke work for each heart. From data obtained during the vena caval occlusion, the relationship between stroke work and end-diastolic volume, termed the preload recruitable stroke work (PRSW) relationship, and the end-diastolic pressure–volume relationship (EDPVR) were determined.

Induction of Brain Death and Donor Management

A Foley catheter was introduced into the donor animal's sub-dural space via a right frontoparietal burr hole. After acquisition of baseline data, the balloon was inflated with water in 3-ml increments every 30 seconds to a total of 21 ml. Fifteen minutes after commencement of balloon inflation, anesthesia was terminated to allow clinical confirmation of brain death. No additional fluid or inotropic support was provided following induction of brain death.

One hour following commencement of balloon inflation, the heart was prepared for harvesting. The left azygos vein, a constant tributary to the coronary sinus in the pig, was ligated outside the pericardium. The superior vena cava was ligated below the right azygos vein. An aortic cross-clamp was applied and the heart arrested by infusion, into the aortic root, of either U74389G-supplemented cardioplegia or carrier

solution–supplemented cardioplegia. The inferior vena cava and left pulmonary vessels were divided to decompress the heart. The heart was excised and then placed in a plastic bag containing cardioplegic solution and submerged in ice for storage.

Orthotopic Transplantation and Recipient Management

A median sternotomy was performed and again the left azygos vein was ligated outside the pericardium. The animal was placed on bypass and actively cooled to 32°C. Orthotopic transplantation of the donor heart was performed using the technique described by Lower and Shumway.[15] Re-warming was commenced during the aortic anastomosis. Once warm, the heart was defibrillated and ventricular demand pacing commenced (120 beats per minute).

A dobutamine infusion (10 μg/kg/min) was commenced 45 minutes post-reperfusion. The first attempt to wean from bypass was made 15 minutes later. No other vasoactive agents were administered. If unsuccessful, further attempts were made over the following hour. Hearts that could not be weaned within 2 hours of reperfusion were considered to have failed to wean from bypass. All animals were killed after post-transplantation data acquisition or upon failure to wean from bypass.

Post-Transplant Left Ventricular Wall Volume/Baseline Steady-State Epicardial End-Diastolic Volume Ratio

At the conclusion of the experiment, the transplanted heart was removed from the recipient. The left ventricle was dissected free of all atrial and valvular tissue and the free wall of the right ventricle. The left ventricular wall volume was determined by volume displacement in a graduated measuring cylinder. The wall volume was divided by the baseline steady-state left ventricular epicardial end-diastolic volume, as determined by sonomicrometry, and expressed as a percentage.

Statistical Analysis

Statistical analyses were performed with SPSS for Macintosh v6.1.1 (SPSS, Inc., Chicago, IL). Differences were considered statistically significant at $p \leq 0.05$. Categoric variables are reported as [the actual incidence of number of hearts in the study group] and compared by Fisher's exact test. Continuous variables are reported as the mean ± the standard deviation with the exception of the regression coefficients, which are the mean ± the standard error. The characteristics of the study groups and the post-transplant left ventricular wall volume/baseline steady-state epicardial end-diastolic volume ratio were compared with the independent-sample *t*-test.

Mean PRSW and EDPVR regression equations were derived for each study group at each timepoint by multiple linear regression (MLR).[16] Only hearts from which pressure–volume loops were obtained post-transplant were included in the baseline analysis. The general linear model used was:

$$Y = b_0 + \sum_{i=1 \text{ to } n-1} p_i \cdot \mathrm{P}_i + b_1 \cdot X \qquad (1)$$

where Y is the normalized stroke work (PRSW) or end-diastolic pressure (EDPVR); P_i is the individual heart dummy variables (effects coding); n is the number of hearts in the study group included in the analysis; X is the normalized epicardial end-diastolic volume; and b_0, b_1 and p_i are the regression coefficients. Regression estimates for X at $Y = 0$ and Y at $X = 1$ were calculated to further characterize the relationships.

To determine whether U74389G altered the effects of preservation on the pressure–volume loop indices, an MLR implementation of analysis of covariance with repeated measures (ANCOVA-RM) was performed.[16] The general linear model used was:

$$Y = b_0 + \sum_{i=1-8} p_i \cdot P_i + b_1 \cdot \mathrm{TP} + b_2 \cdot \mathrm{TG} + b_3 \cdot \mathrm{TP} * \mathrm{TG} + b_4 \cdot X \qquad (2)$$

where Y is the normalized stroke work (PRSW) or end-diastolic pressure (EDPVR); P_i is the individual heart dummy variables (separate effects coding within each study group); TP is the timepoint (effects coding), TG is the treatment group (effects coding); TP * TG is the interaction between TP and TG, X is the normalized epicardial end-diastolic volume; and b_0, b_1, b_2, b_3, b_4 and p_i are the regression coefficients. Interactions were further investigated by performing simplified regressions within study groups or within timepoints.

RESULTS

Two donor hearts were excluded. The first was excluded because it had a large atrial septal defect associated with secondary morphologic ventricular changes (overdeveloped right ventricle and underdeveloped left ventricle). The second donor heart was excluded because it developed a persistent supraventricular tachyarrhythmia after induction of brain death. Both of these hearts had been randomized to the LAZ group, so the final study group sizes were: LAZ, $n = 7$,

TABLE II Characteristics of the 2 study groups

	Lazaroid group	Control group	*p* value
Weights (kg)			
Donor	37 ± 11	37 ± 12	1.000
Recipient	33 ± 11	35 ± 11	0.690
Times (min)			
Brain death to harvest	74 ± 6	73 ± 6	0.756
Warm ischemic time	43 ± 7	47 ± 4	0.271
Total ischemic time	377 ± 32	375 ± 11	0.913
Troponin I (μg/liter)			
At harvest	4.1 ± 3.0	4.0 ± 3.1	0.967

Values are mean ± SD. There were no significant differences between groups.

and CON, $n = 9$. The characteristics of the study groups are detailed in Table II. There were no significant differences between the study groups.

Weaning From Cardiopulmonary Bypass

Overall, 11 of the 16 hearts (69%) were weaned successfully from bypass. A higher proportion of LAZ hearts were weaned successfully, but this difference did not achieve statistical significance (86% [6 of 7] vs 56% [5 of 9]; $p = 0.308$).

Left Ventricular Pressure–Volume Loop Analysis

Representative pressure–volume loops are depicted in Figure 1. The mean regression lines for PRSW and EDPVR for each study group at each timepoint, within the observed data range, are depicted in Figure 2. Coefficients for the mean PRSW and EDPVR regression lines, and the corresponding regression estimates, are presented in Tables III and IV. These were obtained with the MLR model defined in Equation (1). The statistical analysis of the relative effect of storage between groups is presented in what follows and is based on the MLR model defined in Equation (2). Data could not be obtained post-transplant in one of the five CON hearts weaned successfully from bypass because of rhythm instability. The pressure–volume loop analysis is thus based on LAZ, $n = 6$, and CON, $n = 4$.

The PRSW relationship post-transplant had an increased slope and was shifted to the right in both groups, as compared with baseline. The increase in slope was similar in both groups, but the rightward shift in the volume axis intercept was less in the LAZ group. The net effect was a substantial decline in PRSW within each group (both $p < 0.001$), but significantly better preservation of PRSW in the LAZ hearts relative to the CON hearts ($p = 0.271$).

The EDPVR post-transplant had an increased slope and was shifted to the right in both groups, compared with baseline. The increase in slope was less and the rightward shift was longer in the CON group. The net effect was no significant change in compliance in the CON group ($p = 0.2329$) and a significant loss of compliance in the LAZ group ($p < 0.001$), which meant significantly better preservation of compliance in the CON group relative to the LAZ group ($p < 0.001$).

Post-Transplant Left Ventricular Wall Volume/Baseline Steady-State Epicardial End-Diastolic Volume Ratio

Hearts that could not be weaned from cardiopulmonary bypass were macroscopically edematous, consistent with severe ischemia–reperfusion injury. The mean post-transplant left ventricular wall volume in these hearts was 86 ± 3% of the baseline steady-state end-diastolic volume. This was significantly greater than the ratio in hearts that were weaned successfully from bypass, which was 63 ± 5% ($p < 0.001$).

The mean post-transplant left ventricular wall volume/baseline steady-state end-diastolic volume ratio was significantly greater in CON hearts then in LAZ hearts (76 ± 11% vs 64 ± 9%; $p = 0.045$), implying greater edema. The same trend was observed when the analysis was restricted to the 11 hearts successfully weaned from bypass, but it did not reach statistical significance (67 ± 5% vs 61 ± 3%; $p = 0.062$). The difference between groups in the 10 hearts from which post-transplant pressure–volume loops were obtained was minimal (64 ± 3% vs 61 ± 3%; $p = 0.128$).

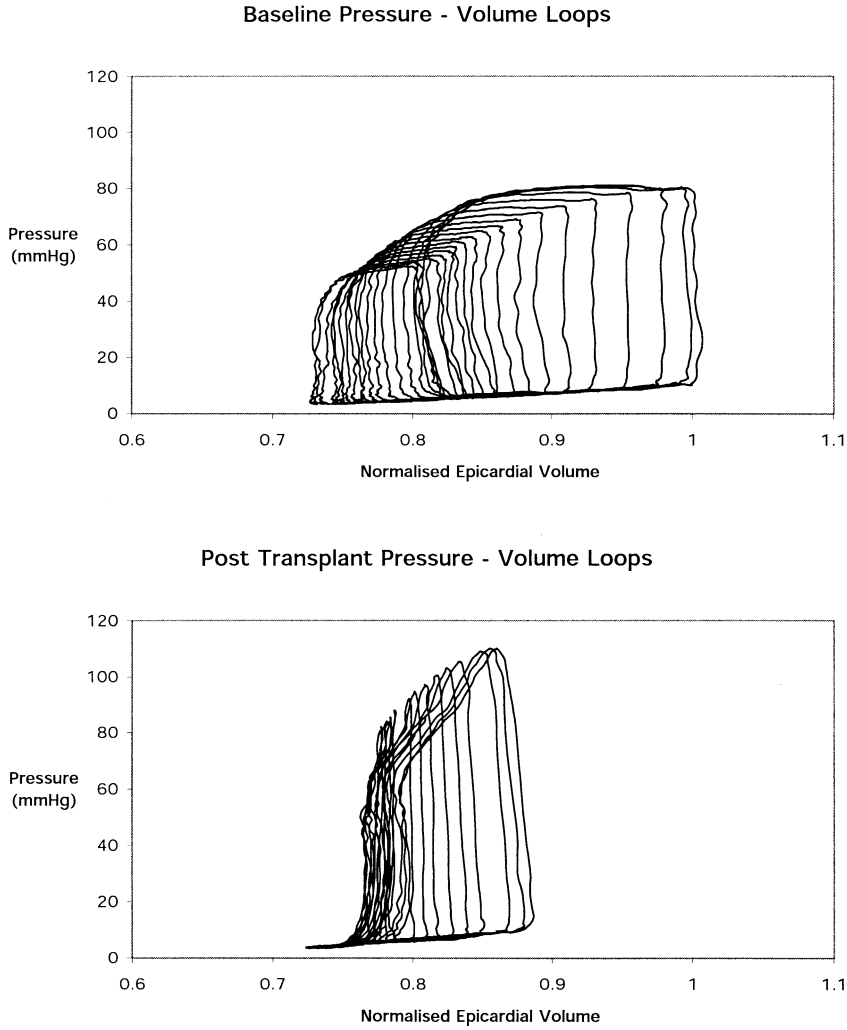

FIGURE 1 Representative left ventricular pressure–volume loops. Pressure–volume loops obtained during transient occlusion of the inferior vena cava at baseline and again post-transplant in the same U74389G-treated heart. The increased dependence on pre-load to produce stroke work is due to the rightward shift in the achievable end-systolic volume. Preload is reduced due to the increased heart rate and the loss of diastolic compliance.

DISCUSSION

This double-blind, randomized, controlled trial, in a clinically relevant large animal model, failed to demonstrate a clear benefit of cardiac allograft preservation in lazaroid (U74389G)-supplemented cardioplegia. This result is in contrast to the available experimental literature, which has consistently shown that lazaroids enhance organ preservation. This may reflect inadequate study power, as the observed difference in weaning from bypass would be of practical significance if substantiated.

Prolonged ischemia predisposes the heart to oxidative stress. This leads, on reperfusion, to a rapid burst in the formation of reactive oxygen species, which cause direct injury to cellular elements. Polyunsaturated fatty acids in biologic membranes are particularly susceptible to injury by reactive oxygen species. Lipid peroxidation compromises membrane integrity and impairs the function of proteins and ion channels associated with the membrane.[3–5] In addition to these direct effects, lipid peroxidation releases inflammatory mediators that act as neutrophil chemotactic factors.[5] Infiltrating neutrophils contribute to the level of reactive oxygen species activity and cause additional damage.[17]

Of particular importance in cardiac ischemia-reperfusion injury is lipid peroxidation involving

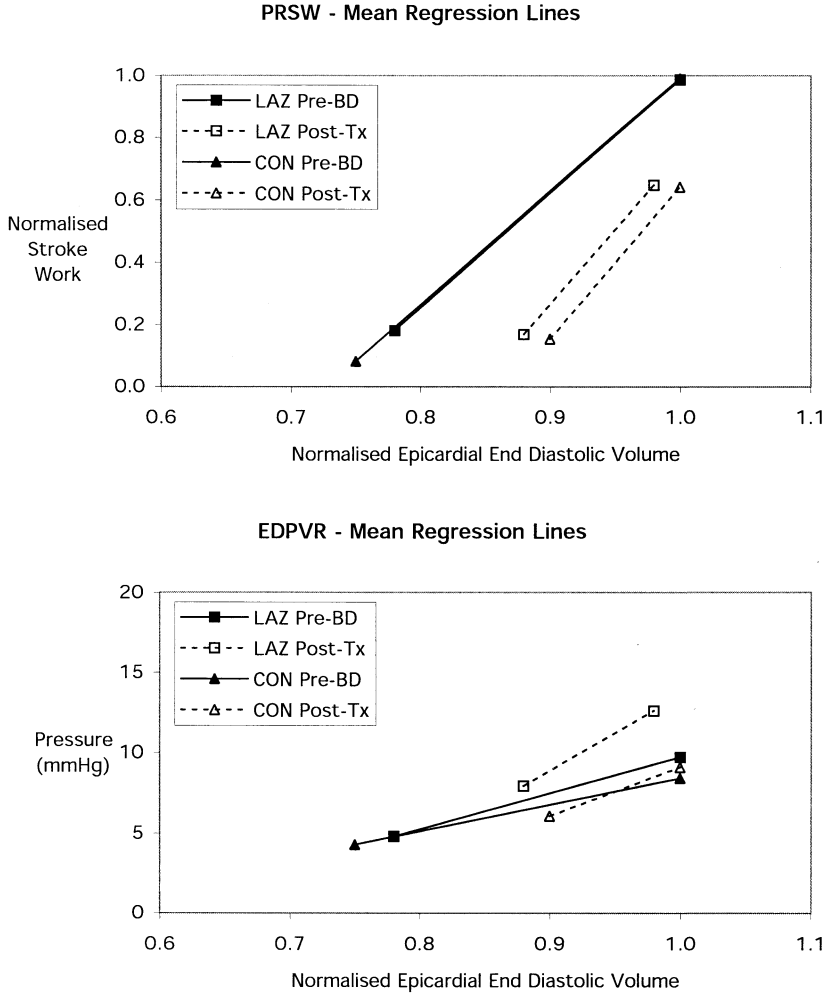

FIGURE 2 Mean PRSW and EDPVR regression lines. Mean regression lines for each study group at each time point are depicted within the observed data range (see Tables III and IV for values). Pre-BD, before brain death; Post-Tx, after transplantation.

membranes associated with cardiac myocytes and coronary microvascular endothelial cells. In the cardiac myocyte, injury to the sarcolemmal membrane, the sarcoplasmic reticulum and mitochondria may lead to intracellular edema, loss of calcium homeostasis and cell death, via both necrotic and apoptotic pathways.[3,4,18] Endothelial cell injury causes interstitial edema, promotes

TABLE III Preload recruitable stroke work relationship

Study group	Time point	Slope	*Y*-axis intercept	*X*-axis intercept	*Y* at *X* = 1
Lazaroid	Baseline	3.66 ± 0.11	−2.68 ± 0.09	0.73	0.99
	Transplanted	4.79 ± 0.15	−4.05 ± 0.13	0.84	0.74[a]
Control	Baseline	3.64 ± 0.12	−2.65 ± 0.10	0.73	0.99
	Transplanted	4.88 ± 0.27	−4.24 ± 0.25	0.87	0.64[ab]

Y, normalized stroke work; *X*, normalized epicardial end-diastolic volume. Regression coefficients (slope and *Y*-axis intercept) are mean ± standard error. Regression estimates (*X*-axis intercept and *Y* at *X* = 1) are calculated from the regression mean.
[a]Significant deterioration in contractility, compared with baseline (both $p < 0.0001$).
[b]Deterioration in control hearts significantly greater than deterioration in lazaroid hearts ($p = 0.0271$).

TABLE IV End-diastolic pressure–volume relationship

Study group	Time point	Slope	*Y*-axis intercept	*X*-axis intercept	*Y* at *X* = 1
Lazaroid	Baseline	22.27 ± 0.71	−12.58 ± 0.62	0.56	9.7
	Transplanted	46.82 ± 1.82	−33.30 ± 1.67	0.71	13.5[a,b]
Control	Baseline	16.5 ± 0.68	−8.10 ± 0.56	0.49	8.4
	Transplanted	30.24 ± 2.63	−21.16 ± 2.43	0.70	9.1

Y, end-diastolic pressure (mmHg); *X*, normalized epicardial end-diastolic volume. Regression coefficients (slope and Y-axis intercept) are mean ± standard error. Regression estimates (*X*-axis intercept and *Y* at *X* = 1) are calculated from the regression mean.
[a]Significant decrease in compliance, compared with baseline ($p < 0.0001$).
[b]Decrease in compliance in lazaroid hearts significantly greater than the decrease in control hearts ($p < 0.0001$).

adhesion and infiltration of activated neutrophils and results in the loss of endogenous nitric oxide production.[17,19]

The 21-aminosteroids, or lazaroids, are a novel class of lipid soluble anti-oxidants. They lack the systemic effects of the corticosteroids from which they were originally derived. They are, however, potent inhibitors of iron-dependent lipid peroxidation.[20]

We have previously demonstrated improved functional recovery of isolated rat hearts, after 6 and 12 hours of hypothermic ischemic preservation, when treated with a lazaroid (U74500A)-supplemented cardioplegia, as compared with hearts treated with conventional cardioplegia.[10] We also demonstrated improved functional recovery after 12 hours of hypothermic ischemic preservation in isolated rat hearts pre-treated with pinacidil (a pharmacologic pre-conditioning agent) and lazaroid (U75400A)-supplemented cardioplegia, when compared with hearts treated with pinacidil and conventional cardioplegia.[11]

Two other groups have investigated the potential for lazaroids to enhance cardiac allograft preservation in large animal models of orthotopic transplantation. Takahashi et al demonstrated a benefit from pre-treatment of the recipient with U74389G before reperfusion of canine hearts that had been stored for 12 hours in University of Wisconsin solution.[12] Tanoue et al demonstrated a benefit from pre-treatment of both the donor and recipient with U74500A, before harvest and reperfusion, respectively, in canine hearts stored for 24 hours in University of Wisconsin solution.[13] The unusual resilience of the canine heart after hypothermic ischemic preservation and the failure to incorporate donor brain death limits the clinical relevance of these models. However, it is important to note that both groups of investigators used a systemic route of administration. It was our hypothesis that cardioplegic supplementation would maximize the amount of drug delivered to the target tissue. Cardioplegic supplementation was the strategy we had successfully applied previously in the isolated rat heart.[10,11] However, the single-pass nature of co-administration with the cardioplegia may not have allowed adequate tissue uptake, limiting the delivered dose to that which was present in the volume of cardioplegia that remained in the vascular space during storage.

The present study was undertaken to assess the cardioplegic supplementation strategy in a more clinically relevant large animal model. This model has several important features that make it an excellent model of clinical cardiac transplantation. First, the porcine heart's intolerance of ischemic preservation is similar to that of the human heart.[21] In addition, the Westran pigs used were sourced from a highly inbred colony within which graft immunotolerance has been demonstrated,[22] removing a potential confounder. Brain death was inflicted on the donor animal, which is known to increase the donor heart susceptibility to ischemia–reperfusion injury.[23,24] Reperfusion in an intact animal incorporates the non-immunologic inflammatory response, which is known to occur with ischemia–reperfusion, bypass and surgery in general.[25] Finally, weaning from cardiopulmonary bypass provides a practical end-point while the pressure–volume loop analyses still allow for quantitative functional comparisons to be made.

It was the practical end-point of weaning successfully from bypass, however, that has made the interpretation of the overall result difficult. The observed difference in weaning successfully from bypass was substantial but not statistically significant because the study lacked the power to detect such a difference. A sample size three times that of the present study would be required to find a difference of the observed magnitude statistically significant. This reflects the fact that, when designing the study, we anticipated few, if any, controls would be weaned successfully from by-

pass. This expectation was based on reports in the literature of weaning rates as low as 3 of 8 orthotopically transplanted porcine hearts, from non–brain-dead donors, after 4 hours of ischemic preservation.[26]

In retrospect, a longer ischemic time may have provided a clearer result in terms of weaning successfully from bypass. Alternatively, a shorter ischemic time may have ensured that all hearts were weaned successfully. This would have avoided the selection bias that was created in the pressure–volume loop analyses by the exclusion of the hearts that could not be weaned. The reported comparison compares only the best 4 control hearts (44% of the study group) to the best 6 U74389G-treated hearts (86% of the study group). This has selected out a group with comparable recovery, and may explain the contradictory findings in left ventricular contractility and compliance.

The observed difference in the mean post-transplant left ventricular wall volume/baseline steady-state end-diastolic volume ratio should be interpreted with caution. First, although the result implies greater edema formation in the control group, it was not validated with a conventional measure of water content. Second, a statistically significant difference was found only when the hearts that failed to wean from bypass were included. This highlights the potential for the difference in the proportion of each group weaned successfully to act as a confounding variable. However, the difference is consistent with a predictable treatment effect and may explain the favorable trend in terms of weaning successfully from bypass.

In conclusion, this double-blind, randomized, controlled trial has demonstrated lazaroid (U74389G)-supplemented cardioplegia to be of marginal benefit over the carrier-supplemented control cardioplegia. In view of the previously reported results supporting the use of lazaroid compounds, further investigation is warranted in this clinically relevant model with larger study groups and with either a longer or shorter ischemic time.

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