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Comparison of University of Wisconsin and University of Pittsburgh solutions for heart transplantation

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Abstract The effectiveness of University of Wisconsin (UW) and University of Pittsburgh (UP) solutions for the preservation of rat hearts was compared. Lewis rat hearts were preserved with UW (group A, $n = 45$) or UP (group B, $n = 45$) solution for 0 or 24 h and then transplanted heterotopically into the recipients' abdomen. Ten recipients in each group were observed to obtain 1-week graft survival rates. Tissue water content and tissue content of adenine nucleotides were measured 2 h after transplantation in six grafts from each group. Six hearts preserved for 0 h and seven hearts preserved for 24 h were taken from each group 24 h after grafting for histopathology. The 1-week graft survival rates of groups A24 and B24 were 60 % and 10 %, respectively. In the 24-h preserved grafts, adenosine triphosphate (ATP) and energy charge [(ATP + adenosine diphosphate/2)/(ATP + adenosine diphosphate + adenosine monophosphate)] of groups A and B were 0.972 ± 0.165 and 0.200 ± 0.123 mg/g wet tissue ($P < 0.05$) and 74.4 % and 61.1 % ($P < 0.05$), respectively. The tissue water content of group A24 was 71.7 %, whereas that of group B24 was 74.1 % ($P < 0.05$). Histopathology revealed more severe muscle edema and necrosis and infiltration of polymorphonuclear cells in group B24 than in group A24. We conclude that UW solution is more appropriate for rat heart preservation than UP solution.

Key words Preservation, heart, rat · Heart, preservation, rat · UW solution, heart, rat · Pittsburgh solution, heart, rat

Introduction

The excellent performance of University of Wisconsin (UW) solution [26] has been demonstrated experimentally and clinically for the preservation of many organs, including the liver [4], kidney [20], and pancreas [5]. Also, the superiority of UW solution to Euro-Collins (EC) [15], St. Thomas Hospital Cardioplegic (ST) [11], and Collins' M (CM) [28] solutions in heart preservation has been assessed. However, recently, Ohkado et al. [18] reported a new preservation solution for the heart containing a high concentration of histidine and lidocaine named University of Pittsburgh (UP) solution. They demonstrated the superiority of the solution to UW solution for heart preservation with the Langendorff heart model [10]. The toxic effect of UW solution on the heart muscle was also demonstrated.

In the present study, we compared the UW and UP solutions using a heterotopic heart transplantation model after prolonged cold preservation.

Materials and methods

Animals

Male Lewis rats (Charles River, Japan) weighing 190–250 g were used as donors and recipients.

Table 1 Composition of University of Wisconsin (UW) and University of Pittsburgh (UP) solutions (*HES*, hydroxyethyl starch)

Ingredient	UW	UP
NA^+ (mM)	20.0	80.0
K^+ (mM)	140.0	22.5
Mg^{2+} (mM)	5.0	6.0
Ca^{2+} (mM)	–	0.1
PO_4^{2-} (mM)	25.0	2.5
Glucose (mM)	–	11.0
Insulin (U/l)	100.0	10.0
Mannitol (mM)	–	20.0
L-Histidine (mM)	–	100.0
Adenosine (mM)	5.0	5.0
Lidocaine (mg/l)	–	100.0
Lactobionate (mM)	100.0	–
Raffinose (mM)	30.0	–
HES (%)	5.0	–
Glutathione (mM)	3.0	–
Allopurinol (mM)	1.0	–
Heparin (IU/l)	1000	–
pH	7.2–7.3	7.8

Table 2 Experimental groups (*CIT*, cold ischemia time; *UW*, University of Wisconsin solution; *UP*, University of Pittsburgh solution)

Group	CIT (hours)	Preservation solution	Experimental number		
			Graft survival	Histopathology	Other
A0	0	UW	10	6	6
A24	24	UW	10	7	6
B0	0	UP	10	6	6
B24	24	UP	10	7	6

Preservation solutions

The compositions of the UW and UP solutions used in the present study are summarized in Table 1. The UW solution was purchased from the DuPont Company (Du Pont Merch Pharmaceutical, Wilmington, Del., USA) and the UP solution was prepared by ourselves according to Ohkado et al.'s specifications [18] within 24 h before the experiment.

Procurement of donor hearts

The donor hearts were prepared according to the method described by Yano et al. [28]. Briefly, donor rats were anesthetized by inhalation of 1 % halothane and 99 % oxygen at a flow rate of 0.2 l/min. After intravenous heparinization (1000 IU/kg) from the penile vein, the abdominal cavity and the thoracic cavity were opened. The superior and inferior vena cava were clamped immediately after the thoracotomy. Hearts were arrested with 5 ml of the respective preservation solutions infused through the aorta, starting from the inferior vena cava, for better flushing of the blood in the cardiac chamber, and care was taken to avoid placing excess pressure on the right atrium. Simultaneously, slushed ice was placed around the heart. After arresting the heart, the superior and inferior vena cava were ligated and the aorta and pulmonary trunk transected. The pulmonary veins were ligated and the hearts were removed; they were immediately placed in 100 ml of the respective solution and preserved for 24 h at 4 °C.

Heterotopic heart transplantation

The hearts were transplanted heterotopically by end-to-side anastomoses with 9-0 nylon sutures between the donor's aorta and the recipient's aorta, and between the donor's pulmonary trunk and the recipient's inferior vena cava using the method of Ono and Lindsey [19]. Recipient rats were given free access to food and water after the operation, and the transplanted hearts were examined daily by palpation for 7 days in order to confirm the presence of a heartheat and to evaluate survival of the grafts.

Experimental groups

The animals were divided into four groups according to preservation solution and preservation time (Table 2).

Histopathology

The recipients selected for histopathological examination were anesthetized and underwent laparotomy 24 h after transplantation. The entire heart was removed, fixed with buffered formaldehyde solution, and stained with hematoxylin-eosin. The extents of muscle edema, muscle necrosis and polymorphonuclear cell infiltration were graded on a 5-point scale from 0 (intact) to 4 (most severe). Grading was done by one pathologist (SK) who had no information about the samples.

Tissue water content

Two hours after transplantation, the anesthetized recipient underwent laparotomy; its heart was removed quickly and placed in liquid nitrogen within 5 s. Equal parts of each of the frozen hearts were dried for 48 h at 110 °C in an oven, and the relative water content was calculated as (wet weight-dry weight)/wet weight, and expressed as a percentage.

Adenine nucleotides

A small, residual part of each of the frozen hearts was used for determination of tissue adenine nucleotides [adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP)]. About 100 mg of the tissue from the apical portion of the hearts was obtained from the frozen tissue. Soon after measurement of the tissue weight, the frozen tissue was homogenized in 1 ml of cold 6 % perchloric acid containing 0.8 mM ethylenediaminetetra-acetic acid with a Polytron homogenizer (Brinkmann, Westbury, N.Y., USA). The homogenates were centrifuged for 10 min at 10,000 *g* and at 4 °C with a refrigerated centrifuge (TOMY, High Speed Micro Refrigerated Centrifuge, MR-150, Tokyo, Japan). The pH of the supernatants was adjusted to 4–6 with 69 % K_2CO_3 solution and centrifuged again for 10 min at 10,000 *g*. Adenine nucleotides of the supernatant were measured by high-performance liquid chromatography (HPLC) using the method described by Hamamoto et al. [8]. Energy charge was calculated as (ATP + 1/2ADP)/(ATP + ADP + AMP).

Statistical analysis

An analysis of variance and a Fischer's exact test as a post-hoc analysis were used for the comparison of energy charge, tissue water content, and adenine nucleotides. A Kruskal-Wallis test, followed by the Mann-Whitney U-test as a post-hoc analysis, was used for the comparison of semiquantitative analysis of tissue injury and graft survival. Data are presented as the mean ± SD with 0.05 as the significance level.

Results

Graft survival

The 1-week graft survival rates of the immediate transplantation groups (A0 and B0) were 100%. One-week survival of grafts in group B was as low as 10% with 24 h of preservation, whereas that in group A hearts was 60% (Table 3).

Table 3 One-week graft survival

Group	Survival (days)	Survival (%)
A0	$>7\times6$	100*
A24	1/2, 3, 5, 4, $>7\times6$	60*
B0	$>7\times6$	100*
B24	$<1\times5$, 1, 1, 1, 4, $>7\times1$	10

* $P<0.01$ vs group B24 (statistical comparison made with the Kruskal-Wallis test followed by Mann-Whitney U-test as a post-hoc analysis)

Histopathology

The semiquantitative assessment of tissue injury is shown in Fig. 1. Tissue injury in groups A0 and B0 was almost the same and slight in comparison with that of 24-h preserved grafts. Muscle edema in group B24 grafts was more severe than that in group A24 grafts, although there was no statistically significant difference. Muscle necrosis was also severe in group B24 grafts compared to those in group A24 ($P<0.05$). The infiltration of polymorphonuclear cells was most severe in group B24 and was significant compared to that in group A24 ($P<0.05$).

Tissue water content

Tissue water content was 68.8% and 69.2% in the two 0-h preserved groups; there was no significant difference between them (Table 4). The 24-h preserved groups showed an apparent and comparable increase in tissue water content to 71.7% and 74.1% in groups A and B, respectively. However, the tissue water content of group B24 grafts was increased and comparable with all of the 0-h preserved groups as well as with the 24-h preserved grafts in group A.

Adenine nucleotides and energy charge

Adenine nucleotides are summarized in Table 4. There was no significant difference in adenine nucleotides and energy charge among the 0-h preserved grafts in the two groups, whereas with the 24-h preserved grafts, UW hearts demonstrated higher levels of ATP than UP hearts: 0.972 ± 0.165 and 0.200 ± 0.123 mg/g wet tissue, respectively, which is statistically significant. In group B, tissue ATP content decreased in 24-h preserved grafts compared with 0-h preserved grafts ($P<0.05$). In contrast, the tissue ATP content in group A did not change significantly after 24-h preservation. The energy charge of both 0-h preserved groups were identical statistically. There was a significant decline in energy charge in the B24 group that was comparable to that in the A24 and B0 groups (Table 4).

Discussion

Our present study demonstrates that UW solution performs better than UP solution in preserving the rat heart using the heterotopic heart transplantation model. The best graft survival rate of heart grafts preserved with UW solution corresponded well with a higher ATP level, a lower tissue water content, and a lesser extent of muscle injury. These findings support the conclusion of the study that showed the superiority of UW solution to Stanford or Collins solutions using the isolated canine heart model for 12 h at 4 °C [26].

UP solution is a highly buffered preservation solution containing a high concentration of histidine [18]. In the report that showed the superiority of UP solution to UW solution, the authors used the Langendorff heart model with an asanguineous perfusate to assess cardiac function after reperfusion following prolonged cold ischemia [18]. UP solution was developed based on the theory that by inhibiting intracellular acidosis, promoting anaerobic glycolysis positively, and removing detrimental end-products chemically during ischemia with histidine, high-energy phosphate compounds, which are indispensable for the maintenance of the integrity of the myocardium, can be produced effectively, leading to an extension of preservation time. However, in our study, we showed that UP solution is less effective for heart preservation.

To obtain maximum graft survival as well as to determine the optimal temperature for both solutions with prolonged organ preservation, several other groups of hearts were preserved at different temperatures (21 °C

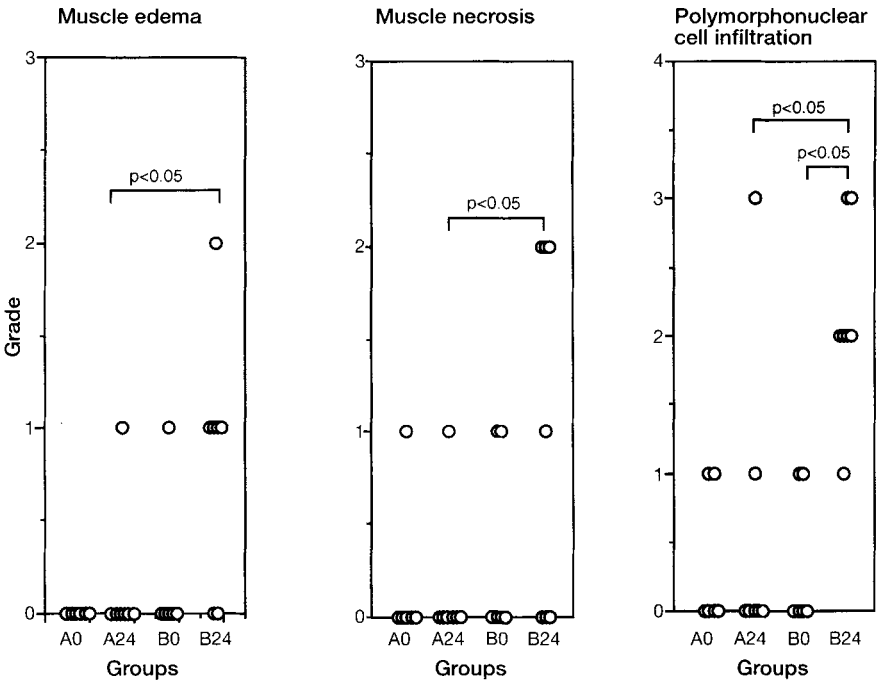

Fig. 1 Semiquantitative assessment of the cardiac muscle 24 h after reperfusion following 24-h preservation. The extents of muscle edema, muscle necrosis, and polymorphonuclear cell infiltration were graded on a 5-point scale ranging from 0 (intact) to 4 (most severe). Statistical comparison was made with the Kruskal-Wallis test, followed by the Mann-Whitney U-test as post-hoc analysis

Table 4 Tissue adenine nucleotides, energy charge, and water content {*EC* energy charge = [(ATP + ADP/2)/(ATP + ADP + AMP)]; *ATP*, adenosine triphosphate; *ADP*, adenosine diphosphate; *AMP*, adenosine monophosphate; *TWC*, tissue water content [(wet weight-dry weight)/(wet weight)]}

Group	EC (%)	ATP (mg/g wet tissue)	ADP	AMP	TWC (%)
A0	85.0 ± 3.39	1.600 ± 0.398	0.433 ± 0.193	0.130 ± 0.189	68.8 ± 1.33
A24	74.4 ± 9.64*,**	0.972 ± 0.165**	0.058 ± 0.022	0.476 ± 0.430**	71.7 ± 1.98**
B0	87.5 ± 4.18	1.733 ± 0.544	0.364 ± 0.162	0.041 ± 0.022	68.5 ± 0.84
B24	61.1 ± 27.2*	0.200 ± 0.123*	0.042 ± 0.043	0.182 ± 0.210	74.1 ± 3.75*

* $P < 0.05$ vs heart preserved for 0 h in the same group; ** $P < 0.05$ vs group B with the same preservation time

and 4 °C) for 6 and 12 h, respectively, in the two solutions. Six hearts were preserved in UP solution at 21 °C for 6 h since superior preservation of high-energy phosphates has been shown with moderate hypothermia (13 °C–21 °C) [18]; one of the six grafts survived for more than 7 days after transplantation (data not shown). Then, several other groups of hearts were preserved in both UW and UP solutions at 4 °C for 6 and 12 h, respectively; 100 % of the grafts survived longer than 7 days (data not shown) until sacrifice. Thus, the present study was carried out at 4 °C with the extension of hypothermic ischemia to 24 h.

Swanson and associates [26], using dog hearts, and Makowka and associates [13], using rat hearts, indicated that hearts stored in UW solution under hypothermic conditions showed significantly higher intracellular ATP levels after reperfusion than those stored in Stanford solution. Southard et al. previously demonstrated, using hypothermically perfused canine kidneys, that adenosine and phosphate together stimulate ATP synthesis and are effective in maintaining ATP at high levels for 3 days [24]. In the present study, ATP appeared to recover better in hearts preserved for 24 h in UW solution than in UP solution, which does not contain adenosine. These facts imply that preservation in UW solution not only reduces the loss of ATP but also protects the ATP-synthesizing system from ischemic injury. During ischemia, calcium accumulates due to enhanced activity of Na^+–Ca^{2+} exchange, decreasing Ca^{2+} uptake by sarcoplasmic reticulum. This accumulation of calcium promotes phospholipase and protease activity, increases intramitochondrial Ca^{2+} concentration, and promotes Ca^{2+}-dependent ATPase activity, leading to myocardial injury [17, 25]. Some 0.1 mmol/l Ca^{++} was added to UP solution since calcium paradox has been shown to occur with histidine in the absence of calcium [21]. However, the calcium in UP solution is might be beneficial to the myocardium only with multiple perfu-

sion of the Langendorff heart model, not in the preservation model with sample immersion in the solution. Hydrogen ion buffering was achieved in the UW solution by adding phosphates, whereas in the UP solution the buffering was obtained by the addition of histidine at a concentration of 100 mmol/l. Adenosine is present in both solutions, primarily to delay the rapid breakdown of high-energy nucleotides into more soluble nucleosides and to prevent the loss of soluble nucleosides during ischemia [6].

Preservation solutions should have the potential to prevent both intracellular and interstitial myocardial edema. Intracellular edema principally occurs due to the failure of the transmembrane Na^+-K^+ pump at hypothermia [12, 14], and interstitial edema results from the infusion of any solution that does not contain any oncotic agents. UW solution contains raffinose, a nonmetabolizable trisaccharide, and lactobionate, an anion; these two impermeants together provide an extracellular osmotic force that limits hypothermic cell swelling in the kidney, pancreas, and liver [27]. In addition, UW solution contains hydroxyethyl starch, which provides a colloidal oncotic pressure to limit the expansion of the interstitial space which, together with cell swelling, leads to tissue necrosis. In contrast, UP solution contains no components that are relatively impermeable across plasma or cell membranes. Thus, the components in UP solution are unlikely to maintain a normal distribution of intracellular and extracellular water. Changes in myocardial water content can alter compliance, and it is possible that a more normal distribution of myocardial water content at the end of the preservation period, as potentially afforded by UW solution, may accelerate the return to normal contractility on reperfusion and, thereby, improve early performance of the hearts.

Oxygen-derived free radicals are believed to play an important role in the genesis of tissue injury in the heart during ischemia and reperfusion when the endogenous protective mechanisms may become overwhelmed [7, 16]. Several studies have demonstrated that the addition of glutathione, an antioxidant, and allopurinol, a competitive xanthine oxidase inhibitor, to myocardial preservation solutions improves the recovery of postischemic function [1–3, 23]. Thus, in light of these studies, the inclusion of both glutathione and allopurinol in UW solution may also contribute to its superior preservation. The striking difference between the results of our study and those of Ohkado's is probably due to the experimental model and to the preservation temperature used in the respective experiments. Yet, in recent reports on reperfusion injury following ischemia, the importance of leukocytes has been pointed out [9, 22]. Since Ohkado et al. used an asanguineous reperfusion system, they could not examine the effect of the leukocytes upon reperfusion.

From the data obtained in this comparison, it can be concluded that the UP solution performed better at 4 °C than at 21 °C. Nevertheless, UW solution is superior to UP solution as it offers better myocardial protection and energy recovery after reperfusion.

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