ELSEVIER

European Journal of Cardio-thoracic Surgery 22 (2002) 738–745

EUROPEAN JOURNAL OF CARDIO-THORACIC SURGERY

www.elsevier.com/locate/ejcts

The preload recruitable stroke work relationship as a measure of left ventricular contractile dysfunction in porcine cardiac allografts

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

Cardiac Mechanics Research Laboratory, St. Vincent's Hospital and the Victor Chang Cardiac Research Institute, Victoria Street, Darlinghurst, Sydney, NSW 2061, Australia

Received 13 May 2002; received in revised form 26 June 2002; accepted 21 August 2002

Abstract

Objective: Paradoxically, it has been reported that after 1.5–4 h of hypothermic ischaemic preservation there is complete recovery of contractile function in canine cardiac allografts, as assessed by the preload recruitable stroke work (PRSW) relationship. This raises questions about the suitability of the canine heart as a model for preservation research and the PRSW relationship as an end-point. The aim of the present study was to evaluate the PRSW relationship as an index of left ventricular contractility in porcine cardiac allografts. **Methods**: Eighteen orthotopic heart transplants were performed in inbred Westran pigs. Brain death was induced in the donor pigs 1 h prior to explantation. The donor hearts were arrested with extracellular cardioplegia, which was stored in ice prior to administration. On explantation, the donor hearts were immersed in cardioplegia and stored in ice. The donor hearts were subjected to either 4 (IT4, $n = 6$), 6 (IT6, $n = 9$) or 14 (IT14, $n = 3$) h of ischaemia. Post-transplant, all hearts were supported with dobutamine (10 mcg/kg per min). The PRSW relationship was derived from pressure-volume loops obtained by epicardial sonomicrometry and transmyocardial micromanometry. Multiple linear regression was used to describe and compare the PRSW relationship before brain death in the donor and after weaning from bypass in the recipient. **Results**: Eleven hearts were weaned successfully from cardiopulmonary bypass: IT4 100% (6/6), IT6 56% (5/9) and IT14 0% (0/3) (IT4 versus IT14: $P = 0.012$). Analysis of the PRSW relationship revealed a reduction in contractility in both the IT4 and IT6 groups (both $P < 0.0001$), but a greater reduction in the IT6 group ($P < 0.0001$). Notably, the volume-axis intercept of the PRSW relationship was found to be a better discriminator of post-preservation contractile dysfunction than the slope of the PRSW relationship. **Conclusions**: The porcine heart's susceptibility to ischaemic injury makes it ideal for evaluating the effect of different preservation strategies on contractile recovery. The PRSW relationship can be used to evaluate the differences in contractile recovery, though the nature of the effect of ischaemic preservation necessitates analysis by multiple linear regression.

Keywords: Ventricular function; Left; Preload recruitable stroke work relationship; Regression analysis; Ischaemia-reperfusion; Myocardial stunning; Heart transplantation

1. Introduction

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 extra-corporeal hypothermic ischaemic preservation. Conventional storage solutions provide only limited protection against ischaemia-reperfusion injury and consequent early graft dysfunction. One-year mortality increases with each hour of ischaemic preservation [2].

Only one group, Bittner and co-workers [3,4], has used a realistic large animal model of donor brain death and orthotopic heart transplantation to systematically investigate the effect of ischaemia, and more specifically the effect of varying the duration of ischaemia, on cardiac contractility. They subjected canine cardiac allografts to either 1.5 or 4 h of ischaemia and assessed contractility with the preload recruitable stroke work (PRSW) relationship, which is the linear relationship between stroke work (SW) and end-diastolic volume (EDV) [5]. Paradoxically, Bittner et al. observed that the PRSW relationship post-transplant was not significantly different to the PRSW relationship pre-explantation. They concluded that ischaemic preservation does not reduce contractility. However, as the duration of ischaemia is known to determine the severity of ischaemia-reperfusion injury [6,7], an alternative interpretation of the results obtained by Bittner et al. is that either methodological or

* Corresponding author. Tel.: +61-412-225-138; fax: +61-2-9922-5991.
E-mail address: j.ryan@garvan.unsw.edu.au (J.B. Ryan).

1010-7940/02/$ - see front matter
PII: S1010-7940(02)00526-2

analytical shortcomings prevented them from identifying the reduction in contractility that probably occurred. Appreciation of such shortcomings has important implications for study design in the field of myocardial preservation research.

Of particular importance is whether or not the PRSW relationship is an appropriate end-point for identifying contractile dysfunction in the transplanted heart. The hypothesis underlying the present study is that the PRSW relationship is a valid end-point but that care must be taken in the way in which it is analysed and interpreted. Bittner et al. based their conclusion on independent comparisons of the effect of ischaemic preservation on the slope (M_w) and volume axis intercept (V_w) of the PRSW relationship. While this approach to regression analysis is commonly used, it is inappropriate for linear relationships in which there are potentially changes in both slope and *x*-axis intercept, as co-variate dependence develops between the slope and *x*-axis intercept [8]. Ischaemia characteristically causes an increase in the volume (*x*) axis intercept of the PRSW relationship [9–12]. This is known as the 'creep' phenomenon and persists after reperfusion [9–12]. In contrast, M_w is reduced during ischaemia but may return to normal or even be increased after reperfusion [9–12]. These simultaneous movements in M_w and V_w make interpretation of changes in the PRSW relationship difficult and necessitate the use of multiple linear regression techniques [8].

The aim of the present study was to assess the PRSW relationship as an index of left ventricular contractility in cardiac allografts after varying periods of ischaemic preservation. In contrast to the studies by Bittner et al. [3,4], a porcine model was used and the changes in the PRSW relationship were examined by multiple linear regression.

2. Materials and 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. Animals and anaesthesia

Thirty-seven highly inbred Westran pigs (22–57 kg) were obtained. 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.

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. Arterial blood gases were obtained as required.

2.2. Experimental design

Experiments were conducted in a porcine model of orthotopic cardiac allograft transplantation. We have previously described this model in detail [13]. It was originally adapted from the canine model used by Bittner et al. [3,4]. In brief, inbred Westran pigs were obtained in pairs with the larger pig in each pair being used as the donor pig. Brain death was induced in the donor pig, so as to subject the donor heart to the catecholamine storm associated with acute brain death. The donor heart was arrested with an extracellular cardioplegia, then explanted and stored in ice. After the period of storage defined in the experimental protocol, the heart was orthotopically transplanted into the recipient pig. Post-reperfusion, all hearts were supported with dobutamine (10 mcg/kg per min) to facilitate weaning from cardiopulmonary bypass (bypass).

Three study groups were defined on the basis of the duration of ischaemic preservation: IT4 (4 h, $n = 6$), IT6 (6 h, $n = 9$), and IT14 (14 h, $n = 3$). (These study groups represent the control groups from three separate studies into novel preservation strategies. As a consequence, differences exist between the protocol used for the IT6 group and the protocol used for the IT4 and IT14 groups. These differences are detailed below at the points where they arise.) Solely for the purposes of illustration, the effect of a dobutamine infusion at 10 mcg/kg per min on a normal heart was studied in an additional animal (IT0, $n = 1$).

2.3. Cardiac instrumentation and data acquisition

The donor heart was exposed via a median sternotomy. Ultrasonic dimension transducers (IT4 and IT14: Sonometrics Corp., Canada; IT6: Vernitron, Inc., USA) 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 digitised (IT4 and IT14: Sonometrics Corp., Canada; IT6: American Data Acquisition Corp., USA).

Data files were recorded in all donors before induction of brain death. However, data files were only recorded in the recipient if the heart could be weaned from cardiopulmonary bypass. Data was obtained at 2 h post-reperfusion in the IT6 hearts and at 3 h post-reperfusion in the IT4 and IT14 hearts. (From our experience of the present model, this difference creates a bias which favours the IT6 group (unpublished data)). These files were recorded immediately before and during transient occlusion of the inferior vena

cava. Mechanical ventilation was suspended during data acquisition.

Customised software (IT4 and IT14: SonoSOFT 3.1.3 Software, Sonometrics Corp., Canada; IT6: Cardiac Data Analysis Software, James W Davis Consultant Inc., USA) 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 ($V = \pi ab^2/6$ where V = left ventricular volume, a = the major axis length and b = the minor axis diameter). Pressure-volume loops were then constructed. End-diastole was determined automatically by the software, using either the left ventricular pressure trace or the first derivative of the pressure trace (dP/dt). The volume at the end diastolic time point was recorded (EDV_{epi}). SW was calculated as the area of the pressure-volume loop for each beat (IT4 and IT14: end-diastole to end-diastole; IT6 end of protodiastole to end of protodiastole). From data obtained during the vena caval occlusion, the PRSW relationship was determined.

2.4. Induction of brain death

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 stimuli after the cessation of anaesthesia and the absence of both pupillary and corneal reflexes were considered adequate confirmation of brain death for the purposes of the study. No additional fluid or inotropic support was provided following induction of brain death.

2.5. Explantation and cardiac allograft preservation

The heart was arrested 1 h after induction of brain death by clamping the ascending aorta and infusing into the aortic root 1000 ml of crystalloid cardioplegia, which had been stored in ice prior to administration. The composition of the cardioplegia administered to IT4 and IT14 hearts was (in mmol/l): Na^+ 150, Cl^- 117, K^+ 19, $MgSO_4$ 5, Ca^{2+} 2, Bicarbonate 28, Apartate 24, Glucose 39, Lactate 27. This extracellular preservation solution is currently used in the clinical transplantation program at our institution [14]. The cardioplegic solution administered to the IT6 study group also contained a lipid based carrier solution which slightly altered the concentrations of the constituents to (in mmol/l): Na^+ 148, Cl^- 119, K^+ 17, $MgSO_4$ 4, Ca^{2+} 2, Bicarbonate 26, Apartate 22, Glucose 35, Lactate 25, Intralipid 0.09%. We have previously demonstrated that the carrier solution does not alter the efficacy of the normal preservation solution [15]. (The reason for adding it was related to treatment group allocation blinding in the study from which this group is taken).

While the cardioplegia was being administered, the heart was decompressed by ligation of the superior vena cava, transection of the inferior vena cava and transection of the left hilar pulmonary vessels. Care was taken during explantation to maximise the length of the great vessels. On explantation, the heart was inspected and a ligature placed on the left azygos vein, a constant tributary of the coronary sinus in the pig. The great arteries were separated. The posterior wall of the left atrium, as defined by the pulmonary veins, was excised. The heart was then immersed in cardioplegic solution within a plastic bag. The plastic bag was then placed in an insulated container and packed in ice for storage.

2.6. Orthotopic transplantation

A median sternotomy was performed and the left azygos vein was ligated. Snares were placed around both the superior and inferior vena cava and 3-0 prolene (Ethicon, NJ, USA) purse-string sutures were placed in the ascending aorta and superior and inferior vena cava. These sites were then cannulated, the aorta cross-clamped and complete bypass instituted. The recipient was actively cooled to 32°C.

Orthotopic transplantation of the donor heart was performed using the technique described by Lower and Shumway [16]. First, the native ventricles were excised by transection of the great arteries close to their emergence from the heart and transection of the heart in the atrioventricular plane. The atrial appendages were then excised, leaving only the posterior walls of the atria and the entry points of the great veins. The donor heart was then removed from storage, orientated and placed in the left chest. All anastomoses were performed with continuous 4-0 prolene. The left atrial anastomosis was performed first. The donor right atrium was then opened vertically, with the atriotomy commencing on the posterior wall at the level of the inferior vena cava but extending into the right atrial appendage superiorly, so as to preserve the sinus node. After completion of the right atrial anastomosis, the donor and recipient pulmonary arteries were trimmed and anastomosed. The aortic anastomosis was performed in a similar manner and the heart reperfused on its completion. During the initial period of reperfusion, the heart was vented through the apex of the left ventricle. Rewarming was commenced during the aortic anastomosis. Once warm, the heart was defibrillated and ventricular demand pacing commenced (120 beats per min).

A dobutamine infusion (10 mcg/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. Hearts that could not be weaned within 2 h of reperfusion were considered to have failed to wean from bypass. No attempt to wean the dobutamine infusion was made prior to post-transplantation data acquisition being undertaken. All animals were sacrificed

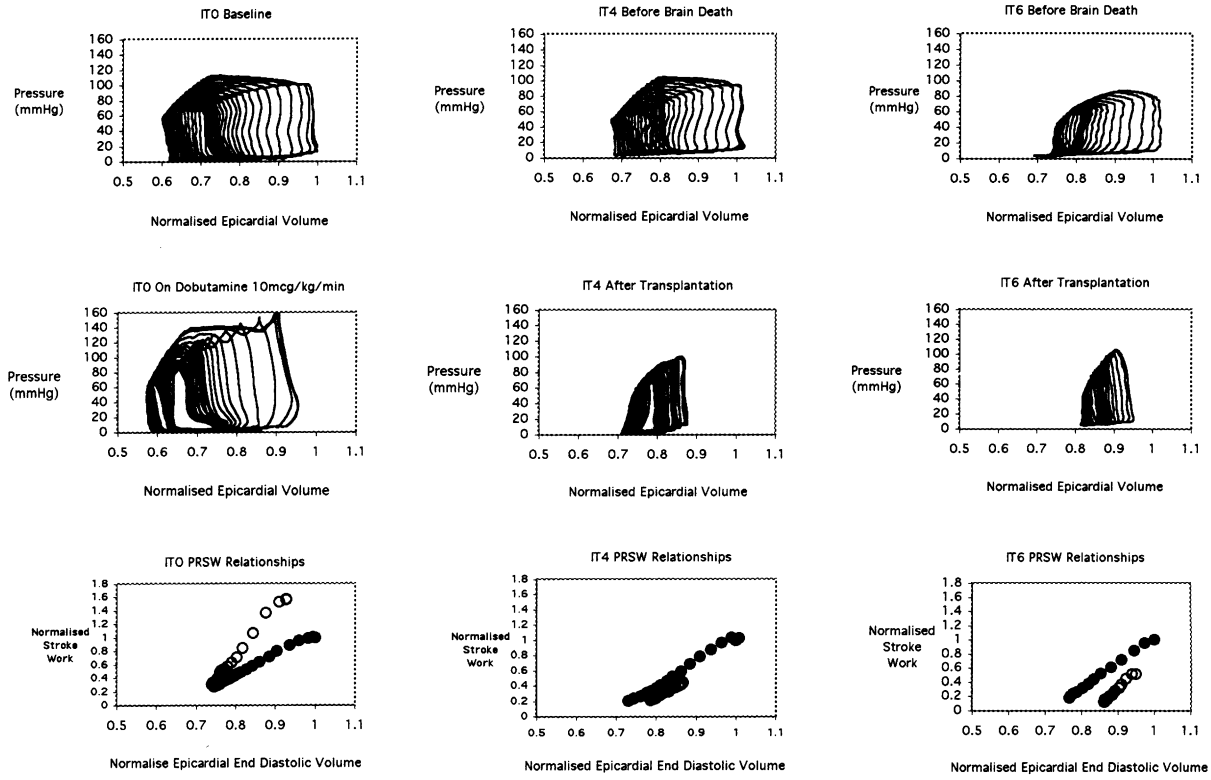

Fig. 1. Representative left ventricular pressure-volume loops and the PRSW relationships derived from the same loops. Data from three hearts belonging to separate groups is displayed: left – IT0; middle – IT4; right – IT6. The pressure-volume loops were obtained during transient occlusion of the inferior vena cava. The upper panels are the baseline loops while the middle panels are the post-intervention loops (dobutamine alone for IT0, post-transplantation for IT4 and IT6). The lower panels depict the PRSW relationship derived from these pressure-volume loops: baseline – closed circles; post intervention – open circles. In the transplanted hearts, the increased dependence on preload to produce stroke work is due to the rightward shift in the achievable end systolic volume and preload is reduced due to the increased heart rate and the loss of diastolic compliance.

following post transplantation data acquisition or on failure to wean from bypass.

2.7. Effect of dobutamine on the normal heart

Baseline pressure-volume loop data was obtained in the one animal in IT 0 as described for the other groups. A dobutamine infusion was then commenced at 10 mcg/kg per min. Fifteen min later, by which time haemodynamic stability had been achieved, pressure-volume loop data were again obtained.

2.8. Statistical analysis

Continuous variables are reported as the mean ± one standard deviation. Categorical variables are reported as the percentage (actual incidence/number of hearts in the study group). Statistical analyses were performed with SPSS for Macintosh 6.1.1 (SPSS Inc., USA). Differences were considered statistically significant at a level of $P < 0.05$.

The proportion of hearts in each group that were weaned successfully from cardiopulmonary bypass was compared with Fisher's exact test, with a Bonferroni correction to account for multiple comparisons.

2.9. Multiple linear regression models

To reduce potential confounding from both heart size and modelling errors in the estimation of ventricular volume from axial dimension measurements, the stroke work and epicardial end-diastolic volume data were normalised, within individual animals, to the baseline steady state values. The 'normalised' PRSW relationships were then examined by a multiple linear regression implementation of analysis of co-variance with repeated measures [8]. This approach was used to overcome the complexities created by concurrent changes in M_w and V_w, which can not be adequately dealt with by simple linear regression and comparison of the values obtained for M_w and V_w from each file [8]. Only hearts from which data could be obtained post-transplantation were included in the analysis.

The multiple linear regression model used to determine the mean normalised PRSW regression equation for each group at each time point was:

$$Y = b_0 + \Sigma_{i=1 \text{ to } n-1} p_i P_i + b_4 X \quad (1)$$

where Y is normalised SW (nSW), X is normalised EDV_{epi} ($nEDV_{epi}$), b_4 is the slope of the relationship (nM_w), b_0 is the y-axis intercept and the term $\Sigma_{i=1 \text{ to } n-1} p_i P_i$ accounts for

Table 1
The effect of ischaemic preservation and the effect of the duration of ischaemic preservation on the PRSW relationship[a]

Study Group	Number of animals	Time Point	nM_w	*y*-axis intercept	nV_w	SWI
IT4	6	Pre-brain death	3.39 ± 0.05	−2.36 ± 0.04	0.70	1.03
		Post-transplantation	4.37 ± 0.12	−3.22 ± 0.10	0.74	1.15
IT6	4	Pre-brain death	3.64 ± 0.12	−2.65 ± 0.10	0.73	0.99
		Post-transplantation	4.88 ± 0.27	−4.24 ± 0.25	0.87	0.64

[a] nM_w = slope of the normalised PRSW relationship, *y*-axis intercept = normalised stroke work axis intercept, nV_w = normalised epicardial end-diastolic volume axis intercept, SWI = stroke work index. Regression coefficients (nM_w and the *y*-axis intercept) are reported as the mean ± one standard error. Regression estimates (nV_w and SWI) are calculated from the mean regression coefficients. Intra-group analyses: pre-brain death contractility is better than post-transplantation contractility in both the IT4 group and IT6 group (both $P < 0.0001$). Inter-group analysis: the deterioration in contractility in the IT4 group is less than the deterioration in contractility in the IT6 group ($P < 0.0001$).

individual animal variability. Two further indices were derived from these parameters for the purpose of describing the group's normalised PRSW relationship at that time point. These were the $nEDV_{epi}$ axis intercept, nV_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 $nEDV_{epi} = 1$ (i.e. at the baseline steady state end-diastolic volume). It represents the interaction between changes in nM_w and nV_w at the normal operating volume of the heart, which is the most physiologically relevant end-diastolic volume.

The effect of ischaemia was determined separately within the IT4 and IT6 groups by the equation:

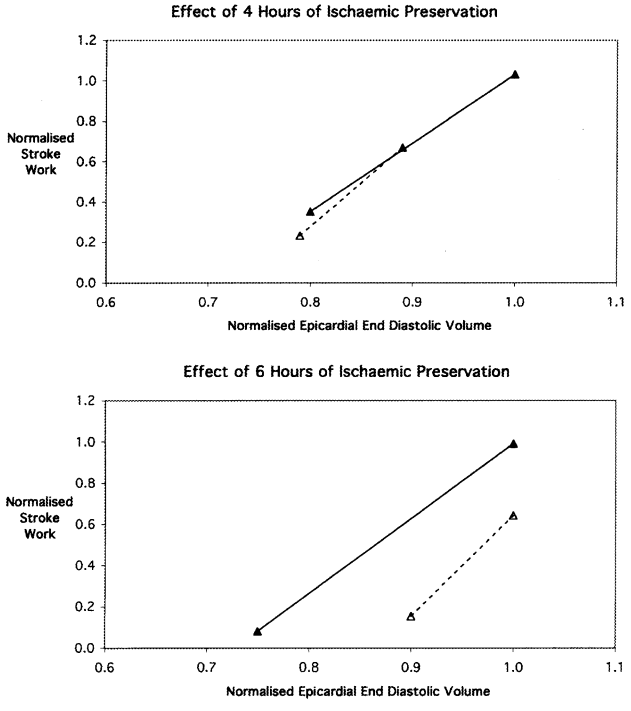

Fig. 2. The PRSW relationship – mean regression lines. Mean regression lines before brain death (closed symbols and unbroken lines) and after transplantation (open symbols and dashed lines) are depicted within the observed data range for the IT4 group (upper panel) and IT6 group (lower panel).

$$Y = b_0 + \Sigma_{i=1 \text{ to } n-1} p_i P_i + b_1 TP + b_4 X \quad (2)$$

where TP is the time point dummy variable (baseline: −1; post-transplant: +1) and the other terms are as defined above.

The effect of the duration of ischaemia was determined by comparing the relative effect of ischaemia on the IT4 and IT6 groups with the equation:

$$Y = b_0 + \Sigma_{i=n-2} p_i P_i + b_1 TP + b_2 SG + b_3 TP \times SG + b_4 X \quad (3)$$

Where SG is the study group dummy variable (IT4: −1; IT6: +1), TP × SG is the time point – study group dummy variable (which is equal to the product of the TP and SG dummy variables) and the other terms are as defined above.

3. Results

3.1. Weaning from cardiopulmonary bypass

Overall, 61% (11/18) of hearts were weaned successfully from bypass. The proportion of hearts weaned successfully from bypass was inversely related to the duration of ischaemic preservation. In the IT4 group, 100% (6/6) were weaned successfully, compared with 56% (5/9) in the IT6 group and 0% (0/3) in the IT14 group. Only the difference between the IT4 and IT14 groups achieved statistical significance ($P = 0.012$).

3.2. Left ventricular contractility

Representative left ventricular pressure-volume loops before brain death and after transplantation from two hearts, one each from the IT4 and IT6 groups, are depicted in Fig. 1. For the purposes of illustration, the effect of dobutamine on the normal heart (IT0) is also depicted in Fig. 1. The PRSW relationships for the IT4 and IT6 groups, both before brain death and after transplantation, are described in Table 1. Only hearts that could be weaned successfully from bypass were included in the analysis (this was because hearts that can not be weaned from bypass are incapable of generating

analysable pressure-volume loops). In addition, one heart in the IT6 group was excluded because pressure-volume loops could not be obtained as a consequence of rhythm and haemodynamic instability. The mean regression lines for each group, before brain death and after transplantation, are depicted within the observed data range in Fig. 2.

Separate intra-group analyses were performed to assess the effect of ischaemic preservation on the PRSW relationship. In the IT4 and IT6 groups, the regression coefficient for the time point dummy variable, b_1, was significantly less than zero (Eq. (2)). This indicates that the normalised PRSW relationship was shifted down/to the right by ischaemic preservation, despite nM_w being greater post-transplantation in both groups. In the case of IT4, nV_w and SWI indicate that the pre-brain death and post-transplantation normalised PRSW relationships intersect when extrapolated within the volume range between $nEDV_{epi} = nV_w$ and $nEDV_{epi} = 1$. The statistical result reflects the relative positions within the common observed data range (see Fig. 2).

To assess the effect of the duration of ischaemic preservation, the relative effect of ischaemic preservation in the IT4 and IT6 groups was determined. The regression coefficient for the study group – study time point interaction dummy variable, b_3, was significantly less than zero (Eq. (3)). This indicates that the IT6 group's normalised PRSW relationship was shifted further down/to the right by ischaemic preservation than was the IT4 group's normalised PRSW relationship. This is reflected by the lower post-transplant SWI in the IT6 group and was a consequence of nV_w increasing by more in the IT6 group than in the IT4 group. The change in nM_w was similar in both groups.

4. Discussion

This study has clearly demonstrated that the PRSW relationship can be used to assess contractile dysfunction in the transplanted heart. However, it is the volume axis intercept of the PRSW relationship, not the slope of the PRSW relationship, that is the better discriminator of the severity of contractile dysfunction. This creep phenomenon has also been described in other experimental models of ischaemic injury [9–12].

4.1. Ischaemia-reperfusion injury and contractile dysfunction

Ischaemia-reperfusion injury involves two separate processes. The severity of the ischaemic injury is dependent on the duration of ischaemia and there is a limit to the duration of ischaemia that the heart can tolerate [7]. This limit is extended by reducing the metabolic requirements of the myocytes, through arresting mechanical activity, removing wall stress, and cooling, and by providing the myocytes with energy substrates at the time of arrest by supplementation of the cardioplegia [17]. The severity of the reperfusion injury is dependent on the severity of the preceding ischaemic injury and thus on the duration of ischaemia [6].

Both myocardial stunning and irreversible functional impairment can occur as a result of ischaemia-reperfusion injury [6]. In clinical cardiac transplantation, the duration of ischaemic preservation is recognised as an independent risk factor for 1- and 5-year mortality [2]. This effect is largely due to an excess mortality in the immediate post-operative period. The relationship between the duration of ischaemic preservation and mortality is linear for recipients of hearts from young organ donors but becomes more exponential as donor age increases [18].

4.2. Susceptibility of porcine hearts to ischaemia-reperfusion injury

On the basis of our early experience with the present model, it is clear that after 6 h of ischaemic preservation porcine cardiac allografts harvested from brain dead donors can not be weaned successfully from bypass without inotropic support [13]. Indeed, after only 4 h of ischaemic preservation and despite inotropic support, Rao et al. were only able to wean successfully from bypass three out of eight porcine cardiac allografts harvested from non-brain dead donors [19]. Further, others have found, as we did in the present study, that porcine cardiac allografts can not be weaned from bypass after 14 h of conventional ischaemic preservation [20,21]. These facts demonstrate that contractile function in porcine cardiac allografts is significantly impaired by ischaemic preservation and that the degree of impairment is related to the duration of ischaemic preservation.

4.3. The effect of ischaemia on the PRSW relationship

The impact of ischaemia on the PRSW relationship in porcine cardiac allografts is even clearer when the effect of dobutamine support is taken into consideration. The dose-response of the PRSW relationship to dobutamine in normal hearts has been documented by Steendijk et al. [22]. Based on the data reported by Steendijk et al., dobutamine at 10 mcg/kg per min should result in an increase in nM_w of 110% and an increase in SWI of 120%. The change in the PRSW relationship for the one heart in IT0, which is depicted in Fig. 1, is consistent with these reported figures. In contrast, the hearts stored for 4 h only had an increase in nM_w of 29% and an increase in SWI of 10% while the hearts stored for 6 h had an increase in nM_w of 34% and a decrease in SWI of 35%. Thus, in both the IT4 and IT6 hearts, the difference between the observed post-transplant SWI and what would be expected from the dobutamine alone, which represents the true effect of ischaemic preservation, was due to a combination of a reduced response in nM_w to dobutamine and to the increase in nV_w.

It is important to note that the post-transplant SWI clearly indicates a difference in recovery of contractile function between the IT4 and IT6 groups. This difference was prob-

ably due to the longer ischaemic time but the contribution of the minor differences in experimental protocols can not be excluded. Regardless, it is the nature of the observed differences in recovery that is particularly important. It was due exclusively due to the greater increase in nV_w. This confirms the observations of others that the volume axis intercept of the PRSW relationship is a better indicator of ischaemic contractile dysfunction then the slope of the relationship [9–12]. Further, the opposing trends in nM_w and nV_w between IT4 and IT6 highlight the need for a multiple linear regression approach to the analysis, rather then independent analyses of the slope and volume axis intercept of the PRSW relationship.

4.4. The Bittner paradox

Bittner and colleagues reported that left ventricular contractility in canine allografts, as measured by the PRSW relationship, is not affected by ischaemic preservation [3,4]. In hearts harvested from non-brain dead canine donors, left ventricular PRSW slope was unchanged in hearts subjected to either 1.5 or 4 h of ischaemia [3]. In hearts harvested from brain dead canine donors, there was a deterioration following brain death but there was no further deterioration following preservation within groups subjected to either 1.5 or 4 h of ischaemia [3]. In contrast, they reported that right ventricular contractility was reduced after 1.5 h of ischaemia in hearts from non-brain dead canine donors that were transplanted with the Shumway technique [4], though not in hearts transplanted with the complete atrio-ventricular technique [3,4]. In hearts from brain dead canine donors, there was deterioration in right ventricular contractility following brain death and a further deterioration following preservation in hearts stored for 4 h though not in hearts stored for 1.5 h [3].

The fact that the present study has demonstrated an effect of ischaemia on allograft contractility, but Bittner's studies did not, may reflect differences between the two models. In particular, the maximum ischaemic time that canine allografts were subjected to by Bittner et al. was the minimum ischaemic time that porcine allografts were subjected to in the present study. This is especially important in view of the remarkable tolerance of the canine heart to ischaemic preservation. Successful transplantation of canine hearts has been reported after 24 h of ischaemic preservation [23,24]. In addition, the fact that they independently compared the values of M_w and V_w obtained from simple linear regressions, rather then using the more appropriate technique of multiple linear regression [8], is also of potential significance.

The other important difference between the present study and the studies by Bittner et al. that included brain death [3] was the duration of brain death prior to harvest. Bittner et al. explanted the donor heart 4 h after induction of brain death, compared with 1 h in the present study. They did observe a reduction in left ventricular contractility between baseline and post transplantation but attributed the deterioration to post-brain death/pre-explantation changes in contractility not ischaemic preservation injury. Notably, the mean slope of the PRSW relationship in allografts subjected to 4 h of ischaemia declined to a greater extent than in allografts subjected to 1.5 h. In addition, half of the eight hearts in the 4 h group required dopamine support to be weaned successfully from bypass compared to none in the 1.5 h group. Bittner et al. attributed these differences to a greater deterioration in left ventricular contractility post-brain death in the hearts subjected to 4 h of ischaemia.

Our observations on the effect of brain death on porcine hearts will be the subject of a separate report. Significantly, we have found that contractility is not reduced 1 h after induction of brain death. Nor was there any evidence in the present study that the brain death experience was different between study groups in the present study (data not shown). Further, Szabo et al. have recently reported that the apparent decrease in left ventricular contractility observed late after brain death actually represents left ventricular adaptation to the altered state of the systemic circulation, through intact ventriculo-arterial coupling [25]. They demonstrated that cross-circulating the heart of a brain dead animal with the circulation of an intact animal abolished the previously observed effect of brain death on left ventricular contractility. This suggests that the greater deterioration in left ventricular contractility in hearts stored for 4 h compared to 1.5 h observed by Bittner et al. [3] may have been due to the longer duration of ischaemic preservation and not due to differences in the brain death experience, as concluded by Bittner et al.

5. Summary

In conclusion, it would appear that the porcine heart is more susceptible to ischaemia-reperfusion injury than the canine heart. The porcine heart is thus a better model in which to determine if novel preservation strategies can significantly enhance recovery of contractile function. More importantly, the PRSW relationship can be used to assess the effect of ischaemic preservation on contractility. However, as a consequence of the creep phenomenon, it is essential that the analysis of the effect of preservation strategies on the changes in the PRSW relationship utilises multiple linear regression techniques and does not rely upon independent comparisons of slope and intercept data.

Acknowledgements

This project was funded by the National Health & Medical Research Council of Australia (Grant 142007) and the National Heart Foundation of Australia (Grant G97S 4862). Dr Ryan is supported by the National Health & Medical Research Council and the Royal Australasian College of Surgeons. The authors would like to acknowledge the tech-

nical assistance provided during the performance of these experiments by the staff of the clinical perfusion service of St. Vincent's Hospital.

References

[1] Copeland JG. Advanced medical therapy does not render heart transplantation obsolete for ambulatory end-stage heart failure patients: a debate. J Heart Lung Transplant 2001;20:725–728.
[2] Hosenpud JD, Bennett LE, Keck BM, Boucek MM, Novick RJ. The Registry of the International Society for Heart and Lung Transplantation: eighteenth Official Report – 2001. J Heart Lung Transplant 2001;20:805–815.
[3] Bittner HB, Kendall SW, Chen EP, Davis RD, Van Trigt P, 3rd. Myocardial performance after graft preservation and subsequent cardiac transplantation from brain-dead donors. Ann Thorac Surg 1995;60:47–54.
[4] Bittner HB, Kendall SW, Chen EP, Davis RD, Van Trigt P, 3rd. Complete atrioventricular cardiac transplantation: improved performance compared with the standard technique. Ann Thorac Surg 1995;60:275–282.
[5] 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.
[6] Bolli R, Marban E. Molecular and cellular mechanisms of myocardial stunning. Physiol Rev 1999;79:609–634.
[7] Bulkley GB. Free radical-mediated reperfusion injury: a selective review. Br J Cancer 1987;8:66–73.
[8] Glantz SA, Slinker BK. Primer of applied regression & analysis of variance. 2nd ed. New York: McGraw-Hill, Inc, 2001.
[9] Glower DD, Spratt JA, Kabas JS, Davis JW, Rankin JS. Quantification of regional myocardial dysfunction after acute ischemic injury. Am J Physiol 1988;255:H85–H93.
[10] Lu L, Xu Y, Greyson CR, Ursell PC, Schwartz GG. Non-elastic deformation of myocardium in low-flow ischemia and reperfusion: ultrastructure-function relations. J Mol Cell Cardiol 1999;31:1157–1169.
[11] Schwartz GG, Xu Y, Greyson C, Cohen J, Lu L. Low-dose inotropic stimulation during left ventricular ischaemia does not worsen post-ischaemic dysfunction. Cardiovasc Res 1996;32:1024–1037.
[12] Matsuwaka R, Matsuda H, Shirakura R, Kaneko M, Fukushima N, Taniguchi K, Nakano S, Kawashima Y. Changes in left ventricular performance after global ischemia: assessing LV pressure-volume relationship. Ann Thorac Surg 1994;57:151–156.
[13] Ryan JB, Wilson MK, Hicks M, Nicholson A, Kesteven SH, Junius F, Feneley MP, Macdonald PS. A brain dead donor model of porcine orthotopic cardiac transplantation for assessment of cardiac allograft preservation. Heart, Lung Circ 2000;9:78–81.
[14] Richens D, Junius F, Hill A, Keogh A, Macdonald P, McGoldrick J, Spratt P. Clinical study of crystalloid cardioplegia vs aspartate-enriched cardioplegia plus warm reperfusion for donor heart preservation. Transplant Proc 1993;25:1608–1610.
[15] Du ZY, Hicks M, Spratt P, Mundy JA, Macdonald PS. Cardioprotective effects of pinacidil pretreatment and lazaroid (U74500A) preservation in isolated rat hearts after 12-h hypothermic storage. Transplantation 1998;66:158–163.
[16] Lower RR, Shumway NE. Studies on orthotopic homotransplantation of the canine heart. Surg Forum 1960;11:18–19.
[17] Tseng EE, Cameron DE. Myocardial protection from ischaemia-reperfusion injury in cardiac surgery. In: Grace PA, Mathie RT, editors. Ischaemia-reperfusion injury, Oxford: Blackwell Science, 1999. pp. 344–356.
[18] Hosenpud JD, Bennett LE, Keck BM, Fiol B, Boucek MM, Novick RJ. The Registry of the International Society for Heart and Lung Transplantation: sixteenth official report – 1999. J Heart Lung Transplant 1999;18:611–626.
[19] Rao V, Feindel CM, Weisel RD, Boylen P, Cohen G. Donor blood perfusion improves myocardial recovery after heart transplantation. J Heart Lung Transplant 1997;16:667–673.
[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-h storage. Transplantation 1998;66:1450–1459.
[21] Kuhn-Regnier F, Fischer JH, Jeschkeit S, Switkowski R, Bardakcioglu O, Sobottke R, de Vivie ER. Coronary oxygen persufflation combined with HTK cardioplegia prolongs the preservation time in heart transplantation. Eur J Cardio-thorac Surg 2000;17:71–76.
[22] Steendijk P, Baan Jr J, Van der Velde ET, Baan J. Effects of critical coronary stenosis on global systolic left ventricular function quantified by pressure-volume relations during dobutamine stress in the canine heart. J Am Coll Cardiol 1998;32:816–826.
[23] Tanoue Y, Morita S, Ochiai Y, Hisahara M, Masuda M, Kawachi Y, Tominaga R, Yasui H. Inhibition of lipid peroxidation with the lazaroid U74500A attenuates ischemia-reperfusion injury in a canine orthotopic heart transplantation model. J Thorac Cardiovasc Surg 1996;112:1017–1026.
[24] Kim YI, Herijgers P, Laycock SK, Van Lommel A, Verbeken E, Flameng WJ. Na + /H + exchange inhibition improves long-term myocardial preservation. Ann Thorac Surg 1998;66:436–442.
[25] Szabo G, Hackert T, Buhmann V, Graf A, Sebening C, Vahl CF, Hagl S. Downregulation of myocardial contractility via intact ventriculoarterial coupling in the brain dead organ donor. Eur J Cardio-thorac Surg 2001;20:170–176.