ELSEVIER

European Journal of Cardio-thoracic Surgery 23 (2003) 898–906

EUROPEAN JOURNAL OF CARDIO-THORACIC SURGERY

www.elsevier.com/locate/ejcts

The initial rate of troponin I release post-reperfusion reflects the effectiveness of myocardial protection during cardiac allograft preservation

Jonathon B. Ryan*, Mark Hicks, Jonathan R. Cropper, Sarah R. Garlick, Scott H. Kesteven, Michael K. Wilson, Michael P. Feneley, Peter S. Macdonald

Heart and Lung Transplant Unit, St. Vincent's Hospital and the Victor Chang Cardiac Research Institute, Sydney, Australia

Received 19 December 2002; received in revised form 10 February 2003; accepted 17 February 2003

Abstract

Objective: To determine if the initial rate of troponin I release post-reperfusion reflects the effectiveness of myocardial protection during cardiac allograft preservation. **Methods**: A porcine model of orthotopic heart transplantation was used. Data from two control groups (CON_4 and CON_{14}) and two treatment groups (CAR_4 and CAR_{14}) were analysed. Hearts in CON_4 ($n = 6$) and CAR_4 ($n = 6$) were subjected to 4 h of ischaemia while hearts in CON_{14} ($n = 3$) and CAR_{14} ($n = 6$) were subjected to 14 h of ischaemia. All hearts were arrested and stored in the same extracellular preservation solution. Both donor and recipient animals in the CAR_4 and CAR_{14} groups received a single intravenous dose of cariporide (2 mg/kg), prior to explantation and reperfusion, respectively. **Results**: Mean (SEM) plasma troponin I levels (μg/ml) 3 h post-reperfusion were: CON_4 210 ± 52, CAR_4 68 ± 21, CON_{14} 633 ± 177, CAR_{14} 346 ± 93. On multiple linear regression analysis, the rate of troponin I release over the first 3 h post-reperfusion was significantly lower in hearts stored for 4 h compared to hearts stored for 14 h ($P < 0.0001$) and in hearts treated with cariporide compared to control hearts ($P = 0.0017$). Early graft function was superior in hearts treated with cariporide, when compared to control hearts stored for the same period of time. All of the CAR_{14} hearts could be weaned from cardiopulmonary bypass whereas none of the CON_{14} could be weaned (6/6 vs. 0/3; $P = 0.012$). While all hearts stored for 4 h could be weaned, contractility, as measured by the preload recruitable stroke work (PRSW) relationship, was significantly better preserved in CAR_4 hearts than in CON_4 hearts ($P < 0.0001$). **Conclusions**: The initial rate of troponin I release post-reperfusion is determined by the duration of cardiac allograft ischaemia. Altering the myocardial preservation strategy can reduce the rate of release. Such reductions are associated with improvements in early graft function. These findings validate the initial rate of troponin I release post-reperfusion as an end-point when comparing cardiac allograft preservation strategies. In addition, the present study provides indirect evidence that troponin I degradation during ischaemia-reperfusion is related to the accumulation of intracellular calcium.

Keywords: Troponin I; Heart transplantation; Myocardial ischemia; Myocardial reperfusion injury; Myocardial stunning; Sodium–hydrogen antiporter

1. Introduction

The inherent logistics of cadaveric organ donation subject the donor heart to a period of extra-corporeal hypothermic ischaemic preservation. Conventional preservation solutions provide only limited protection against the consequent ischaemia–reperfusion injury, as evidenced by the direct relationship between ischaemic time and recipient mortality [1]. As a result, cardiac allograft preservation remains an area of active research. It is critical that such research uses relevant end-points and that these end-points are subjected to appropriate statistical analysis.

We have previously demonstrated [2] that left ventricular contractile dysfunction in orthotopically transplanted cardiac allografts can be assessed by the preload recruitable stroke work (PRSW) relationship [3]. However, the utility of functional end-points such as the PRSW relationship is limited by the fact that functional data can only be obtained from hearts that are successfully weaned from cardiopulmonary bypass (bypass). In contrast, biochemical end-points are not subject to such limitations.

On the basis of an observational study in 14 patients,

* Corresponding author. P.O. Box 194, Milsons Point, NSW 2061, Australia. Tel.: +61-4-1222-5138; fax: +61-2-9922-5991.

E-mail address: j.ryan@garvan.unsw.edu.au (J.B. Ryan).

1010-7940/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S1010-7940(03)00114-3

Carrier et al. have proposed the initial slopes of the time-activity curves of creatine kinase MB isoenzyme (CK-MB) and cardiac troponin T (cTnT) as biochemical end-points for assessing cardiac allograft preservation injury and comparing preservation strategies [4]. The present analysis, which utilizes data from two related studies that we have recently undertaken, was performed to determine if the initial rate of release of cardiac troponin I (cTnI) is a valid end-point for assessing the effectiveness of myocardial protection during cardiac allograft preservation. Specifically, its validity was tested by determining: (i) whether the initial rate of release of cTnI is related to the duration of cardiac allograft ischaemia, (ii) whether modification of the preservation strategy alters the initial rate of release of cTnI, and (iii) whether there is concordance between inter-group differences in the initial rate of release of cTnI and inter-group differences in post-transplant contractile dysfunction.

2. Methods

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

2.1. Experimental design

We utilized a porcine model of orthotopic heart transplantation, which we have previously described in detail [2]. Two control groups (CON_4 and CON_{14}) and two treatment groups (CAR_4 and CAR_{14}) were studied. Hearts in CON_4 ($n = 6$) and CAR_4 ($n = 6$) were subjected to 4 h of ischaemia while hearts in CON_{14} ($n = 3$) and CAR_{14} ($n = 6$) were subjected to 14 h of ischaemia. All hearts were arrested and stored in the extracellular preservation solution that we currently use in our clinical transplantation program (composition (mmol/l): Na^+ 124.8, Cl^- 120.4, K^+ 19.2, Mg^{2+} 4.7, SO_4^{2-} 4.7, Ca^{2+} 2.3, HCO_3^- 28.2, aspartate 24.1, glucose 39.2, lactate 25.1). Both donor and recipient animals in the CAR_4 and CAR_{14} groups received a single intravenous dose of the sodium-hydrogen exchanger inhibitor cariporide (2 mg/kg), prior to explantation and reperfusion, respectively.

Two different ischaemic times were used so as to determine whether the initial rate of release of cTnI was related to the duration of cardiac allograft ischaemia. Two different preservation strategies were used so as to determine whether modification of the preservation strategy could alter the initial rate of release of cTnI and, if so, whether differences in the initial rate of release of cTnI were associated with corresponding differences in contractile dysfunction. Cariporide was chosen as the preservation strategy intervention so as to test the hypothesis that cTnI degradation during ischaemia-reperfusion is related to the accumulation of intracellular calcium, as sodium-hydrogen exchanger inhibition has been shown to reduce the accumulation of intracellular calcium [5]. The 2×2 nature of the experimental design enabled each comparison to be made under two different sets of experimental conditions.

The preservation solution was prepared on the day of the experiment by our clinical perfusion service and stored in ice until required. Similarly, each dose of cariporide was individually prepared in our laboratory on the day of the experiment. Cariporide powder was weighed, mixed with 0.1–0.2 ml of dimethyl sulfoxide to form a slurry then dissolved in 10 ml of normal (0.9%) saline by vortexing. Aventis Pharma provided the cariporide free of charge, under an independent external investigator agreement.

2.2. Animals and anaesthesia

Forty-two highly inbred Westran pigs (36–68 kg) were obtained in pairs. The larger pig in each pair was used as the donor animal. Each animal was pre-medicated with an intramuscular injection of ketamine (10 mg/kg), midazolam (1 mg/kg) and atropine (1.2–1.8 mg). General anaesthesia was induced with intravenous thiopentone (50 mg boluses, to effect), and maintained by inhaled isoflurane (1–3% 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. Prophylactic lignocaine (1 mg/kg) was administered prior to sternotomy. Arrhythmias were treated with internal DC countershock (10–30 J) and additional lignocaine (1 mg/kg). The donor animal received heparin (5000 units) in preparation for harvest. The recipient animal received heparin (10 000 units) prior to going on cardiopulmonary bypass ('bypass'). Additional heparin (10 000 units) was added to the pump prime and a repeat dose (10 000 units) was given 3 h after the initial dose. Recipient animals also received methylprednisolone on induction (500 mg) and 15 min prior to reperfusion (500 mg).

Pulse, cardiac rhythm, arterial pressure, expired CO_2 and core temperature were monitored continuously in all animals. Left atrial pressure was also monitored in the transplanted heart.

2.3. Cardiac instrumentation and data acquisition

The donor heart was exposed via a median sternotomy. Ultrasonic dimension transducers (2 mm diameter, Sonometrics Corp., Canada) 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., USA) within the left ventricle. This was removed prior to harvest and re-introduced after transplantation. Dimension and pressure

data were obtained at a sampling rate of 200 Hz and digitized (Sonometrics Corp.).

Data files were recorded before induction of brain death and again 3 h post-reperfusion, if the heart was weaned successfully from bypass. These files were recorded immediately before and during transient occlusion of the inferior vena cava. Mechanical ventilation was suspended during data acquisition.

SonoSOFT 3.1.3 software (Sonometrics Corp.) was used to acquire and analyse the data files. The prolate ellipsoid model was used to calculate epicardial left ventricular volume from the dimension data ($LVV = \pi.a.b^2/6$ where LVV is 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 left ventricular pressure trace. The volume at the end-diastolic time point was recorded. Stroke work was calculated as the area of the pressure-volume loop for each beat (end diastole to end diastole). From data obtained during the vena caval occlusion, the PRSW relationship was determined.

2.4. Induction of brain death and donor management

A Foley catheter was introduced into the donor animal's subdural space via a right fronto-parietal burr hole. After acquisition of baseline data, the balloon was inflated with water in 3-ml increments every 30 s to a total of 21 ml. Fifteen minutes after commencement of balloon inflation, anaesthesia was terminated. Typical haemodynamic changes, the absence of responses to painful stimuli after the cessation of anaesthesia and the absence of both pupillary and corneal reflexes were considered adequate 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 1000 ml of cold crystalloid cardioplegia (see above). The inferior vena cava and left pulmonary vessels were divided to decompress the heart. The heart was excised then placed within a plastic bag containing cardioplegic solution and submerged in ice for storage.

2.5. 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 [6]. Rewarming was commenced during the aortic anastomosis. Once warm, the heart was defibrillated and ventricular demand pacing commenced (120 beats/min).

A dobutamine infusion (10 μg/kg per min) was commenced 45 min post-reperfusion. The first attempt to wean from bypass was made 15 min later. No other vasoactive agents were administered. If this was unsuccessful, a second attempt was made at 2 h. If this attempt was also unsuccessful, the dobutamine infusion was increased to 20 μg/kg per min and a final attempt was made at 3 h. All animals were killed following post-transplantation data acquisition or on failure to wean from bypass.

2.6. Troponin I release

The plasma concentration of troponin I was determined at specific time points in both the donor and recipient. In the donor, the blood samples were obtained on induction of anaesthesia, immediately prior to induction of brain death and 1 h after induction of brain death. In the recipient, the blood samples were obtained on induction of anaesthesia and hourly post-reperfusion for 3 h.

At each designated time point, 6 ml of blood was drawn from the arterial line and placed in a lithium heparin tube (Beckton-Dickinson, North Ryde, Australia). The samples were gently mixed by inversion then placed on ice. Within 10 min of collection, the samples were centrifuged at 2000 rpm for 10 min in a refrigerated centrifuge (4 °C). The plasma supernatant was pipetted into two 1.5-ml Eppendorf tubes (1 ml in each) and stored at −20 °C. Within 24 h of completion of each experiment, one of the plasma aliquots was thawed and centrifuged at $12\,000 \times g$ for 10 min, so as to ensure removal of all particulate matter and residual fibrin. The concentration of cTnI was measured using the AxSYM microparticle enzyme immunoassay platform (Abbott Laboratories, Abbott Park, IL, USA) [7]. To ensure the concentration of cTnI fell with the measurement range, post-reperfusion samples were diluted 1:50 with AxSYM matrix buffer.

2.7. Statistical analysis

Statistical analyses were performed with SPSS for Macintosh 6.1.1 (SPSS Inc., USA). Differences were considered statistically significant at a level of $P < 0.05$; Bonferroni corrections were applied where appropriate. Categorical variables are reported as their actual incidence while continuous variables are reported as the mean ± standard error of the mean (SEM). The characteristics of the study groups were compared by analysis of variance or Student's t-test for independent samples, as appropriate. Weaning successfully from cardiopulmonary bypass was compared by Fisher's exact test.

The effect of lengthening the ischaemic time and the effect of modifying the preservation strategy on the initial rate of release of cTnI were analysed simultaneously by a multiple linear regression (MLR) implementation of

analysis of co-variance with repeated measures (ANCOVA-RM) [8], with time post-reperfusion being the co-variate. The general linear model used was:

$$\text{TROP} = b_0 + \Sigma_{i=1-17}\, p_i.P_i + b_1.\text{IT} + b_2.\text{TG} + b_3.\text{IT} * \text{TG} + b_4.\text{TPR} \quad (1)$$

where TROP is the recipient plasma cTnI level, IT is a dummy variable defined by the ischaemic time (4 h = −1, 14 h = +1), TG is a dummy variable defined by the treatment group (control = −1, cariporide = +1), IT * TG is a dummy variable that assesses the interaction between ischaemic time and treatment group allocation (the value being the product of the IT and TG dummy variables), TPR is the time post-reperfusion and $\Sigma_{i=1-17}\, p_i.P_i$ accounts for individual animal variability.

Stroke work and epicardial end-diastolic volume data were normalized, within individual animals, to their respective baseline steady-state values, so as to reduce potential confounding from both heart size and modelling errors in the estimation of ventricular volume from axial dimension measurements. The 'normalized' PRSW relationships were then examined by MLR implementations of ANCOVA-RM [8], with the normalized end-diastolic volume being the co-variate. This approach was used to overcome the complexities created by concurrent changes in the slope (M_w) and volume axis intercept (V_w) of the PRSW relationship, which cannot be adequately dealt with by simple linear regression and comparison of the values obtained for M_w and V_w from each file [2].

The general linear model used to analyse the normalized PRSW relationship for each group at each time point was:

$$\text{nSW} = b_0 + \Sigma_{i=1 \text{ to } n-1}\, p_i.P_i + b_4.\text{nEDV}_{\text{epi}} \quad (2)$$

where nSW is normalized stroke work, nEDV_{epi} is normalized epicardial end-diastolic volume, b_4 is the slope of the relationship ($\text{n}M_w$), b_0 is the nSW-axis intercept and $\Sigma_{i=1 \text{ to } n-1}\, p_i.P_i$ accounts for individual animal variability. Two further indices were derived from these parameters for the purpose of describing the group's normalized PRSW relationship at that time point. These were the nEDV_{epi}-axis intercept, $\text{n}V_w$ ($-b_0 / b_4$) and the stroke work index, SWI ($b_0 + b_4$). SWI is the regression estimate of the group's mean nSW when $\text{nEDV}_{\text{epi}} = 1$ (i.e. at the baseline steady state end-diastolic volume). It represents the interaction between changes in $\text{n}M_w$ and $\text{n}V_w$ at the normal operating volume of the heart, which is the most physiologically relevant end-diastolic volume.

The general linear model used to analyse the relative effect of ischaemic preservation on the study groups was:

$$\text{nSW} = b_0 + \Sigma_{i=n-2}\, p_i.P_i + b_1.\text{TP} + b_2.\text{SG} + b_3.\text{TP} * \text{SG} + b_4.\text{nEDV}_{\text{epi}} \quad (3)$$

where TP is a dummy variable defined by the time point (baseline: −1; post-transplant: +1), SG is a dummy variable defined by the study group (for CON_4 vs. CAR_4: $\text{CON}_4 = -1$, $\text{CAR}_4 = +1$; for CAR_4 vs. CAR_{14}: $\text{CAR}_4 = -1$, $\text{CAR}_{14} = +1$), TP * SG is a dummy variable that assesses the interaction between the time point dummy variable and the study group dummy variable (the value being the product of the TP and SG dummy variables) and the other terms are as defined above.

3. Results

The characteristics of the study groups are detailed in Table 1. There were no significant differences between the study groups.

3.1. Post-induction of anaesthesia cTnI

At induction of anaesthesia, cTnI was not detected in the plasma of 40 of the 42 pigs. In the remaining two pigs the level detected was 0.2 μg/ml, which is within the normal reference range.

3.2. Post-instrumentation and post-induction of brain death cTnI

Immediately prior to the induction of brain death, cTnI was detected in the plasma of 20 of the 21 donor pigs, with a mean concentration of 1.27 ± 0.25 μg/ml. One hour after induction of brain death, cTnI was detected in the plasma of all 21 donor pigs with a mean concentration of 2.54 ± 0.37 μg/ml.

3.3. cTnI release post-reperfusion

Mean recipient plasma cTnI levels for the first three hours post reperfusion are depicted in Fig. 1 (effect of ischaemic time) and Fig. 2 (effect of cariporide treatment). The initial rate of release of cTnI was significantly lower for recipients of hearts stored for 4 h, compared to recipients of hearts treated with the same preservation strategy but stored for 14 h ($\text{CON}_4 < \text{CON}_{14}$ and $\text{CAR}_4 < \text{CAR}_{14}$; $P < 0.0001$). Similarly, the initial rate of release of cTnI was significantly lower in the recipients of cariporide treated hearts, compared to recipients of control hearts that had been stored for the same period of time ($\text{CAR}_4 < \text{CON}_4$ and $\text{CAR}_{14} < \text{CON}_{14}$; $P = 0.0017$). There was no evidence of an interaction between ischaemic time and treatment group allocation ($P = 0.3986$).

3.4. Weaning from cardiopulmonary bypass

All hearts stored for 4 h were successfully weaned from bypass. All of the CAR_4 hearts were weaned at the first attempt whereas only half of the CON_4 hearts could be weaned at the first attempt. The three CON_4 hearts that failed at the first attempt were successfully weaned at the

Table 1
Characteristics of the four study groups[a]

	CAR_4	CON_4	CAR_{14}	CON_{14}	*P*-value
Weights (kg)					
Donor	51 ± 4	44 ± 2	53 ± 3	49 ± 3	0.28
Recipient	50 ± 4	43 ± 2	51 ± 4	45 ± 2	0.28
Times (min)					
Brain death to harvest	71 ± 1	71 ± 1	73 ± 2	75 ± 0	0.12
Implantation time	37 ± 1	40 ± 3	38 ± 2	37 ± 2	0.80
Total ischaemic time	250 ± 5	245 ± 4	847 ± 5	848 ± 5	0.94, 0.39
Troponin I (μg/l)					
At harvest	2.6 ± 0.8	3.7 ± 0.6	2.0 ± 0.5	1.2 ± 0.3	0.14

[a] The group values are the mean ± standard error of the mean. With the exception of the total ischaemic time, the group data was compared by ANOVA. Because differences in the total ischaemic time were created by the experimental protocol, the groups were compared as two pairs CAR_4 vs. CON_4 and CAR_{14} vs. CON_{14}, using Student's *t*-test for independent samples.

second attempt and so were weaned on the same level of dobutamine support as the others.

Of the hearts stored for 14 h, only the CAR_{14} hearts could be weaned from bypass. Half of the CAR_{14} hearts were successfully weaned at the first attempt. The other three failed at both the first and second attempt but were successfully weaned after the level of dobutamine support was increased.

The ability to wean from bypass was thus significantly different between the CON_4 and CON_{14} groups (6/6 vs. 0/3; $P = 0.012$) and between the CAR_{14} and CON_{14} groups (6/6 vs. 0/3; $P = 0.012$). These inter-group differences in post-transplant contractile dysfunction are in agreement with the equivalent inter-group differences in the initial rate of release of cTnI.

3.5. PRSW relationship

Coefficients for the mean PRSW regressions for each study group at each time point, and the corresponding regression estimates, are presented in Table 2. Representative pressure-volume loops and the derived PRSW relationships are depicted in Fig. 3. There are no data available for

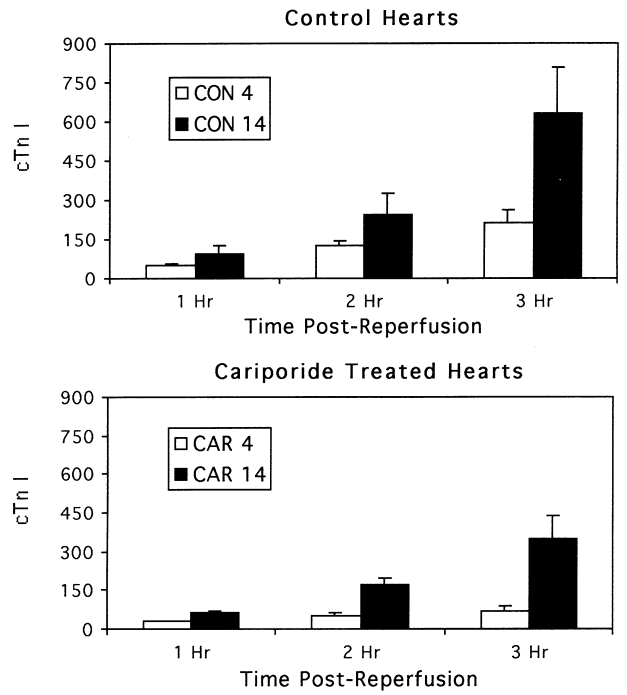

Fig. 1. Effect of ischaemic time on troponin I release post-reperfusion. Recipient plasma cTnI (mean ± standard error of the mean) hourly post-reperfusion. Hearts separated by preservation strategy: upper panel, CON_4 vs. CON_{14}; lower panel, CAR_4 vs. CAR_{14}. The initial rate of cTnI release post-reperfusion was significantly greater in hearts stored for 14 h compared to hearts stored for 4 h ($P < 0.0001$).

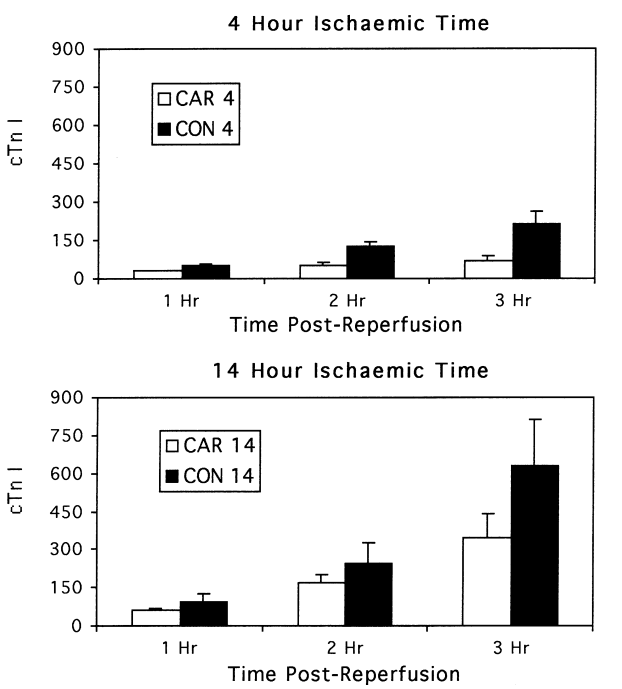

Fig. 2. Effect of cariporide treatment on troponin I release post-reperfusion. Recipient plasma cTnI (mean ± standard error of the mean) hourly post-reperfusion. Hearts separated by ischaemic time: upper panel, CON_4 vs. CAR_4; lower panel, CON_{14} vs. CAR_{14}. The initial rate of cTnI release post-reperfusion was significantly greater in control hearts compared to cariporide treated hearts ($P = 0.0017$).

Table 2
Preload recruitable stroke work (PRSW) relationship[a]

Study group	Time point	nM_w	nSW axis intercept	nEDV$_{epi}$ intercept	SWI
CAR_4*‡	Baseline	3.44 ± 0.07	−2.42 ± 0.07	0.70	1.03
	Post-transplant	5.65 ± 0.16	−4.24 ± 0.14	0.75	1.41
CON_4*	Baseline	3.39 ± 0.05	−2.36 ± 0.04	0.70	1.03
	Post-transplant	4.37 ± 0.12	−3.22 ± 0.10	0.74	1.15
CAR_{14}‡	Baseline	2.99 ± 0.03	−1.96 ± 0.03	0.66	1.03
	Post-transplant	3.48 ± 0.10	−2.59 ± 0.09	0.75	0.88
CON_{14}	Baseline	3.05 ± 0.04	−2.06 ± 0.03	0.68	0.99
	Post-transplant	N/A	N/A	N/A	N/A

[a] Relative recovery of left ventricular contractile function post-transplant: *$CAR_4 > CON_4$ ($P < 0.0001$) and ‡$CAR_4 > CAR_{14}$ ($P < 0.0001$). nM_w, slope of the normalized PRSW relationship; nSW, normalized stroke work; nEDV$_{epi}$, normalized epicardial end-diastolic volume; SWI, stroke work index. Regression coefficients (nM_w and nSW-axis intercept) are mean ± standard error of the mean. Regression estimates (nEDV$_{epi}$-axis intercept and SWI) are calculated from the regression mean.

the CON_{14} hearts post transplant because none of these hearts could be weaned from bypass.

On pair-wise MLR analysis, left ventricular contractility was significantly better preserved in CAR_4 hearts compared to CON_4 hearts ($P < 0.0001$) and in CAR_4 hearts compared to CAR_{14} hearts ($P < 0.0001$). Again, these inter-group differences in post-transplant contractile dysfunction are in agreement with the equivalent inter-group differences in the initial rate of release of cTnI.

4. Discussion

Troponin I (TnI) is a protein found exclusively in striated muscle. Along with troponin T (TnT) and troponin C (TnC), it forms part of the troponin complex, which regulates the calcium sensitivity of the contractile apparatus. Muscle injury, including non-lethal ischaemia, results in the release of TnI into the circulation. As cTnI, the troponin I isoform expressed by cardiac muscle, is readily distinguished from the isoforms expressed by fast and slow twitch skeletal muscle, cTnI is a sensitive and specific marker of myocardial injury [9].

In individuals in whom an acute coronary syndrome is suspected, elevated serum cTnI is diagnostic as myocardial injury can usually only be attributed to myocardial ischaemia. In contrast, the interpretation of elevated serum cTnI levels following cardiac surgery is complex as myocardial injury can be attributed to direct surgical trauma, ischaemia-reperfusion injury and, in some patients,

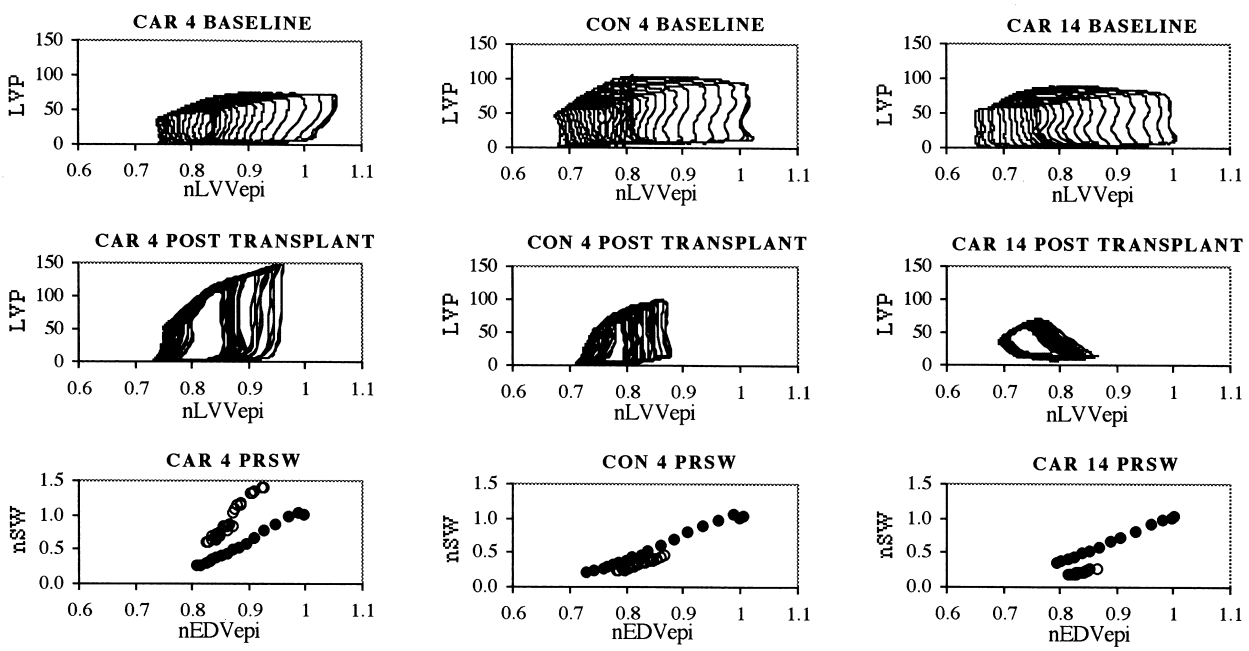

Fig. 3. Left ventricular pressure–volume loops and derived relationships. One heart from each group is depicted before (●) and after transplantation (○). Data obtained during transient occlusion of the inferior vena cava. Stroke work and volume have been normalized to the baseline steady state stroke work and end-diastolic volume for the individual heart.

peri-operative infarction. However, the cTnI release that is due to ischaemia-reperfusion injury is of great practical significance as cTnI degradation within viable myocytes is central to the pathophysiology of myocardial stunning [10].

Despite the above limitations, it is not surprising that cTnI and cTnT are often used to assess the relative effectiveness of myocardial protection strategies during cardiac surgical procedures, including cardiac transplantation, and in models thereof. However, their validity as end-points has largely been assumed. The first assumption is that the severity of the intra-operative ischaemic injury is the primary determinant of troponin release. In the present study, analysing the effect of ischaemic time on the initial rate of cTnI release tested this assumption. The second assumption is that, for any given ischaemic time, the myocardial protection strategy is the primary determinant of the severity of the intra-operative ischaemic injury. On the basis of the first assumption, this implies that the myocardial protection strategy is the primary determinant of troponin release. In the present study, analysing the effect of cariporide treatment on the initial rate of cTnI release tested this assumption. The third assumption is that a reduction in troponin release has practical significance in terms of a reduction in the severity of post-operative dysfunction. In the present study, looking for concordance between the effect of ischaemic time and cariporide treatment on the initial rate of cTnI release and post-transplant contractile dysfunction tested this assumption. While the present study validated each of these assumptions, previous reports have produced conflicting results.

4.1. Relative contribution of intra-operative ischaemia and surgical trauma to troponin release

Bennetts et al. have reported that off-pump coronary artery bypass graft (CABG) surgery reduces cTnT release by 60%, compared to conventional on-pump CABG surgery [11]. This provides clear evidence that troponin release due to intra-operative ischaemia is greater than troponin release due to surgical trauma, as the surgical trauma is similar with both techniques. It does not, however, reflect a reduction in the severity of ischaemic injury. The reduction in intra-operative ischaemia related troponin release in off-pump surgery is achieved by reducing both the volume of myocardium at risk and the duration of intra-operative ischaemia. Indeed the severity of the ischaemic conditions in the myocardium that is rendered ischaemic intra-operatively is actually greater as it is normothermic, contracting and subjected to normal wall tension.

4.2. Troponin release and the duration of intra-operative ischaemia

Others investigators have used the relationship between troponin release and ischaemic time to determine if cTnI or cTnT release reflects the severity of intra-operative ischaemia, as was done in the present study. Carrier et al. [4] reported that the rate of cTnT release, but not peak cTnT, significantly correlated with ischaemic time in clinical heart transplantation. However, they also reported that the correlation between ischaemic time and the rate of cTnT release was not as strong as the correlation between ischaemic time and the rate of CK-MB release. In contrast, following routine CABG surgery, Koh et al. [12] found that the correlation between ischaemic time and cTnT release was stronger than the correlation between ischaemic time and CK-MB. The latter result is consistent with what would be expected on theoretical grounds, as CK-MB is not as specific for myocardial injury as cTnI and cTnT, and is thus generally considered a poor marker of post-operative myocardial injury (for review see Adams [13]).

Not all studies have found a correlation between troponin release and ischaemic time. The difficulty in demonstrating a correlation in clinical studies may be due in part to clustering of ischaemic times and the presence of confounding variables. For example, Carrier et al. also found a positive correlation between the initial rate of cTnT release and donor age [4]. Hirsch et al. [14] found that cTnI release following paediatric cardiac surgery did not correlate significantly with ischaemic time when analysed within procedure-specific cohorts. However, there were marked differences between the procedure-specific cohorts, which correlated with the mean ischaemic times for the procedure-specific cohorts. Thus a significant correlation between ischaemic time and cTnI release became evident when the data were pooled. Etievent et al. [15] found a significant correlation between ischaemic time and cTnI levels at 6 h post-reperfusion following aortic valve replacement (AVR) surgery but not following CABG surgery. They attributed the failure to demonstrate a correlation in the CABG surgery group to confounding factors such as variable protection of the myocardium with antegrade cardioplegia in the presence of coronary artery disease.

4.3. Troponin release and clinical outcome

Consistent with the findings of the present study, Koh et al. [12] reported a negative correlation between post-reperfusion left ventricular contractility and cTnT release. More importantly, others have shown that there is a correlation between troponin release and clinical outcomes. Hirsch et al. [14] correlated cTnI release with inotrope requirements, duration of intubation, duration of intensive care unit stay and duration of hospitalization. Immer et al. [16] correlated cTnI release following paediatric cardiac surgery with inotrope requirement, severity of renal dysfunction and the duration of intubation.

4.4. Troponin release as an end-point for comparing myocardial protection strategies

Overall, the present study and the above evidence

support the principle of troponin release as an end-point for comparing myocardial protection strategies. However, the more practical test is whether or not there is concordance between troponin release data and functional data when myocardial protection strategies are compared. While concordance was observed in the present study, there are a number of examples in the literature in which it has not been. Significant differences in cTnI and cTnT release have been observed in the absence of a significant difference in functional recovery [17–19]. Inversely, significant differences in functional recovery have been observed in the absence of significant differences in cTnT release [20–22].

4.5. Troponin degradation is related to the accumulation of intracellular calcium

cTnI degradation during ischaemia-reperfusion involves both cleavage of cTnI and cross-linking between the troponin complex elements. On the basis of indirect evidence, the cleavage of cTnI has been attributed to calpain, a cysteine protease, while the cross-linking of troponin complex elements has been attributed to a cardiac tissue transglutaminase (TGase) isoform. Both of these enzymes are calcium dependent, which has led previous investigators to hypothesize that cTnI release reflects the intracellular accumulation of calcium [10,23].

The present study provides further indirect evidence for the role of calcium. First, calcium accumulation is related to the duration of ischaemia [24]. Thus the increase in cTnI release from hearts stored for 14 h, compared to the hearts stored for 4 h, can be attributed to the increase in the intracellular accumulation of calcium. Second, sodium-hydrogen exchanger inhibition with cariporide reduces the intracellular accumulation of calcium [5]. Accordingly, the reduction in cTnI release from hearts treated with cariporide, compared to control hearts stored for the same period of time, can be attributed to the reduction in the intracellular accumulation of calcium.

4.6. Conclusion

This study has clearly demonstrated that the initial rate of cTnI release post-reperfusion reflects the effectiveness of myocardial protection during cardiac allograft preservation. This validates its use as an end-point when comparing cardiac allograft preservation strategies. In addition, this study provides indirect evidence that troponin I degradation during ischaemia–reperfusion is related to the accumulation of intracellular calcium.

Acknowledgements

This project was funded by the National Health and Medical Research Council of Australia (Grant 142007). J.B.R. is supported by the National Health and Medical Research Council and the Royal Australasian College of Surgeons. The authors would like to acknowledge the technical assistance provided by the staff of the clinical perfusion service of St Vincent's Hospital.

References

[1] Hertz M, Taylor D, Trulock E, Boucek M, Mohacsi P, Edwards L, Keck B. The registry of the international society for heart and lung transplantation: nineteenth official report – 2002. J Heart Lung Transplant 2002;21:950–70.
[2] Ryan JB, Hicks M, Cropper JR, Garlick SR, Kesteven SH, Wilson MK, Macdonald PS, Feneley MP. The preload recruitable stroke work relationship as a measure of left ventricular contractile dysfunction in porcine cardiac allografts. Eur J Cardiothorac Surg 2002;22:738–45.
[3] Glower DD, Spratt JA, Snow ND, Kabas JS, Davis JW, Olsen CO, Tyson GS, Sabiston Jr DC, Rankin JS. Linearity of the Frank–Starling relationship in the intact heart: the concept of preload recruitable stroke work. Circulation 1985;71:994–1009.
[4] Carrier M, Solymoss BC, Cartier R, Leclerc Y, Pelletier LC. Cardiac troponin T and creatine kinase MB isoenzyme as biochemical markers of ischemia after heart preservation and transplantation. J Heart Lung Transplant 1994;13:696–700.
[5] Stromer H, de Groot MC, Horn M, Faul C, Leupold A, Morgan JP, Scholz W, Neubauer S. Na(+)/H(+) exchange inhibition with HOE642 improves postischemic recovery due to attenuation of Ca(2 +) overload and prolonged acidosis on reperfusion. Circulation 2000;101:2749–55.
[6] Lower RR, Shumway NE. Studies on orthotopic homotransplantation of the canine heart. Surg Forum 1960;11:18–19.
[7] Apple FS, Maturen AJ, Mullins RE, Painter PC, Pessin-Minsley MS, Webster RA, Spray Flores J, DeCresce R, Fink DJ, Buckley PM, Marsh J, Ricchiuti V, Christenson RH. Multicenter clinical and analytical evaluation of the AxSYM troponin-I immunoassay to assist in the diagnosis of myocardial infarction. Clin Chem 1999;45:206–12.
[8] Glantz SA, Slinker BK. Primer of applied regression and analysis of variance, 2nd ed. New York: McGraw-Hill, 2001.
[9] Collinson PO, Boa FG, Gaze DC. Measurement of cardiac troponins. Ann Clin Biochem 2001;38:423–49.
[10] Bolli R, Marban E. Molecular and cellular mechanisms of myocardial stunning. Physiol Rev 1999;79:609–34.
[11] Bennetts JS, Baker RA, Ross IK, Knight JL. Assessment of myocardial injury by troponin T in off-pump coronary artery grafting and conventional coronary artery graft surgery. Aust NZ J Surg 2002;72:105–9.
[12] Koh TW, Hooper J, Kemp M, Ferdinand FD, Gibson DG, Pepper JR. Intraoperative release of troponin T in coronary venous and arterial blood and its relation to recovery of left ventricular function and oxidative metabolism following coronary artery surgery. Heart 1998;80:341–8.
[13] Adams JE. The use of troponins to detect cardiac injury after cardiac and noncardiac surgery. Markers in cardiology: current and future clinical applications. Armonk, NY: Futura, 2001. p. 193–202.
[14] Hirsch R, Dent CL, Wood MK, Huddleston CB, Mendeloff EN, Balzer DT, Landt Y, Parvin CA, Landt M, Ladenson JH, Canter CE. Patterns and potential value of cardiac troponin I elevations after pediatric cardiac operations. Ann Thorac Surg 1998;65:1394–9.
[15] Etievent JP, Chocron S, Toubin G, Taberlet C, Alwan K, Clement F, Cordier A, Schipman N, Kantelip JP. Use of cardiac troponin I as a marker of perioperative myocardial ischemia. Ann Thorac Surg 1995;59:1192–4.
[16] Immer FF, Stocker F, Seiler AM, Pfammatter JP, Bachmann D, Printzen G, Carrel T. Troponin-I for prediction of early postoperative

course after pediatric cardiac surgery. J Am Coll Cardiol 1999;33: 1719–23.
[17] Nediani C, Perna AM, Liguori P, Formigli L, Ibba-Manneschi L, Zecchi-Orlandini S, Fiorillo C, Rizzuti G, Nassi P. Beneficial effects of the 21-aminosteroid U 74389G on the ischemia-reperfusion damage in pig hearts. J Mol Cell Cardiol 1997;29:2825–35.
[18] Caputo M, Modi P, Imura H, Pawade A, Parry AJ, Suleiman MS, Angelini GD. Cold blood versus cold crystalloid cardioplegia for repair of ventricular septal defects in pediatric heart surgery: a randomized controlled trial. Ann Thorac Surg 2002;74:530–4.
[19] Krejca M, Skiba J, Szmagala P, Gburek T, Bochenek A. Cardiac troponin T release during coronary surgery using intermittent cross-clamp with fibrillation, on-pump and off-pump beating heart. Eur J Cardiothorac Surg 1999;16:337–41.
[20] Fischer JH, Kuhn-Regnier F, Jeschkeit S, Switkowski R, Bardakcioglu O, Sobottke R, Rainer de Vivie E. Excellent recovery after prolonged heart storage by preservation with coronary oxygen persufflation: orthotopic pig heart transplantations after 14-hr storage. Transplantation 1998;66:1450–9.
[21] Carrier M, Leung TK, Solymoss BC, Cartier R, Leclerc Y, Pelletier LC. Clinical trial of retrograde warm blood reperfusion versus standard cold topical irrigation of transplanted hearts. Ann Thorac Surg 1996;61:1310–4.
[22] Ericsson AB, Kawakami T, Vaage J. Intermittent warm blood cardioplegia does not provide adequate myocardial resuscitation after global ischaemia. Eur J Cardiothorac Surg 1999;16:233–9.
[23] McDonough JL, Arrell DK, Van Eyk JE. Troponin I degradation and covalent complex formation accompanies myocardial ischemia/reperfusion injury. Circ Res 1999;84:9–20.
[24] Pierce GN, Czubryt MP. The contribution of ionic imbalance to ischemia/reperfusion-induced injury. J Mol Cell Cardiol 1995;27: 53–63.