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Thoracic

PBSH: A New Improved Cardiac Preservation Solution in Comparison With Three Clinically Proven Solutions

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ABSTRACT

Introduction. A solution under development at our institute, based on phosphate-buffered sucrose, has shown good preservation for kidneys and livers. This work used a refined version of this solution (PBSH—phosphate-buffered sucrose for the heart) in heart preservation, comparing it to solutions already widely used (University of Wisconsin, St. Thomas's, and Celsior solutions).

Methods. Following an initial washout phase and control working mode on a Langendorff system, hearts were flushed with preservation solution and after 6 hours at 4°C were then reperfused for 15 minutes followed by working heart mode for a further 30 minutes. Hemodynamic parameters were measured and compared with their preischemic values and expressed as percentage recovery. Enzyme measurement came from the collection of the initial 1.5 mL of the coronary effluent after the storage period. This was to test for creatine phosphokinase (CPK) and lactate dehydrogenase (LDH). Hearts were then placed immediately into liquid nitrogen for adenosine triphosphate (ATP), lactate, and creatine phosphate (CP) testing. Spectrophotometric analysis was used to assess both the release of CPK and LDH into the coronary effluent, and the level of ATP, lactate, and CP in the frozen heart tissue.

Results. These results show that hearts that are preserved in PBSH are hemodynamically as well preserved as the hearts preserved in other solutions tested and their enzyme and lactate content is lower while having higher levels of energy compounds in these hearts.

Conclusion. Overall these results show that PBSH is at least as effective in cardiac preservation in the rat model of 6 hours of cold ischemia as these other widely used solutions tested.

ONE of the ideals of transplantation is to prolong the period of effective organ preservation so that the transplantation can be planned and carried out as a routine surgical procedure, which would allow the recipient to be immunologically manipulated prior to receiving the organ. Currently, cardiac preservation is limited to 4–6 hours, which is much shorter than that for abdominal organs, with

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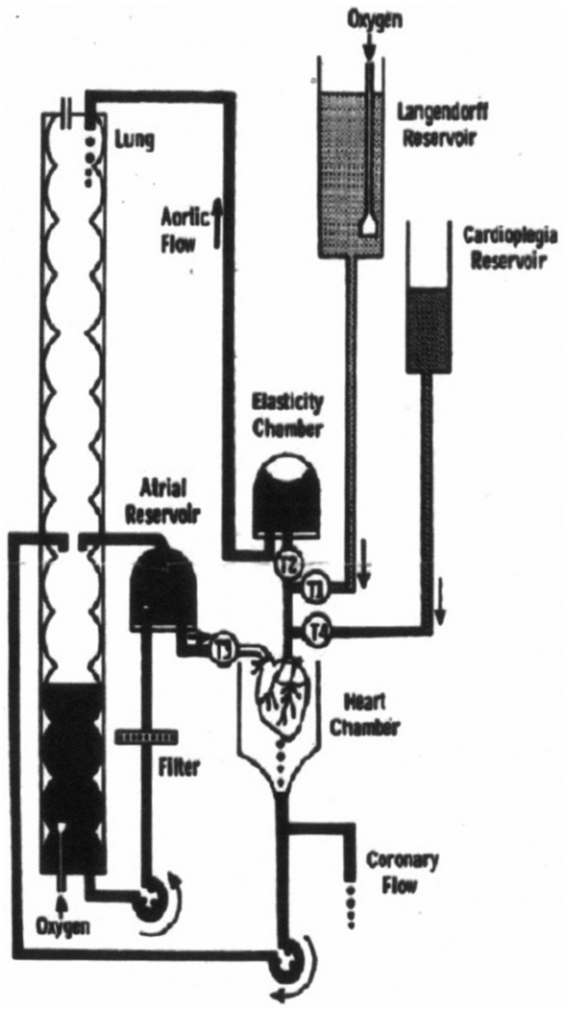

Fig 1. The Working Heart Model.

kidneys being safely preserved for 24 hours, livers for 16 hours, and pancreata for 12 hours.

Integral to this ideal is the need for the organ to function immediately and effectively following the prolonged period of preservation. Thus the quality of organ preservation must not be compromised by the lengthening storage times.

A solution has been under development at our institute, based on phosphate-buffered sucrose, which has shown good preservation for kidneys and livers.[1,2] This work used a refined version of this solution, PBSH (Phosphate-Buffered Sucrose for the Heart), to be used in heart preservation and compared it with solutions that are already widely used.

METHOD

Figure 1 shows the Langendorff model in the Working Heart Mode, which was used in all of the experiments for this research.

These experiments were carried out under Home Office licence according to standard guidelines for animal care. Male Wister rats (280–340 g) were anesthetized with a single intraperitoneal injection of pentobarbitone (0.6 μg/g). Following midline and subcostal laparotomy, hearts were removed and mounted on the initial Langendorff model for a washout and equilibrium period of 2–3 minutes.[3] During this period cannulation of the left atrium took place.

The perfusion solution used throughout the experiment when in conjunction with the Langendorff system was Krebs-Henseleit Buffer.

The heart was then switched to working mode for 15 minutes, during which time hemodynamic parameters were measured so that each heart could act as its own preischemic control.

Hearts were flushed with 25 mL of the preservation solution and left in the same solution at 4°C. The solutions used to compare against PBSH (n = 7), were ones that are used for heart preservation in a clinical setting (Table 1). They were as follows: St. Thomas's Hospital solution (STH; n = 8), University of Wisconsin solution (UW; n = 7), and Celsior solution (CS; n = 7). Table 1 shows a comparison of the compositions of the solutions used in this research.

After 6 hours the hearts were then reperfused initially in a Langendorff model for 15 minutes followed by working perfusion for 30 minutes.[4] Hemodynamic parameters were measured, compared with their preischemic values, and expressed as percentage recovery. Aortic flow (AF) was measured using timed collection into a graduated container prior to return to the oxygen chamber. Coronary flow (CF) was returned from the heart chamber through an opening in the base and measured using timed collection of the effluent in a graduated cylinder (Table 2).

For the measurement of the enzymes the initial 1.5 mL of the coronary effluent was collected immediately after the storage period. This was to test for creatine phosphokinase (CPK) and lactate dehydrogenase (LDH). The hearts were then placed immediately into liquid nitrogen so that they could be tested for the

Table 1. Components of Preservation Solutions

Components	PBSH	UW	CS	STH2
Phosphate (mmol/L)	60	25		
Sodium (mmol/L)	116	30	100	120
Potassium (mmol/L)	16	125	15	16
Magnesium (mmol/L)	5	5	13	16
Calcium (mmol/L)	0.6		0.25	1.2
Bicarbonate (mmol/L)				10
Chloride (mmol/L)			41.5	149.2
Sucrose (mmol/L)	100			
Lactobionate (mmol/L)		100	80	
Mannitol (mmol/L)			60	
Glucose (mmol/L)				10
Raffinose (mmol/L)		30		
Histidine (mmol/L)			30	
Glutamate (mmol/L)			20	
Hydroxyethyl starch (mmol/L)		50		
Allopurinol (mmol/L)		1		
Diltiazem (mmol/L)	0.5			
Adenosine (mmol/L)		5		
Glutathione (mmol/L)		3		
Dexamethasone (mmol/L)		16	3	
Insulin (unit/L)		40		
Osmolarity	310	320	360	320
pH	6.9	7.4	7.3	7.8

Table 2. Preischemic Hemodynamic Values

	STH2	UW	CS	PBSH
No.	6	6	6	6
HR (beats/min)	283.2 ± 5.2	281.4 ± 3.2	288.6 ± 4	281.4 ± 3.7
SP (mm Hg)	118.6 ± 1.5	123.6 ± 0.8	123.1 ± 2.5	130 ± 5.4
AF (mL/min)	55.4 ± 2.7	58.7 ± 2.8	63.1 ± 5.3	55.5 ± 4.4
CF (mL/min)	21.2 ± 0.7	24.3 ± 0.6	22.9 ± 0.9	23.7 ± 1.7
CO (mL/min)	76.6 ± 2.6	83.0 ± 3.2	86.0 ± 4.7	79.2 ± 5.3

Abbreviations: HR, heart rate; SP, systolic pressure; CO, cardiac output; STH2, St. Thomas's Hospital solution.

adenosine triphosphate (ATP), lactate, and creatine phosphate (CP).

Spectrophotometric analysis was used to assess both the release of CPK and LDH into the coronary effluent, and the level of ATP, lactate, and CP in the frozen heart tissue.[5]

Statistics

All data was expressed as mean ± SEM for the group in each of the experiments. The statistical tests used were Student *t* test and one-way analysis of variance (ANOVA) using Dunnett test for multiple comparisons with a control and Tukey test for multiple comparisons. Statistical significance was assumed when $P < .05$. The program software used was MINITAB version 12.

RESULTS

Preischemic control values were compared to see if there was any difference between the 4 groups of hearts, and no significant difference was found in terms of heart rate (HR), systolic pressure (SP), AF, CF, or cardiac output (CO). Therefore, all 5 groups were comparable in terms of preischemic hemodynamic function.

No significant difference was found between any of the groups in regard to the percentage recovery of the HR (Fig 2). The percentage recovery of the SP was significantly higher in PBSL than in STH, UW, and CS ($P < .001$) but there were no significant differences between these 3 ($P = .63$).

PBSH has a significantly higher recovery of AF ($P < .02$) than STH, UW, and CS solutions, but there were no significant differences between these 3 ($P = .83$; Fig 3). PBSH has a statistically higher recovery of CF ($P < .01$) than UW and CS, but does not reach significance in comparison with STH. CO is probably the most clinically relevant of the hemodynamic functions and in this parameter PBSH has a statistically higher recovery ($P < .01$) than STH, UW, and CS, but there were no significant differences between these 3 ($P = .83$).

The release of CPK was found to be significantly higher in the coronary effluent of hearts preserved in UW ($P < .01$) then those hearts preserved in STH, CS, or PBSH (Fig 4). Although it did not reach significance when compared with STH or CS, there was a trend toward lower release of CPK from hearts preserved in PBSH. LDH release was found to be highest in hearts preserved in UW and this reached significance compared with those hearts preserved in CS and PBSH, but not those preserved in STH.

ATP and CP were found to be significantly higher in unpreserved heart tissue than when the hearts were preserved in any of the preservation solutions (Fig 5). ATP content of heart tissue preserved in PBSH was higher than in those preserved in other solutions, but only reached a significant difference when compared with those preserved in UW solution ($P < .02$). CP content of heart tissue preserved in PBSH or CS is higher than those preserved in the other solutions, but only reached significance between PBSH and STH solutions ($P < .02$).

These results showed that hearts that were preserved in any of the 4 preservation solutions tested had statistically higher levels of lactate in their tissue than normal heart tissue because the level found in control hearts was statistically lower than any of the others.

Although not reaching significance, there was a trend toward a lower level of lactate found in PBSH-perfused hearts than the others.

DISCUSSION

These results show that, overall, hearts that are preserved in PBSH are in a hemodynamically superior condition to hearts preserved in other solutions tested and their enzyme and lactate content is lower while having higher levels of

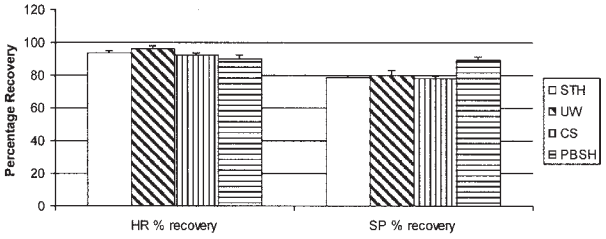

Fig 2. Percentage recovery of HR and SP.

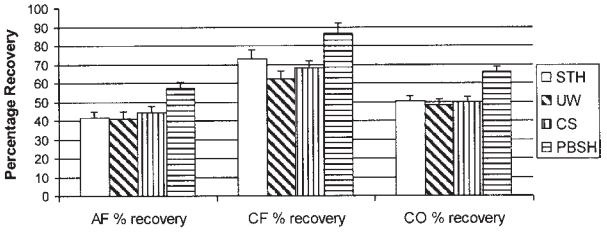

Fig 3. Percentage recovery of AF, CF, and CO.

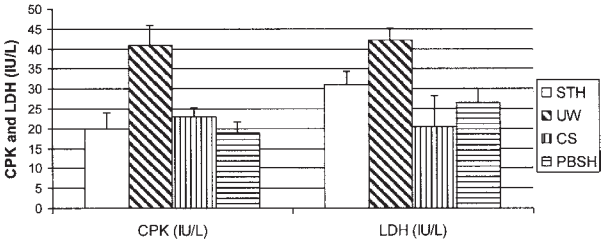

Fig 4. CPK and LDH release.

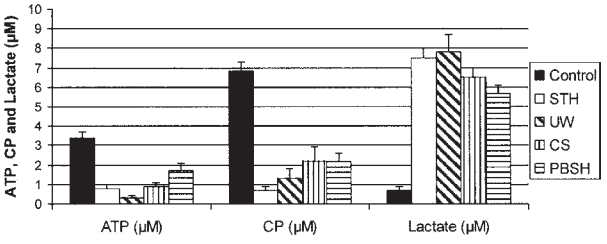

Fig 5. ATP, CP, and lactate levels in heart tissue.

energy compounds in these hearts. Hemodynamically PBSH is clearly a superior preservation solution to STH, UW, and CS.

High-energy phosphate compounds, including ATP and CP, are markers of energy preservation within the cardiac muscle after preservation. The higher the level of these compounds the better the quality of the organ preservation, because the more energy remaining in the heart the higher the probability that it will start working immediately. ATP was found to be higher in hearts preserved in PBSH than in any other solution, and this reached significance in the UW-preserved hearts.

Overall, these results show that PBSH is at least as effective in cardiac preservation in the rat model of 6 hours of cold ischemia as the other widely used solutions tested.

More work now needs to follow to look at retransplanting the hearts and using large animal models to see if this effectiveness continues to be seen posttransplantation.

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