Prolonged Rat Cardiac Preservation with UW Lactobionate Solution

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THE MOST significant limiting factor in the further expansion of clinical cardiac transplantation consists of the availability of suitable donors and the duration of viability of the organ following harvest. Currently, the safe limits of static cold cardiac preservation are considered to be four to six hours using the standard Stanford preservation solution as listed in Table 1. Belzer and colleagues have developed a preservation solution known as the University of Wisconsin (UW) lactobionate cold-storage solution (Table 1) which has demonstrated consistent 72 hour and 30 hour or longer preservation of the pancreas and liver, respectively.[1] Prompted by these successes and significant advances in static cold preservation, we compared UW-solution and the traditional Stanford solution for cardiac preservation in a heterotopic heart transplant model in rats. Additional pharmacologic manipulation with an antagonist of Platelet-Activating Factor (PAF), which has been shown to ameliorate ischemic liver injury,[2] and with an oxygen free radical scavenger (SOD) were evaluated.

MATERIALS AND METHODS

Animals

Inbred male Lewis (RT1[l]) rats (220-250 grams) were purchased from Harlan Sprague Dawley, Indianapolis, Indiana. Syngeneic heterotopic heart transplants were performed in all studies and animals were randomly assigned to the study groups.

Rats were acclimatized in a central animal facility for at least two weeks prior to investigation and received standard rat chow and water ad libitum.

Surgical Procedures

Rats were anesthetized with 40 mg/kg sodium pentobarbital (IP) and maintained with inhalational methoxyflurane.

Donor

Organs were prepared by entering the chest cavity, clamping the inferior vena cava, venting the heart and perfusing through the aortic arch with 2 cc of heparinized (200 u/ml) group specific preservation solution. The inferior and superior vena cavae were ligated and divided, and the other major vessels were divided simultaneously. A ligature was secured around the heart and the organ was then removed and placed in cold (4°C) group specific preservation medium for 12 or 18 hours of cold ischemic preservation time (CIT).

Recipient

The technique for surgical implantation of the primarily vascularized heterotopic cardiac transplant in rats has been described previously.[3] Briefly, an end-to-side anastomosis is performed between the donor and recipient aorta, as well as the donor pulmonary artery to recipient vena cava.

Graft Evaluation

Impulses of the transplanted hearts were monitored daily by palpation through the abdominal wall for a period of 3 days or until hearts ceased to function. The intensity of the cardiac impulse was graded on a scale of 0-4 (4 being a maximal impulse).

Experimental Groups

Animals were randomly divided into: Group 1: UW—12 hours CIT; Group 2: UW plus SRI 63-441—12 hour CIT; Group 3: UW plus SRI 63-441 plus SOD—12 hour CIT; Group 4: UW—18 hour CIT; Group 5: Stanford—12 hour CIT; Group 6: Stanford—18 hour CIT.

Pharmacologic Administration

SRI 63-441: The specific receptor antagonist of PAF, SRI 63-441, was supplied by Sandoz Research Institute (East Hanover, New Jersey) and its properties and pharmacology have been described elsewhere.[4] The powdered form (MW 662) was reconstituted in 0.68% sodium acetate buffer and 0.9% sodium chloride (pH 5.3) to a final concentration of 10 mg/ml, and placed in a warm water bath (26-28°C) for a period of 5 minutes. A single bolus injection of 20 mg/ml was administered to the donor via the inferior vena cava 4 minutes prior to harvest.

Superoxide Dismutase (SOD): Human recombinant SOD was supplied by Pharmacia AB, Uppsala, Sweden. It was diluted in 0.9% sodium chloride to a final concentration of 8 mg/ml and administered intravenously to the recipient at a dosage of 15 mg/kg at 2 minutes prior to revascularization of the heart.

Pathologic and Biochemical Evaluation

Biventricular segments of heart were formalin fixed and submitted for routine paraffin embedding. Hematoxylin and eosin (H + E) slides were prepared and morphological assessment of cardiac injury was performed using a semi-quantitative scale of 0 to 4 (4 being most severe). Hearts were assigned a score of 5 if they failed to function immediately. In functioning cardiac grafts, the myocardium was assessed for the following; myocytolysis (dissolution of myocytes), myocardial necrosis and inflammatory infiltrate. The degree of injury was measured by the extent of myocardium involved in the biventricular section, 1 < 10%, 2 ≥ 10% and ≤ 30%, 3 ≥ 30% and < 60%, 4 ≥ 60%.

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Table 1. Composition of Cold Storage Solutions

UW-Lactobionate		Standard	
Dilute to 1 liter with sterile water		To 1 liter of 5% Dextrose	
K-lactobionate*	100 mmol	Mannitol	12.5 grams
		Sodium bicarbonate	25 MEQ
Raffinose	30 mmol	KCL	20 MEQ
KH_2PO_4	25 mmol		
$Mg SO_4$	5 mmol		
Adenosine	5 mmol		
Glutathione	3 mmol		
Insulin	100 U		
Bactrim	0.5 ml		
Dexamethasone	8 mg		
Allopurinol	1 mmol		
Hydroxethyl starch solution*	50 grams		

*A generous gift from American Critical Care, McGraw Park, IL.

Measurements of ATP levels were performed for the 12-hour CIT group at 3 and 7 hours following revascularization of the heart and at postoperative day 3. At sacrifice, the heart was excised and rapidly frozen with a Wallenberger clamp precooled in liquid nitrogen. The frozen sample was used to determine the concentration of ATP according to the methodology described by Lamprecht and Trautschold.[5] The animal's own heart served as a control.

Statistics

Graft survival times and ATP levels for each of the experimental groups were expressed as a mean and standard error of the mean and were compared using Student's t-test. For histologic analysis, the group ischemic scores were tested for differences in distribution using a non parametric rank sum. A p value of <0.05 was considered statistically significant.

RESULTS

Cardiac survival after 12 and 18 hours of CIT is summarized in Table 2.

Twelve Hour Preservation

Grossly, upon revascularization of the transplanted organ, all hearts including those UW-grafts treated with either SRI 63-441 alone or in combination with SOD, developed an immediate and effective impulse. No difference was noted between any of the groups for the interval between revascularization and the initial contraction. In all but one case (Group 5), hearts maintained a 3-4+ impulse until sacrifice at postoperative day 3. While these grafts continued to function as determined by palpation, abnormalities such as edema, hemorrhagic areas and paleness in color of the grafts were evident at the time of revascularization, and were more pronounced in the hearts stored in the Stanford solution.

18 Hour Preservation

Hearts preserved in the Stanford solution, for the most part, failed to function immediately. One graft survived with a slight impulse for 5 hours, while all others became severely edematous and hemorrhagic immediately following revascularization. Conversely, 66% of the cardiac grafts stored in UW survived until the time of sacrifice at postoperative day 3. Generally, the interval between revascularization and initial impulse was prolonged in hearts preserved in UW-lactobionate and all hearts failed to achieve a 4+ impulse.

It is evident from the graft survival data in Table 2, that between 12 and 18 hours of preservation there is a significant influence of the UW solution on graft viability in the heterotopic position. Administration of SRI 63-441 either alone or in combination with SOD did not further augment the effect of the UW solution on cardiac preservation either in terms of graft survival (Table 2) or as exhibited by the degree of morphologic injury present (Fig 1).

The ability of UW to maintain myocardial energy levels is also apparent for the 12 hour CIT group as evidenced by the ATP levels measured at 3 hours postoperatively (UW: 1.89 ± 0.24 versus S: 0.62 ± 0.16, $p < 0.001$). Values for the UW and Stanford grafts at three days were not statistically different as compared to native hearts.

Table 2. Cardiac Survival after CIT

Group	Solution	N	Cold Ischemic Time (hours)	Graft Survival (days)
1	UW	14	12	3.00 ± 0.00
2	UW + SRI 63-441	7	12	3.00 ± 0.00
3	UW + SRI 63-441 + SOD	7	12	3.00 ± 0.00
4	UW	8	18	2.50 ± 0.27*
5	Stanford	13	12	2.80 ± 0.1
6	Stanford	8	18	0.03 ± 0.03

*$p < 0.001$ for UW compared to Stanford at 18 hours CIT (t-test).

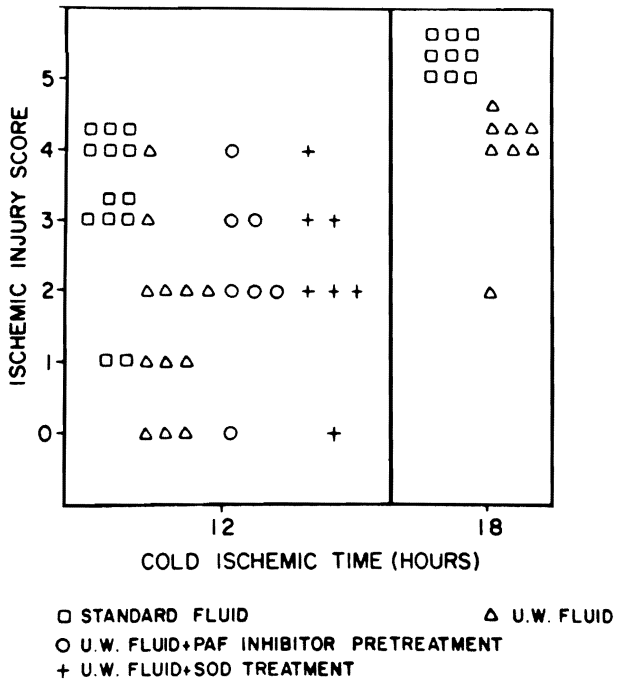

Fig 1. The ischemic scores (y axis 0-5) are summarized at two time intervals; 12 and 18 hours of preservation. At both time points the ischemia score is significantly different ($p < 0.01$) when comparing UW solution to the Stanford solution. No additional improvement was noted in SOD and/or SRI 63-441 treatment as compared to UW preservation alone.

All cardiac grafts were evaluated in a blind manner and ischemic scores were assessed. The nature of the myocardial injury did not appear to be influenced by the preservation protocols. The extent of injury was less in the hearts preserved in UW-lactobionate solution as shown in Figure 1. SOD or SRI 63-441 treatment did not further enhance the preservation as reflected in the ischemic score received (Fig 1).

COMMENTS

These studies demonstrate a significant difference in the degree of cardiac injury after 12 and 18 hours of static cold preservation in the UW and Stanford solutions as determined by cardiac survival, ATP levels and histologic evaluation. However, these represent less significant improvements when one considers the degree of morphologic myocardial injury present at 3 days post transplant. This raises considerable doubt as to whether hearts preserved for this time period could support the circulation in an orthotopic position. Nevertheless, the comparison of the traditional Stanford solution to the new UW solution in this model, allows some insight into the quality of cardiac preservation that can be achieved. The results presented suggest that with further modification of the UW preservation solution, extended cardiac preservation and viability for transplantation may be achieved.

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