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Safe orthotopic transplantation of hearts harvested 24 hours after brain death and preserved for 24 hours

Stig Steen[a], Audrius Paskevicius[a], Qiuming Liao[a] and Trygve Sjöberg[a]

Department of Cardiothoracic Surgery, Skåne University Hospital, and Lund University,, Lund, Sweden

ABSTRACT

Objectives. The aim of this study was to demonstrate safe orthotopic transplantation of porcine donor hearts harvested 24 hours after brain death and preserved for 24 hours before transplantation. *Design.* Circulatory normalization of brain dead (decapitated) pigs was obtained using a new pharmacological regimen (n = 10). The donor hearts were perfused at 8 °C in cycles of 15 min perfusion followed by 60 min without perfusion. The perfusate consisted of an albumin-containing hyperoncotic cardioplegic nutrition solution with hormones and erythrocytes. Orthotopic transplantation was done in 10 recipient pigs after 24 hours' preservation. Transplanted pigs were monitored for 24 hours, then an adrenaline stress test was done. *Results.* All transplanted pigs were stable throughout the 24-hour observation period with mean aortic pressure around 80 mmHg and normal urine production. Mean right and left atrial pressures were in the range of 3– 6 and 5–10 mmHg, respectively. Blood gases at 24 hours did not differ from baseline values. The adrenaline test showed a dose dependent response, with aortic pressure increasing from 98/70 to 220/150 mmHg and heart rate from 110 to 185 beats/min. *Conclusion.* Orthotopic transplantation of porcine hearts harvested 24 hours after brain death and preserved for 24 hours can be done safely.

Introduction

The present standard of clinical heart preservation for transplantation was developed by Hearse and Braimbridge at St Thomas Hospital in London in the 1970s and consists of flushing the coronary vessels with a cold cardioplegic solution followed by static ischemic storage at 4 °C.[1] Data from the ISHLT registry on 10473 patients receiving heart transplants between 2006 and 2011 reveals an increase in hazard ratio for 1-year mortality from 1.0 at 3 hours to 1.9 (95% confidence interval 1.5 to 2.5) at six hours of cold ischemia.[2] Other data suggest that human donor hearts preserved with cold static preservation are safe for 4–6 hours,[3–5] the shorter preservation times preferably to be used for older donors or recipients with risk factors.[2]

Successful orthotopic transplantation with donor hearts from brain dead animals is a convincing method to demonstrate safe heart preservation. However, this is not a satisfactory method to find the limits of different heart preservation techniques, because if the preservation is not adequate, heart failure with resulting hypo-perfusion of the body will occur followed by metabolic acidosis, which will negatively affect heart function. To monitor the on-going function of inadequately preserved hearts, we have developed a model where the porcine "donor heart" works *ex-vivo*, perfused with arterial blood from a heparinized normothermic "recipient" pig.[6,7] In brief, the ascending aorta of the donor heart is perfused by means of a pressure-controlled roller pump with blood from the femoral artery of the recipient pig. The perfusion pressure is set at 90 mmHg and the coronary flow and coronary vascular resistance are monitored continuously. The deoxygenated blood from the donor heart (with vena cava superior and inferior ligated) is pumped by the right heart through the pulmonary artery into a reservoir from which it is pumped back with a second roller pump into the femoral vein of the recipient pig. A flexible latex balloon is fixed in the left ventricle by means of a patch sutured to the mitral valve annulus. A large Y-tube with inlet and outlet valves is connected to the balloon. With this system we have compared 2 and 12 hours cold (4 °C) ischemic preservation of hearts from 60-kg pigs with St Thomas solution.[6,7] After 24 hours of reperfusion, the hearts preserved for 12 hours had statistically significantly reduced function (results for hearts preserved for 2 hours in brackets): left ventricular developed pressure 77 ± 7 mmHg (145 ± 9), cardiac output 3.9 ± 0.6 L/min (5.0 ± 0.2), coronary flow 143 ± 12 mL/min (246 ± 30), coronary vascular resistance 49,762 ± 5250 dyn s cm^{-5} (28,444 ± 3800), and O_2 consumption 3.7 ± 0.5 mL/min/100g (5.6 ± 0.4). After 24 hours of reperfusion, ring segments of coronary arteries were investigated for endothelium-dependent relaxation in organ baths. Compared to fresh coronary arteries the endothelium-dependent relaxation was reduced by 6% (p < 0.001) in the 2-hour group and by 58% (p = 0.001) in the 12-hour group; the difference between the 2- and 12-hour preserved hearts was statistically significant (p < 0.01). A regression analysis showed that a high coronary vascular resistance correlated with a low endothelium dependent relaxation (p < 0.001). Thus, 12 hours of cold ischemic

preservation with St Thomas solution causes significant deterioration in myocardial contractility and in coronary endothelium dependent relaxation resulting in a progressive increase of coronary vascular resistance with a corresponding decrease in coronary flow and cardiac output.[6,7] Endothelial cells produce a variety of substances which control vascular permeability, vessel tone, coagulation, fibrinolysis and inflammatory response. The endothelium is antithrombogenic when well preserved, and yet promotes platelet aggregation and coagulation if injured. Vasoconstriction, intimal hyperplasia and accelerated atherosclerosis may also occur as a result of bad endothelial preservation.[8]

We have developed a non-ischemic (non-hypoxic) method to keep hearts vital for prolonged periods of time, and the aim of the present study was to use this new method to demonstrate safe orthotopic transplantation of porcine hearts harvested 24 hours after brain death and preserved for 24 hours before being transplanted.

Materials and methods

Animals, anesthesia and basic fluid support

Thirty Swedish pigs of native breed with a median body weight of 47 kg (range 35–63 kg) were used in the study group (10 blood donors, 10 brain dead heart donors and 10 recipients). Additionally, six healthy pigs of similar size were used as a control group (3 donors with intact brain and 3 recipients). After weaning from the mother's milk, the pigs (then around 20 kg) were transported to a farm where they could move freely out-of-doors. All pigs were blood grouped and cross matched, and the body weight of the donor pigs was matched to be similar to that of the recipients. The pigs stayed at that farm until they were transported to the laboratory where the experiments took place. The animals were treated in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (Eight Edition, revised 2011) and the directive "On the protection of animals used for scientific purposes" of The European Parliament and of the Council (Directive 2010/63/EU). The study was approved by the local Ethics Committee for Experimental Research.

The design of the study is shown in Table 1. Anesthesia was induced with intramuscular ketamine hydrochloride 25 mg/kg (Ketalar; Pfizer AB, Stockholm, Sweden) and xylasin 4 mg/kg (Rompun Vet.; Bayer AB, Solna, Sweden). Atropine sulphate 0.5 mg (Atropine; Mylan AB, Stockholm, Sweden) and sodium thiopental 5–8 mg/kg (Pentothal; Inresa Arzneimittel GmbH, Freiburg, Germany) were given intravenously before

tracheostomy. Anesthesia and muscular relaxation were maintained with a continuous 20 mL/hour infusion of a mixture of ketamine hydrochloride 16 g and pancuronium bromide 600 mg (Pavulon; Organon Teknika, Boxtel, the Netherlands) dissolved to 1000 mL with glucose 10%. Krebs solution was given intravenously (3 mL/kg/h) for basic fluid support. Krebs solution was made in house and has the following composition in mmol/L: K^+ 4.6, Na^+ 135, Mg^{2+} 1.2, Ca^{2+} 1.5, Cl^- 119, $HCO3^-$ 25, $H_2PO_4^- + HPO_4^{2-}$ 1.2, and D-glucose 5.5. The animals were ventilated with a Servo Ventilator 300 (Siemens AB, Solna, Stockholm) using volume-controlled pressure-regulated ventilation with a positive end expiratory pressure of 5 cm H_2O. Central venous and aortic pressure catheters were established through the neck vessels. The aortic and central venous pressures were displayed on a fluoroscope (Spacelabs Healthcare, Snoqualmie, WA). Temperature corrected blood gases and other chemical parameters were measured by means of a Radiometer ABL 725 (Copenhagen, Denmark).

Brain death

Brain death was caused by decapitation between cervical vertebrae C2 and C3.[9] Decapitation was accomplished with a minimum of blood loss within 60 minutes. Thirty minutes after decapitation, continuous intravenous infusion of a "brain-death-cocktail" was started (Table 2). The cocktail consists of: cocaine (a catecholamine neuronal re-uptake blocker, purchased as powder and dissolved to 1 mg/mL in distilled water, Sigma-Aldrich Sweden AB, Stockholm, Sweden); noradrenaline (ampoules 1 mg/mL, Abcur AB, Helsingborg, Sweden), adrenaline (ampoules 1 mg/mL, Mylan AB, Stockholm, Sweden), desmopressin (Minirin, ampoules 4 µg/mL, Ferring Läkemedel AB, Malmö, Sweden), triiodothyronine (T3), thyroxine (T4) (purchased as powder and dissolved to 0.1 mg/mL in distilled water, Sigma-Aldrich Sweden AB) and cortisol (100 mg, Solu-Cortef, Pfizer, Sollentuna, Sweden). A basic Krebs solution fluid supply of 3 mL/kg/h was given. Additionally, 480 mL/24 h were used for the anesthesia infusion. For a detailed description of the decapitation and pharmacological normalization of circulation after acute brain death, see work by Steen et al.[9]

Preparation of the heart preservation system

Table 1. Study design.

Day I:	Induction of brain death by decapitating the heart donor pig.
Day II:	a. Preparation of clean erythrocytes from the blood donor pig. b. Heart preservation system made ready for use. c. Heart harvested from the donor pig (24 hours after brain death) and preserved in the heart preservation system at 8 °C.
Day III:	Orthotopic transplantation of the preserved donor heart (24 hours after harvesting) into the recipient pig.
Day IV:	Adrenaline test of the transplanted heart (24 hours after transplantation).

Table 2. Pharmacological normalization of circulation after acute brain death.

Substances	Diluted in NaCl to 50 mL	Doses (µg/kg/min)
Cocaine	1 mg	0.003–0.014
Noradrenaline	1 mg	0.003–0.014
Adrenaline	1 mg	0.003–0.014
Cortisol	300 mg	1.00–4.25
Triiodothyronine	0.3 mg	0.001–0.004
Thyroxine	0.3 mg	0.001–0.004
Desmopressin	36 µg	0.12–0.51 ng/kg/min

All drugs and saline were mixed in a 50 mL syringe and placed in a syringe pump. A continuous intravenous infusion was started 30 min after brain death. During the first 10 hours 1.7 mL/h was given, then the pump speed was gradually reduced to 0.4 mL/h to avoid hypertension.

Figure 1. The heart preservation system. The size of the hardware is 55 × 40 × 44 cm and the weight is 26 kg (left). The disposable part (2.8 kg) is shown to the right. The heart is kept submerged in the preservation medium, making it insensitive to mechanical shocks and air emboli during transport. The system can be transported in regular airplanes, tolerating the angles that occur during take-off and landing.

Table 3. The perfusion medium used for 24-hour heart preservation.

Na^+	136 mmol/L
K^+	23 mmol/L
Ca^{2+}	1.3 mmol/L
Mg^{2+}	8.0 mmol/L
Cl^-	142 mmol/L
HCO_3^-	25 mmol/L
PO_4^{2-}	1.3 mmol/L
D-Glucose	6.3 mmol/L
Albumin	75 g/L
Cocaine	6 pmol/L
Noradrenaline	6 pmol/L
Adrenaline	6 pmol/L
T3	3 pmol/L
T4	2 pmol/L
Cortisol	420 pmol/L
Insulin	8 U/L
Imipenen	20 mg/L
Erythrocytes (Hct)[a]	15%
96% O_2 + 5% CO_2[b]	0.2 L/min

[a]When all drugs and erythrocytes have been added and mixed and the PCO_2 has stabilized, pH is adjusted to 7.40 by means of sodium bicarbonate.
[b]Administered through the oxygenator.

The heart preservation system, which has been developed in-house, can be described as a portable heart–lung machine (Figure 1). The main components are: an automatic pressure and flow-controlled perfusion system, an automatic gas exchange system, a leucocyte filter, an arterial filter, a heater-cooler unit, an autonomous power unit, and a programmable sequencer. The system was filled with 3.5 L of perfusate medium (Table 3), developed in house, consisting of an albumin-containing (Albumin, CSL Behring GmbH, Marburg, Germany) hyperoncotic cardioplegic nutrition solution with hormones according to Table 3 (Insulin, Actrapid, Novo Nordisk, Malmö, Sweden), the antibiotic imipenem (Cilaststin, Fresenius Kabi, Uppsala, Sweden) and erythrocytes to a hematocrit of 15%. A Medtronic Autolog cellsaver (Medtronic Inc, Minneapolis, MN) and a Pall Leucocyte Filter (Pall Corp. Port Washington, NY) were used to get clean erythrocytes, which were mixed into the medium. The medium was circulated in the heart preservation system at a temperature of 8 °C.

Donor operation and heart preservation

Twenty-four hours after decapitation, sternotomy was done and the ascending aorta was exposed. After systemic heparinization, the ascending aorta was cannulated with a cardioplegic cannula which was connected to a 3/16-inch tube from the heart preservation system. The superior and inferior caval veins were clamped. When the heart was empty, the distal ascending aorta was cross-clamped and 600 mL of oxygenated 8 °C preservation medium were given into the ascending aorta directly from the preservation system with a perfusion pressure of 60 mmHg. The appendices of the right and left atrium were cut to keep the heart decompressed. The cardioplegic heart was excised and submersed in the cold (8 °C) perfusate medium. After inspecting and testing the sufficiency of the aortic valve, the distal ascending aorta was cannulated with a special double lumen cannula for easy de-airing. Antegrade coronary perfusion (8 °C) was started within 5 minutes to avoid hypoxia. The hole left by the cardioplegic cannula was closed with a suture. A baby feeding tube was placed in the coronary sinus for blood gas sampling. The mitral valve was made insufficient with a soft longitudinally split 3/8-inch silicon tube put through the mitral valve into the left ventricle and fixed with a suture to the opened left atrium. This was done to prevent inflation of the left ventricle if leakage of perfusate medium through the aortic valve should occur during perfusion. The caval veins and pulmonary artery were left open for a free outlet of perfusate from the coronary sinus. The double lumen cannula supplying the aorta with the preservation medium was fixed in a vertical position and the heart was completely submerged in the preservation medium. A perfusion pressure in the range of 20–25 mmHg was chosen with the intention of obtaining a minimum flow of 100 mL/min. The heart was perfused 20% of the time using a pattern of 15 minutes of perfusion and 60 minutes of non-perfusion. Before each perfusion, the preservation medium was automatically mixed for 2 minutes and the perfusion lines were recirculated to remove erythrocyte sequestration. Each perfusion cycle included pressure sensor zero calibration. The pressure-flow pattern and temperature were shown on the screen of the heart preservation system and stored in its computer.

Recipient pig

After sternotomy and cystostomy, heparin was given and the ACT time controlled. The arterial cannula was placed in the distal ascending aorta and bicaval cannulation was done. The heart-lung machine was primed with 1000 mL Ringer and 250 mL Mannitol. Extra-corporeal circulation (ECC) was
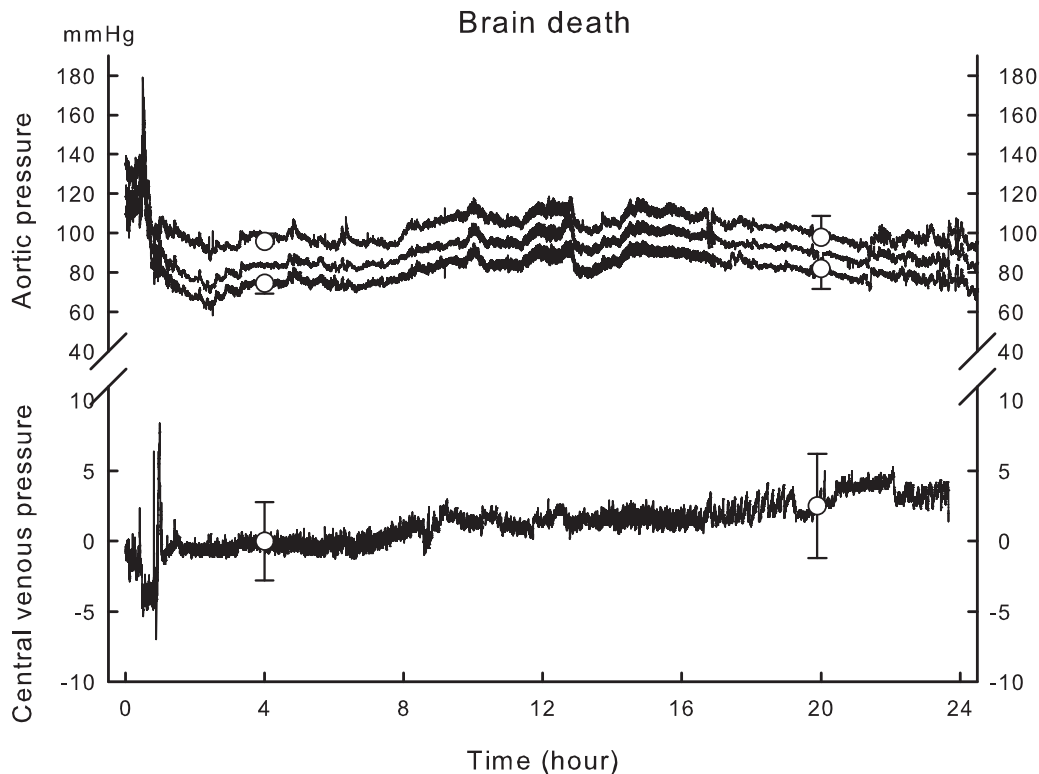
Figure 2. Aortic and central venous blood pressure during 24 hours after brain death (decapitation). The peak pressure seen at the beginning is due to the catecholamine storm occurring during the first 30 minutes after decapitation. Results are given as mean ± SEM, n = 10.

established at 30 °C. Warming started 20 minutes before estimated reperfusion.

Standard orthotopic heart transplantation was done with the four anastomoses sewn in the following order: left atrium, right atrium, pulmonary artery and aorta. After each of the first 3 anastomoses, cardioplegic preservation medium (8 °C) was given from the heart preservation system into the ascending aorta of the donor heart. The pigs were given methylprednisolone 1 g (Solu-Medrol, Pfizer, Sollentuna, Sweden) intravenously before the aorta was de-clamped. The transplanted hearts started spontaneously or after 1 or 2 defibrillations. All hearts regained sinus rhythm and there was no need for pacing. Before weaning from ECC, adrenaline 10 µg was given into the left atrium and an intravenous infusion of adrenaline 0.05 µg/kg/min was started, gradually reduced, and stopped within 6 hours. If the hematocrit fell below 30%, erythrocyte concentrate was given. The thorax was closed with drainage as for a routine heart operation. After 24 hours, bolus doses of 10, 20 and 100 µg adrenaline were given intravenously and the peak responses for blood pressure and heart rate were registered. Thereafter, the hearts were preserved with St Thomas cardioplegic solution, and sent by express delivery to Stockholm for further analyses at the cellular muscle physiology laboratory at the Karolinska Institute.

Control group

In three donor pigs, the hearts were preserved for 24 hour at 4 °C with St Thomas solution (Plegisol, Hospira, Stockholm,

Sweden) and orthotopic transplantation was performed as described above.

Statistics

Student's t-test for paired data was used for statistical calculations. Results are given as mean ± standard error of the mean, n = 10.

Results

Brain dead donor

The decapitated pigs had a stable circulation (Figure 2) and a urinary production of 70–100 mL/hour throughout the 24-hour observation period. There was no need for extra fluid therapy beyond the basic need. Before harvesting of the hearts, the arterial blood had the following values: PaO_2/FiO_2: 63.7 ± 2.8, $PaCO_2$: 4.7 ± 0.3 kPa, pH: 7.48 ± 0.02, Base excess: 2.3 ± 0.9 mmol/L, Hb: 103 ± 5 g/L, Hct 32 ± 1%, and glucose: 6.1 ± 0.3 mmol/L.

Donor heart

The pre-decided perfusion pressure between 20 and 25 mmHg gave a coronary flow in the range of 120 and 200 mL/min. At the start of the perfusion, blood gases in the medium leaving the oxygenator were: PaO_2: 44.1 ± 7.3 kPa, SaO_2: 97.8 ± 0.2%, $PaCO_2$: 5.6 ± 1.1 kPa, base excess: −0.4 ± 2.1, pH: 7.29 ± 0.06, glucose: 6.7 ± 2.4 mmol/L, and lactate: 0.9 ± 0.2 mmol/L. After 24 hours the values were: PaO_2: 56.8 ± 20 kPa,

SaO2: $98.0 \pm 0.1\%$, PaCO$_2$: 6.9 ± 0.9 kPa, base excess: -0.6 ± 1.5 mmol/L, pH: 7.23 ± 0.09, glucose: 6.4 ± 2.4 mmol/L, and lactate: 1.5 ± 0.6 mmol/L. At the beginning of each perfusion phase, the oxygen saturation in the coronary sinus had decreased to between 23% to 30%. After 5–7 minutes of perfusion, the saturation in the coronary sinus had stabilized in the range of 96–97%.

Recipient

There was no problem in weaning from ECC. The median (range) aortic clamp time was 66 (52–72) min, the reperfusion time from aortic de-clamping until weaning from ECC was 25 (17–35) min, and the total time on ECC was 96 (82–112) min. All 10 recipient animals had stable circulation throughout the 24-hour observation period. The mean aortic pressure was around 80 mmHg and the central venous pressure varied between 4 and 7 mmHg (Figure 3).

The mean pulmonary artery pressure was slightly elevated to around 20 mmHg whereas the left atrial pressure was normal and in the range of 5–10 mmHg (Figure 4). The median urinary production during the 24 hours was 1790 (range 1380–2460) mL.

The arterial blood gases before transplantation and during 24 hours of reperfusion are given in Table 4. There were no statistically significant differences between the baseline values and the values 24 hours after transplantation except for haemoglobin, where the values were 121 ± 3 and 105 ± 5 g/L, respectively ($p = 0.0015$) (Table 4).

The results of the adrenaline stress test are shown in Figure 5. The heart rate increased in a dose-dependent way from 110 min^{-1} before adrenaline to 185 min^{-1} at the highest dose of adrenaline given. The systolic and diastolic blood pressures also increased dose-dependently from 98/70 mmHg to 220/150 mmHg (Figure 5).

Controls

After being transplanted, the hearts developed edema during reperfusion while still on the heart-lung machine and after weaning from bypass, the pigs died within 1 hour in spite of maximal inotropic support. Autopsy of the hearts showed minimal ventricular lumen due to massive edema (Figure 6).

Discussion

The preservation of a potential donor heart should start with optimization of the circulation in the brain dead donor. We have studied the consequences of total brain and brain stem death in decapitated pigs.[9,10] After decapitation, there is a catecholamine storm, followed by vascular collapse due to the sudden eclipse of the vasomotor centre in the brain stem. The consequences of the abolished pituitary gland function show themselves as reduced concentrations of T3, T4, antidiuretic hormone and cortisol.[9] When noradrenaline is given in high doses, blood pressure is not elevated as expected, since the sympathetic nerve terminals suck up the noradrenaline without releasing it again, due to the abolished stimulation
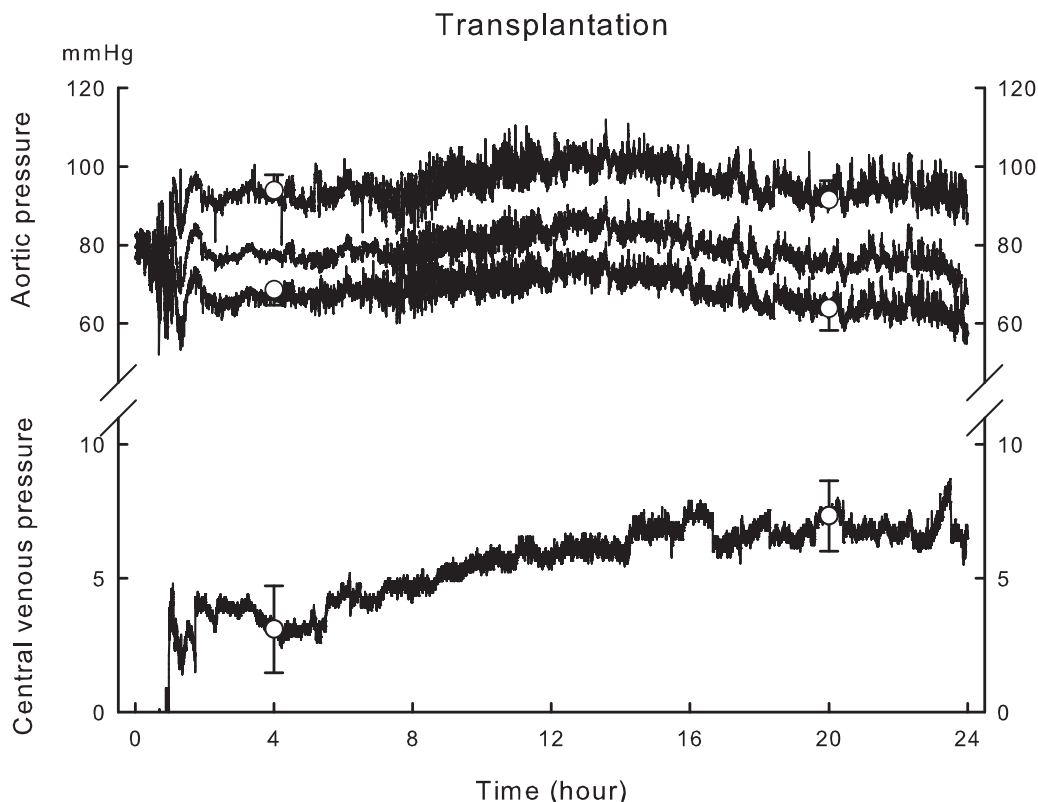

Figure 3. Systolic, mean and diastolic aortic pressures and central venous pressure during 24 hours post transplantation. Results at 4 and 20 hours are given as mean $\pm$ SEM, n = 10.
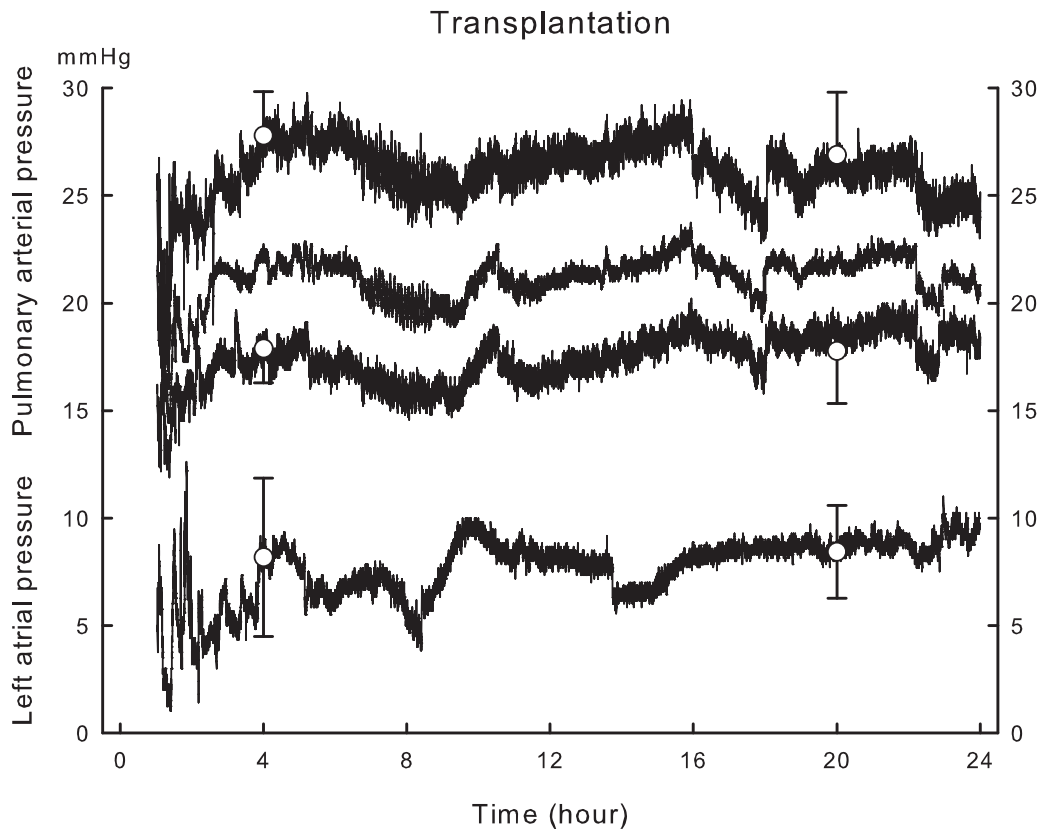
Transplantation

Figure 4. Systolic, mean and diastolic pulmonary arterial pressures (upper part) and left atrial pressure (lower part) during 24 hours after transplantation. Results at 4 and 20 hours are given as mean ± SEM, n = 10.

Table 4. Arterial blood gases before transplantation and during 24 hours of reperfusion.

	Baseline	Start of reperfusion	6 Hours	12 Hours	24 Hours
Temperature (°C)	36.9 ± 0.1	31.8 ± 0.6	35.3 ± 0.5	36.7 ± 0.4	36.8 ± 0.4
Hemoglobin (g/L)	121 ± 3	99 ± 4	125 ± 44	116 ± 6	105 ± 5
PaO$_2$/FiO$_2$	60.3 ± 2.6	47.7 ± 5.6	51.3 ± 3.9	50.5 ± 5.7	52.7 ± 5.5
PaCO$_2$ (kPa)	5.13 ± 0.26	4.50 ± 0.28	5.35 ± 0.14	5.48 ± 0.24	5.33 ± 0.25
pHa	7.41 ± 0.03	7.42 ± 0.02	7.35 ± 0.01	7.36 ± 0.02	7.38 ± 0.03
ABE (mmol/L)	−0.1 ± 1.2	−2.3 ± 0.7	−2.4 ± 0.5	−2.0 ± 0.6	−1.2 ± 0.9
Glucose (mmol/L)	7.4 ± 0.6	11.4 ± 1.0	8.9 ± 0.8	8.5 ± 1.1	7.1 ± 1.3

When comparing the baseline values with the 24-hour values, there were no statistically significant differences, except for hemoglobin (Student's *t*-test, p = 0.0015).

from the vasomotor centre. By blocking the noradrenaline uptake using the reuptake-1 blocker cocaine, the blood pressure can be normalized with low doses of noradrenaline (0.003–0.014 µg/kg/min, see Table 2), doses that do neither affect circulation through the kidneys nor liver function.[9] Furthermore, there is no need for extra fluid supply beyond basic need, which protects the lungs from edema.[10]

Experimental and clinical studies suggest that brain death-associated cardiac dysfunction is related to the neurohormonal storm and subsequent circulatory collapse.[11] Therefore, we used donor hearts from brain dead pigs to make the study more relevant. Brain death in experimental animal models is most commonly induced by increasing the intracranial pressure through inflation of an intracranial, supratentorial balloon.[12,13] When the intracranial pressure becomes higher than the systolic arterial pressure, the brain and brain

stem are assumed to be without circulation. However, according to Barklin, who has written a PhD thesis on brain death in pigs,[13] the pig has ascending spinal arteries which provide an extracranial blood supply to the brain stem; the pig also has a very strong tentorium cerebelli. An inflated balloon in the supratentorial intracranial space will therefore not guarantee complete brain stem death. The extent of brain stem damage is related to the catecholamine response. According to Barklin, there are no specified ways to determine the extent of brain stem death in pigs, which is a serious concern and reduces the external validity of different porcine models of brain death.[13]

Decapitated pigs are subject to a catecholamine storm, with an increase in plasma concentration of noradrenaline from a base value of 600 pmol/L to 47,000 pmol/L, and adrenaline from 115 pmol/L to 34,000 pmol/L.[9] Barklin induced brain death by gradual inflation over 1 hour of a supratentorially placed balloon.[13] This caused a catecholamine storm with a peak increase in plasma concentration of noradrenaline to 2500 pmol/L and adrenaline to 5500 pmol/L, i.e., a much lower response than after decapitation, indicating that the brain stem was not completely dead. To ensure total brain stem death, we decapitated the donor pigs between cervical vertebrae 2 and 3.

Stoica and coworkers studied human brain dead donors which they grouped according to the dose of noradrenaline given by the referring hospital in response to donor hypotension.[11] They found that the use of noradrenaline at doses higher than 0.05 µg/kg/min was associated with increased cardiac graft dysfunction, particularly affecting right ventricular performance, with higher early and late mortality. In the

present study, due to the blockade of the noradrenaline neuronal reuptake with cocaine, the doses of noradrenaline needed to normalize the arterial blood pressure of the brain dead donors were in the range of 0.003–0.014 µg/kg/min, i.e., 30–60 times lower than 0.05 µg/kg/min (Table 2).

To a certain extent, a circulated heart in a decapitated body may be compared to a circulated and oxygenated heart in a preservation box, where the heart with its nerve terminals and receptors is surrounded by the components needed to keep a decapitated body circulatory stable (Tables 2 and 3).
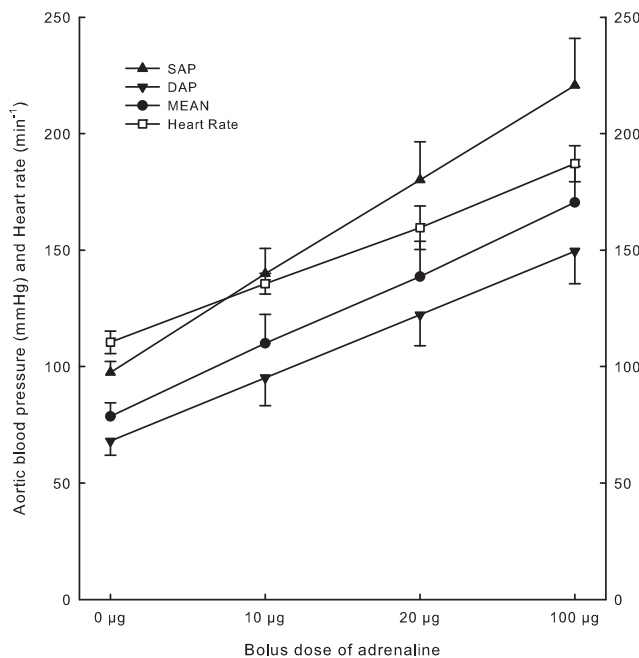

Figure 5. The heart rate and aortic blood pressure response after three different bolus doses of adrenaline given intravenously. Results are given as mean ± SEM, n = 10.

Hypothermic and cardioplegic perfusion was used to reduce the metabolic need of the heart. Because of this, the donor hearts could be perfused intermittently only 20% of the total preservation time, without being exposed for hypoxia. To avoid edema when perfusing a cardioplegic heart, it is necessary to have an oncotic pressure in the preservation medium that is higher than the perfusion pressure used and this was accomplished using albumin (75 g/L) in the preservation medium. At the end of each perfusion cycle, the heart was filled with saturated erythrocytes and during the non-perfusion phase, the heart received oxygen from the de-saturating erythrocytes in the capillaries.

At the end of the present experiment, 24 hours after transplantation, all pigs were stable with normal blood pressure without inotropic support, normal urine production, and normal blood gases, indicating adequate body perfusion. The adrenaline test showed that all transplanted hearts had the capacity to develop a dose-dependent increase in aortic pressure, indicating a well-functioning myocardium (Figure 5).

Recently, a prospective, randomized non-inferiority trial was published comparing standard cold cardioplegic storage of human donor hearts with beating hearts perfused ex-vivo with blood from the donor at normothermia with The Organ Care System.[14] In the standard group, the cold cardioplegic storage time was 3 hours and 15 minutes and the 30 day patient and graft survival rate was 97% (n = 61 patients). In the Organ Care System group, the cold ischemia time was 1 hour and 53 minutes and the time with normothermic perfusion was 3 hours and 31 minutes. The 30 day patient and graft survival in this group was 94% (n = 63). Five donor hearts were deemed unacceptable for transplantation after preservation with the Organ Care System and were discarded and not included in the analysis, which focused only on recipient outcomes. In a comment from Freed and White in the Lancet [15], the discarded hearts all met study inclusion
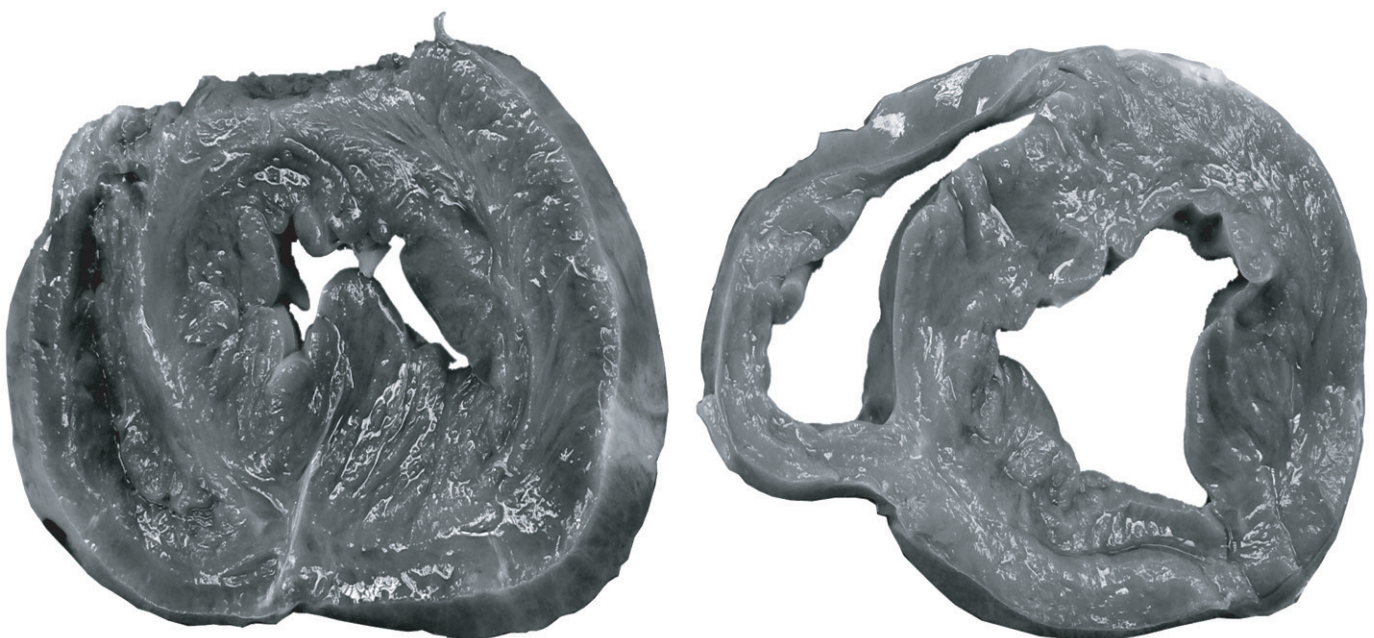

Figure 6. Transverse section through the ventricles of a heart preserved for 24 hours in St Thomas solution, after failed weaning from the heart-lung machine (left). A normal heart of the same size (the recipient's explanted heart) is shown for comparison (right).

criteria and would have been transplanted if they had not been randomized into the Organ Care System group, which makes the results difficult to interpret.

To conclude, the present study shows that orthotopic transplantation of porcine hearts harvested 24 hours after acute total brain and brain stem death and then preserved for 24 hours can be done safely.

Disclosure statement

Funding information

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