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Low-flow Perfusion Preservation Versus Static Preservation for Isolated Rat Heart: Effects on Recovery of Myocardial Function

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ABSTRACT

Background. Clinically, donor hearts cannot be preserved for >6 hours between explantation and recipient implantation. A better approach is needed to preserve donor hearts for a longer time. We tested whether low-flow perfusion (LFP) could satisfactorily preserve isolated rat hearts with histidine-tryptophan-ketoglutarate (HTK) solution or Fuwai modified (FWM) solution.

Methods. We divided 32 male Sprague-Dawley rats randomly into 4 groups ($n = 8$): H1, H2, F1, and F2. The Langendorff heart model immersed isolated hearts in the H1 and F1 groups in HTK or FWM solution for 8 hours at 4°C. Isolated hearts in the H2 and F2 groups were low-flow perfused with HTK solution and FWM solution for 8 hours at a pressure of 10 cmH_2O at 4°C. After 60 minutes reperfusion, we measured recovery of cardiac function, myocardial water content, and leakage of myocardial enzymes.

Results. After reperfusion, no cardiac rebeating was observed among F1 group hearts; in addition, they showed significantly higher myocardial water content and lactate dehydrogenase leakage compared with the other 3 groups ($P < .05$). The recovery rates of cardiac function among H2 hearts were better than the other 3 groups ($P < .05$); their myocardial water content and enzyme leakage were less than the other 3 groups ($P < .05$).

Conclusions. Hypothermic LFP was better than static storage to preserve isolated rat hearts. HTK solution afforded better myocardial protection than FWM.

PRESERVATION of the isolated heart is important for successful transplantation. The approaches and solutions should be carefully chosen to achieve good preservation. In recent years, histidine-tryptophan-ketoglutarate (HTK) and University of Wisconsin solutions have greatly improved myocardial preservation, but their protective effects do not last for 6 hours when used traditionally at low-temperature.[1,2] A survey of 111 heart transplant cases in China[3] showed 98% of heart transplant patients to experience survival and a good quality of life. The long-term survival rate was 95.2% with 3 subjects surviving >10 years. For these transplantations, donor hearts were preserved in static condition from 52 to 310 minutes (>6 hours). However, China has a vast territory; quite often, the locations of the donor heart and the recipient are distant. The success of transplantation may be further improved if better approaches can be implemented to preserve donor hearts.

Continuous perfusion has been used to preserve organs for >10 years,[4,5] as implemented in kidney transplantation.[6] Unfortunately, this endeavor is still a laboratory study for heart preservation.[4,7–10] Theoretically, continuous perfusion enables the organ to maintain myocardial aerobic metabolism to provide energy substrates, to sustain transmembrane ion gradients and to eliminate myocardial edema. Therefore, we compared the effects of HTK and Fuwai modified (FWM) solutions during static versus low-flow perfusion to preserve isolated rat hearts.

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MATERIALS AND METHODS

All experimental procedures and protocols in our study were reviewed and approved by our Animal Care and Use Committee, conforming to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (1996 revision).

Animals and Drugs

Adult male Sprague-Dawley (SD) rats (350–450 g) purchased from our Experimental Animal Center were reared with 12-hour light–dark cycles in a temperature-controlled room with ad lib access to food and water. HTK was purchased from Dr F. Kohler Chemie (Germany, 063181); Histidine, tryptophan, ketoglutarate, mannitol, raffinose, and glutathione, from Sigma-Aldrich (St. Louis, Mo).

Langendorff Heart Preparation

SD rats anesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg) as confirmed by no response to tail clamping were heparinized (1000 U/kg) to permit rapid excision of their hearts and placement in ice-cold Krebs-Henseleit (KH) buffer before aorta cannulation for mounting on a modified, noncirculating Langendorff apparatus. The hearts were perfused at a constant pressure of 100 cm H_2O with continuously gassed (95% oxygen/5% carbon dioxide) KH buffer: NaCl, 118.0 mmol/L; KCl, 4.7 mmol/L; $MgSO_4$, 1.2 mmol/L; KH_2PO_4, 1.2 mmol/L; $CaCl_2$, 1.8 mmol/L; $NaHCO_3$, 25.0 mmol/L; glucose, 11.1 mmol/L; and EDTA, 0.5 mmol/L, pH = 7.4. A small latex balloon connected to a pressure transducer was inserted through the mitral valve of the left atrium into the left ventricle. The balloon was filled with saline to achieve an end-diastolic pressure of 8–12 mm Hg, which was maintained at a consistent balloon volume.

Preservation Solutions

Two cardioplegic solutions—HTK and FWM—were used in this study. FWM, a calcium-free in solution, was prepared by Fuwai Hospital (Beijing, China). The Mannitol in HTK is replaced with raffinose in FWM. Glutathione is added to the FWM solution (Table 1).

Experimental Protocols

The heartbeats reached a steady state after 30 minutes of balanced perfusion at which time we measured hemodynamics. The hearts were arrested by perfusion with about 50–70 mL cold (4°C) HTK or FWM solution for 5 minutes. H1 group hearts arrested with HTK were immersed still in HTK for 8 hours at 4°C. H2 hearts arrested with HTK were preserved using continuous low-flow HTK perfusion at a pressure of 10 cmH_2O for 8 hours at 4°C. F1 hearts were treated with FWM similar to the H1 group hearts. F2 hearts were treated with FWM similar to H2 hearts. After 8 hours, all rat hearts were transferred to the Langendorff apparatus and restored slowly to 37°C over 5–8 minutes for 60 minutes reperfusion.

Table 1. Composition of HTK and FWM

Content (mmol/L)	HTK	FWM
NaCl	15	15
KCl	10	10
$MgCl_2 \cdot 6H_2O$	4	4
$CaCl_2$	0.015	—
Histidine-buffer	198	198
Tryptophan	2	2
Ketoglutarate	1	1
Mannitol	30	—
Raffinose	—	30
Glutathion	—	3
pH	7.3	6.85
Osmolarity (mOsM)	310	313

Hemodynamic Recordings

Analog signals digitized with BIOPAC150 (BIOPAC Systems Inc., Goleta Calif) were continuously recorded at 1000 Hz with Acq Knowledge version 4.0 software (BIOPAC Systems Inc.). Characteristic data derived from left ventricular pressure (LVP) measurements included left ventricular developed pressure (LVDP), maximal and minimal first derivatives of LVP (dP/dt_{max} and dP/dt_{min}) as indices of contractility and relaxation, as well as heart rate. Coronary flow (CF) was measured by serial collections of coronary effluent. These variables calculated after 30 minutes equilibration (baseline) were expressed as recovery percentages (%).

Determination of Myocardial Damage

To assess myocardial damage, lactate dehydrogenase (LDH) was determined from collected coronary effluents using an automated biochemistry analyzer (Hitachi 7600, Tokyo, Japan) with commercial LDH assay kits (Roche Diagnostics, Mannheim, Germany). Coronary effluent (1 mL) spot collected from hearts at baseline and at the end of preservation were coded for blinded analysis.

Myocardial Water Content

To evaluate myocardial edema, left ventricular anterior wall tissue samples were weighed before and after drying in an over 80°C for 24 hours. The myocardial water content was calculated by the formula: {(wet weight − dry weight)/wet weight} × 100%.

Statistical Analysis

Data were expressed as mean values ± standard deviation. Statistical analysis among the groups was assessed by 1-way analysis of variance followed by a Newnam-Keul test for multiple comparisons. The chi-square test was used for categorical variables. $P < .05$ was considered to be significant. All statistical analyses were performed using SPSS 13.0 software (SPSS Inc., Chicago, Ill).

RESULTS

Effect on Hemodynamics

The cardiac function parameters of all isolated hearts were similar at baseline (Table 2). During reperfusion, ventricular fibrillation was observed usually, but sinus rhythm was not regained in any heart of the F1 group (Fig 1). These hearts appeared to be pale and stone-like. After reperfusion, regular rebeats were observed in all hearts of the other 3 groups, but there were differences in recovery of cardiac function. Representative recordings of LVDP are presented in Fig 2. Before arrest, the LVDP measurements of all isolated hearts were strong and regular, but the pressures of hearts in the H1, H2, and F2 groups displayed only partial recovery during reperfusion. The heart rates were slowed and accompanied by irregular rhythms.