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

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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 (CON4 and CON14) and two treatment groups (CAR4 and CAR14) were analysed. Hearts in CON4 (n = 6) and CAR4 (n = 6) were subjected to 4 h of ischaemia while hearts in CON14 (n = 3) and CAR14 (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 CAR4 and CAR14 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: CON4 210 ± 52, CAR4 68 ± 21, CON14 633 ± 177, CAR14 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 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 CAR4 hearts could be weaned from cardiopulmonary bypass whereas none of the CON14 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 CAR4 hearts than in CON4 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,
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 (CON4 and CON14) and two treatment groups (CAR4 and CAR14) were studied. Hearts in CON4 (n = 6) and CAR4 (n = 6) were subjected to 4 h of ischaemia while hearts in CON14 (n = 3) and CAR14 (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, Mg2+ 4.7, SO42− 4.7, Ca2+ 2.3, HCO3− 28.2, aspartate 24.1, glucose 39.2, lactate 25.1). Both donor and recipient animals in the CAR4 and CAR14 groups received a single intravenous dose of the sodium-hydrogen exchange 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 CO2 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 reintroduced 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 \( \mu \)g/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 \( \mu \)g/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:

\[
TROP = b_0 + \sum_{i=1}^{17} p_i P_i + b_1 IT + b_2 TG + b_3 IT * TG + b_4 TPR
\]

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 \(\sum_{i=1}^{17} p_i P_i\) 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:

\[
nSW = b_0 + \sum_{i=1}^{n-1} p_i P_i + b_4 nEDV_{epi}
\]

where nSW is normalized stroke work, \(nEDV_{epi}\) is normalized epicardial end-diastolic volume, \(b_2\) is the slope of the relationship (\(M_w\)), \(b_0\) is the nSW-axis intercept and \(\sum_{i=1}^{n-1} p_i P_i\) 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, \(nV_w\) (\(-b_0/b_2\)) and the stroke work index, SWI (\(b_0 + b_2\)). SWI is the regression estimate of the group’s mean nSW when \(nEDV_{epi} = 1\) (i.e. at the baseline steady state end-diastolic volume). It represents the interaction between changes in n\(M_w\) and 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:

\[
nSW = b_0 + \sum_{i=-2}^{n-1} p_i P_i + b_1 TP + b_2 SG + b_3 TP * SG + b_{4i} nEDV_{epi}
\]

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 CON4 vs. CAR4: CON4 = −1, CAR4 = +1; for CAR4 vs. CAR14: CAR4 = −1, CAR14 = +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 (CON4 < CON14 and CAR4 < CAR14; 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 (CAR4 < CON4 and CAR14 < CON14; 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 CAR4 hearts were weaned at the first attempt whereas only half of the CON4 hearts could be weaned at the first attempt. The three CON4 hearts that failed at the first attempt were successfully weaned at the
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 CAR14 hearts could be weaned from bypass. Half of the CAR14 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 CON4 and CON14 groups (6/6 vs. 0/3; \[ P = 0.012 \]) and between the CAR14 and CON14 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

<table>
<thead>
<tr>
<th>Characteristics of the four study groups</th>
<th>Control (CON4)</th>
<th>Control (CON14)</th>
<th>CAR (CAR4)</th>
<th>CAR (CAR14)</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weights (kg) Donor</td>
<td>51 ± 4</td>
<td>44 ± 2</td>
<td>53 ± 3</td>
<td>49 ± 3</td>
<td>0.28</td>
</tr>
<tr>
<td>Weights (kg) Recipient</td>
<td>50 ± 4</td>
<td>43 ± 2</td>
<td>51 ± 4</td>
<td>45 ± 2</td>
<td>0.28</td>
</tr>
<tr>
<td>Times (min) Brain death to harvest</td>
<td>71 ± 1</td>
<td>71 ± 1</td>
<td>73 ± 2</td>
<td>75 ± 0</td>
<td>0.12</td>
</tr>
<tr>
<td>Times (min) Implantation time</td>
<td>37 ± 1</td>
<td>40 ± 3</td>
<td>38 ± 2</td>
<td>37 ± 2</td>
<td>0.80</td>
</tr>
<tr>
<td>Total ischaemic time</td>
<td>250 ± 5</td>
<td>245 ± 4</td>
<td>847 ± 5</td>
<td>848 ± 5</td>
<td>0.94, 0.39</td>
</tr>
<tr>
<td>Troponin I (µg/l) At harvest</td>
<td>2.6 ± 0.8</td>
<td>3.7 ± 0.6</td>
<td>2.0 ± 0.5</td>
<td>1.2 ± 0.3</td>
<td>0.14</td>
</tr>
</tbody>
</table>

\* 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 CAR4 vs. CON4 and CAR14 vs. CON14, using Student’s \( t \)-test for independent samples.

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**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, CON4 vs. CON14; lower panel, CAR4 vs. CAR14. 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, CON4 vs. CAR4; lower panel, CON14 vs. CAR14. The initial rate of cTnI release post-reperfusion was significantly greater in control hearts compared to cariporide treated hearts (\( P = 0.0017 \)).
the CON14 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 CAR4 hearts compared to CON4 hearts ($P_{0.0001}$) and in CAR4 hearts compared to CAR14 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.

### 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,
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 cTnI 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.

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