The UW solution has greater potential for longer preservation periods than the Celsior solution: comparative study for ventricular and coronary endothelial function after 24-h heart preservation

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Abstract

Objective: Improvement of long-term heart preservation methods would potentially increase the donor pool and improve survival. We compared the efficacies of the University of Wisconsin (UW) and Celsior solutions on ventricular and endothelial functions after 24-h preservation. Methods: We used an isolated heart preparation perfused with blood. The heart was excised from a rabbit, stored for 24 h in the UW or Celsior solution, and then perfused with blood from a support-rabbit. We evaluated cardiac output and coronary endothelial function. Results: The Frank—Starling curve showed a significant left and upward shift in the UW group compared with that in the Celsior group (p < 0.01). There were no significant differences between the groups for the coronary blood flow in response to sodium nitroprusside or acetylcholine. The serum creatine kinase MB level after reperfusion was significantly lower in the UW group than in the Celsior group (10.7 ± 1.4 ng/mL vs 30.4 ± 5.4 ng/mL, p < 0.01), whereas lipid peroxide levels did not differ significantly between the two groups. Conclusions: The UW group showed better left ventricular function than the Celsior group, indicating that the UW solution has greater potential for long-term preservation than Celsior solution.

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Keywords: Heart transplantation; Preservation; Endothelium

1. Introduction

Cardiac transplantation has been performed in 63,000 patients around the world and is accepted as a therapy for end-stage heart disease [1]. However, the number of heart transplant procedures has reached a ceiling since the beginning of 1990s. One of the reasons for this is a shortage in the number of donors. We have previously investigated the potential of long-term preservation using animal models [2–5], since improvement of long-term myocardial preservation methods would potentially increase the donor pool and improve both early and late survivals [6]. Transplantation models of long-term preservation clearly reveal the advantages and disadvantages of preservation methods, including those of preservation solutions.

The University of Wisconsin (UW) solution is one of the most widely used preservation solutions, and many investigators have reported effective myocardial protection by the UW solution in experimental and clinical transplantations [7,8]. However, the UW solution has also been reported to have the disadvantage of causing endothelial dysfunction.

Since it was first reported in 1994 [9], cardiac surgeons have been attracted to using the Celsior solution, which was originally designed for heart preservation. Some investigators have shown that the Celsior solution has the advantages of being able to prevent free radical injury [10], coronary endothelial damage [11,12], and depletion of adenine nucleotides [13]. However, other investigators have reported the necessity of a higher dose of catecholamines post-operatively [14] and higher frequency of necessary defibrillations [15,16].

The efficacies of the Celsior solution on ventricular or coronary endothelial function after long-term preservation have not yet been investigated. Using isolated rabbit hearts perfused with cross-circulated blood, we compared the efficacies of the UW and Celsior solutions on ventricular and endothelial function after 24-h preservation in order to investigate their potentials for longer preservation periods and reveal the advantages and disadvantages of these solutions.
2. Materials and methods

2.1. Experimental animals

Japanese white rabbits weighing 2.9—3.2 kg were used (21 as heart donors, 17 as support-rabbits, and 17 as blood donors). The experimental protocol was reviewed by the Committee on the Ethics of Animal Experiments of the Faculty of Medicine, Kyushu University, and carried out under the Guidelines for Animal Experiments of the Faculty of Medicine, Kyushu University, and the Law (No. 105) and Notification (No. 6) of the Japanese Government.

2.2. Donor heart management

The rabbits were anesthetized using sodium thiamylal (20 mg/kg) and intubated with a tracheal tube connected to a mechanical ventilator (model SN-480-5; Shimano, Tokyo, Japan) with 100% oxygen. For further anesthesia, vecuronium bromide (1 mg/kg) and fentanyl citrate (60 mg/kg) were given intravenously. After performing a median sternotomy, the heart and aortic arch were exposed. After heparinization (1000 units/kg), the innominate artery was cannulated in order to administer a cardioplegic solution. After the inferior vena cava was transected to decompress the heart, the aortic arch was quickly excised and preserved for 24 h at 1°C. The temperature was maintained by a heat exchanger (model RTE-210; Neslab Instruments Inc., Newington, NH, USA).

In the UW group (n = 6 for LV function, n = 5 for endothelial function), cardiac arrest was induced with an infusion of 5 mL/kg of a 4°C cardioplegic solution (the Kyushu University cardioplegic solution; 2.5% glucose, Na⁺ 87 mM, K⁺ 20 mM, Cl⁻ 97 mM, HCO₃⁻ 10 mM, Ca²⁺ 0.1 mM, insulin 5 units/L, lidokine 100 mg/L). The University of Wisconsin solution (ViaSpan; DuPont Pharmaceutical, Wilmington, DE, USA) was then used for coronary vascular washout and immersion. In the Celsior group (n = 7 for LV function, n = 5 for endothelial function), the Celsior solution (Pasteur Merieux Serums et Vaccins, Lyon, France) was used to induce cardiac arrest and wash out the coronary vascular beds, and also for immersion.

2.3. Support-rabbit and cross-circulation system

We used an isolated rabbit heart preparation perfused with support-rabbit blood, as previously described [2,3,17]. The support-rabbits were anesthetized and pretreated in the same manner as donor rabbits. Anesthesia was maintained with constant infusion of fentanyl citrate (100 mg/h) and vecuronium bromide (1 mg/h). After heparinization (1000 units/kg), the common carotid artery and external jugular vein were exposed and cannulated. Oxygenated blood from the common carotid artery of the support-rabbit was introduced to a cannula connected to the ascending aorta of the donor heart using a microtube pump (model MP-3; Tokyo Rikakikai Inc., Tokyo, Japan). The blood draining from the system was returned to the jugular vein by another microtube pump (Fig. 1). During the use of this system, the hematocrit of the perfusion blood was maintained at 28% using a blood donor rabbit. Arterial blood gas analyses of the support-rabbit were performed with a pH/blood gas analyzer (model IL-1304; Instrumentation Laboratory, Barcelona, Spain). Femoral arterial pressure of the support-rabbit was also continuously monitored.

2.4. Measurement of left ventricular function in the working preparation

We measured LV function after 24 h of preservation using the working preparation. The donor heart was perfused
through the aorta and then the Langendorff preparation was initiated (Fig. 1A). The perfusion pressure was maintained at 60 mmHg and blood temperature was maintained at 37 °C. After the pulmonary veins were closed, a double-lumen cannula was inserted into the left atrium. One lumen of the double-lumen cannula was connected to a pressure transducer to measure left atrial pressure (LAP). The other lumen was connected to an atrial reservoir. The aortic flow (AoF) rate was measured by an in-line electromagnetic flow probe (model 2N764; Transonic System Inc., New York, NY, USA) connected to a flow meter (model T206; Transonic Systems Inc.). Aortic pressure was measured from a sidearm in the aortic tract. All signals (pressures and flows) were continuously monitored on an oscillograph (polygraph 360 system; NEC Sanei, Tokyo, Japan), digitized on-line at 200 Hz with an analog-to-digital converter (MacLab System; ADlnstruments Ltd., Dunedin North, New Zealand), and recorded on a digital computer (PowerBook 550C; Apple Computer Inc., Cupertino, CA, USA).

After 60 min of Langendorff perfusion, the working preparation was started (Fig. 1B). In the working preparation, the heart was paced atrially at 250 beats/min and aortic afterload pressure was fixed at 60 mmHg. After stabilization of the working preparation for 30 min, the aortic flow rates were measured at points of varied LAP. Systolic and diastolic pressures, variables determined by the cardiac output and stiffness or compliance of the afterload system, varied according to increases in LAP whereas the mean pressure was kept stable (60 mmHg) throughout the measurements. Based on these data, we determined the Frank-Starling curve. We measured serum creatine kinase MB (CK-MB) and lipid peroxide (LPO) levels in the coronary effluents 120 min after reperfusion.

2.5. Evaluation of coronary endothelial function in the Langendorff preparation

The hearts were perfused in the Langendorff preparation at 80 mmHg perfusion pressure. We measured the coronary blood flow (CBF) 60 min after reperfusion. We tested the vasodialatory responses 60 min after reperfusion by using sodium nitroprusside (SNP; 100 μg) and acetylcholine chloride (Ach; 10 μg). We administered these drugs in the aortic tract for 1 min via a syringe infusion pump. The CBF was measured during the infusion of these drugs by an in-line electromagnetic flow probe connected to a flow meter. All hearts were paced atrially at 250 bpm during the experiment.

2.6. Serum chemistry measurements

The serum CK-MB level was measured using a chemiluminescent immunoassay (SRL Inc., Tokyo, Japan). The serum LPO level was measured by a modification of the Yagi method (Determiner LPO; SRL Inc.).

2.7. Statistical analysis

The results for each group are expressed as mean ± standard deviation. The unpaired Student’s t-test was used to examine the differences in each parameter. The relationship between LAP and aortic flow was analyzed by a multiple regression model using a dummy variable technique to investigate intergroup differences. A significance was designated as a probability value of less than 0.05.

3. Results

No significant differences were observed between the two groups for the body weights of the donor rabbits. During the experiment, hemodynamics of the support-rabbits were stable, and systolic blood pressure was maintained over 100 mmHg. Minimal alterations in the arterial carbon dioxide tension, pH and arterial bicarbonate levels were observed in the support-rabbits.

In the UW group, all hearts started to beat without ventricular fibrillation and could be evaluated functionally in the working preparation, whereas all hearts in the Celsior group spontaneously recovered sinus rhythm after several minutes of ventricular fibrillation and two hearts could not be evaluated functionally in the working preparation because they could not produce an output against the afterload (60 mmHg).

3.1. Left ventricular function: Frank-Starling curves

After 90 min of reperfusion, the fitted Frank-Starling curve obtained by a multiple linear regression model showed a significant left and upward shift (p < 0.01) in the UW group (y = −1.97x^2 + 36.03x − 45.22; R = 0.95) compared with that in the Celsior group (y = −0.09x^2 + 8.39x − 56.92; R = 0.98) (Fig. 2).

3.2. Coronary endothelial function

Sixty minutes after reperfusion the CBF did not differ significantly between the two groups (UW: 14.9 ± 3.0 mL/min vs Celsior: 14.5 ± 2.8 mL/min).

![Fig. 2. Frank-Starling curve data obtained in this study. The thick black line represents the fitted Frank-Starling curve of the UW group (n = 6), while the broken black lines show the individual curves in the UW group. The thick gray line represents the fitted Frank-Starling curve of the Celsior group (n = 5), while the broken gray lines show the individual curves in the Celsior group. The curve shows a significant left and upward shift (p < 0.01) in the UW group compared with that in the Celsior group. AoF: aortic flow; LAP: left atrial pressure.](image)
Table 1
Maximum CBF during administration of SNP or Ach

<table>
<thead>
<tr>
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<th>SNP</th>
<th>Ach</th>
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<tr>
<td>UW group (n = 5)</td>
<td>33.6 ± 2.2</td>
<td>29.3 ± 7.8</td>
</tr>
<tr>
<td>Celsior group (n = 5)</td>
<td>32.8 ± 4.6</td>
<td>31.1 ± 5.9</td>
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Table 2
CK-MB and LPO levels at 120 min after reperfusion

<table>
<thead>
<tr>
<th></th>
<th>CK-MB (ng/mL)</th>
<th>LPO (nmol/mL)</th>
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</thead>
<tbody>
<tr>
<td>UW group (n = 6)</td>
<td>10.7 ± 1.4</td>
<td>2.2 ± 0.7</td>
</tr>
<tr>
<td>Celsior group (n = 7)</td>
<td>30.4 ± 5.4</td>
<td>2.3 ± 0.3</td>
</tr>
</tbody>
</table>

CK-MB: creatine kinase MB; LPO: lipid peroxide.

The vasodilatory responses to Ach and SNP are shown in Table 1. No significant differences were observed between the two groups for the maximum CBF in response to SNP (UW: 33.6 ± 2.2 mL/min vs Celsior: 32.8 ± 4.6 mL/min) and Ach (UW: 29.3 ± 7.8 mL/min vs Celsior: 31.1 ± 5.9 mL/min).

3.3. Serum chemistry

As shown in Table 2 the serum CK-MB level in the coronary effluent after reperfusion was significantly higher in the Celsior group than in the UW group (30.4 ± 5.4 ng/mL vs 10.7 ± 1.4 ng/mL, p < 0.01). The LPO level in the coronary effluent after reperfusion did not differ significantly between the two groups (Celsior: 2.3 ± 0.3 nmol/mL vs UW: 2.2 ± 0.7 nmol/mL).

4. Discussion

In this study, the UW group showed better left ventricular function and lower frequency of ventricular fibrillation compared with the Celsior group after 24 h of cold preservation. No difference in endothelial function was observed between the two groups. The serum CK-MB level after reperfusion was significantly lower in the UW group than in the Celsior group, although LPO levels did not differ significantly between the two groups.

4.1. UW solution and Celsior solution

The UW solution was originally developed for preservation of the pancreas [18]. Many of the components are principally added to decrease cellular swelling. Lactobionate and raffinose are included to increase the extracellular oncotc pressure and further reduce cellular edema. Hydroxyethyl starch is a nontoxic colloid that is added to reduce interstitial edema [18]. In our previous studies on long-term heart preservation, we basically used the UW solution as the preservation solution because its advantages have been demonstrated in many reports [7,8,19].

On the contrary, the Celsior solution was designed to fulfill two major objectives: (1) to combine the general principles of hypothermic organ preservation with those specific for the myocardium, and (2) to offer the possibility of use not only as a storage medium but also as a perfusion fluid during initial donor heart arrest, post-storage graft reimplantation and early reperfusion [9]. Since it was first reported in 1994, several studies have documented the advantages of the Celsior solution [10—13]. However, the efficacies of the Celsior solution on ventricular or coronary endothelial function after long preservation periods, such as 24 h, have not yet been investigated.

4.2. Left ventricular function after preservation

Several studies reported that the Celsior solution effectively preserved myocardial function, protected against free radical-mediated cell injury [10] and prevented ischemia—reperfusion-induced pulmonary microvascular permeability [20]. In this study, the Celsior group showed less left ventricular function compared with the UW group. Some investigators reported that Celsior-preserved hearts required more inotropic support in the early phase after transplantation [14], and these investigators assumed a reversible dysfunction as the underlying phenomenon.

In our results, LPO levels in the coronary effluents after reperfusion did not differ significantly between the two groups. However, the serum CK-MB level in the coronary effluent after reperfusion was significantly higher in the Celsior group than in the UW group. At reperfusion, the ability to protect against free radical-mediated cell injury may be the same for these two solutions. However, during preservation, the myocardial protective effects of the UW solution were superior to those of the Celsior solution. The previous studies would not have been able to clarify this action owing to their short preservation periods.

4.3. Arrhythmia after reperfusion

Reperfusion arrhythmias are commonly observed during postischemic reperfusion after open-heart surgery and transplantation. The difference in the rate of ventricular fibrillation immediately after reperfusion between the two groups was a remarkable finding in this study. All UW-preserved hearts started to beat without ventricular fibrillation, whereas all Celsior-preserved hearts recovered sinus rhythm after several minutes of ventricular fibrillation. These findings may be attributable to the intracellular composition of electrolytes in the UW solution that decrease flux across the membrane during preservation and help to maintain a physiologic electrolyte concentration within the cells of the conduction system and myocardium. On the contrary, some studies reported that the incidence of arrhythmias in Celsior-preserved hearts tended to be high [15,16]. Since the Celsior solution is an extracellular-type solution, a higher K+ gradient across the membrane, triggering arrhythmia as in hypokalemia, may be responsible for this consequence. The characteristics of Celsior-preserved hearts would be much more clearly expressed in the current study due to the longer preservation period evaluated.
4.4. Coronary endothelial function after preservation

Endothelial dysfunction causes changes in microcirculation after reperfusion due to cellular swelling, platelet accumulation, impairment of procoagulant and anticoagulant properties, and leukocyte adherence. In addition, vasomotor dysfunction of the epicardial endothelium may precede and predict the allograft vasculopathy seen in vasomotor dysfunction of the epicardial endothelium. For example, the accumulation, impairment of procoagulant and antioxidant properties, and leukocyte adherence. In addition, it has been reported that the reduced glutathione in the Celsior solution improves the preservation of coronary endothelial function [12]. However, in the current study, no difference in endothelial function was observed between the two groups. One of the reasons for this may be that we used a cardioplegic solution to induce arrest as our clinical strategy. Our institute previously reported [23,24] that the use of an extracellular-type solution to induce arrest before using an intracellular-type solution for storage attenuated the increase in coronary vascular resistance and decrease in high-energy phosphate observed with the use of an intracellular-type solution for both inducing arrest and storage. The use of the Kyushu University cardioplegic solution (extracellular-type solution) to induce arrest may compensate for the defects of the UW solution (intracellular-type solution).

4.5. Blood-perfused rabbit heart model

As an animal model, we used a blood-perfused isolated working rabbit heart model developed in our laboratory [2,3,17]. Many studies have demonstrated that polymorphonuclear leukocytes and platelets play an important role in mediating ischemia–reperfusion injury. In our additional studies, the CBF was markedly dependent on the perfusate [2,25]. Therefore, our blood-perfused model is well suited to investigate the process of ischemia–reperfusion and study the vasodilatory responses in the whole heart compared to isolated heart preparations perfused with crystallloid solutions. Furthermore, since we cannot avoid running extracorporeal circulation during heart transplantation, this model is suitable for the evaluation of cardiac function after preservation.

5. Conclusions

To the best of our knowledge, this is the first study to investigate ventricular or coronary endothelial function of Celsior solution-preserved hearts after long-term preservation. In this situation, the UW solution is superior to the Celsior solution for left ventricular function and frequency of ventricular fibrillation. The serum CK-MB level after reperfusion was significantly lower in UW solution-preserved hearts than in Celsior solution-preserved hearts. The UW solution, therefore, has greater potential for longer preservation periods than the Celsior solution.

References


