Heart Donation After Cardiac Death: Preliminary Study on an Isolated, Perfused Swine Heart After 20 Minutes of Normothermic Ischemia

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ABSTRACT

Background. We measured the functional and metabolic status of hearts submitted to normothermic ischemia before preservation through the use of an ex vivo pig heart model to assess the feasibility of donation after cardiac death (DCD) in heart transplantation.

Methods. Ten pigs were separated into 2 groups: control ($n = 6$, brain-dead group) and DCD ($n = 4$, heart donation after cardiac death). In the control group, hearts were excised 20 minutes after the brachiocephalic trunk cross-clamping and were immediately reperfused. In DCD, hearts were excised 20 minutes after exsanguination and asphyxia, stored in the Centre de Résonance Magnétique Biologique et Médicale (CRMBM) solution for 2 hours, and then were reperfused. Cardioplegic arrest was induced with the use of 1 L of CRMBM solution (4°C) and the heart was reperfused for 60 minutes through the use of an ex vivo perfusion system in Langendorff mode with normothermic autologous blood. During reperfusion, functional parameters were analyzed. Biochemical assays were performed in myocardial effluents and freeze-clamped hearts.

Results. No electromechanical activity was found in DCD compared with control. Creatine kinase (CK) was higher at 2 minutes of reperfusion in DCD versus control ($P = .005$). Adenosine triphosphate was lower in DCD versus control ($P = .0019$). Malondialdehyde, an oxidative stress index, was present only in DCD. The nitric oxide (NO) pathway was impaired in DCD versus control, with lower eNOS expression ($P < .0001$) and total nitrate concentration content ($P = .04$).

Conclusions. We reported no cardiac functional and metabolic recovery in the DCD group after normothermic ischemia and reperfusion, which indicates that a single immersion of the cardiac graft during storage does not provide an optimal protection. New strategies in heart preservation are necessary for recruiting heart donation after cardiac death.

LACK of donor organs currently limits cardiac transplantation. The use of heart donation after cardiac death (DCD) could increase cardiac graft availability and expand the donor pool [1]. Nevertheless, these hearts are exposed to inevitable warm cardiac ischemia and require special myocardial protection to minimize tissue damage [2]. Successful transplantation of hearts after normothermic ischemia has already been shown in dogs [3] and primates [4] pretreated with cardioprotective drugs when a controlled reperfusion with blood cardioplegia was performed. In contrast, pig hearts that did not undergo any pre-treatment with cardioprotective

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drugs failed to be successfully reanimated when excised 10 minutes or more after animal exsanguination [5].

To assess the feasibility of donation after cardiac death in heart transplantation and to evaluate the extent of tissue damage in these conditions, we measured the functional and metabolic status of hearts submitted to 20 minutes of normothermic ischemia before preservation without any pretreatment with the use of an ex vivo pig heart model. Heart rate and developed pressure were measured as an index of myocardial performance. Metabolic status was evaluated by use of adenine nucleotides and phosphocreatine contents. Malondialdehyde (MDA) was used to evaluate lipid peroxidation and oxidative stress. Caspase-3 expression was studied as an index of myocardial apoptosis. The expression of endothelial and inducible nitric oxide synthase (NOS) isoforms and total nitrate concentration (NOx) were determined as markers of the NO pathway. A preliminary form of this study was published recently as an abstract [6].

METHODS

Animals

Experiments were conducted on 10 large White pigs (weight, 62 ± 3 kg). All procedures were performed at the Animal House Unit of the LaSalle Beauvais Polytechnic Institute (policy agreement No. A60) and received prior approval both from the Animal Protocol Review Committee of the institution and by the Animal Welfare Committee of the Picardie Council Veterinary Office. All animals received humane care in compliance with the Principle of Laboratory Animal Care formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1996).

Pigs were sedated by injection of Zoleptil (8 mg/kg) (Movianto, United Kingdom). Spontaneous breathing was allowed with an oxygen mask with 10 L/min^{-1} oxygen flow. General anesthesia was then induced with atropine (0.5 mg), buprenorphine (10 mg), propofol (2–2.5 mg/kg), and ketamine (3 mg/kg). After intubation and a bolus of cisatracrium (1 mg/kg) for curarization of the animal, volume-controlled ventilation (minute volume: 8 mL/kg body weight; adaptation according blood gas analysis) was applied. Continuous hemodynamic monitoring was performed for each pig with electrocardiogram, heart rate, and invasive blood pressure monitoring. Pulse oxymetry, capnometry, and temperature were also measured continuously. Pigs were separated into 2 groups: control ($n = 6$, brain-dead group) and DCD ($n = 4$, heart donation after cardiac death).

Experimental Protocol

After median sternotomy, heparin (300 UI/kg^{-1}) was given intravenously. In the control group, 20 minutes after brachiocephalic trunk cross-clamping, 1 L of Centre de Résonance Magnétique Biologique et Médicale (CRMBM) cardioplegic solution [7] at 4°C was infused before excising the hearts. After explantation, hearts were immediately reperfused at 50 mL/min for 5 minutes before increasing flow to 140 mL/min. In the DCD group, controlled exsanguination (500 mL/min) was induced in pigs. After cardiac arrest was achieved with standstill or ventricular fibrillation, asphyxia was induced by turning the ventilator off. After 20 minutes of ischemia, 1 L of CRMBM cardioplegic solution [7] at 4°C was injected and hearts were excised and stored in the CRMBM solution for 2 hours and were then reperfused. During the first 20 minutes of reperfusion, hearts were perfused at 50 mL/min of flow for 5 minutes before increasing flow to 140 mL/min. In both groups, hearts were reperfused for 60 minutes through the use of an ex vivo perfusion system in Langendorff mode with normothermic autologous blood. During reperfusion, functional parameters were analyzed by use of an intraventricular balloon inserted into the left ventricle. An initial pressure value within the balloon was set to 5 mm Hg. Left ventricular developed pressure and heart rate were measured.

Biochemical Assays in Myocardial Effluents and Tissues

CK was determined in myocardial effluents by use of an enzymatic kit (Randox Laboratories Limited, United Kingdom) during reperfusion. At the end of the experiments, hearts were rapidly freeze-clamped and kept at −80°C for biochemical assays. Adenine nucleotides, phosphocreatine, and MDA contents were measured by use of high-performance liquid chromatography [8]. Caspase-3 expression was studied as an index of apoptosis by Western blot with the use of a specific antibody against cleaved caspase-3 (Ozyme, France). Endothelial (e) and inducible (i) NOS protein expression were determined as previously described [9,10]. Tissue NOx was determined with the use of a nitrate/nitrite colorimetric assay kit (Cayman Chemical, Ann Arbor, Mich, United States). Water content was calculated by means of the ratio heart weight after explantation over heart weight after reperfusion and expressed as a percentage.

Data Analysis

The results are expressed as mean values ± SEM. Comparisons between groups were analyzed by use of unpaired *t* tests (function) and 1-way analysis of variance followed by a Scheffe's post hoc test (biochemical assays). A value of $P < .05$ was considered statistically significant.

RESULTS

No electromechanical activity was found in DCD compared with control. Heart rate and developed pressure were measured only in the control group, with 52 ± 20 beats/min and 71 ± 9 mm Hg, respectively ($P < .05$ versus DCD group). CK (UI/L) was significantly higher ($P = .005$) in DCD versus control (8569 ± 636 versus 3205 ± 172) only after 2 minutes of reperfusion. Adenosine triphosphate (ATP) content and total adenine nucleotides were significantly lower in DCD versus control ($P = .0019$ and $P = .0038$, respectively) (Table 1). No significant differences were found in phosphocreatine content and

Table 1. Phosphocreatine and Adenine Nucleotides in Control and DCD Freeze-Clamped Hearts

	PCr µmol/g Proteins	ATP µmol/g Proteins	TAN	AEC
Control	54.8 ± 10.4	33.8 ± 3.8	39.8 ± 4.5	0.92 ± 0.01
DCD	26.4 ± 6.9	5.9 ± 2.9	13.3 ± 3.0	0.58 ± 0.13
P value	.0629	.0019	.0038	.0629

Abbreviations: DCD, donation after cardiac death; PCr, phosphocreatine; ATP, adenosine triphosphate; TAN, total adenine nucleotides (ATP+ADP+AMP); AEC, adenylate energy charge ([ATP+0.5 ADP]/[ATP+ADP+AMP]*10).