Ex Vivo Evaluation of the Biventricular Cardiac Function for Donation After Circulatory Death Model: An Experimental Study

Abstract

Background: Few reports on a biventricular working heart model with *ex vivo* perfusion exist owing to the complexity of establishing a circuit. Hence, we investigated it for donation after circulatory death.

Material and Methods: The heart in six juvenile pigs (~20 kg) was arrested by asphyxiation. After 30 min of global ischemia, the heart was harvested, reperfused with normoxemic blood cardioplegia for 20 min, and subsequently perfused with hyperxemic blood. After 70 min of controlled reperfusion, the system was switched to the biventricular working mode. Cardiac function was assessed before anoxia and during the biventricular mode.

Results: Left and right ventricular functions worsened during the biventricular mode, as compared to those before anoxia (dP/dt_{max}, 673 ± 120 vs. 283 ± 95 and 251 ± 35 vs. 141 ± 21 mmHg/s, respectively; $P < 0.001$). Systemic (resistance/100 g net heart weight) and pulmonary vascular resistance indexes during the biventricular mode were similar

to those before anoxia (829 ± 262 vs. 759 ± 359, $P = 0.707$, and 167 ± 57 vs. 158 ± 83 dynes·sec·cm^{-5} - l-100-g net heart weight, $P = 0.859$, respectively).

Conclusion: The biventricular working heart model with *ex vivo* perfusion was feasible, exhibiting stable hemodynamics, and has the potential to be a powerful tool for direct cardiac function assessment.

Keywords: Donation after circulatory death, Ex vivo, Heart function tests, Heart transplantation, Myocardial reperfusion

Background

An increasing number of patients remain in need of heart transplantation. Consequently, donation after circulatory death (DCD) is reconsidered for these patients in order to increase the donor pool. DCD heart transplantation has not been regularly performed due to concerns about the severity of injury sustained during hypoxemic cardiac arrest and the warm ischemic standoff period that ethically define death. Considering that the heart is particularly susceptible to ischemia, DCD heart transplantation has not yet

achieved widespread clinical application, despite estimations that the use of DCD hearts has the capacity to increase the number of heart transplantations by 17–30% [1–3]. In the nascent stages of heart transplantation, strategies for donor organ preservation are limited. Cold storage has been found to be simple, inexpensive, and reproducible and could be relatively easily undertaken in the clinical setting. Although universally adopted, cold ischemic storage is recognized to have limitations, including low levels of anaerobic metabolism continuing in the background with subsequent depletion of adenosine triphosphate stores and an increase in acidosis [4]. Continuous machine perfusion using hypothermic preservation solutions has been attempted and shown to provide superior systolic function with preserved adenosine triphosphate levels, as compared to that of cold storage [5]. Several experimental studies have described alternative techniques for organ preservation [5–8]. Nonetheless, there is a concern that the diastolic function may be impaired owing to the development of significant myocardial edema [9]. The main challenge in adopting this technique for poorly contracting donor hearts is that there is currently no means of functional assessment during preservation. Furthermore, as right ventricular (RV) failure frequently occurs after cardiopulmonary bypass or heart transplantation, RV systolic and diastolic

function assessment is essential in any prospective model [10,11]. Without the reassurance of *ex vivo* assessment following organ retrieval, there will be continued reluctance to any further expansion of the donor pool.

The significance of initial controlled reperfusion and acute post-transplant graft function using a 30-min warm ischemic porcine DCD model has been described [12,13]. However, there exist few reports on a biventricular (BV) working heart model with *ex vivo* perfusion owing to the complexity of setting up a circuit. The present study aimed to investigate a novel method of *ex vivo* functional evaluation based on the BV working mode using a porcine DCD model.

Material and Methods

Animal preparation

All experimental animals were cared for in accordance with the institutional guidelines and the *Guide for the Care and Use of Laboratory Animals* provided by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1996). The experimental protocol was approved by the experimental animals committee at our university. Six female Yorkshire pigs (mean

68 weight: 20 ± 3 kg) were used. Another pig was used for blood collection in each
69 experiment. Following premedication with an intramuscular injection of ketamine
70 hydrochloride (10 mg/kg), an ear vein was cannulated. Anesthesia was induced with
71 thiamylal sodium (50 mg) and atropine sulfate (0.5 mg) and was maintained with
72 isoflurane inhalation (0.5–2.0%) and pancuronium (0.2 mg/kg). An endotracheal tube
73 was inserted through a tracheotomy, and mechanical ventilation was commenced with a
74 tidal volume of 10 mL/kg. Heparin (500 U/kg) was intravenously administered after
75 median sternotomy was performed. Hemodynamics, including the heart rate, aortic root
76 pressure (AoP), pulmonary arterial pressure (PAP), left atrial pressure (LAP), and right
77 atrial pressure (RAP), were monitored. A 7-Fr pressure-tip catheter and a conductance
78 catheter (CD Leycom, Hengelo, the Netherlands) were inserted from the apex of the LV
79 and RV. Cardiac function was assessed before the induction of anoxia as the control.
80
81 *Experimental protocol*
82 The experimental timeline is illustrated in Figure 1. Cardiac arrest was induced by
83 turning off the ventilator. The heart was excised and weighed after it was arrested. At 30
84 min after asphyxiation, a perfusion cannula was inserted into the aortic root, and

isolated continuous myocardial perfusion was initiated by cardiopulmonary bypass with blood cardioplegic solution at 20 °C, with a perfusion pressure of 40 mmHg for 20 min (Figure 2). This solution included leukocyte-depleted normoxemic oxygenated blood mixed with modified St. Thomas' Hospital solution (100 mL of 20% D-mannitol, 19 mEq of sodium bicarbonate, 18.5 mEq of potassium chloride, and 37 mEq of magnesium sulfate in 1 L of 5% glucose) in a 4:1 ratio. Subsequently, myocardial perfusion was converted to leukocyte-depleted hyperxemic oxygenated blood at 20 °C, with a perfusion pressure of 40 mmHg (i.e., initiation of blood reperfusion). After 20 min, the perfusion pressure was increased to 60 mmHg, while the temperature was gradually increased to 37 °C for 30 min. MCI-186 (3-methyl-1-phenyl-2-pyrazolin-5-one), a hydroxyl radical scavenger with antioxidant effects, was administered from the reperfusion for 30 min (i.e., until 10 min after the initiation of blood reperfusion). The appropriate infusion duration and doses of MCI-186 based on our dose-response study of this agent in reperfusion injury were used [13].

After 70 min of controlled reperfusion, i.e., the non-working heart mode, the system was gradually switched into the LV working mode over the next 60 min (Figure 3). The LV working mode was easily switched to the BV working mode by clamping a tube

(asterisk in Figure 3) connecting the reservoir to a damping chamber and unclamping the tubes (double asterisk in Figure 3) of both the inflow of the right atrium (RA) and the outflow of the RV. Cannulas were inserted into the left atrium, descending aorta, superior vena cava, and main pulmonary artery. Continuous epinephrine infusion (0.1 μg/kg/min) was initiated. Subsequently, the working mode was commenced by gradually filling both ventricles. First, the clamp occluding the inflow line of the left atrium was opened and the left atrium was gradually filled by the pump until LAP reached the same pressure as that measured before cardiac arrest. Second, the outflow line of the descending aorta was accurately regulated with the pinchcock until AoP reached the same pressure as that measured before cardiac arrest. The right heart *ex vivo* system was then established, while the RAP and PAP were monitored. After establishing the BV working mode, both LV and RV functions were evaluated with the conductance catheter.

Cardiac function, lactate level, and myocardial edema

The maximum and minimum of the first derivative of both ventricular pressures (dP/dt_{max} and dP/dt_{min}, respectively) were attained from a pressure curve acquired from

a micromanometer catheter. Calibration for blood conductivity was performed just before each measurement. The LV end-systolic pressure-volume relationship (LV-ESPVR) and LV end-diastolic pressure-volume relationship (LV-EDPVR) were calculated using the pressure-volume relation curves obtained by changing the volume *via* inferior vena cava occlusion prior to the induction of anoxia or *via* a reduction in the flow of the pump during the BV working mode. The RV end-systolic pressure-volume relationship (RV-ESPVR) and RV end-diastolic pressure-volume relationship (RV-EDPVR) were calculated similarly. Blood samples were collected from the aortic root and coronary sinus to measure lactate concentrations at 5 min after reperfusion was initiated and every 10 min thereafter until the end of controlled reperfusion. Blood gas analysis was performed, and lactate concentrations were measured using an i-STAT analyzer (Abbott Inc., Abbott Park, IL). The oxygen and lactate extraction ratios (E) were calculated using the following equation: $E\ (\%) = (A - V)/A \times 100$, where A is the concentration of the arterial substrate and V is the concentration of the coronary sinus substrate. Myocardial edema was evaluated using the wet weight of the heart, including the aortic arch measured at two time points—namely, after recovery of the organ

(control value) and at the end of the protocol. The myocardial perfusion flow was measured by calculating the amount of coronary effluent.

Resistance

Systemic vascular resistance (SVR) was calculated using the following equation: SVR = (AoP - RAP)/cardiac output (CO). On the other hand, pulmonary vascular resistance (PVR) was calculated using the following equation: PVR = (PAP - LAP)/CO. To provide a proper afterload, SVR index (SVRI) and PVR index (PVRI) were defined as each value divided by the wet weight of the heart after recovery of the organ, respectively.

Statistical analysis

Continuous variables are reported as means (± standard deviation) and were compared using the Wilcoxon signed-rank test. The statistical significance level was set at $P < 0.05$. All statistical analyses were performed using SPSS version 22 (Chicago, IL).

Results

Procedural variables

The mean agonal period (time from turning off the ventilator to cardiac arrest) was 8 ± 1 min, the mean warm ischemic time was 22 ± 1 min, and the mean heart weight was 196 ± 38 g. The percentage change in heart weight at the end of the protocol, as compared to that after recovery of the heart, was 10.6 ± 5.8%.

Controlled reperfusion

The myocardial perfusion rate is shown in Figure 4. The flow rate decreased gradually and reached the lowest value at 30 min (i.e., 10 min after blood reperfusion; $P = 0.015$). After the perfusion pressure was increased to 60 mmHg, the flow rate returned to the initial level.

The oxygen extraction course is shown in Figure 5. Approximately 30% of oxygen delivered to the myocardium was consumed during the first 5 min of controlled reperfusion. However, the high extraction rate immediately decreased to 15% at 30 min after reperfusion ($P = 0.009$).

The lactate extraction course is presented in Figure 6. A massive release of lactate was observed at the beginning of controlled reperfusion; nevertheless, the difference in

169 transmyocardial lactate decreased gradually and reached negligible levels at 30 min
170 after the initiation of controlled reperfusion ($P = 0.002$).
171
172 *Hemodynamics and cardiac function*
173 The baseline hemodynamic values measured before anoxia and during the BV mode are
174 summarized in Table 1, whereas the catheterization data are presented in Table 2. The
175 LV function (dP/dt_{max}, ESPVR, and EDPVR) during the BV mode was significantly
176 lower than that before anoxia (LV-dP/dt_{max}: 673 ± 120 vs. 283 ± 95 mmHg/s, $P < 0.001$;
177 LV-ESPVR: 1.81 ± 0.46 vs. 1.09 ± 0.27 mmHg/mL, $P = 0.008$; LV-EDPVR: 0.22 ± 0.11
178 vs. 0.57 ± 0.33 mmHg/mL, $P = 0.031$, respectively). Similarly, the RV function during
179 the BV mode was significantly lower than that before anoxia (RV-dP/dt_{max}: 251 ± 35 vs.
180 141 ± 21 mmHg/s, $P < 0.001$; RV-ESPVR: 1.35 ± 0.35 vs. 0.81 ± 0.29 mmHg/mL, $P =$
181 0.017; RV-EDPVR: 0.27 ± 0.12 vs. 0.49 ± 0.09 mmHg/mL, $P = 0.005$, respectively).
182 SVRI and PVRI during the BV mode were not significantly different compared to those
183 before anoxia (829 ± 262 vs. 759 ± 359 dynes·sec·cm^{-5} - l-100-g net heart weight, $P =$
184 0.707; 167 ± 57 vs. 158 ± 83 dynes·sec·cm^{-5} - l-100-g net heart weight, $P = 0.859$,
185 respectively) (Table 3).

186

187 **Discussion**

188 DCD donors may have the potential to be an additional source of organs for heart
189 transplantation. A reliable method for the evaluation of donor heart viability prior to
190 transplantation is essential before the donor heart can be utilized clinically. In the
191 present study, we employed a large animal model of DCD to investigate the heart's
192 tolerance to normothermic global ischemia. Both LV and RV systolic and diastolic
193 functions during the BV mode deteriorated, as compared to those before anoxia. SVRI
194 and PVRI during the BV mode did not differ through *ex vivo* perfusion, indicating the
195 feasibility of the *ex vivo* BV assessment of the marginal donor hearts. Taken together,
196 this study showed that the assessment of the BV function was feasible with a simple *ex*
197 *vivo* perfusion system under the appropriate setting of preload and afterload, suggesting
198 that this novel heart preparation has the potential of being a powerful tool for direct RV
199 function assessment.

200

201 *BV working mode*

Demmy et al. first reported an isolated BV working mode using rats in 1992 [14], and Abicht et al. described the procedure using pigs in 2018 [15]. In the meantime, no reports on the BV working mode were published because of its complex circuit and difficulty in adjusting the preload and afterload. Our *ex vivo* perfusion system of porcine hearts has been developed with several important features. First, a separate reservoir for the left atrium was not equipped, arranging our circuit in series. Alternatively, the flow to the left atrium was directly controlled by the pump connected to the outflow of the RV (Figure 3). We matched the CO of both LV and RV equivalent leads to mimic the *in vivo* conditions, but it differed from most other BV working mode heart models, which use two univentricular systems—one for each side of the heart. Second, the LV working mode can be easily switched to the BV working mode by clamping a tube connecting the reservoir to a damping chamber and unclamping both the inflow of the RA and the outflow of the RV (Figure 3). Monitoring each pressure allows the adjustment of proper preload and afterload for both ventricles in this system, thereby resulting in achievement of conditions comparable to the SVRI and PVRI during *in vivo* conditions. In a clinical situation, the assessment of cardiac function before retrieval would not be practical in DCD cases unlike in donation after brainstem death. However, considering the

219 characteristics of DCD hearts that potentially have severe myocardial damage from
220 hypoxic myocardial perfusion during the agonal period and subsequent warm ischemic
221 injury, an elaborate evaluation of the DCD hearts is indispensable prior to heart
222 transplantation.
223
224 *RV failure*
225 Several studies have reported impaired LV function with DCD hearts using the
226 univentricular working mode [8, 12–14]. RV failure in heart transplant recipients is
227 attributable to multiple factors. Most commonly, it results from coupling of a donor
228 heart that is not adapted to the elevated pulmonary artery pressure and resistance to the
229 increased afterload due to pulmonary hypertension and increased pulmonary resistance
230 in the recipient [16]. Additionally, the cardiac function of the donor heart may be
231 impaired by ischemia and reperfusion injury. Raina et al. described that combining the
232 parameters, such as preoperative lower RV fractional area change and higher estimated
233 RAP, allowed prediction of RV failure after LV assist device placement, which affected
234 the PVR [11]. White et al. reported the transition in RV pressure and volume of DCD
235 hearts using conductance catheter examination and magnetic resonance imaging during

236 the agonal period [17]. A rapid decrease in arterial oxygen partial pressure coincided
237 with an increase in RV systolic pressure and in diastolic and systolic functions, leading
238 to a decline in the RV function and an eventual extension in the RV end-diastolic phase.
239 This observation suggests that DCD hearts may not be compatible for recipients with
240 elevated PVR. Therefore, an RV functional assessment during *ex vivo* perfusion must be
241 carried out prior to transplantation, particularly for the marginal heart such as in DCD
242 donor heart transplantation.
243
244 *Significance of controlled reperfusion*
245 Myocardial metabolic products play a key role in the evolution of ischemic damage.
246 Increased lactate levels and the associated increase in cytosolic reduced nicotinamide
247 adenine dinucleotide were shown to inhibit glycolysis and reduce anaerobic adenosine
248 triphosphate production [18]. Iyer et al. investigated the lactate concentration course in
249 the coronary circulation of DCD hearts and concluded that the lactate profile applied
250 during *ex vivo* perfusion was a valid marker of DCD cardiac allograft viability [8].
251 Neely et al. demonstrated that the decrease in the contractile force of the ischemic hearts
252 was associated with increased tissue lactate and that the onset of irreversible damage

253 was also related to the continued presence of high lactate levels [19]. Thus, adequate
254 washout of increased tissue lactate concentrations during reperfusion may be an optimal
255 goal to obtain better functional recovery. Furthermore, the adequacy of perfusion is
256 principally based on oxygen supply, and a viable myocyte extracts oxygen very
257 efficiently compared to other cells in the body (myocytes: 70%; other body cells: 40%)
258 [20]. Once the oxygen supply meets the myocardial tissue demand, a steady state is
259 attained, and further increase in the oxygen supply does not result in additional oxygen
260 uptake. In the present study, a massive release of lactate was observed at the beginning
261 of controlled reperfusion; subsequently, the difference in transmyocardial lactate
262 gradually decreased and reached negligible levels by 30 min. Similarly, oxygen
263 extraction has been shown to rapidly attain peak levels at 5 min and to gradually return
264 to baseline levels by 30 min. This result was comparable to the finding in a previous
265 study [12]. The results indicate that initial controlled reperfusion for 20 min results in
266 greater preservation of the cardiac function, thus preventing myocardial edema.
267 Therefore, lactate extraction, oxygen extraction, or both would be effective indicators
268 during *ex vivo* heart perfusion and should accordingly be monitored.
269

Limitations

The present study has some limitations. First, the sample size was small, which can potentially skew the results of statistical analysis. Second, heart transplantation was not performed. Hemodynamics and the value of the afterload appeared similar to the condition before anoxia; however, it is unclear whether the measurement during our *ex vivo* perfusion was the same as that after the transplantation. Finally, the cardiac function was only evaluated during the acute phase; hence, it should be evaluated after the transplantation.

Conclusions

The BV working heart model with *ex vivo* perfusion was established with stable hemodynamics. Resuscitation of porcine DCD hearts is viable by virtue of continuous myocardial perfusion instead of cardioprotective pretreatment. Pretransplant assessment of DCD hearts using the *ex vivo* perfusion system can be an effective tool in the clinical setting, particularly with marginal hearts.

Disclosure

The authors declare no conflict of interest.

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349 circulatory death” in murine heart model. ASAIO J. 2018;64:225-31.
350

Tables

Table 1

Hemodynamic variables

	Pre	Post	*P*-value
Heart rate (beats/min)	78 ± 11	95 ± 7	0.018
Mean arterial pressure (mmHg)	44 ± 8	38 ± 11	0.260
Mean pulmonary arterial pressure (mmHg)	12 ± 3	13 ± 2	0.591
Left atrial pressure (mmHg)	4 ± 3	7 ± 2	0.115
Right atrial pressure (mmHg)	3 ± 2	7 ± 3	0.059

All values are expressed as mean ± SD. Pre: before anoxia; post: during biventricular mode.

Table 2

Catheterization data

LV	Pre	Post	*P*-value
dP/dt_{max} (mmHg/s)	673 ± 120	283 ± 95	< 0.001
dP/dt_{min} (mmHg/s)	739 ± 136	287 ± 99	< 0.001
ESPVR (mmHg/mL)	1.81 ± 0.46	1.09 ± 0.27	0.008
EDPVR (mmHg/mL)	0.22 ± 0.11	0.57 ± 0.33	0.031
RV	Pre	Post	*P*-value
dP/dt_{max} (mmHg/s)	251 ± 35	141 ± 21	< 0.001
dP/dt_{min} (mmHg/s)	258 ± 66	135 ± 28	< 0.001
ESPVR (mmHg/mL)	1.35 ± 0.35	0.81 ± 0.29	0.017
EDPVR (mmHg/mL)	0.27 ± 0.12	0.49 ± 0.09	0.005

All values are expressed as mean ± SD. Pre: before anoxia; post: during biventricular mode; LV: left ventricle; RV: right ventricle; dP/dt_{max}: maximum of the first derivative of both ventricular pressures; dP/dt_{min}: minimum of the first derivative of both ventricular pressures; ESPVR: end systolic pressure-volume relationship; EDPVR: end diastolic pressure-volume relationship.

Table 3

Adjustment of systemic and pulmonary vascular resistance

	Pre	Post	*P*-value
Systemic vascular resistance index (dynes·sec·cm^{-5} - l-100-g net heart weight)	829 ± 262	759 ± 359	0.707
Pulmonary vascular resistance index (dynes·sec·cm^{-5} - l-100-g net heart weight)	167 ± 57	158 ± 83	0.859

All values are expressed as mean ± SD. Pre: before anoxia; post: during biventricular mode

Figure Legends

Figure 1. Experimental timeline

Red arrow: blood gas analysis was performed at regular intervals.

MCI-186, hydroxyl radical scavenger.

Figure 2. Controlled reperfusion

LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle.

Figure 3. Biventricular working mode

The LV working mode can be easily switched to the BV working mode by clamping a tube (*) connecting the reservoir to a damping chamber and unclamping tubes (**) of both the inflow of the RA and the outflow of the RV. BV, biventricular; LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle.

Figure 4. Myocardial perfusion speed at 5 and 10 min, and every 10 min to the end of controlled reperfusion. *$P = 0.015$

384 Figure 5. Oxygen extraction ratio at 5 and 10 min, and every 10 min to the end of

385 controlled reperfusion. *P = 0.009

386

387 Figure 6. Lactate extraction ratio at 5 and 10 min, and every 10 min to the end of

388 controlled reperfusion. *P = 0.002